MHV-RESISTANT SJL/J MICE EXPRESS A NON-FUNCTIONAL HOMOLOG TO THE MHV RECEPTOR GLYCOPROTEIN

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ABSTRACT

MHV-A59 recognizes a 100-110 K glycoprotein receptor on intestinal and liver plasma membranes. The receptor protein was affinity purified using an anti-receptor monoclonal antibody. Purified receptor was sequenced from the 1st to the 15th amino acids. Antiserum raised against a synthetic peptide having this sequence bound to the 110 K receptor from BALB/c intestine and a 58 K fragment of the receptor. SJL/J mice are resistant to MHV-A59 and their plasma membranes were previously shown to lack binding to MHV. However, anti-peptide antibody bound to a 105 K protein and a 53 K protein fragment from SJL/J plasma membranes. These SJL/J proteins did not bind MHV or to the blocking monoclonal anti-receptor antibody. Genetic resistance of SJL/J mice to MHV-A59 appears to be due to mutation of the receptor glycoprotein resulting in a molecule that does not bind MHV.

INTRODUCTION

Infection of a host animal with a coronavirus requires specific host functions. Susceptibility to mouse hepatitis virus (MHV) is under host genetic control. Different outcomes of inoculation with MHV are seen in different genetically pure strains of mice[1-7]. Of 12 mouse strains tested only SJL/J mice were resistant to CNS infection with JHM strain of MHV[5]. This resistance is due to a recessive locus, *hv-2*, that is independent of H2 haplotype and Ir genotype[5,6]. Resistance of SJL/J macrophages to MHV strain A59 is also due to a recessive gene that is independent of the H2 complex[7]. Resistance to MHV-A59 is found at early ages and macrophages isolated from SJL/J mice as young as one week old are resistant. The resistance locus for MHV-A59 in SJL/J mice maps near the centromere on chromosome 7.

Coronavirus MHV binds to a glycoprotein receptor found in plasma membrane fractions from susceptible tissues[8,9]. This MHV receptor is 100 K in liver and 110 K in intestine. Polyclonal and monoclonal antibodies directed against the receptor block infection of cultured cells by MHV-A59 indicating that this is the biological receptor for MHV-A59 on these cells[10].

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Genetic control of susceptibility to MHV-A59 is strongly correlated with presence or absence of this receptor. Plasma membranes isolated from susceptible mouse strains have the 100-110 K receptor glycoprotein[8]. SJL/J mouse tissues have no detectable MHV receptor activity. SJL/J mice immunized with crude preparations of receptor developed strong immune responses against the receptor glycoprotein. Polyclonal antibodies and monoclonal antibodies from these immunized mice react with the receptor glycoprotein from susceptible mice such as BALB/c but not with any proteins in the SJL/J mice[10]. The SJL/J immune system therefore sees the receptor protein as foreign. It was not known whether SJL/J mice entirely lack the protein that serves as the receptor for MHV or if they express a variant protein that does not have the ability to bind virus. If a variant of the receptor protein is present in the SJL/J mouse, this protein must lack the binding sites for virus and for the polyclonal and monoclonal anti-receptor antibodies.

In order to characterize the MHV receptor we affinity purified the protein and began sequencing. We used a partial sequence to produce a synthetic peptide derived from the amino terminal region (NTR) of the receptor. Antibody raised against this synthetic peptide (anti-NTR) was used to look for an SJL/J protein homologous to the MHV receptor from BALB/c mice.

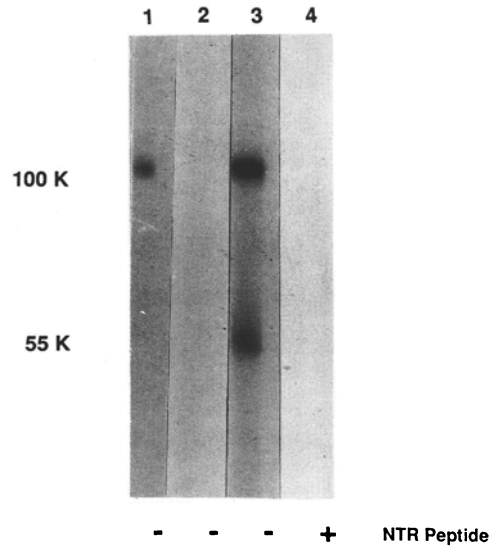

Figure 1. Immunoblot of affinity purified MHV receptor protein with antibody directed against the amino terminal peptide of the MHV receptor. Affinity purified receptor was treated with 2-mercaptoethanol, run on an SDS gel and blotted to nitrocellulose. Blot strips were reacted with polyclonal anti-receptor antibody (lane 1), rabbit pre-immune serum (lane 2), anti-NTR antibody (lane 3) or with anti-NTR antibody which had been pre-absorbed with excess NTR peptide before applying to the blot (lane 4). Molecular weight of the receptor in daltons calculated from standard proteins is shown to the left.

METHODS and RESULTS

Monoclonal antibody CC1 (MAb) directed against the receptor protein was produced by immunizing SJL/J mice with BALB/c intestinal brush border membrane as described[10]. MAb CC1 recognizes receptor protein on Western blots and live cells and blocks virus binding to receptor. Affinity purification of the receptor was accomplished using this monoclonal antibody. A crude membrane fraction from susceptible mouse liver was produced by homogenization and differential centrifugation. Receptor was solubilized from the membranes using nonionic detergent in the presence of protease inhibitors. Solubilized receptor was then allowed to bind to the monoclonal antibody and eluted with 6 M sodium thiocyanate.

Purity of the receptor protein increased 3,000 to 10,000 fold in this single affinity purification step. Final purification of receptor was achieved by preparative SDS-gel electrophoresis followed by electroblotting the receptor to PVDF membrane (Immobilon P, Millipore Corp.). Receptor on PVDF was then stained for protein and the receptor band was cut out and used directly for amino sequencing using the method of Matsudaira[11]. The preliminary sequence for the amino terminus of the intact receptor was determined for amino acids 1 through 15 of the receptor. A single first amino acid was found indicating complete purity of the receptor.

Solid phase synthesis was used to produce a synthetic peptide designated NTR representing the first 15 amino acids of the receptor protein. Antibodies directed against this peptide were produced in rabbits by coupling the peptide to keyhole limpet hemocyanin. The antiserum produced was tested for reactivity with the receptor purified from susceptible mouse liver. Anti-NTR antibody reacted with the affinity purified receptor at 100 K and with a 55 K protein fragment of the receptor (Fig. 1). The specificity of this reaction is shown by

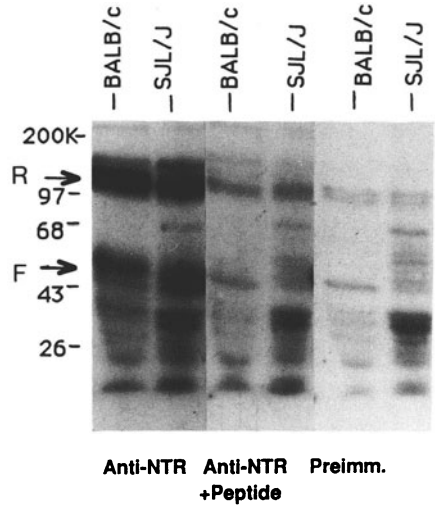

Figure 2. Immunoblot of intestinal brush border membranes fromsusceptible BALB/c mice and resistant SJL/J mice. Proteins blots were reacted with the anti-NTR antibody or pre-immune serum as indicated. Anti-NTR + peptide indicates that this blot was reacted with Anti-NTR antibody that had been pre-absorbed with excess NTR peptide. R and F mark the position of the BALB/c receptor and the fragment of the receptor respectively.

the ability of the NTR peptide to block the reaction of the anti-NTR antibody with the receptor. The 100 K receptor protein and the 55 K protein therefore share a common amino terminal region. These data confirm the sequence for the amino terminal region of the receptor and suggest that the 55 K protein is a fragment of the receptor generated by the loss of approximately half of the molecule from the middle to the carboxy terminal end.

To determine if the intestinal receptor, like the hepatocyte receptor, would react with the anti-NTR antibody, intestinal brush border membranes from BALB/c mice were immunoblotted with anti-NTR antibody or pre-immune rabbit serum. The anti-NTR antibody specifically bound to the intact receptor (R in Fig. 2) which is 110 K in BALB/c intestine and a 58 K fragment of the receptor (F in Fig. 2). Pre-absorption of the anti-NTR antibody with excess NTR peptide eliminated the binding to both the 110 K and 58 K bands. Binding of the antibody to several background bands was non-specific and occurred with the pre-immune rabbit serum and with the anti-NTR serum that had been pre-absorbed with the NTR peptide.

The anti-NTR antibody was used to test if the SJL/J membranes have a protein that shares the NTR sequence. In SJL/J intestinal brush border membranes (Fig 2) an upper band of about 105 K and a fragment at about 53 K reacted specifically with the anti-NTR antibody. As was found in the BALB/c proteins the binding of anti-NTR with these two specific SJL/J bands could be blocked by pre-absorption of the serum with the NTR peptide. These data show that polypeptides antigenically related to the MHV receptor of BALB/c mice are found in the SJL/J mouse intestine membranes.

We can begin to map the functional and antigenic domains of MHV receptor protein using anti-NTR, MAb CC1 and virus binding as probes to compare the BALB/c MHV receptor with the homologous inactive SJL/J protein. These data, summarized in Figure 3, suggest that the BALB/c receptor, its fragment, the

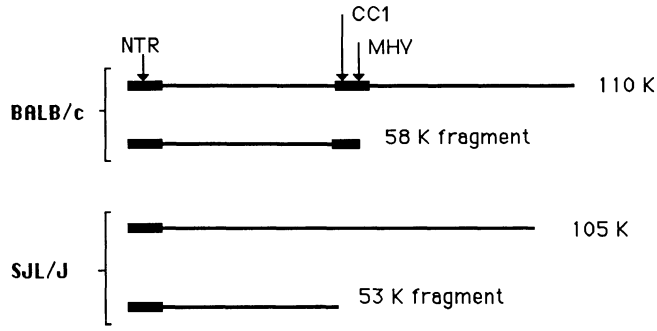

Figure 3. Model for the BALB/c intestine receptor for MHV and its inactive homolog from SJL/J mice and their naturally occurring protein fragments. The location of the NTR peptide and the proposed binding sites for the CC1 monoclonal antibody and for MHV are indicated.

SJL/J variant protein and the SJL/J fragment are all co-terminal at the amino end. The blocking monoclonal antibody CC1 binds to the BALB/c receptor and its 58 K fragment but not to the SJL/J variant proteins. MHV binds to the large 110 K receptor from BALB/c and to the BALB/c receptor fragment. Blocking of virus binding to the receptor by MAb CC1 suggests that the MAb binding site and the virus binding site are near each other in the receptor molecule. Immunoblots containing approximately equal amounts of the 58 K fragment and the intact 110 K receptor, as determined by binding of anti-NTR and MAb CC1 show much more binding of MHV to the 110 K receptor than to the 58 K fragment (data not shown). This result suggests that the MHV binding site overlaps the end of the 55 K fragment in BALB/c intestine.

DISCUSSION

We have found that the intestinal brush border membranes from SJL/J mouse which lacks a functional receptor for MHV expresses a variant protein that shares its amino terminal region with the receptor found in BALB/c mice This variant SJL/J protein is approximately 5 K smaller on than the BALB/c receptor. We have also found that the SJL/J mouse has a similar lower band at about 53 K in intestine which appears to be a naturally occurring fragment. This reduction in molecular weight from 110 K in the BALB/c receptor protein to 105 in the SJL/J variant protein appears to be located within the first half of the variant molecule between the NTR sequence and the middle of the molecule since the fragment of the variant protein is also reduced 5K. We have not ruled out the formal possibility, however, that the lower receptor band is made from a smaller mRNA. The smaller size seen in the SJL/J bands may be due to deletion of part of the coding region of the receptor gene or to splicing out of an exon in the SJL/J mRNA. Alternatively, the smaller size could be due to a mutation that removes a site for glycosylation of the receptor core protein. We will now be able to examine these possibilities by directly comparing the MHV receptor and the non-binding variant protein.

The cellular function of the MHV receptor protein is not yet known. Presumably this protein has an important function in the host animal. The finding that the SJL/J mouse does not entirely lack this protein but expresses a variant molecule suggests that the variant protein serves the same cellular function as the related BALB/c protein. Further information on the structure of the MHV receptor protein and the variant SJL/J protein, including the complete amino acid sequence will depend on the analysis of cDNA clones for the receptor gene. Comparison of the complete amino acid sequence of the BALB/c and its SJL/J homolog should permit identification of the domain of the receptor protein that binds to MHV.

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REFERENCES

1. Bang, F. B., and A. Warwick. 1960 Mouse macrophages as host cells for the hepatitis virus and the genetic basis of their susceptibility. Proc. Natl. Acad Sci. USA 46: 1065.

2. Kantock, M., A. Warwick, and F. B. Bang. 1963. The cellular nature of genetic susceptibility to a virus. J. Exp. Med. 117:781.

3. Weiser, W., I. Vellisto, and F. B. Bang. 1976 Congenic strains of mice susceptible and resistant to mouse hepatitis virus. Proc. Soc. Exp. Biol. Med. 152:499.

4. Levy-Leblond, E., D. Oth, and J. M. Dupuy. 1979. Genetic study of mouse sensitivity to MHV-3 infection: influence of the H2 complex. J. Immunol. 122: 1359.

5. Stohlman, S. A. and J. A. Frelinger. 1978. Resistance to fatal central nervous system disease by mouse hepatitis virus, strain JHM. I. Genetic analysis. Immunogenetics 6: 277.

6. Knobler, R. L., M. V. Haspel, and M. B. A. Oldstone. 1981. Mouse hepatitis virus type 4 (JHM strain)-induced fatal central nervous system disease. I. Genetic control and the murine neuron as the susceptible site of disease. J. Exp. Med. 153:832.

7. Smith, M. S., R. E. Click, and P. G. W. Plagemann. 1984. Control of mouse hepatitis virus replication in macrophages by a recessive gene on chromosome 7. J. Immunol. 428.

8. Boyle, J. F., D. G. Weismiller, and K. V. Holmes. 1987. Genetic resistance to mouse hepatitis virus correlates with absence of virus binding activity on target tissues. J. Virol. 61:185.

9. Holmes, K. V., J. F. Boyle, D. G. Weismiller, S. R. Compton, R. K. Williams, C. B. Stephensen, and M. F. Frana. 1987. Adv. Exp. Med. Biol. 181:197.

10. Holmes, K. V., R. K. Williams, and C. B. Stephensen. 1989. Coronavirus receptors. In Concepts in viral pathogenesis, A. Notkins and M. B. A. Oldstone (Eds). Springer Verlag, New York.

11. Matsudaira, P.1987. Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. 10035.