IDENTIFICATION OF A RECEPTOR FOR MOUSE HEPATITIS VIRUS

K. V. Holmes, J. F. Boyle, D. G. Weismiller, S. R. Compton, R. K. Williams, C. B. Stephensen, and M. F. Frana

Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

There has recently been renewed interest in the study of virus receptors[1,2]. This stems from several causes: increased understanding about interactions of cell surface molecules with other ligands such as toxins and hormones; improved methodologies for studying molecules present on the plasma membrane in very small amounts; comprehensive information about the structure of virus attachment proteins which interact with receptors on the plasma membranes of susceptible cells; and enhanced interest in the potential role of virus receptors in determining the tissue tropisms and pathogenicity of virus infections in vivo.

Our laboratory is particularly interested in studying receptors for coronaviruses because of the very interesting biology and pathogenesis of this virus group. Coronaviruses, which cause a variety of diseases in man and domestic animals, exhibit a wide variety of patterns of tissue tropism, virulence and pathogenicity[3]. Even within a single coronavirus such as mouse hepatitis virus (MHV), there are many strains which differ markedly from each other[4]. Susceptibility to murine coronaviruses varies markedly among different strains of mice and is determined by host genes[5]. Susceptibility to MHV is dominant, and the gene determining host susceptibility has been mapped to mouse chromosome 7[6]. We undertook studies of MHV receptors in order to characterize early events in the virus replicative cycle, to identify the domain of the E2 glycoprotein which interacts with receptors and to explore the role of receptors in the tissue and species tropisms and pathogenicity of coronaviruses.

We focussed first on identifying coronavirus receptors in tissues which would be natural targets for coronavirus infection in vivo. We developed new methods to detect interactions of MHV virons or isolated E2 glycoprotein with plasma membranes of intestines and livers from genetically susceptible or resistant strains of mice[7]. These experiments were designed to test the hypothesis that genetic resistance to MHV is due to absence of a specific virus receptor on cell membranes of resistant mouse strains.

Binding of MHV to brush border membranes isolated from intestines of genetically susceptible BALB/c mice was assayed in a solid phase assay[7]. Briefly, membranes immobilized on nitrocellulose were incubated with MHV-A59, and bound virus was detected with antibody directed against the E2 glycoprotein and radiolabeled Staphylococcal protein A. This assay

M. M. C. Lai et al. (eds.), *Coronaviruses*

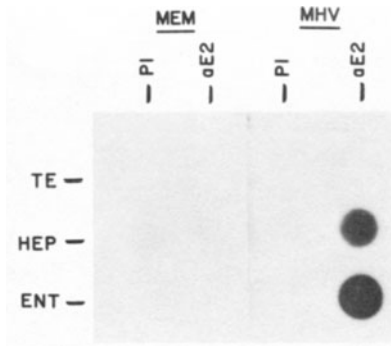

Fig. 1. Solid phase assay for MHV receptor. Membranes from BALB/c hepatocytes (HEP) or enterocytes (ENT) or buffer (TE) on nitrocellulose were incubated with medium (MEM) or MHV, then pre-immune serum (PI) or anti-MHV E2 (aE2)glyco-protein, followed by ^{125}I Staphylococcus aureus protein A. (Reproduced from 7 with permission.)

proved to be highly specific and sensitive enough that virus binding activity could be detected readily in only lug of membrane protein (Figure 1).

To determine whether MHV would bind to brush border membranes from genetically resistant SJL mice[6,8], we used this solid phase virus binding assay. We found that intestinal brush border membranes from SJL mice had only 5% of the MHV-binding activity of membranes from BALB/c mice. Similar differences were observed between binding of MHV-A59 to isolated hepatocyte plasma membranes from SJL and BALB/c mice[7]. These observations support the hypothesis that SJL mice are resistant to MHV because they lack a specific virus receptor on the membranes of the normal target cells for this virus.

We further characterized the cell surface molecule that bound MHV-A59 by incubating the BALB/c brush border vesicles with detergents prior to incubation with MHV in the solid phase virus binding assay. We found that virus binding activity was not inhibited by incubation of the membranes with a with variety of detergents, including 0.1% Tween 20 or SDS. The observation that SDS did not inactivate MHV binding activity suggested that we might be able to determine the molecular weight of the virus-binding molecule if we blotted BALB/c membrane proteins to nitrocellulose after SDS-PAGE and then used a virus overlay and immunodetection system like that done for the solid phase virus binding assay. This new assay is called a virus-overlay protein blot assay (VOPBA). Figure 2 shows a VOPBA comparing virus binding activities of membrane components from BALB/c and SJL mice. Although a large number of membrane proteins was detected by Coommassie blue staining of the gels, only one 100K band of BALB/c enterocyte membranes showed virus binding activity (Fig 2). Virus binding activity was not detected in membranes from SJL mice by VOPBA but was detected in membranes from semi-susceptible C3H mice. Similarly, membranes from hepatocytes of BALB/c and C3H mice had a virus-binding band

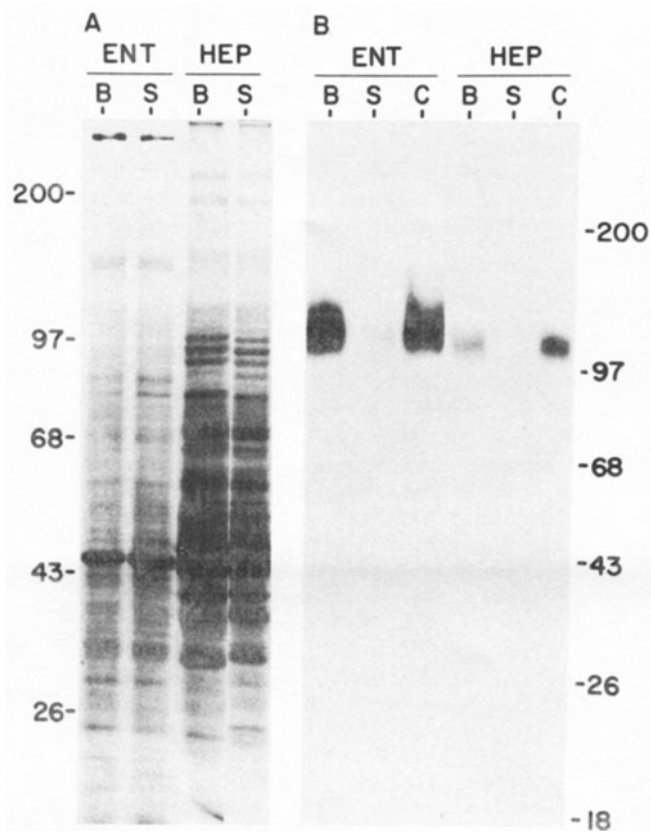

Fig. 2. A. Coomassie blue stain of SDS-PAGE of BALB/c (B) or SJL/J (S) mouse enterocyte (ENT) or hepatocyte (HEP) membranes. B. VOPBA assay blot of these and C3H (C) membranes was overlaid with MHV virions and detected with antiviral antibody and ^{125}I Staphylococcus aureus protein A. (Reproduced from 7 with permission.)

at about 100K, while membranes from SJL hepatocytes had none. These observations confirm the absence of virus binding activity in membranes from SJL mice which was initially demonstrated with the solid phase virus binding assay.

Furthermore, the VOPBA described above provided a simple way to purify the virus binding entity from the complex mixture of components in the BALB/c membranes. Preparative polyacrylamide gel electrophoresis was used to isolate molecules of approximately 100K which contained virus binding activity. This material was treated with enzymes to help characterize the receptor. The results indicate that the MHV receptor is inactivated by treatment with proteolytic enzymes, but not by neuraminidase (Table 1), or other glycosidases. Thus, the coronavirus receptor is clearly distinct from the neuraminic acid-containing receptors for orthomyxoviruses and paramyxoviruses. Figure 3 shows that virus binding activity can be removed from detergent- solubilized membranes by incubation with lectins including Concanavalin A and ricin 120. These observations are compatible with the hypothesis that the 100K virus binding entity is a glycoprotein, and suggest that the protein component

Table 1

Inactivation of Virus Binding Activity of BALB/c Membranes

Treatment	Virus Binding Activity
None	+++
Trypsin	-
Chymotrypsin	-
Neuraminidase	+++

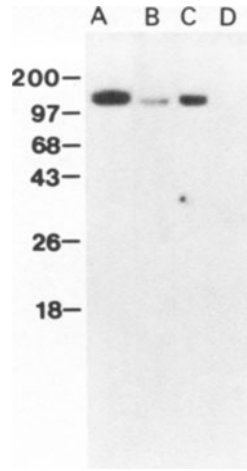

Fig. 3. Lectin binding of MHV receptor. Detergent-solubilized proteins of BALB/c intestinal brush border membranes were reacted with lectins conjugated to agarose. Bound membrane proteins were solubilized with SDS, separated by SDS-PAGE and virus binding activity was determined by the VOPBA procedure. Lane A shows 10 ug membrane proteins; lane B, proteins bound to Concanavalin A; lane C, proteins bound to Riciuus communis lectin; lane D, proteins bound to agarose without lectins. Molecular weight standards are shown at left.

of the molecule may contain the virus binding domain. Additional studies on the properties of the virus binding molecule from BALB/c membranes using two-dimensional gel electrophoresis showed that the receptor had an isoelectric point of approximately 3.5. Thus, two dimensional gel electrophoresis provides an additional step for the purification of the virus receptor molecule. Further characterization of this molecule is in progress.

We would like to determine whether the 100K virus binding molecule which we have described above actually functions as the virus receptor

Table 2

Species Specificity of Binding of MHV-A59

Origin of Brush Border Membranes*	Binding of MHV-A59
BALB/c mouse	++++
Dog	-
Cat	-
Cotton rat	-
Human**	-

* From 0.3 to 25ug of protein from brush border vesicles purified from different species were tested in a solid phase assay to detect binding of MHV-A59.

**Human brush border vesicles were purified from human colon adenocarcinoma cell line, Caco-2[9].

in vivo. This is a difficult problem. The virus receptor is present in extremely low amounts relative to other membrane proteins, and it has not yet been cloned or purified to homogeneity. We are attempting to develop polyclonal and monoclonal antibodies directed against the receptor. If such antibodies inhibited virus infection of the mouse intestine, hepatocytes or cultured cells, that would be strong support for a direct role of the 100K glycoprotein in virus infection. These antibodies would also be a valuable aid for cloning the receptor, and examining its distribution on different murine tissues and on the livers and intestines of other species.

An alternative way to determine whether receptors play a critical role in the species specificity of MHV-A59 has been undertaken. Brush border membranes have been purified from intestines of many species, and their virus binding activities have been compared with that of BALB/c membranes using the solid phase virus binding assay. Some results of these ongoing studies are shown in Table 2. To date, no species other than the mouse has been found to have virus binding activity for MHV-A59 in its intestinal brush border membranes. This observation supports the hypothesis that species specificity of coronavirus infections may depend upon availability of specific virus receptors on the membranes of target tissues.

A summary of the characteristics of the receptor for MHV-A59 is shown in Table 3. It appears that the receptor is a glycoprotein of about 100K, and that it can be found on several tissues of susceptible strains of mice, but not on tissues from genetically resistant strains of mice or from other species.

The studies described here represent a new approach to investigating the pathogenesis and tissue and species tropisms of coronaviruses. It will be of considerable interest to learn whether other coronaviruses bind to similar molecules in their normal host species. Further studies of binding of various coronaviruses, or virus strains with altered tissue tropism, host range and/or virulence to membrane components from tissues

Table 3

Properties of the MHV Receptor

Type of molecule:	Glycoprotein
Molecular weight:	Approx. 100K
Isoelectric point:	Approx. 3.5
Effect of detergents:	None
Present on tissues:	BALB/c liver and intestine
Absent on tissues:	SJL liver and intestine Liver and intestines of several other species

of many species will help to elucidate the molecular basis of selective infection of specific tissues in certain species by coronaviruses.

ACKNOWLEDGEMENTS

The authors thank E. C. Bauer and T. R. Scheiner for excellent technical assistance. This work was supported in part by grant #AI 18997 from the National Institutes of Health, grant #2H-13 from U. S. Agency for International Development, and grant #RO7403 from USUHS. The opinions or assertations contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

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