CHAPTER 12

Coronavirus Receptors

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Genetics of Mouse Strain Susceptibility to MHV

One of the classic examples of host resistance to virus infection is mouse hepatitis virus (MHV), a murine coronavirus. In susceptible mouse strains, various strains of MHV cause enteric, respiratory, and neurological diseases. Bang and his colleagues showed that MHV could cause a fatal infection in one strain of mice but not in another. Peritoneal macrophages isolated from these mouse strains showed susceptibility or resistance to MHV corresponding to that of the intact animal [1]. This difference in host susceptibility was due to a single autosomal gene, *hv-1,* and the allele for resistance to the fatal disease was recessive [2].

Many additional strains of mice have since been tested for susceptibility to various strains of MHV. The best-characterized host resistance system for MHV is the SJL/J mouse, which is profoundly resistant to both the JHM and A59 strains of MHV. These animals have an LD_{50} more than 1,000-fold higher than susceptible BALB/c mice [3], and macrophages and glial cells isolated from SJL/J mice are resistant to infection with MHV-JHM or MHV-A59 in vitro [4,5]. Genetic analysis of the SJL/J mouse showed that resistance to MHV is determined by a single autosomal recessive allele, *hv-2,* located on mouse chromosome 7 [4–6]. Cellular resistance to viral infection appears to be determined at a very early step in virus replication, such as adsorption, penetration, or primary translation of the genome.

Recent studies in our laboratory suggest that the molecular basis for the genetic resistance of SJL/J mice to MHV is the failure of these animals to express a specific receptor for MHV on the normal target cells for virus replication [7]. This chapter will describe the identification and character-

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ization of the MHV receptor and demonstrate its role in determining host susceptibility to virus infection.

Identification and Characterization of the 110-K Glycoprotein Receptor for MHV

A solid-phase virus-receptor assay was developed to detect binding of MHV to plasma membranes purified from the natural target tissues for MHV [7]. Brush border membranes isolated from the small intestine of susceptible BALB/c or resistant SJL/J mice were immobilized on nitrocellulose in a dot blot apparatus and then incubated with MHV-A59 virus. Virus bound to the intestinal brush border membranes was detected immunologically with antibody directed against the peplomer glycoprotein E2 and radioiodinated staphylococcal protein A. Virus binding to brush border membranes from BALB/c mice was directly proportional to the amount of membranes used, but no binding of virus to brush border membranes from SJL/J mice was detected. Similar findings were made with hepatocyte membranes from BALB/c and SJL/J mice. These data suggest that SJL/J mice are resistant to MHV infection because they fail to express a specific receptor for MHV on the membranes of the normal target cells for this virus.

To determine the molecular weight of the MHV receptor on BALB/c membranes, a virus-overlay protein blot assay (VOPBA) was used [7]. Membrane proteins from BALB/c or SJL/J intestinal brush borders or hepatocytes were solubilized in SDS, separated by SDS-PAGE, and blotted onto nitrocellulose sheets. The sheets were then incubated sequentially with bovine serum albumin to block nonspecific binding, MHV, anti-E2 antibody, and radioiodinated staphylococcal protein A. With BALB/c brush border membranes or hepatocyte membranes, MHV bound only to a single broad band with a molecular weight of approximately 100K to 110K. No virus-binding activity was found in SJL/J membrane proteins. The 110K protein from BALB/c brush borders was specific for MHV and did not bind other viruses that can also infect murine enterocytes, such as mouse polio or rotavirus.

Additional characteristics of the MHV receptor were determined. Treatment of BALB/c brush border membranes with deoxycholate solubilized MHV-binding activity, which could then be quantitatively adsorbed to beads coated with any of several lectins including concanavalin A or ricin 120 [8]. This showed that the MHV receptor is a glycoprotein. The 110K glycoprotein was excised from SDS-PAGE gels of BALB/c brush border membrane and treated with endoglycosidase-F to release N-linked oligosaccharides. Endo-F treatment reduced the apparent molecular weight of the receptor to approximately 70K, without substantially reducing virus-binding activity [9]. Receptor eluted from the 100K to 110K region of an SDS-PAGE gel was very sensitive to proteases, and treatment with proteases destroyed virus-binding activity. However, virus-binding activity was not inhibited by

treatment of the receptor with neuraminidase [8]. Taken together, the data show that MHV bound to an SDS-insensitive part of the 110K glycoprotein that was not removed by endo-F. Thus, the domain of the receptor that is recognized by the E2 glycoprotein of MHV is likely to be a linear sequence of amino acids.

Characterization of Antireceptor Antibodies

A second approach to the characterization of the MHV receptor was the development of antibodies directed against the receptor. A highly specific antireceptor antibody was developed by immunization of SJL/J mice, which do not express the MHV receptor, with extracts of intestinal brush border membranes from BALB/c mice, which do express the receptor [9]. In Western blots this polyclonal antibody recognized only the 110K receptor in BALB/c brush border membrane proteins, and the antibody did not bind to any proteins of SJL/J membranes. Pretreatment of MHV-susceptible mouse fibroblast cell lines with the polyclonal antireceptor antibody protected them from infection with MHV [9].

Hybridoma cell lines that produce monoclonal antibodies (MAbs) directed against the MHV receptor were derived from the spleens of mice producing antireceptor antibodies. More than 30 MAbs, which reacted in an enzyme-linked immunoassay with 110K receptor eluted from SDS-PAGE gels, were identified and tested for their ability to recognize the 110K receptor by immunoblot and the ability to protect L2 cells from infection with MHV-A59 [9]. Only three of the MAbs protected cells from infection. One of these MAbs, CC1, was studied extensively. It blocked infection of mouse fibroblasts with MHV-A59, MHV-JHM, MHV-3, MHV-S, and MHV-1 [9]. This indicates that, on the mouse fibroblast cell lines, the 110K glycoprotein receptor is the only receptor for all five of these prototype strains of MHV.

MAb CC1 recognized the 110K MHV receptor in Western blots of BALB/c brush border membranes, but it did not detect any protein band in SJL/J brush border membranes. This antireceptor MAb also reacted in dot immunoassays and in immunoblots with murine intestinal brush border membranes, but not with comparable brush border membrane preparations from other species [9]. Fluorescent antibody labeling of murine intestine showed that MAb CC1 specifically recognized the apical brush border of intestinal epithelial cells of BALB/c mice but failed to react with membranes of SJL/J intestine (Figure 12.1) [9]. Thus, all our results based on immunoblotting, inhibition of biological activity, and distribution of receptor activity on cells and tissues indicate that the antireceptor MAb CC1 recognizes the 110K glycoprotein to which MHV binds. Furthermore, the ability of MAb CC1 to inhibit MHV-binding activity indicates that the domain of the 110K glycoprotein recognized by MAb CC1 may be either close to or identical with the domain to which the E2 glycoprotein of MHV binds.

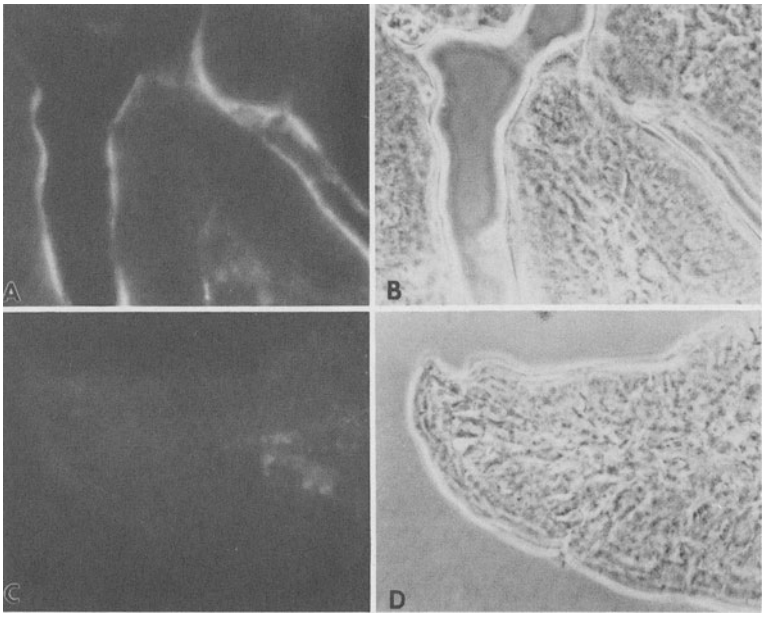

Figure 12.1. Expression of the glycoprotein receptor for mouse hepatitis virus on brush border membranes of mouse small intestine correlates with susceptibility of the mice to MHV. Monoclonal antireceptor antibody CC1 was used to detect the MHV receptor on frozen sections of mouse small intestine. BALB/c mice, which are highly susceptible to MHV infection, expressed the MHV receptor on brush border membranes **A** In contrast, SJL/J mice, which are resistant to MHV infection, did not express the receptor on their intestinal brush border membranes **C** Panels **B** and **D** are phase-contrast images of the same sections.

Affinity Purification of the Receptor Glycoprotein

MAb CC1 was used for affinity purification of MHV receptor from hepatocyte membranes from an MHV-susceptible mouse strain [9]. The amino-acid sequence of the first 15 amino acids from the N-terminus of this affinity-purified receptor was determined. A synthetic peptide corresponding to these 15 amino acids was conjugated with keyhole limpet hemocyanin and used to raise antiserum that recognized the 110K receptor from BALB/c intestinal brush border membranes in a Western blot, indicating that the derived sequence was indeed the MHV receptor [9].

The normal cellular function of the MHV receptor is not yet known. Receptors for viruses in several other groups have been identified, and these are often molecules that function as membrane receptors for such ligands as growth factors, hormones, or other cells [10]. Cloning, sequencing, and expression of the MHV receptor gene should help to identify both its normal cellular function and the domain that binds the E2 glycoprotein of MHV.

Role of Receptors in Coronavirus Species Specificity

One of the central questions about coronavirus receptors is whether they play a role in determining the marked species specificity of coronaviruses. Most coronaviruses infect only one species or a few closely related species [11]. For example, MHV causes natural infection only in mice and can be made to infect rats only by intracerebral inoculation of infant animals. Several experimental approaches were used to determine whether the species specificity of MHV infection is the consequence of the E2 glycoprotein of MHV binding selectively to a domain of the 110K glycoprotein that is only expressed on murine tissues. Immunofluorescence experiments on cell lines of different species showed that antireceptor antibody bound only to MHV-susceptible murine cell lines and not to cell lines from humans, hamsters, cats, or dogs [12]. Solid-phase, MHV-receptor assays using brush border membranes from nine species demonstrated that MHV-A59 bound only to brush border membranes from BALB/c mice and not to brush border membranes from human, pig, cat, dog, rat, or cow [12]. These experiments suggest that the species specificity of MHV is due to absence of the MHV-binding moiety of the receptor on cells of other species.

Coronavirus Hemagglutination

For some virus groups, receptors have been characterized by studying virus interactions with erythrocytes. For example, orthomyxoviruses and paramyxoviruses bind to glycolipids or glycoproteins on erythroctye membranes by an interaction of the viral hemagglutinin glycoprotein with N-acetyl neuraminic acid residues on the cellular macromolecules [10]. Although most coronaviruses do not cause hemagglutination, several coronaviruses, including bovine coronavirus (BCV), hemagglutinating encephalomyelitis virus of swine (HEV), human respiratory coronavirus (OC43), and some strains of infectious bronchitis virus (IBV), can cause hemagglutination [13]. For BCV, hemagglutination is caused by a 140K viral glycoprotein called HE, which is a disulfide-linked dimer of a 65K glycoprotein [14]. This hemagglutinin is not expressed in most strains of MHV, although an open reading frame homologous to the BCV hemagglutinin but lacking an initiator methionine has been found in the genome of MHV-A59 [15]. Surprisingly, this coronavirus hemagglutinin shares about 30% homology with the single glycoprotein of influenza C [15]. The hemagglutinating glycoproteins of both BCV and influenza C bind specifically to 9-O-acetylated neuraminic acid residues on erythrocytes and have esterase activity that inactivates 9-O-acetylated neuraminic acid–containing receptors on erythrocyte membranes [16]. This viral enzymatic activity has therefore been termed *receptor-destroying activity*. It is not yet clear, however, whether infection of susceptible cells in vitro or in vivo results from binding of BCV by means of

the interaction of the HE glycoprotein with 9-O-acetylated neuraminic acid residues on the cell or from the interaction of the E2 glycoprotein with a specific glycoprotein analogous to the 110K MHV receptor. Coronavirus-induced membrane fusion is a function of the E2 glycoprotein [17,18]. Therefore, even if the BCV virion should bind to susceptible cells via the HE glycoprotein, interaction of the E2 glycoprotein with some component of the cell membrane may be required for penetration of the viral nucleocapsid.

Several lines of evidence show that MHV-A59 does not bind to susceptible cells by recognizing 9-O-acetylated neuraminic acid–containing receptors. First, MHV-A59 does not express the HE glycoprotein. Second, MHV-A59 binds to only a single glycoprotein in VOPBA, whereas OC43, a serologically related human coronavirus that expresses an HE glycoprotein, binds to multiple proteins that presumably all bear 9-O-acetylated neuraminic acid moieties [19]. Third, endo-F treatment of purified 110K MHV receptor, which should remove any N-linked oligosaccharides bearing 9-O-acetylated neuraminic acid, does not prevent binding of MHV. Finally, neuraminidase treatment of murine cells, which should remove the 9-O-acetylated neuraminic acid residues, fails to prevent binding of MHV-A59.

In some other virus groups, the virus–membrane interaction associated with hemagglutination is not necessarily identical to the interaction that leads to infection of a susceptible cell. For example, the erythrocyte-binding hemagglutinin of polyomavirus is found on noninfectious particles, whereas a different domain of the viral capsid protein binds to a component of the plasma membrane of susceptible cells that is not present on erythrocytes [20]. Possibly the hemagglutinin of some coronaviruses may, like that of polyomavirus, be irrelevant in the infectious process.

Nevertheless, it is interesting to speculate upon the biological significance of the observation that two different viral glycoproteins can mediate attachment of some coronaviruses to cell membranes. Those coronaviruses, such as MHV-A59, canine coronavirus, feline infectious peritonitis virus, or human coronavirus 229E, that do not express the HE glycoprotein are nevertheless quite capable of causing disease and being transmitted naturally, so the HE glycoprotein is not essential for infectivity of a coronavirus. It would be quite interesting to induce expression of an HE glycoprotein in such a virus in order to study what effect it may have on the pathogenesis and epidemiology of the virus. Possibly the virulence, tissue tropism, or species specificity of these viruses would be altered significantly.

The two membrane-binding activities of coronavirus glycoproteins may provide some coronaviruses with a choice of two different ways to interact with cells in order to initiate infection. Alternatively, the hemagglutinin could be essential for some coronaviruses if their E2 glycoprotein fails to recognize a glycoprotein receptor on host cells. It would be interesting to delete or mutagenize the gene for the HE glycoprotein of a coronavirus that normally expresses it, such as BCV or OC43, in order to determine how absence of this protein affects viral replication and pathogenesis.

Sensitive assays to detect binding of many different coronaviruses to glycoprotein receptors, or 9-O-acetylated neuraminic acid receptors on their normal target cells, and clones of the viral glycoprotein genes that can be sequenced and expressed either separately or in concert are becoming available. In the near future, these will be used to elucidate the mechanism(s) by which coronaviruses bind to cells and initiate infection.

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