Chapter C10

CORONAVIRUS RECEPTORS

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Abstract: The major receptor for murine coronavirus, mouse hepatitis virus (MHV), is identified as a protein, cell-adhesion molecule 1 in the carcinoembryonic antigen family (CEACAM1), which is classified in the immunoglobulin superfamily. There are four CEACAM1 isoforms, with either four or two ectodomains, resulting from an alternative splicing mechanism. CEACAM1 is expressed on the epithelium and in endothelial cells of a variety of tissues and hemopoietic cells, and functions as a homophilic and heterophilic adhesion molecule. It is used as a receptor for some bacteria as well. The N terminal domain participates in mediating homophilic adhesion. This domain is also responsible for binding to the MHV spike (S) protein; the CC' face protruding in this domain interacts with an N terminal region of the S protein composed of 330 amino acids (called S1N330). The binding of CEACAM1 with MHV S protein induces S protein conformational changes and converts fusion-negative S protein to a fusion-positive form. The allelic forms of CEACAM1 found among mouse strains are thought to be an important determinant for mouse susceptibility to MHV.

Key words: CEACAM1, cell adhesion molecule, carcinoembryonic antigen, mouse hepatitis virus

1. INTRODUCTION

The Coronavirus family includes a number of different viruses that infect a variety of animal species, causing numerous diseases, mainly in organs of the enteric, respiratory and central nervous systems. They are

classified into three distinct groups in terms of serological cross-reactivity and sequence homology. Group 1 consists of porcine transmissible gastroenteritis virus, human coronavirus (HCoV) 229E, feline infectious peritonitis virus and so on. Group II includes mouse hepatitis virus (MHV), HCoV-OC43, bovine coronavirus and some others. Group III is comprised of avian coronaviruses; infectious bronchitis virus and turkey coronavirus. All of these viruses infect animals in a highly species-specific fashion, although some of them can experimentally infect animals different from their natural hosts. The receptor protein for group I viruses has been revealed to be an aminopeptidase N, while the receptor for MHV in group II is a protein classified in the immunoglobulin (Ig) superfamily. The receptors for other viruses in group II, as well as for viruses in group III, have not yet been identified. In this chapter, I describe the receptor for MHV as well as the interaction of MHV receptor and the virus spike (S) protein. The receptors of other coronaviruses can be found in a review article (1).

2. RECEPTORS FOR MHV

2.1 Discovery of MHV receptor proteins

MHV infects mice, but few other species. The major target organs are the liver, intestine and central nervous system. This host-species specificity or organ tropism of MHV has been thought to be determined mainly by the cellular receptor for MHV. A series of studies carried out by Kay Holmes and her colleagues, which began with analysis of differential susceptibility to MHV infection among mouse strains, has led to the finding of a major MHV receptor molecule. Boyle et al. found that the plasma membranes isolated from MHV-susceptible BALB/c mouse hepatocytes or enterocytes contained a 110 to 120-kDa protein that binds to MHV particles, but those derived from MHV-resistant SJL mice lacked such a protein (2). This finding suggested that the difference in MHV susceptibility among mouse strains is determined by this protein, presumably the MHV receptor. By using monoclonal antibody (MAb) CC-1 specific to 110-120 kDa protein from BALB/c, they purified a protein of ca. 110 kDa and determined the amino acid sequence in its N terminal region, from which the 110-kDa protein was postulated to be a glycoprotein classified in the carcinoembryonic antigen (CEA) family (3, 4). Finally, they isolated a gene encoding this protein, which was revealed to be cell adhesion molecule 1 in the CEA family of the Ig superfamily [formerly called biliary glycoprotein1 (Bgp1) and currently termed CEACAM1] (5). MHV non-permissive BHK cells transfected with this gene were converted to MHV-susceptible cells, indicating that this

molecule is the receptor for MHV. It was also found that MHV-resistant SJL mice express a homologous protein (6, 7).

Two other species of glycoprotein, Bgp2 (8) and pregnancy-specific glycoprotein (9), both of which belong to CEA family members, were thereafter found to serve as the MHV receptor in mouse species. However, none of these are as highly efficient as CEACAM1 in terms of receptor functionality or receptor utility by MHV strains. Human CEA glycoprotein works as an MHV receptor as well (10).

2.2. Structure and functional regions of major MHV receptor proteins

CEACAM1 is a member of the Ig superfamily and its prototypical 120-kDa glycoprotein consists of four ectodomains (in the order of N, A1, B and A2 from the N terminus), a transmembrane region (TM) and a cytoplasmic tail (Cy) (Fig. 1, 11). The N domain is similar to an Ig-variable domain, and the three other domains resemble a C2 Ig-constant domain. Four different isoforms of CEACAM1 are known to exist, and have been produced by alternative splicing (Fig. 1). Two of the isoforms have 4 ectodomains and the other two have 2 domains, consisting of an N terminal and A2 domains, one of which has either a short or long Cy. The two-domain protein is 48 to 58 kDa in size. CEACAM1 has two allelic forms, CEACAM1^a and CEACAM1^b (Fig. 1). The former is expressed in most laboratory mouse strains, while the latter, insofar as is currently known, is expressed only in MHV-resistant SJL mice (12). In wild mice, however, both of those forms are widely distributed (13). The major structural differences between CEACAM1^a and CEACAM1^b lie in the N domain, which differs in 29 of its 108 amino acids (6, 7). CEACAM1^a is 10- to 100-fold higher than CEACAM1^b in terms of receptor function (14, 15). There is no apparent difference in virus-binding activity as examined by a neutralization test between the 4-ectodomain isoform and the 2-domain CEACAM1^a (16). However, mice deleting the 4-domain CEACAM1^a and expressing the 2-domain isoform alone are more resistant to MHV than those expressing both of the 4- and 2-domain isoforms (17). Thus, there could be a difference between them in terms of MHV receptor function in mice. On the contrary, CEACAM1^b isoform containing 4 domains neutralizes MHV-A59 strain more efficiently than does the isoform containing 2 domains (N and A2 domains), while both of these isoforms showed similar neutralization activity to MHV-JHM strain (16), suggesting a virus-strain specificity in the interaction with CEACAM1.

The N domain is responsible for receptor function (18). Since the CEACAM1 splice variant deleting the A1 and B domains is functional, then it is evident that these domains are not necessary for receptor function. The CEACAM1 isoform containing the N and second A1 domains is also

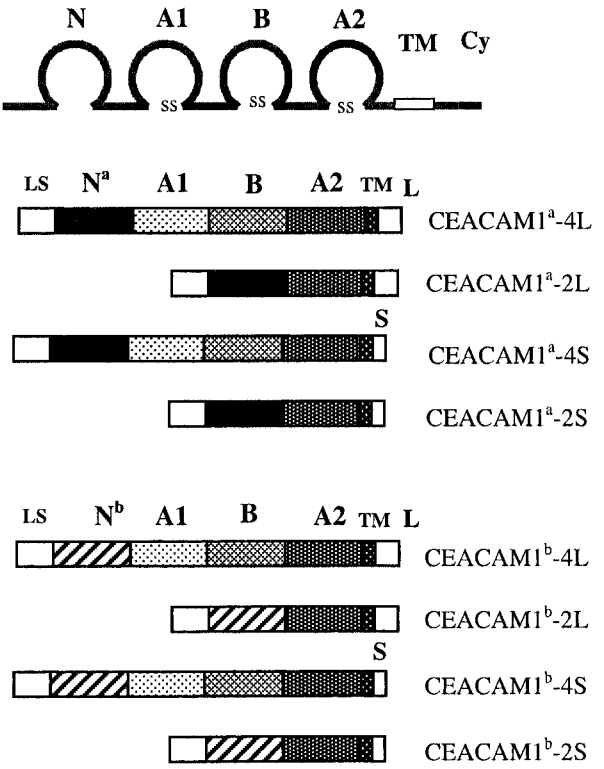

Fig. 1. Schematic structure of a major MHV receptor CECACM1: Four distinct isoforms of CECAM1exist in each CEACAM1[a] and CECAM1[b] allelic forms. There are four or two ectodomains resembling Ig-V or -C domain. Also, long (L) or short (S) cytoplasmic tail (Cy) is known. LS; leader sequence, TM; transmembrane domain.

functional, indicating that the fourth A2 domain is not absolutely critical. Although CEACAM1 consisting of N domain alone bound MHV, it did not work as a functional receptor when expressed on CEACAM1-negative cells (18). However, the chimeric CEACAM1 having the N domain linked to the mouse poliovirus receptor homolog deleting N domain that has a binding specificity to poliovirus served as a functional receptor for MHV (19). As a result of these reports, it is believed that the N domain alone is sufficient for receptor function; however, when expressed on the cell surface, CEACAM1 containing the N domain alone was buried among the various molecules expressed on the cell surface because of its shortness and hence it failed to bind to viruses (19). By using a soluble form of

CEACAM1, we have recently found that CEACAM1 with N domain alone converted MHV S protein from a fusion-negative to fusion-positive form (20). Collectively, the N domain is sufficient for the MHV receptor function. Detailed analysis using N domain deletion mutants of CEACAM1 showed that a stretch composed of 19 amino acids (aa34 to 52) in the N domain is particularly important for receptor function (21). The stretch is located in the CC' loop in the N domain composed by β-strands and is supposed to protrude from the N domain (22, 23). The difference in receptor function observed between CEACAM1^a and CEACAM1^b results from a 6-amino-acid difference in the above-described 19 amino acids in the N domain (14).

2.3. Distribution and biological function of CEACAM1

CEACAM1 is reported to be distributed in various cells in different organs, not only in the target organs of MHV, but also in those in which MHV infection has not been detected. A high level of CEACAM1 expression is reported on epithelium and endothelial cells of a variety of tissues, and in hemopoietic cells, such as monocytes, macrophages, granulocytes and their precursors, the B cells, activated T cells and thymic stromal cells (11, 24). It is also demonstrated on both apical membranes of epithelial cells as well as on sites of cell-cell contact (e.g., between hepatocytes, stratified epithelia, junctional epithelium that forms a transition zone between gingival epithelium and teeth, and between pericytes and endothelial cells of blood vessel walls). Furthermore, during early mouse embryonic development, CEACAM1 is abundantly expressed in endodermal and mesenchymal derivatives (25), but is not detected by immune histochemistry in any type of cells in the brain (26). However, MHV infection in the brain was blocked by anti-CEACAM1 MAb CC-1 (27). Also, a CEACAM1 isoform with 2 domains was detected by RT-PCR in the brain (28). These findings suggest that the CEACAM1 molecule is expressed, albeit in very small amounts, in some cell populations of the brain.

The major biological function of CEACAM1 is cell adhesion. It serves as both a homophilic and heterophilic adhesion molecule (11). Homophilic adhesion, confirmed by in vitro studies of rodent and human CEACAM1, is thought to be important in the embryonic organization of the intestinal epithelium and liver hepatocytes , in placental trophoblasts, during muscle and tooth development and vascularization of the central nervous system (24). CEACAM1 also plays an important role in neutrophil activation and adhesion during inflammatory responses (29), lymphoregulation and immunosurveillance (30), angiogenesis (31), and the negative regulation of cell proliferation (11, 24). Heterophilic adhesion of CEACAM1 to other

CEACAM family members has been shown (32). Also, heterophilic adhesion to *Opa* proteins of *Neisseria gonorrhoeae*, *Neisseria meningitis* and *Haemophilus influenzae* mediates their infections (33, 34), indicating that CEACAM1 is a receptor for those bacteria. This also facilitates bacterial colonization of the gut and bacterial phagocytosis by neutrophils and is involved in the initial tethering of granulocytes to E-selectin on the endothelium prior to their transendothelial migration during inflammatory responses. It was recently shown that homophilic adhesion of CEACAM1 involves N-terminal domain interactions. The GFCC'C" face of the N domain, which includes the MHV binding site CC' region, is responsible for homophilic interaction (22).

3. INTERACTION OF CEACAM1 AND MHV S PROTEIN

The MHV S protein comprising a petal-like projection on virion surface is the ligand for the CEACAM1 molecule. The projection is composed of two or three molecules of the S1-S2 heterodimer derived from S protein. S protein is a type I glycoprotein. It is synthesized and co-translationally glycosylated as a 150-kDa, protein, becomes as 180-200 kDa protein after modification of glycans and cleaved by a host-derived proteinase into two subunits, N terminal S1 and C terminal S2 (35). S1 comprises an outer knob-like structure of the spike, and S2 consists of the stem-like part beneath the knob (36). S1 and S2 units are associated by non-covalent linkage, and they can be easily dissociated from each other by denaturing reagents or even during a purification process. Alpha-helices constructed by the heptad repeats in the S2 play an important role for oligomerization of S1-S2 heterodimers, though there is another determinant in the S1 for oligomerization (37). Following its synthesis, the S protein is incorporated into the envelope of viral particles after interaction with viral integral membrane protein in the internal compartments from endoplasmic reticulum to the Golgi apparatus. The S protein is also transported to the plasma membrane.

The N terminal region in the S1 composed of 330 amino acids (S1N330) is responsible for binding to CEACAM1 (38). Among S proteins of a variety of MHV strains, there are three conserved regions in S1N330 (S1N330-1, -II and -III) composed of 10 or more identical amino-acid stretches (39). Site-directed mutagenesis analysis suggested that two of these regions, S1N330-1 and –II, located far from one another, are involved in receptor binding (39). Studies using MHV variants containing mutations in S1N330-I confirmed the significance of this region in receptor binding (40). S1N330-III was recently suggested to be responsible for

virus entry into the cell in combination with a region in the S2 (41). Denaturing of S1N330 abolished the receptor-binding activity, indicating that the tertiary structure composed of different regions in the S1N330 or/and the dimerization of S1 which takes place within S1N330 (37) is important.

The interaction of MHV S protein and CEACAM1 leads to the S protein functional conversion from a fusion-negative to a fusion-positive form (42). Recently, it was also shown that this functional activation is accompanied with conformational changes in the S protein; receptor-bound S protein has a fraction resistant to proteinase digestion, while receptor-unbound S protein is susceptible (43). These functional and structural changes of the S protein greatly resemble those of the envelope protein of retroviruses that take place after they bind to their receptors (44, 45), suggesting that MHV enters cells in a fashion similar to that of retroviruses.

4. MHV RECEPTOR AND ITS IMPLICATIONS FOR MOUSE SUSCEPTIBILITY TO MHV

A number of investigators have reported that BALB/c, C57BL and most other mouse strains, later revealed to have a *Ceacam1*^{a} (*1*^{a}) gene, are susceptible, while SJL mice with a *Ceacam1*^{b} (*1*^{b}) gene are resistant (46, 47). Genetic analyses indicated that a single dominant gene located on chromosome 7 is responsible for susceptibility to MHV (47). The *Ceacam1* could be a gene determining susceptibility, since 1) the 1^a makes mice susceptible and 2) *Ceacam1* is also mapped on chromosome 7 (48). The expression of either 1^a or 1^b in CEACAM1-negative cells converted them to MHV susceptible, suggesting that allelic differences in receptor proteins were not sufficient to explain the differences in mouse susceptibility to MHV (6, 7). In detailed studies, however, cells transiently expressing 1^a were 10 to 100 times more sensitive to MHV than were cells expressing 1^b (14, 15), indicating a small, but significant difference between 1^a and 1^b. This also suggested that the MHV receptor expressed in SJL is still functional. If the receptor allele controls MHV susceptibility, then SJL should be relatively, but not completely, resistant to MHV. SJL mice are, in fact, resistant to MHV when challenged with a low dose of virus, but susceptible when inoculated with a high dose of virus (49, 50). These observations suggest that *Ceacam1* is a gene controlling MHV susceptibility. Of 120 mice of (BALB/c x SJL) F2 and backcrossed mice to SJL, all mice with *1*^{a}/*1*^{a} and *1*^{a}/*1*^{b} genotypes were susceptible, while all mice with *1*^{b}/*1*^{b} genotype were resistant after infection with a low dose of virus (12). This implies the MHV receptor gene and MHV-susceptibility

gene are identical, and if not, they are located within 0.86 cM on chromosome 7. To finally examine whether the receptor gene is identical to the gene that controls MHV susceptibility, gene replacement is a useful strategy. The MHV susceptibility of BALB/c mice in which $1^a/1^a$ is replaced by $1^b/1^b$ and SJL mice in which $1^b/1^b$ is replaced with $1^a/1^a$ will conclusively establish whether the MHV receptor gene is the gene which controls the MHV susceptibility of mice.

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