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VIRUS-RECEPTOR INTERACTIONS IN THE ENTERIC TRACT

Virus-Receptor Interactions

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SUMMARY

Expression of specific virus receptors on the surface of intestinal epithelial cells or M cells can determine whether or not a animal is susceptible to infection with an enterotropic virus. Receptors for many animal viruses have been identified. The specificity of virus-receptor interactions clearly affects the species specificity of virus infection, and in some instances may be an important determinant of viral tissue tropism. In this paper, the specificity of coronavirus-receptor interactions is summarized. Porcine and human coronaviruses utilize aminopeptidase N as their receptors, but in a species-specific manner. Mouse hepatitis virus uses several rodent glycoproteins in the carcinoembryonic antigen family as receptors. In addition, some coronaviruses can interact with carbohydrate moieties on the cell surface. Understanding the molecular mechanisms of virus-receptor interactions may lead to development of novel strategies for the control of enteric viral diseases.

INTRODUCTION

The gastrointestinal and respiratory tracts are important sites of entry for many viruses. Some viruses replicate only in the epithelial tissues or underlying lymphoid tissues of the respiratory and/or GI tracts, causing local infections such as colds or gastroenteritis. Other viruses enter through the GI or respiratory tracts, and then disseminate to infect other tissues and cause systemic diseases.

To establish infection in the GI or respiratory tracts, a virus must first bind to an appropriate receptor on the cells of these tissues. Many different types of cellular macro-

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Table 1. Factors that affect the tissue tropism of enteric viruses

Tissue tropism of enteric viruses depends upon:
Virus survival to reach intestinal tract
Tissue specific expression of specific virus receptor
Cell and virus factors required for virus penetration
Cell factors required for virus replication and release
Cell and virus factors that affect cytopathic effects

molecules and carbohydrate moieties that are expressed on the apical surfaces of enteric and respiratory epithelial cells could potentially serve as specific virus receptors. Each virus encodes capsid proteins or envelope glycoproteins that have been optimized by natural selection to bind selectively to a specific receptor on the cell surface. The specificity of the virus-receptor interaction determines, at least in part, the species specificity and tissue tropism of virus infection. Among many factors that may also play a role in tissue tropism are virus requirements for a specific transcription factor, protease, or transport mechanism found only in differentiated target cells, age-dependent factors that affect tissue maturation and immunological response, and/or specific stages of the cell cycle (Table 1).

Many types of viruses can infect the gastrointestinal tract. In this paper, we will discuss receptor interactions of viruses that cause diarrhea in animals and/or man. Many of the principal viruses that cause enteric infections and their cellular receptors are shown in Table 2. For some other important enteric viruses, including caliciviruses, specific recep-

Table 2. Receptors for Some enterotropic viruses

Virus	Receptor
Transmissible gastroenteritis virus	Porcine aminopeptidase N (Delmas *et al.*1992)
Mouse hepatitis virus	Murine biliary glycoproteins (Dveksler *et al.*1991; Dveksler *et al.*1993)
Bovine coronavirus	9-O-acetylated neuraminic acid (Fenner & Woodroofe, 1965)
Human parvovirus B19	Erythrocyte P antigen (globoside) (Brown *et al.*1994)
Canine parvovirus	Sialic acid-containing oligosaccharides (Parrish, 1991)
Rotavirus SA11	Sialic acid containing oligosaccharides(Rolsma *et al.*1994)
Adenovirus	Integrins αvβ3, αvβ5, (for internalization) (Roivainen *et al.*1994)
Human cytomegalovirus	Heparan sulfate (Compton *et al.*1993)Human aminopeptidase N (for internalization) (Soderberg *et al.*1993)
Murine CMV	MHC Class I (Wykes *et al.*1993)
Echovirus 1 and 8	Alpha 2 subunit of integrin VLA-2 (Colston & Racaniello, 1994)
Echovirus 7, 13, 21, 29, 33	Decay-accelerating factor CD55 (GPI linked complement regulatory protein) (Clarkson *et al.*1995; Ward *et al.*1994; Bergelson *et al.*1994)
Coxsackievirus A9	Integrin αvβ3 (vitronectin receptor) (Roivainen *et al.*1994)
Coxsackie B viruses	100 kd protein in some cell types (Xu *et al.*1995) Decay-accelerating factor (Shafren *et al.*1995; Bergelson *et al.*1995)
Foot and mouth disease virus	Integrin (Berinstein *et al.*1995)
Poliovirus	Poliovirus receptor (Ig superfamily) (Mendelsohn *et al.*1989; Koike *et al.*1990)

tors have not yet been identified. Characterization of a virus receptor that is a cellular glycoprotein is often done by identification of specific binding of the virus to the cellular protein, cloning of the cDNA encoding the candidate receptor, expression of the cDNA in cells of a species normally resistant to infection, and demonstration that the putative receptor confers virus susceptibility upon the transfected cells (Dveksler *et al.*1991). Characterization of a specific carbohydrate moiety as a receptor for a virus requires a different strategy (Schultze & Herrler, 1994; Schultze & Herrler, 1993; Vlasak *et al.*1988b). Virus should bind to the purified receptor candidate, and the purified carbohydrate should interfere with binding of the virus to susceptible cells. Enzymatic removal of the carbohydrate moiety from glycoproteins or glycolipids on the cell membrane should protect cells from infection, whereas virus susceptibility should be restored by adding the moiety to the cell membrane using a glycosyl transferase or insertion of specific gangliosides into the membrane.

In viral diarrhea, the target tissue is usually the epithelial cells of the enteric epithelium. Viruses that infect these cells in the small intestine can exhibit still further tissue tropisms, in that they may selectively replicate in and destroy either cells at the tips of the villi (eg. coronaviruses, caliciviruses or rotaviruses) or cells in the villus crypt (eg. parvoviruses). Host responses to the virus infection and subsequent death of these cells include rapid repair of epithelial integrity by formation of new tight junctions between epithelial cells, infiltration of mononuclear inflammatory cells into the lamina propria, and development of local and systemic antibodies and cytotoxic T cells directed against viral proteins. Virus infections that kill epithelial cells in the villus cause shortening and blunting of the villi, leaving villi covered principally with immature epithelial cells that absorb nutrients and secrete fluids differently from the mature, highly specialized absorptive cells that normally cover the upper third of the villus. This results in viral diarrhea, by mechanisms which are not yet fully understood.

THE ROLE OF RECEPTORS IN CORONAVIRUS INFECTIONS

In this paper, we will summarize current information about specific virus-receptor interactions in the gut which are exhibited by different coronaviruses. Coronaviruses are an important cause of diarrhea in many species of domestic animals, particularly in young animals (Holmes & Lai, 1996). In adult animals, coronavirus infection of enterocytes often results in a mild or inapparent infection associated with shedding of the virus in the feces. Coronaviruses cause diarrheal disease in young pigs, cattle, dogs, cats and mice. As shown in Table 2, three different receptors for enterotropic coronaviruses have been identified. Murine coronavirus MHV utilizes several mouse biliary glycoproteins in the carcinoembryonic antigen family of the immunoglobulin superfamily (Dveksler *et al.*1991; Dveksler *et al.*1993; Nedellec *et al.*1994; Yokomori & Lai, 1992a); porcine transmissible gastroenteritis virus TGEV and human respiratory coronavirus HCV-229E use aminopeptidase N (APN) a membrane-bound metalloprotease (Yeager *et al.*1992; Delmas *et al.*1992); and bovine coronavirus BCV and hemagglutinating encephalomyelitis virus of swine (HEV) utilize a carbohydrate moiety, 9-O acetylated neuraminic acid as their receptors (Vlasak *et al.*1988b; Schultze & Herrler, 1994; Schultze *et al.*1993; Schultze & Herrler, 1993). The coronavirus receptor glycoproteins are expressed abundantly on the apical membranes of epithelial cells of the small intestine of the natural host species (Tables 3 and 4), an optimal site for invasion by a virus that enters the GI tract, as well as on other cell types (Godfraind *et al.*1995; Coutelier *et al.*1994; Huang *et al.*1990).

Table 3. Localization of Biliary glycoprotein isoforms

Fetus	Maternal-fetal interface in placenta
	Mesenchymal, then epithelial surfaces
Adult	Brush border membranes of small intestine, bile canaliculus, thyroid, kidney proximal tubule
	Respiratory epithelium, colon crypts and apical membranes, pancreas, prostate, salivary glands, lacrimal glands, and others
	Endothelial cells
	B lymphocytes, macrophages, thymic stromal cells

Most enterotropic coronaviruses cause disease only in one species (Holmes & Lai, 1996). For example, canine coronavirus (CCV) causes diarrheal disease only in dogs, and enterotropic strains of mouse hepatitis virus (HV), cause diarrhea only in mice. TGEV of swine causes epizootic diarrhea in newborn pigs, but not in other species.

For many coronaviruses, the spike (S) glycoprotein, a 150–200 kDa trimer, is the receptor-binding component of the viral envelope (Godet *et al.*1994; Delmas & Laude, 1990; Taguchi, 1995). In addition, a hemagglutination-esterase (HE) glycoprotein is found on the envelopes of some coronaviruses including bovine coronavirus (BCV), human coronavirus HCV-OC43, porcine hemagglutinating encephalomyelititis virus (HEV), and certain strains of MHV or rat coronavirus (RCV) (Gagneten *et al.*1995; Vlasak *et al.*1988a; Schultze & Herrler, 1994; Schultze *et al.*1993; Yokomori *et al.*1993). Both the S and the HE glycoproteins of BCV bind to 9-O-acetylated neuraminic acid moieties on glycoproteins or glycolipids on the cell surface (Schultze *et al.*1991). This paper will focus on the interactions of coronavirus S glycoproteins with cellular glycoprotein receptors Bgp and APN.

The first coronavirus receptor to be identified was MHVR (also called Bgp1a), a receptor for mouse coronavirus MHV (Dveksler *et al.*1991). A monoclonal antibody (MAb-CC1) that blocks infection of murine cells *in vitro* with the A59 strain of MHV (MHV-A59) was generated by immunization of MHV-resistant SJL/J mice with purified intestinal brush border membranes from MHV-susceptible BALB/c mice. Treatment of infant mice with anti-MHVR MAb-CC1 inhibits MHV infection *in vivo* (Smith *et al.*1991). Thus, the development of appropriate new drugs that block viruses from binding to specific receptors on cells of the enteric tract may provide new ways to control virus-induced diarrhea.

Affinity purification of the MHV receptor glycoprotein (MHVR) from the livers of MHV-susceptible mice showed that the receptor is a member of the carcinoembryonic antigen (CEA) family of glycoproteins (Williams *et al.*1991; Williams *et al.*1990). MHVR, or Bgp1a, has four immunoglobulin-like domains, a transmembrane region and a short cytoplasmic tail (Dveksler *et al.*1991). Expression of the recombinant MHVR glycoprotein in MHV-resistant human or hamster cells made them susceptible to infection with many

Table 4. Localization of aminopeptidase N

Intestinal brush border
Granulocyte membranes
Synaptic junctions
Respiratory epithelium

strains of MHV, and infection was blocked by pre-treatment of the MHVR-transfected cells with anti-MHVR MAb-CC1. Several isoforms of MHVR are generated by alternative splicing of Bgp1a transcripts, and each of these serves as a functional MHV receptor when the cloned cDNA is expressed in hamster cells (Dveksler *et al.*1993; Yokomori & Lai, 1992b).

Deletion mutagenesis of MHVR showed that MHV and MAb-CC1 both bind to the N-terminal Ig-like domain of MHVR (Dveksler *et al.*1993). A chimeric protein in which the N-terminal domain of MVHR was substituted for the N-domain of the mouse poliovirus receptor homolog (mph), another murine glycoprotein in the immunoglobulin superfamily, serves as a functional receptor for MHV-A59 when expressed in hamster cells (Dveksler *et al.*1995). Analysis of mutant MHVR glycoproteins showed that N-linked glycosylation of the N-terminal domain of MHVR is not required for MHV receptor activity (Dveksler *et al.*1995).

In addition to the gene encoding MHVR, mice express a related glycoprotein called Bgp2 which is encoded by another gene, located near Bgp1 on mouse chromosome 7 (Nedellec *et al.*1994). Expression of recombinant murine Bgp2 glycoprotein in hamster cells makes them susceptible to MHV-A59 infection. Some murine cell lines such as CMT93 cells express both MHVR (Bgp1a) and Bgp2 (Nedellec *et al.*1994). Although both of these glycoproteins can serve as functional MHV receptors when expressed in MHV-resistant hamster cells, treatment of CMT93 cells with MAb-CC1 which binds to MHVR but not to Bgp2, completely blocks MHV-A59 infection (Nedellec *et al.*1994). This observation suggests that MHVR and Bgp2 may interact and/or be co-localized in the membrane so that blocking MHVR by MAb-CC1 causes steric inhibition of virus binding to Bgp2.

SJL/J mice, which are highly resistant to infection with MHV-A59, express a variant allele of MHVR (Bgp1a), called Bgp1b (Dveksler *et al.*1993; Yokomori & Lai, 1992a). The N-terminal domains of Bgp1a, Bgp2, and Bgp1b differ markedly in their amino acid sequences (Nedellec *et al.*1994). For example, 29 of the 108 amino acids in Bgp1b are different from those in Bgp1a (Dveksler *et al.*1993). Anti-MHVR MAb-CC1 fails to bind to Bgp1b. When the recombinant Bgp1b glycoprotein is expressed in hamster cells, they become susceptible to infection with MHV (Dveksler *et al.*1993). Therefore, recombinant Bgp1b is a functional MHV receptor when expressed at high levels in hamster cells. However, for reasons that are not yet clear, Bgp1b appears to be a less efficient receptor than Bgp1a when expressed under its natural promoter at lower levels in murine cells, since Bgp1b on SJL/J intestinal brush border membranes fails to bind MHV-A59 virions, although this virus binds well to Bgp1a on intestinal brush border membranes from MHV susceptible BALB/c mice (Boyle *et al.*1987; Compton *et al.*1992). These observations suggest that a second host cell-dependent factor in addition to specific virus receptors may affect coronavirus-induced membrane fusion or virus uncoating (Yokomori *et al.*1993).

Two additional CEA-related glycoproteins of mice have been cloned and tested for receptor activity. They are Cea10, an anchorless, secreted glycoprotein cloned from the Cl 1D line of cells derived from C3H mice (J.-H. Lu, G. Dveksler, , N. Beauchemin, W. Zimmermann, and K.V. Holmes in preparation), and brain CEA (bCEA) (Chen *et al.*1995), an anchorless glycoprotein cloned from mouse brain. Cea 10 has no virus receptor activity, and substitution of its N-terminal domain for that of MHVR yielded a glycoprotein with no receptor activity (J.-H. Lu and colleagues, in preparation). In contrast, bCEA is a functional receptor for several strains of MHV, but, curiously, not for the neurotropic strain MHV-JHM (Chen *et al.*1995).

The localization of expression of the murine biliary glycoprotein Bgp1a on different tissues has been studied in mouse embryos and adult mice (Huang *et al.*1990; Godfraind

*et al.*1995; Coutelier *et al.*1994; Williams *et al.*1991). Table 3 shows that this MHV receptor is present in epithelial tissues of the gut and respiratory tract that represent common portals of entry for infectious agents. The receptor glycoprotein is detectable on all cell types that are susceptible to MHV, except those in the brain (Godfraind *et al.*1995). It is also present on macrophages and on endothelial cells throughout the body, which could facilitate spread of the virus by viremia. In addition, the receptor is expressed on some cells, such as the proximal tubules of the kidney, which are not susceptible to MHV infection, demonstrating that expression of a specific virus receptor on a particular tissue is necessary but not sufficient to permit virus infection of that tissue.

In marked contrast to MHV which is in coronavirus serogroup II, coronaviruses in serogroup 1, including HCV-229E, TGEV, CCV and feline coronaviruses FIPV and FeCV, do not use Bgp-related glycoproteins as receptors. Several monoclonal antibodies directed against intestinal brush border membrane proteins were found to block infection of porcine or human tissue culture cells with TGEV or HCV-229E, respectively (Delmas *et al.*1992; Yeager *et al.*1992). These MAbs bind to aminopeptidase N (APN, also called CD13), a metalloprotease that is a class II membrane glycoprotein expressed on intestinal brush border membranes and other tissues (Table 4). Expression in virus-resistant rodent cells of recombinant porcine or human APN makes the cells susceptible to infection with TGEV or HCV-229E, respectively (Delmas *et al.*1992; Yeager *et al.*1992). Interestingly, although porcine coronavirus TGEV can bind to intestinal brush border membranes of humans as well as pigs (S.R. Compton and K.V. Holmes, in preparation), TGEV infects pig cells but not human cells. Similarly, human coronavirus HCV-229E infects human cells but not porcine cells, although the virus binds to brush border membranes from both species. Although porcine cells are resistant to HCV-229E infection, porcine cells transfected with HCV-229E genomic RNA, an approximately 30 kb single stranded, plus sense RNA, become infected with the human virus and develop virus-specific antigens in the cytoplasm. These data suggest that a species-specific block in serogroup I coronavirus infection occurs after the stage of virus binding to the APN receptor glycoproteins, and prior to replication of viral RNA and expression of viral proteins.

Using chimeric porcine and human APN glycoproteins, a 100 amino acid long segment derived from porcine APN was found to be required for infection of transfected cells with TGEV. The mechanism by which this segment of the APN glycoprotein confers species-specificity for TGEV infection is not yet understood.

From studies of the receptor specificity of coronaviruses and other viruses, it is now becoming clear that the specificity of virus receptor interactions can be determined at one or more levels of the virus replicative cycle, including virus binding, penetration and uncoating, RNA replication or protein synthesis. Identification of a specific receptor for an enterotropic virus is just the first step in determining how the species specificity and tissue tropism of virus infection may be determined. Mapping of the domain of the receptor recognized by the virus attachment protein is necessary to determine whether the virus-binding site on the receptor is also required for the cellular function of the glycoprotein. Understanding of the specificity of virus-receptor interactions in the enteric tract may ultimately lead to development of new receptor-blocking drugs to prevent or treat enteric virus infection.

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