CHAPTER 4

Coronavirus Receptors

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I. CORONAVIRUS SPECIES AND TISSUE TROPISMS

Coronaviruses are highly species-specific in that they generally cause disease in only one host species (Möstl, 1990; Wege *et al.*, 1982). However, experimental inoculation of other species with several coronaviruses, either by artificial routes such as intracerebral inoculation or in the highly susceptible neonatal period, can result in mild or asymptomatic infection as shown in Table I. In general, coronaviruses only infect cells from their normal host species or from species that are susceptible to infection with an antigenically related coronavirus (Table II). Host-dependent differences in susceptibility to coronavirus infection can be demonstrated within a species. For example, different strains of inbred mice vary greatly in their susceptibility to infection with various murine hepatitis virus (MHV) strains (Bang and Warwick, 1960; Stohlman *et al.*, 1980; Wege *et al.*, 1982).

Coronaviruses exhibit strong tissue tropisms *in vivo*. While coronavirus infections are usually initiated in the respiratory and/or enteric epithelium and some coronavirus infections are limited to these tissues, several coronaviruses can spread to specific other organs such as the liver, lymphoid organs, brain, peritoneum, or kidney. Different isolates of the same or closely related coronaviruses may exhibit differing degrees of tropism for enteric or respiratory epithelium. For example, more than 20 isolates of MHV are divided into two biotypes based on their initial site of replication in either the respiratory or enteric tract (Barthold, 1986).

In this chapter, we will discuss the role of virus receptors as determinants of the species specificity, host strain specificity, and tissue tropism of corona-

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The Coronaviridae, edited by Stuart G. Siddell, Plenum Press, New York, 1995.

TABLE I. Infection of Animals with Coronavirus

Coronavirus	Mouse	Rat	Cow	Turkey	Human	Pig	Dog	Cat	References
MHV	**d, D**[a]	b[b]							a
SDAV	d, A	**d, D**							b, c
BCV	d	b	**d, A**	a[c]	A				b, d, e, f, g
TCV				**d, D**					
HEV	b					**d, A**			h
HCV-OC43	d				**d, D**				b, i
HCV-229E					**d, D**			A	j
TGEV						**d, D**	a	A	k, l, m
CCV						a, A	**d, A**	a, A	n, o, p, q, r
FIPV						d, A		**d, D**	n, s

[a]Boldface indicates infection of natural host; nonboldface indicates infection of a foreign species.
[b]Lower case letters indicate neonatal animal; upper case letters indicate weanling/adult animal.
[c]a or A indicates asymptomatic infection following natural route of exposure; b or B indicates disease following intracerebral inoculation only; d or D indicates disease following natural route of exposure.
References: a, Cheever *et al.*, 1949; b, Barthold *et al.*, 1990; c, Bhatt *et al.*, 1977; d, Kaye *et al.*, 1975; e, Akashi *et al.*, 1981; f, Dea *et al.*, 1991; g, Storz and Rott, 1981; h, Hirahara *et al.*, 1992; i, McIntosh *et al.*, 1967; j, Barlough *et al.*, 1985; k, Larson *et al.*, 1979; l, McClurkin *et al.*, 1970; m, Reynolds *et al.*, 1979; n, Woods *et al.*, 1981; o, Woods and Wesley, 1986; p, Barlough *et al.*, 1984; q, Stoddart *et al.*, 1988; r, McArdle *et al.*, 1992; s, Woods and Pederson, 1979.

virus infection. It is important to note, however, that susceptibility to coronavirus infection and disease depends on many other host-dependent factors in addition to receptor availability and specificity, including intracellular determinants of virus replication and immunological responses to virus infection.

II. CORONAVIRUS ENVELOPE GLYCOPROTEINS THAT INTERACT WITH RECEPTORS

Coronavirus envelopes may exhibit either one or two envelope glycoproteins that interact with different cellular receptors. The virus attachment proteins are the spike glycoprotein (S), which forms the large peplomers characteristic of coronaviruses, and the hemagglutinin esterase (HE) glycoprotein, which forms short spikes in some coronaviruses in the MHV/OC43/BCV (bovine coronavirus) serogroup (Spaan *et al.*, 1988; Sturman and Holmes, 1985; Vlasak *et al.*, 1988a,b). Aspects of the S and HE glycoproteins that may be important for receptor interactions are summarized briefly here. These envelope glycoproteins will be discussed in detail in other chapters of this volume.

The S glycoprotein forms a trimeric spike that is responsible for virus attachment to specific receptor glycoproteins, mediates virus-induced membrane fusion, and induces neutralizing antibody and cell-mediated immune responses (Daniel *et al.*, 1993; Delmas and Laude, 1990; Rasschaert *et al.*, 1987). The S glycoprotein of BCV, like its HE glycoprotein, can bind to 9-*O*-acetylated sialic acid residues on host cell macromolecules (Schultze *et al.*, 1991a). Monoclonal antibodies to S inhibit virus infection and/or virus-induced cell fusion, and virions that lack S protein are not infectious (Daniel *et al.*, 1993; Holmes *et al.*, 1981; Rasschaert *et al.*, 1987). Sequencing of the genes encoding the S

TABLE II. Infection of Cultured Cells with Coronaviruses

	Species from which cell lines were derived									
Coronavirus	Mouse	Rat	Cow	Turkey	Human	Pig	Dog	Cat	Monkey	References
MHV	**+**[a]	+			+					a, b
SDAV	+	**+**								c
BCV			**+**		+	+	+		+	d, e, f, g, h
TCV				**+**	+					i
HEV					+	**+**	+			h, j
HCV-OC43					**+**				+	k
HCV-229E					**+**					
TGEV						**+**	+	+	+	l, m, n, o, p
CCV					+		**+**	+		p, q, r
FIPV								**+**		

[a]Boldface indicates infection of cells from normal host species; nonboldface indicates infection of cells from a foreign species. References: a, Lucas *et al.*, 1977; b, McIntosh *et al.*, 1969; c, Percy *et al.*, 1989; d, Gerna *et al.*, 1981; e, King and Brian, 1982; f, Schultze and Herrler, 1992; g, Dea *et al.*, 1980; h, Schultze *et al.*, 1990; i, Dea *et al.*, 1989; j, Vautherot *et al.*, 1992; k, Kapikian *et al.*, 1972; l, Ishii *et al.*, 1992; m, Welter, 1965; n, Barlough *et al.*, 1983; o, Horzinek *et al.*, 1982; p, Woods, 1982; q, LaPorte *et al.*, 1980; r, Evermann *et al.*, 1981.

glycoproteins of many coronaviruses indicates that there is a high degree of variability between different viruses and between different strains of the same virus (Cavanagh *et al.*, 1988). Newly synthesized S proteins of many coronaviruses can be cleaved at a cluster of basic amino acids near the center of the protein by trypsin or related host proteases associated with the Golgi apparatus to yield two subunits called S1 and S2 (Lai, 1990; Spaan *et al.*, 1988). S1, the amino-terminal subunit located on the tip of the spike, exhibits a high degree of variability. S2, the carboxy-terminal subunit, is predicted to include the stalk of the spike, the transmembrane, and intracytoplasmic domains. The extent of S cleavage depends on the type of coronavirus, the virus strain, and the host cell used to grow the virus. For several coronaviruses including BCV and rat coronavirus (RCV), addition of trypsin to the culture medium increases cell-fusing activity, infectivity, and/or plaquing ability (Gaertner *et al.*, 1991; Storz *et al.*, 1981). Mutations at the cleavage site of the S glycoprotein of MHV are associated with reduced virulence, delayed cell fusion, and virus persistence (Gallagher *et al.*, 1991; Kant *et al.*, 1992). Large insertions or deletions that occur near specific sites in S1 of some coronaviruses have been associated with altered receptor interactions, different tissue tropism, and altered virulence (Gallagher *et al.*, 1990; La Monica *et al.*, 1991; Wang *et al.*, 1992). For example, two closely related porcine coronavirus strains, transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV), that differ markedly in the lengths of their S1 glycoproteins cause either enteric or respiratory disease, respectively (Laude *et al.*, 1993).

The HE glycoprotein found on some coronavirus envelopes is a 120 to 140-kDa disulfide-linked dimer of 60 to 65-kDa monomers that forms a fringe of short spikes (Herrler *et al.*, 1991; Vlasak *et al.*, 1988a). HE is encoded in the genomes of coronaviruses in the serogroup that includes MHV, BCV, OC43, hemagglutinating encephalitis virus (HEV), and turkey coronavirus (TCV), but not in the genomes of the avian infectious bronchitis virus (IBV) or HCV229E groups of coronaviruses (Dea *et al.*, 1989; Hogue and Brian, 1986; Schultze *et al.*, 1990; Vautherot *et al.*, 1992). Whether or not full-length HE protein is expressed in cells infected with a particular virus strain is determined by the availability of an mRNA with a 5′-terminal HE gene and by initiation and termination codons in the gene. Thus, HE protein is found on envelopes of some MHV strains but not others (Yokomori *et al.*, 1991). Coronavirus HE is closely related in amino acid sequence to the HE glycoprotein of influenza C (Herrler *et al.*, 1991; Luytjes *et al.*, 1988). These HE proteins bind to 9-*O*-acetylated sialic acid receptor moieties, Neu5,9Ac_2, that are found on glycoproteins, glycolipids, and gangliosides on membranes of erythrocytes, enterocytes, brain, and other cell types *in vivo* and on cell lines from several species (Herrler *et al.*, 1987, 1991; Varki, 1992, 1993). Acetylation at the 9 position of sialic acid is developmentally regulated in various cell types of different species. The acetyl-esterase of HE releases the acetyl groups from the neuraminic acid, destroying virus-binding activity (Schultze *et al.*, 1991b). Treatment of virions with pronase selectively removes HE and inhibits hemagglutination (Dea *et al.*, 1989; Hogue and Brian, 1986; King *et al.*, 1985). Monoclonal antibodies to the HE glycoproteins can inhibit hemagglutination, neutralize virus infectivity, and inhibit esterase ac-

tivity (Parker *et al.*, 1989, 1990; Storz *et al.*, 1991; Vautherot *et al.*, 1992; Yoo *et al.*, 1992).

III. CORONAVIRUS BINDING AND PENETRATION

Coronavirus envelope glycoproteins may interact with specific cell membrane receptors at several steps during the virus replicative cycle. To initiate infection, virions bind to cell membrane receptors by either the S or HE glycoprotein, or possibly by sequential binding of HE followed by binding of S. Next, conformational changes in either the viral attachment glycoprotein, S, or the receptor, or both may be induced by virus binding and lead to fusion of the viral envelope with host cell membranes. This membrane fusion event has similar properties to the cell–cell fusion that occurs in cell cultures or tissues of animals infected with some coronaviruses (Barthold, 1986). MHV-A59-induced fusion is optimal at neutral or mildly alkaline pH (Sturman *et al.*, 1990), like that induced by Sendai and HIV, which fuse at the plasma membrane (White, 1990). For other coronaviruses, such as HCV-229E, IBV, or some MHV variants, infected cells generally do not fuse at neutral pH, and the viral envelope may fuse with endosomal membranes rather than the plasma membrane (Gallagher *et al.*, 1991). After synthesis of viral genomic RNA and proteins, virions mature by budding at intracellular membranes in a special pre-Golgi compartment and are transported through the Golgi apparatus into post-Golgi vesicles that probably fuse with the plasma membrane to release virions from infected cells (Griffiths and Rottier, 1992; Tooze and Tooze, 1985). Newly synthesized cellular glycoproteins that serve as virus receptors are probably also present in the endoplasmic reticulum and Golgi membranes, so that interactions of envelope glycoproteins with nascent receptors could occur in these compartments. Coronavirus virions tend to adhere to the plasma membrane of the host cell following release, forming dense mats of adsorbed virions (Oshiro, 1973), which suggests that elution of virions from receptors is inefficient. The viral spike glycoprotein, S, which is not incorporated into virions at intracellular membranes, is transported to the plasma membrane where it can bind to specific virus receptors on adjacent uninfected cells and initiate cell–cell fusion.

Attachment and penetration are complex processes that have not yet been studied in great detail for most coronaviruses. Initial binding of a single HE or S glycoprotein to a receptor may be quickly followed by the recruitment of additional receptors beneath the virion. Cooperative interactions of many envelope glycoproteins with receptors could prolong adherence of the virion to the plasma membrane. Subsequent fusion of the viral envelope with the plasma membrane or endosomal membrane may depend on the affinity of S for its receptor, the fusing activity of the S glycoprotein, and the lipid composition of the cell membrane. The early events in coronavirus–cell interactions are technically difficult to study due to the high ratio of noninfectious to infectious virions in coronavirus preparations and the tendency of S1 to detach from the virions (Boursnell *et al.*, 1989; Cavanagh *et al.*, 1986; Sturman *et al.*, 1990). Studies on the interactions of purified virus attachment proteins with purified

recombinant receptor proteins will provide additional useful information about receptor affinities.

IV. CARCINOEMBRYONIC ANTIGEN-RELATED RECEPTORS FOR MHV

As shown in Table I, MHV naturally infects only mice, although young rats can be infected following intracerebral inoculation. Inbred mouse strains show varying degrees of susceptibility to different MHV strains. Princeton mice (PRI) are fully susceptible and die from MHV-2 infection (Bang and Warwick, 1960). Similarly, BALB/c, C57Bl/6, and DBA mice are fully susceptible to fatal hepatitis from MHV-3 infection (Le Prevost *et al.*, 1975). In contrast, C3H mice are semiresistant to MHV-2, which produces lower virus titers and only mild or inapparent disease, and to MHV-3, which gives adult C3H mice chronic neurological disease but not hepatitis (Virelizier *et al.*, 1975). A/J mice, which are also semiresistant to MHV-3, do not develop disease, although virus titers in the liver are comparable to those of BALB/c mice (Dindzans *et al.*, 1986; Le Prevost *et al.*, 1975). Of all inbred mouse strains tested, only SJL mice are resistant to MHV-JHM-induced encephalomyelitis, and peritoneal macrophages from SJL but not other mouse strains are resistant to infection with MHV-JHM and MHV-A59 (Knobler *et al.*, 1984; Stohlman and Frelinger, 1978).

To determine whether receptors play a role in the mouse strain specificity and tissue tropism of MHV infections, Boyle *et al.*, (1987) studied binding of MHV virions to membranes from murine enterocytes or hepatocytes, which are the natural target tissues of MHV. In a solid-phase assay, MHV-A59 bound to membranes from MHV-susceptible BALB/c mice, but not to membranes from MHV-resistant SJL mice. This assay also showed that binding of MHV-A59 to intestinal brush border membranes is highly species specific, and no virus binding could be detected on membranes from the rat, cat, dog, pig, human, rabbit, or chicken (Compton *et al.*, 1992). A virus-overlay protein blot assay showed that in BALB/c liver and intestine only one 110 to 120-kDa BALB/c membrane glycoprotein, called the mouse hepatitis virus receptor (MHVR), bound MHV-A59 virions (Boyle *et al.*, 1987). Virus binding to proteins from SJL membranes was not detected. An anti-receptor monoclonal antibody, MAb-CC1, was generated by immunization of SJL mice with BALB/c intestinal brush border membranes. MAb-CC1 bound to the 100 to 120-kDa MHVR and to a 55 to 58-kDa glycoprotein in immunoblots of BALB/c liver and intestine membranes, blocked binding of MHV to cultured BALB/c or C3H fibroblasts, and protected them from infection with MHV-A59 and other strains of MHV (Dveksler *et al.*, 1991, 1993a; Williams *et al.*, 1990). Binding of radiolabeled MAb-CC1 to membrane preparations from different mouse tissues showed that the highest levels of MHVR protein were on colon, small intestine, and liver (Williams *et al.*, 1991). MHVR was not detected on other BALB/c tissues or on any SJL/J tissues by this MAb-CC1 binding assay. Thus, the small intestine and liver, which are major target tissues for MHV infection *in vivo*, express the highest levels of

MHVR. The mouse strain-dependent expression of MHVR also correlates with the susceptibility of different mouse strains to MHV infection.

Immunoaffinity chromatography of Swiss-Webster mouse liver using MAb-CC1 yielded a mixture of a 100- to 120-kDa and a 55- to 58-kDa glycoprotein (Williams *et al.*, 1990). Comparison of the N-terminal amino acid sequence of the 100- to 120-kDa glycoprotein, called MHVR, with known sequences revealed strong homology with members of the carcinoembryonic antigen (CEA) family of glycoproteins in the immunoglobulin superfamily (Williams *et al.*, 1991). The sequence of a full-length cDNA clone encoding MHVR predicted a mature MHVR protein of 424 amino acids with 4 immunoglobulinlike domains, a transmembrane domain, and a short intracytoplasmic domain (Dveksler *et al.*, 1991). The protein is highly glycosylated, with 16 predicted N-linked glycosylation sites. The coding sequence of MHVR is identical to that of mmCGM1 (BgpA), a murine CEA-related glycoprotein in the biliary glycoprotein (BGP) subgroup (Beauchemin *et al.*, 1989a). Expression of MHVR in a vaccinia virus vector yielded a recombinant glycoprotein that binds MHV and MAb-CC1. Human cells and hamster cells are resistant to infection with MHV-A59 virions, but when transiently or stably transfected with cDNA encoding MHVR, they become susceptible to MHV-A59 and all other strains of MHV tested. Infection of these MHVR-transfected cells can be blocked by pretreatment of the cells with MAb-CC1 (Dveksler *et al.*, 1991, 1993a). These data show conclusively that MHVR is a functional receptor for MHV and suggest that the block in MHV replication in cells of non-murine species is in receptor-dependent virus attachment and entry.

On the MHVR glycoprotein, the binding sites for the S glycoprotein of MHV and for MAb-CC1 are located on the N-terminal Ig-like domain (Dveksler *et al.*, 1993b). Several other members of the immunoglobulin superfamily of glycoproteins serve as receptors for viruses, including ICAM-1 for human rhinoviruses, CD4 for HIV, and poliovirus receptor for polioviruses (White and Littman, 1989). For each of these receptors, as for MHVR, the virus binds to determinants on the N-terminal immunoglobulinlike domain.

In addition to MHVR, liver and enterocyte membranes from BALB/c mice and other MHV-susceptible strains contain a 55- to 58-kDa glycoprotein that is recognized by MAb-CC1 (Williams *et al.*, 1990). From these tissues, cDNAs derived from several alternatively spliced transcripts of MHVR were cloned and sequenced. The 55 to 58-kDa glycoprotein is encoded by one of these transcripts which includes only the N-terminal and fourth Ig-like domain and transmembrane and cytoplasmic domains (Dveksler *et al.*, 1993a). The second and third Ig-like domains were deleted by splicing. Therefore, this protein was called MHVR(2d) to distinguish it from the original receptor, which is now called MHVR(4d) (Dveksler *et al.*, 1993a). Additional splice variants generated 2 and 4 domain proteins with longer cytoplasmic domains. When transfected into hamster fibroblasts, each of these new MHVR-related cDNAs encodes an MHVR glycoprotein isoform that is recognized by MAb-CC1 and that makes the cells susceptible to infection with MHV-A59 and other strains of MHV, as evaluated by development of viral antigens in the cytoplasm of cells, or by synthesis and release of infectious virions (Dveksler *et al.*, 1993a; Yokomori and

Lai, 1992a,b). Thus, in MHV-susceptible strains of mice there are at least four alternatively spliced transcripts of the *Bgp* gene that encode functional MHV receptors.

MHV-resistant adult SJL/J mice were studied to determine whether they express proteins homologous to MHVR. A polyclonal rabbit antibody directed against the 15 amino acids at the amino-terminus of MHVR and a polyclonal antibody directed against human CEA both recognized 55-kDa and 105- to 115-kDa glycoproteins in SJL/J intestinal brush border membranes (Williams *et al.*, 1990). In addition, reverse transcriptase polymerase chain reaction (RTPCR), Northern blot, and RNase protection assays were used to characterize cDNAs from SJL/J mice and outbred CD-1 mice that encode glycoproteins homologous to MHVR (Dveksler *et al.*, 1993a; Yokomori and Lai, 1992a,b). Sequencing of these cDNAs revealed major differences in the amino acid sequence of the N-terminal domain between MHVR and its SJL/J homologue, as well as minor differences in other domains. One of three potential N-linked glycosylation sites from the N-terminal Ig domain of MHVR was missing in the SJL homologue, which could result in the slightly smaller size of the resulting glycoproteins. The coding sequence of the SJL homologue is identical to that of mmCGM2 derived from outbred CD-1 mice (Beauchemin *et al.*, 1989b; McCuaig *et al.*, 1993). Both 2 domain and 4 domain isoforms of mmCGM2 were identified, and a transcript encoding an mmCGM2 with a longer cytoplasmic domain was cloned. No MHVR transcripts or genes could be found in SJL tissues by RTPCR or RNase protection assays, which indicates that MHVR and mmCGM2 are alleles of the same murine *Bgp* gene (Dveksler *et al.*, 1993a; Nedellec *et al.*, 1993). None of the mmCGM2 isoforms was recognized by MAb-CC1, and none bound MHV-A59 virions in solid phase binding assays or virus overlay protein blot assays (Dveksler *et al.*, 1993a; Williams *et al.*, 1990). Therefore, it was surprising that each of the recombinant mmCGM2 isoforms when expressed in hamster fibroblasts made the cells susceptible to infection with MHV-A59 (Dveksler *et al.*, 1993a). Differences in the characteristics of the N-terminal Ig domains of the BALB/c and SJL isoforms could account for the observed differences in virus and MAb-CC1 binding and receptor activity between MHVR and mmCGM2 isoforms. Transfection of mmCGM2 cDNA into SJL cell lines made them susceptible to MHV-A59 infection (Dveksler *et al.*, 1993a), suggesting that the block in coronavirus replication in these cells is at an early step in virus attachment or entry. The Bgp glycoproteins expressed in adult SJL mice may have weaker MHV-binding activity than the MHVR glycoprotein, and overexpression of a recombinant SJL protein may at least partially compensate for its weak binding activity. Direct measurement of affinity between MHV spike glycoproteins and receptor isoforms will be required to test this hypothesis.

Several cellular functions of the Bgp protein isoforms of mice and rats have been identified. These proteins may bind calmodulin and can serve as calcium-independent or calcium-dependent homophilic cell adhesion molecules (Culic *et al.*, 1992; Turbide *et al.*, 1991). The rat Bgp protein has ecto-ATPase activity (Lin and Guidotti, 1989), and Bgp proteins act as bile acid transporters in the liver (Sippel *et al.*, 1993). The expression of Bgps and other CEA-related glyco-

proteins is regulated in a tissue-dependent manner during development, and is often elevated in transformed cells (Beauchemin *et al.*, 1987). Consequently, a single cell type from an outbred CD-1 mouse or an SJL X BALB/c hybrid mouse may express several isoforms of MHVR as well as several isoforms of mmCGM2. How the expression of each of these related glycoproteins is regulated, how the proteins are targeted, and how they interact with each other or with other cell membrane molecules are not yet known. Immunolabeling of BALB/c tissues with MAb-CC1 shows that large amounts of MHVR antigen are expressed in BALB/c mice on the intestinal brush borders membranes, respiratory epithelium, bile canaliculi, endothelial cells, and proximal tubules of the kidney (Godfraind, in preparation). MHVR has also been detected by fluorescence activated cell sorting (FACS) analysis on B cells, macrophages, and fibroblast cell lines (Coutelier *et al.*, 1994). Thus, large amounts of receptor glycoproteins are expressed on the membranes of the respiratory and enteric tracts where virus enters the body and on hepatocytes which are a major target tissue for MHV infection, and receptor is also expressed on macrophages and endothelial cells which could play a role in spread of infection by viremia. It is not yet clear whether the expression of different levels of receptor isoforms in different tissues affect the tissue tropisms of diverse MHV strains.

V. AMINOPEPTIDASE N RECEPTORS FOR TGEV AND HCV- 229E

Coronaviruses serologically related to HCV-229E include canine coronavirus (CCV), feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV), and TGEV and PRCV. Unlike MHV, which binds only to intestinal brush border membranes from MHV-susceptible strains of mice, HCV-229E and serologically related coronaviruses bind to receptors on brush border membranes of several unrelated species (Table III) (Compton, 1988; Compton *et al.*, 1992). The species specificity of infection with these viruses appears to be determined by a step subsequent to virus binding.

The strategy used to identify cellular receptors for members of the HCV-229E group of coronaviruses was similar to that used to identify the MHV receptors. Monoclonal antibodies directed against membranes of virus-susceptible cells were prepared and tested for the ability to block virus infection, and the protein precipitated by that antibody was identified, cloned, and expressed in foreign species to be tested for virus receptor activity (Delmas *et al.*, 1992; Yeager *et al.*, 1992). Several MAbs directed against porcine cell membrane proteins were found to prevent infection with TGEV (Delmas *et al.*, 1992). These antibodies immunoprecipitated a 130- to 150-kDa membrane glycoprotein. N-terminal amino acid sequencing of the immunoprecipitated protein identified it as porcine aminopeptidase N (APN), a metalloprotease that is a class 2 glycoprotein (Ashmun and Look, 1990; Look *et al.*, 1986). Direct binding of TGEV to purified porcine APN (pAPN) was demonstrated (Delmas *et al.*, 1992). When the cDNA encoding pAPN was cloned and expressed in TGEV-resistant canine cells, the cells became susceptible to TGEV infection (Delmas *et al.*, 1992). Thus, pAPN is a functional receptor for TGEV.

TABLE III. Binding of Coronaviruses to Intestinal Brush Border Membranes of Different Species

Virus	Source of brush border membranes				
	Mouse	Human	Dog	Cat	Pig
MHV	**++**[a]	—	—	—	—
HCV-229E	±	**++**	+	+	+
CCV	—	++	**++**	++	++
FIPV	—	++	++	**++**	++
TGEV	—	++	++	+	**++**

[a]Boldface indicates binding to membranes from normal host; nonboldface indicates binding to membranes from a foreign species. ++, Strong binding; +, moderate binding; ±, weak binding; —, no detectable binding.

Similarly, a monoclonal antibody, MAb-RBS, directed against membrane glycoproteins on human cell lines was found to block HCV-229E infection of human WI-38 fibroblasts and HL-60 cells (Yeager *et al.*, 1992). This MAb also immunoprecipitates a 150-kDa glycoprotein that is recognized by MAbs directed against human APN (hAPN), which is also called CD13 (Look *et al.*, 1989). Murine fibroblasts are normally resistant to infection by HCV-229E, but they become susceptible to the virus following transfection with a cDNA clone that encodes hAPN, and infection is blocked by MAb-RBS (Yeager *et al.*, 1992). Interestingly, although hAPN is a functional receptor for HCV-229E, it is not a good receptor for TGEV which is serologically related (Holmes, in preparation). This observation suggests that other species-specific host factors may block critical steps in virus replication that follow virus binding to APN to determine the species specificity of infection with this group of coronaviruses.

The amino acid sequences and domain structures of APN and Bgp are unrelated, although both cellular glycoproteins can serve as receptors for one or more coronaviruses. APN is a zinc-binding protein that is found on the plasma membrane as a homodimer with its N-terminal domain anchored in the cytoplasm (Ashmun and Look, 1990). A short transmembrane region is followed by a stalk domain, and the catalytic site of the enzyme is on its globular carboxy-terminal domain near a HELAH consensus sequence.

In vivo, APN is found on enterocyte brush border membranes where it removes the N-terminal amino acids from oligopeptides generated in the gut lumen by endopeptidases. APN is expressed on myeloid tumor cells and may play a role in metastasis of melanoma, and APN at synaptic junctions helps to degrade neuroactive peptides (Ashmun and Look, 1990; Hersh, 1985). Because the S glycoproteins of some coronaviruses require protease cleavage to activate cell fusion and/or viral infectivity, it was important to determine whether the protease activity of APN plays a role in its receptor function. Several lines of evidence suggest that the S glycoprotein of HCV-229E may bind at or near the catalytic site of hAPN. MAb-RBS, which blocks virus binding to hAPN, also inhibits enzyme activity (Yeager *et al.*, 1992). A cDNA that encodes hAPN containing a 39 amino acid deletion that includes the HELAH sequence en-

coded a protein that was not recognized by MAb-RBS and could not serve as a receptor for HCV-229E when expressed in mouse fibroblasts (Ashmun *et al.*, 1992; Yeager *et al.*, 1992). Finally, chelation of zinc inactivated both the enzymatic activity of hAPN and its HCV-229E-receptor activity (Yeager *et al.*, 1992). Other evidence strongly suggests that the protease activity of hAPN or pAPN is not required for its coronavirus receptor function. Small inhibitors of the protease, actinonin and bestatin, did not block HCV-229E or TGEV infection (Delmas *et al.*, 1992; Yeager *et al.*, 1992), and point mutations that abolish enzyme activity did not reduce virus receptor activity (Yeager *et al.*, 1992; Ashmun and Holmes, in preparation). Recently, it has been shown that determinants that are essential for the TGEV-receptor interaction reside within a domain of pAPN that is distinct from the enzymatic site (Delmas *et al.*, 1994). In summary, the serologically related coronaviruses TGEV and HCV-229E, which cause disease in porcine intestine and human respiratory tract, respectively, both utilize APN glycoproteins of their normal host species as receptors. The APN glycoproteins appear to be used as receptors because of their expression on respiratory and intestinal epithelial cell membranes, and their enzymatic activity does not appear to play a role in coronavirus infection.

VI. CARBOHYDRATE RECEPTORS FOR CORONAVIRUSES

The observation that the HE glycoproteins of several coronaviruses in the MHV/BCV/OC43 serogroup bind to Neu5,9Ac_2 residues on erythrocytes like the HE glycoprotein of influenza C, suggested that this carbohydrate moiety might serve as a receptor determinant for these coronaviruses (Schultze *et al.*, 1990, 1991b; Vlasak *et al.*, 1988a,b). The acetyl esterase activities of the HE glycoproteins of BCV, HCV-OC43, and HEV, like that of influenza C virus, cleaves acetyl groups from Neu5,9Ac_2, rendering the erythrocytes resistant to agglutination by these viruses. Because 9-*O*-acetylated neuraminic acid moieties are found on a variety of cell types in many species (Varki, 1992, 1993), any coronaviruses that use this carbohydrate as their only receptor would be expected to have broader host range and tissue tropism than those of coronaviruses that use species-specific determinants of a particular glycoprotein such as murine CEA-related glycoproteins or APN. Several observations on BCV support this hypothesis. BCV, which expresses high levels of HE and esterase activity (Schultze and Herrler, 1992; Vlasak *et al.*, 1988a,b), infects cultured cells and tissues of more species than most other coronaviruses (Tables I and II). Pretreatment of several BCV-susceptible cell lines with neuraminidase or acetylesterase rendered the cells resistant to BCV infection, and resialylation with Neu5,9Ac_2 but not with nonacetylated sialic acids, restored susceptibility to BCV infection (Schultze and Herrler, 1992). Treatment of BCV virions with diisopropyl fluorophosphate, which inhibits the esterase activity of the HE glycoprotein, drastically reduces BCV infectivity (Vlasak *et al.*, 1988b).

Although coronavirus HE may facilitate virus binding to cells, interaction of the viral S glycoprotein with a specific glycoprotein receptor may be required for virus penetration. This hypothesis has not yet been tested directly because

viruses that express HE but not S glycoprotein are not available. However, MHV variants that express S but not HE are infectious *in vitro* and *in vivo*, indicating that HE is not essential for MHV infectivity (La Monica *et al.*, 1991; Yokomori *et al.*, 1991). The role of HE expression in determining coronavirus virulence is not yet understood. Because the purified S glycoproteins of BCV and HCV-OC43, in addition to the HE glycoproteins, bind $Neu5,9Ac_2$ (Kunkel and Herrler, 1993; Schultze *et al.*, 1991a), the experiments on modulation of virus susceptibility by removal or replacement of $Neu5,9Ac_2$ must be interpreted with caution. It is possible that some coronavirus virions first bind to cells by interaction of HE with 9-*O*-acetylated neuraminic acid residues on any macromolecule, and then their S glycoproteins bind to $Neu5,9Ac_2$ or another moiety on a specific receptor glycoprotein, leading to virus penetration and uncoating.

The expression of HE glycoprotein could affect the entry of coronaviruses into cells in several ways. HE might mediate initial attachment to cells and stabilize virus binding until S interacts with protein receptors to initiate membrane fusion. If the esterase activity of HE can cleave $Neu5,9Ac_2$ on mucins or on cells such as erythrocytes that lack the capability to internalize virus, then expression of HE would increase the probability that a virion would reach receptors on susceptible cells. HE in the Golgi apparatus of infected cells might cleave acetyl residues from $Neu5,9Ac_2$ on nascent cell membrane molecules, resulting in cells deficient in $Neu5,9Ac_2$. This might facilitate release of virions from the infected cells.

The coronaviruses, like picornaviruses and retroviruses, have evolved to utilize a variety of different receptor determinants for entry into susceptible cells. The characterization of receptor-binding sites on viral envelope glycoproteins and the selection and characterization of mutant viruses that utilize different receptors may elucidate the molecular mechanisms that determine coronavirus tissue tropism and species specificity.

VII. REFERENCES

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