# 5

# VIRUS-RECEPTOR INTERACTIONS AND INTERSPECIES TRANSFER OF A MOUSE HEPATITIS VIRUS

Lisa E. Hensley,[1] Kathryn V. Holmes,[2] Nicole Beauchemin,[3,4] and Ralph S. Baric[1,5]

[1]Department of Epidemiology
[5]Department Of Microbiology And Immunology
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599
[2]Department of Microbiology
University of Colorado Health Sciences Center
Denver, Colorado
[3]Department of Biochemstry
[4]Department of Medicine
McGill Cancer Centre
McGill University
Montreal, Quebec, Canada

## 1. ABSTRACT

Molecular mechanisms regulating virus xenotropism and cross-species transmission are poorly understood. Host range mutants (MHV-H2) of mouse hepatitis virus (MHV) strains were isolated from mixed cultures containing progressively increasing concentrations of nonpermissive Syrian baby hamster kidney (BHK) cells and decreasing concentrations of permissive murine astrocytoma (DBT) cells. MHV-H2 was polytrophic, replicating efficiently in normally nonpermissive BHK cells, Syrian and Chinese hamster (DDT-1 and CHO) cells, human adenocarcinoma (HRT), primate kidney (VERO) and in murine 17Cl-1 cell lines. Little if any virus replication was detected in feline kidney (CRFK), and porcine testicular (ST) cell lines. To study the effects of xenotropic spread on virus receptor-interactions in the original host, murine DBT cells were pretreated with a monoclonal antibody (MAb) CC1, directed against the MHV receptor, MHVR, a biliary glycoprotein (Bgp1$^a$). Under treatment conditions that completely ablated the replication of the parental MHV strains, CC1 antireceptor antibodies did not block MHV-H2 replica-

*Coronaviruses and Arteriviruses*, edited by Enjuanes *et al.*
Plenum Press, New York, 1998

tion. Following expression of MHVR in normally nonpermissive ST and CRFK cells, infection with the parental MHV strains, but not MHV-H2 was observed. To characterize the molecular basis preventing the interaction between MHV-H2 and MHVR, revertants of MHV-H2 (MHV-H2R6, MHV-H2R11) were isolated following a persistent MHV-H2 infection in DBT cells. These revertant viruses efficiently recognized MHVR, however infection of murine cells was resistant to MAb CC1 blockade. In addition, MHV-H2 and the revertant viruses efficiently recognized other Bgp receptors for docking and entry. These data suggest that interspecies transfer may remodel normal virus-receptor interactions that may result in altered virulence, tropism or pathogenesis in the original host.

## 2. INTRODUCTION

Emerging virus are frequently defined as either newly recognized viruses, or viruses that are rapidly increasing in incidence or expanding in geographic range (Morse, 1995). Although some new viral diseases may have resulted from mutations that altered tissue tropism, virulence or pathogenesis in the normal host, most new human viruses probably arose by cross species transmission from animal reservoirs (Morse, 1995; Kilbourne 1991). Recent examples include equine morbillivirus, human immunodeficiency viruses, hantavirus, hemorrhagic fever viruses, arboviruses, bovine spongiform encephalopathy, canine distemper, and influenza viruses. Factors that precipitate zoonotic virus transference to alternative hosts are extremely difficult to predict, hampering efforts to prevent the emergence and dissemination of new viral diseases in animals, humans, and plants (Baric et al., 1997).

MHV is highly species specific and tissue tropic, and is therfor an excellent model to investigate the fundamental molecular mechanisms that regulate virus xenotropism and cross-species transmission. Similar to many emerging RNA viruses, MHV host range is predominantly mediated at the level of entry as the MHV genomic RNA is infectious in nonpermissive baby hamster kidney cells and also human cells, and expression of MHVR permits viral docking and entry in nonpermissive hosts (Dveskler et. al., 1991; Schochetman et. al., 1977). In this paper, we investigate whether cross-species transmission of MHV has altered virus interactions in the original host.

## 3. MATERIALS AND METHODS

### 3.1. Viruses and Cells

Briefly, murine astrocytoma (DBT), murine 17Cl-1, baby hamster kidney (BHK), Chinese hamster ovary (CHO), hamster smooth muscle (DDT-1), feline kidney (CRFK), porcine testicular (ST), primate kidney (VERO), and human colorectal (HRT) cells were maintained as previously described (Baric et al., 1997). CHO cells stably expressing recombinant Bgp2 (CHO-Bgp2) and BHK cells stably expressing $Bgp1^b$ (BHK-$1^b$) were maintained in standard tissue culture medium containing genetecin (800 μg/ml). These Bgp genes contain the Ig-like domains 1 and 4, the transmembrane domain, and the cytoplamic domain.

MHV-A59, MHV-JHM, MHV-H2, H2R6 and H2R11 were plaque purified and propagated in DBT cells or BHK cells. The TRSB strain of sindbis virus (kindly provided by Dr. Robert E. Johnson, University of North Carolina at Chapel Hill) was propagated in DBT cells.

## 3.2. Virus Growth Curves

Cultures of BHK, DDT-1, CHO, 17Cl-1, VERO, CRFK, MDCK, ST, HRT, BHK-$1^b$ and CHO-Bgp2 cells in 60$mm^2$ dishes or in LabTek chamber slides (Nunc. Inc., Naperville, Ill.) were infected at an MOI of 10. After 1 hour at room temperature the innocula were removed and the monolayers were washed 3 times with phosphate buffered saline (PBS). The cultures were overlaid with complete media and maintained at 37°C. Samples of virus were harvested at various times post-infection and stored at -70°C. Virus titers were determined by plaque assay in DBT cells.

## 3.3. Detection of Viral Antigens by Immunofluorescence

Different cell lines grown on LabTek chamber slides (Nunc. Inc., Naperville, Ill.) were infected with MHV-A59 or MHV-H2 at room temperature at a MOI of 10 for 1 hour.. The innocula were removed and the monolayers were washed 3 times with PBS. Fresh media were added and the cells were maintained at 37°C. Infected cells were fixed and prepared for examination by Immunofluorescence as previously described (Chen, W. et. al., 1997).

## 3.4. Stable Expression of MHVR

A plasmid containing MHVR under the control of the cytomegalovirus (CMV) promoter in the pcDNA3 (Invitrogen) expression vector was transfected into CRFK and ST cells and stable expressing cells were selected with geneticin (Chen, W. et al., 1997). Stable cell lines were infected with H2R6, H2R11, MHV-JHM, MHV-H2 and MHV-A59.

## 3.5. Blockade of the $Bgp1^a$ Receptor

Blockade of MHV entry using the anti-MHVR MAb CC1 was performed as previously described (Chen et. al., 1997). As controls, additional blockade experiments were performed using two commercially antibodies, a monoclonal (kat4c), directed against human CEA gene family members: Bgp, CEA, CGM6, CGM1a and NCA, and a polyclonal (CEA) directed against all human CEA gene family members (DAKO).

## 3.6. Transient Expression of Bgp2

Bgp2 under the control of the CMV promoter in the prCMV expression system (5μg) (Invitrogen), was cotransfected with the pHook2 expression vector (2.5μg) (Invitrogen) by electroporation. Transfected cells were selected at 40 hours following electroporation. Briefly the cells were incubated with pHook magnetic beads for 1–2 hours, scraped and separated with a magnet. The procedure was repeated 24 hours later. Selected cells were seeded onto LabTek slides (Nunc Inc.). Cells were innoculated with MHV-H2 at an MOI of 10 for 1 hour and examined by immunofluorescence at 30 hours post-infection.

## 3.7. Scanning Electron Microscopy

Cultures of DBT cells were infected with MHV-A59, MHV-JHM, MHV-H2, H2R6 and H2R11 at an MOI of 10 for 1 hour at room temperature. At 14 hours post infection cultures were prepared for examination according to standard techniques (Bozzola, 1992).

**Table 1.** Virus replication in different mammalian cell lines

| | Virus Titer (Pfu / ml) | | |
|---|---|---|---|
| Cell lines | MHV - A59 | MHV-JHM | MHV - H2 |
| Murine DBT | $5.0 \times 10^7$ | $1.0 \times 10^7$ | $9.5 \times 10^6$ |
| Murine 17Cl-1 | $7.4 \times 10^7$ | $9.5 \times 10^6$ | $2.8 \times 10^6$ |
| Hamster BHK | $< 1.0 \times 10^2$ | $< 1.0 \times 10^2$ | $2.0 \times 10^8$ |
| Hamster DDT-1 | $< 1.0 \times 10^2$ | $< 1.0 \times 10^2$ | $7.5 \times 10^7$ |
| Hamster CHO | $1.0 \times 10^3$ | $< 1.0 \times 10^2$ | $7.5 \times 10^6$ |

# 4. RESULTS

## 4.1. Host Range Phenotype of MHV-A59 and MHV-H2

As previously reported MHV-H2 replicated efficiently in cultures of hamster (BHK, and CHO) and murine cells (DBT and 17Cl-1) (Baric et al., 1997). Under identical conditions MHV-A59 and MHV-JHM failed to replicate in the hamster cell lines. Although the replication of MHV-H2 was less efficient than the parental viruses in DBT cells (Table 1) significant synctium formation were noted within 16 hours of MHV-A59, MHV-JHM and MHV-H2 infection (Figure 1). These data demonstrated that the cross-species transmission of MHV-2 had not ablated the capacity of the virus to replicate in cells derived from the original murine host. Similar findings have been reported with primate-adapted strains of MHV (Murray, 1992).

## 4.2. MHV-H2 Resistance to MHVR Receptor Blockade

As coronavirus host range specificity is mediated at the level of entry, it seemed likely that expanded xenotropism may have altered specific interactions between the cellular receptor and the MHV-H2 viral attachment protein. To address this question murine DBT cells were pretreated with CC1 anti-MHVR MAb for 1 hour prior to infection. Anti-MHVR MAb CC1 has been demonstrated to block infection by binding to the N-terminus of MHVR (Dveksler et. al., 1993b). Although CC1 pretreatment significantly reduced MHV-A59 viral titers and the percentage of infected cells as observed by immunofluorescence, pretreatment failed to block MHV-H2 infection and did not reduce the percentage of infected cells (Table 2 and Table 3). As a control pretreatment of DBT cells with commercially available antibodies kat4c and a polyclonal CEA antibody directed against all human CEA glycoproteins did not block MHV-A59 or MHV-H2 infection (Table 2). These data suggested that MHV-H2 had either remodeled its normal interactions with MHVR so that it was no longer susceptible to blockade by the anti-MHVR MAb CC1 or suggested that MHV-H2 was no longer efficiently utilizing MHVR as a receptor for entry.

## 4.3. MHV-H2 Does Not Efficiently Utilize MHVR for Entry

To distinguish among these possibilities, MHVR in the pcDNA3 vector was transfected into nonpermissive ST cells. Although the parental strains MHV-A59 and MHV-JHM replicated efficiently in MHVR expressing ST cells, with titers approaching $1 \times 10^7$ PFU/ml in 24 hours, MHV-H2 failed to replicate above background levels (Table 4).

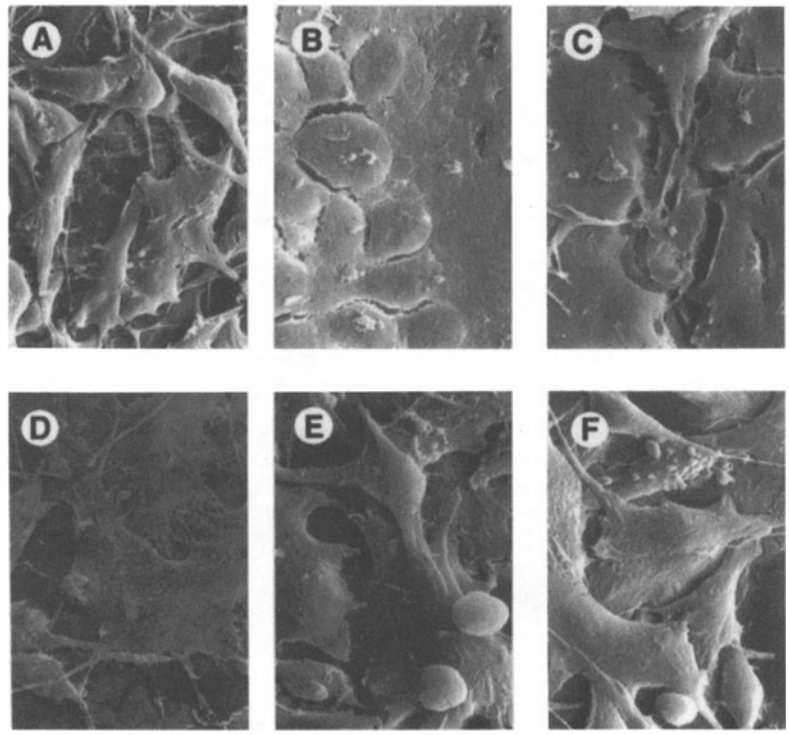


**Figure 1.** Synctium formation in DBT cells. DBT cells were infected with MHV-A59, MHV-JHM, MHV-H2, H2R6 and H2R11 at an MOI of 10 for 1 hour. At 16 hours post infection cells were examined with a scanning electron microscope. Panel A: Uninfected DBT cells; Panel B: DBT cells infected with MHV-A59; Panel C: DBT cells infected with MHV-JHM; Panel D: DBT cells infected with MHV-H2; Panel E: DBT cells infected with H2R6; Panel F: DBT cells infected with H2R11.

**Table 2.** Blockade of MHV infection in DBT cells

| | Virus Titers (Pfu / ml ) | | | |
|---|---|---|---|---|
| Virus | Untreated | Anit-MHVR CC1* | CEA$^{\Delta}$ | Kat4c$^{+}$ |
| MHV-A59 | $2.5 \times 10^6$ | $1.5 \times 10^2$ | $5.6 \times 10^6$ | $5.6 \times 10^6$ |
| MHV-JHM | $1.5 \times 10^6$ | $< 1.0 \times 10^2$ | N/D$^{\ddagger}$ | N/D$^{\ddagger}$ |
| MHV-H2 | $1.5 \times 10^6$ | $6.5 \times 10^5$ | $2.7 \times 10^5$ | $1.3 \times 10^6$ |

*A monoclonal antibody directed against MHVR.

$^{\Delta}$A commercially available (DAKO©) polyclonal antibody directed against human CEA and CEA related glycoproteins.

$^{+}$A commercially available (DAKO©) monoclonal antibody directed against human cd66abde glycoproteins.

$^{\ddagger}$Not done.

**Table 3.** MHV-H2 resistance to CC1 blockade

| | Percentage of infected cells[2] | |
|---|---|---|
| Virus | Untreated | Anit- MHVR CC1 |
| MHV-A59 | > 98.0 % | < 1.0 % |
| MHV-H2 | > 98.0 % | > 98.0 % |

[1]DBT cells were pretreated for 1hour with the Mab CC1. Cells were then challenged with either MHV-A59 or MHV-H2 at an MOI of 10. Cells were fixed at 22 hours post infection and examined by immunofluorescence.
[2]As measured by immunofluorescence at 22 hours post infection.

While significant numbers of infected cells were observed in cultures infected with MHV-A59 by immunofluorescence, there was no observable increase in the number of MHV-H2 infected cells (Table 5). Importantly, CC1 treatment of the ST-MHVR cell lines blocked MHV-A59 and MHV-JHM infection (Table 4). In addition, infection of cells expressing $Bgp1^b$ allowed for low levels of MHV-A59 and MHV-H2 replication. These data indicated that MHV-H2 no longer efficiently recognized MHVR as a receptor for entry.

## 4.4. MHV-H2 Usage of Bgp2

DBT cells, which were derived from outbred CD1 mice, express MHVR, $Bgp1^b$ and encode Bgp2 (Nedellec et al., 1994). In order to determine if MHV-H2 utilized different Bgp genes for entry into DBT cells, Bgp2 was transiently transfected into nonpermissive ST cells. Transfected cells, selected by the pHook system, were susceptible to infection by both MHV-A59 and MHV-H2 (Table 5). These data suggested that MHV-H2 may enter into murine cells by recognizing Bgp2, and to a lesser extent the N terminus of Bgp1b as a receptor.

## 4.5. Bgp Receptor Usage by the H2R6 and H2R11 Revertants

To further investigate the inability of MHV-H2 to utilize MHVR for entry into cells, revertants (H2R6 and H2R11) were isolated on day 25 and day 93 post-infection respectively, from a culture of DBT cells persistently-infected with MHV-H2. Importantly these revertants replicated efficiently in DBT cells but little if any replication was observed in

**Table 4.** MHV replication in ST-MHVR cells

| | Virus Titer (Pfu / cell)[1] | | |
|---|---|---|---|
| | MHV - A59 | MHV-JHM | MHV - H2 |
| ST cells (prior to transfection) | $2.5 \times 10^2$ | $< 1.0 \times 10^2$ | $2.5 \times 10^2$ |
| ST-MHVR cells | $7.1 \times 10^7$ | $1.7 \times 10^5$ | $< 1.0 \times 10^2$ |
| ST-MHVR cells + Mab CC1 | $4.1 \times 10^4$ | $2.5 \times 10^2$ | $< 1.0 \times 10^2$ |

[1] At 22 hours post infection

**Table 5.** Virus infection in ST cells expressing MHVR or Bgp2[1]

| | Percentage of infected cells | | |
|---|---|---|---|
| Virus | ST cells | MHVR stable expressing ST cells | ST cells transientsly expressing BGP2 |
| MHV-A59 | < 0.01% | 40 – 50% | ~30 % |
| MHV-H2 | < 0.01 % | < 0.01% | ~25 % |

[1] At 30 hours post infection

BHK and CHO cells. Thus persistent MHV-H2 infection in DBT cells selected for variants which had lost their capacity to replicate efficiently in hamster cells. Interestingly, these virus also failed to produce syncytium, rather significant cell rounding was noted in infected cultures (Figure 1). While these revertants infected less than 5% of BHK cells under normal conditions, >95% of the BHK cell lines stably expressing Bgp1b or MHVR were susceptible to H2R6 and H2R11 infection. Similarly, while less than 5% of the control CHO cells were infected under normal conditions, > 95% of CHO cell lines expressing high levels of Bgp2 were susceptible to infection with H2R6 and H2R11 (Table 6). These data suggested that H2R6 and H2R11 had remodeled their interactions with the murine Bgp glycoprotein receptors and now utilized MHVR, $Bgp1^b$ and Bgp2 receptors for entry into murine cell lines.

## 5. DISCUSSION

Gene-environment interactions that govern virus cross-species transmission and xenotropism are poorly understood. For RNA viruses, host range specificity is often mediated at level of the virus-receptor interaction. For example, the host range expansion of canine parvovirus type 2 from a feline parvovirus in 1978 was likely attributed to a few changes in the region of the virus capsid where three protein monomers interact (Parish, 1994). We have suggested that mutations affecting virus xenotropism may result in new diseases by either promoting virus adaptation and dissemination into a new host species, or by altering normal virus-receptor interactions that result in new tissue tropisms, altered virulence or pathogenicity in the original host. In this paper we have used MHV as a model to study the consequence of virus interspecies traffic and xenotropism on virus-receptor interactions in the original host.

**Table 6.** MHV-H2 revertants H2R6 and H2R11 replication in murine and hamster cells and Bgp receptor usage

| | Virus Titer (Pfu / cell) | |
|---|---|---|
| Cell line | H2R6 | H2R11 |
| DBT | $1.0\ 10^8$ | $1.5 \times 10^8$ |
| BHK | $1.0 \times 10^4$ | $1.0 \times 10^4$ |
| CHO | $< 1.0 \times 10^4$ | $< 1.0 \times 10^4$ |
| BHK-MHVR | $1.0 \times 10^8$ | $1.1 \times 10^8$ |
| BHK - Bgp1 | $1.5 \times 10^8$ | $1.0 \times 10^8$ |
| CHO - Bgp2 | $2.5 \times 10^6$ | $1.0 \times 10^7$ |

The host range mutant, MHV-H2, was resistant to CC1 antibody blockade, suggesting that MHV-H2 may either be recognizing a portion of MHVR which was not accessible to CC1 blockade, had a decreased affinity for MHVR, or was recognizing alternative receptors for entry into murine cells. The failure of MHVR expression in nonpermissive cells to support MHV-H2 infection, while allowing for MHV-A59, MHV-JHM infection suggested that MHV-H2 no longer efficiently recognized MHVR as a receptor for docking and entry into murine cells. Although it remains possible that ST cells may not express some unidentified cofactors necessary for MHV-H2 entry and replication, the ability of MHV-A59 and MHV-JHM to replicate efficiently in ST cells expressing MHVR make this unlikely. To our knowledge this is the first MHV variant described which has lost its capacity to efficiently recognize MHVR as a receptor for docking and entry.

In addition to demonstrating that MHVR was no longer recognized efficiently for MHV-H2 entry we have shown that different Bgp genes or alleles expressed in DBT cells are likely acting as the principle receptors for virus docking and entry. These data suggest that interspecies traffic of viruses may subtly alter virus-receptor interactions which may result in altered tissue tropism, pathogenesis or virulence in the original host. Interestingly, virus adaptation to alternative host species in vitro has been used to isolate attenuated vaccine variants of measles and polioviruses. While attenuating mutations in these viruses have been identified, in most cases the functions of these mutations in virus replication in the original and the adopted hosts has not been elucidated. It will be of interest to determine whether the virulence, tissue tropism and pathogenesis of MHV-H2 has been altered in B6, Balb-c and SJL mice. If so, the MHV-H2 host range variant may provide an interesting model system to investigate the mechanisms by which virus-host interactions attenuate virulence and pathogenicity.

In conclusion, the isolation of MHV host range variants has selected for viruses which have remodeled their normal interactions with cellular receptors. The availability of these mutants may allow us to identify virus residues which promote or abrogate S glycoprotein-MHVR interactions and promote high affinity interactions with Bgp2.

## REFERENCES

Baric, R. S., Yount, B., Hensley, L., Peel, S., and Chen W., 1997, Episodic Evolution and Interspecies Transfer of a Murine Coronavirus, *J. Virol.* **71**:1946–55.

Bozzola, Russel. Electron Microscopsy, Jones and Bartlett Publishers, Boston. 1992.

Chen, C.S., Asanaka, M., Yokomori, K., Wang, F-I., Hwang, S.B., Li, H-P., and Lai, M.M.C., 1995, Pregnancy-specific glycoprotein is expressed in the brain and serves as a receptor for mouse hepatitis virus, *Proc. Natl. Acad. Sci. USA.* **71**:1688–1691.

Chen, D.S., Asanaka, M., Chen, F.S., Shively, J.E., and Lai, M.M.C., 1997, Human carcinoembryonic antigen and biliary glycoprotein can serve as mouse hepatitis virus receptors, *J. Virol.* **71**:1688–91.

Chen, W., and Baric, R.S., 1996, Molecular anatomy of mouse hepatitis virus persistence: coevolution of increased host cell resistance and virus virulence, *J. Virol.* **70**:3947–3960

Dveksler, G.S., Pensiero, Cardellichio, C.B. Williams, J.-K. Jiang, G.S. Holmes, K.V.,and Dieffenbach, C.W., 1991, Cloning of the mouse hepatitis virus (MHV) receptor: expression in human and hamster cell lines confers susceptibility to MHV, *J. Virol.* **65**:6881–6891.

Dveksler, G.S., Pensiero, C.W., Cardellichio, C.B., McCuaig, K., Pensiero, M.N., Jiang, G.-S., Beauchemin, N., and K.V. Holmes, 1993a, Several members of the mouse carcinoembryonic antigen-related glycoprotein family are functional receptors for the coronavirus mouse hepatitis virus-A59, *J. Virol.* **67**:1–8.

Dveksler, G.S., Pennsiero, M.N., Dieffenbach, C.W., Cardellichio, C.B., Basile, A.A., Elia, P.E., and Holmes, K.V., 1993b, Mouse hepatitis virus strain A59 and blocking antireceptor monoclonal antibody bind to the N-terminal domain of cellular receptor, *Proc. Natl. Acad. Sci. USA.* **90**:1716–1720.

Kilbourne, ED., 1991, New viruses and new disease: mutation, evolution and ecology, *Curr. Opin. Immunol.*, **3**:518–524.

Morse, SS., 1995, Factors in the emergence of infectious diseases, *Emerging Infect. Dis.* **1**:7–15.

Murray, Cai, G., Hoel, K. Zhang, J.-Y., Soike, K. F., Cabirac, G.F., 1992, Coronavirus Infects and Causes Demylination in Primate Central Nervous System, *Virology*, **188**: 274–278.

Nedellec., Dveksler, G. S., Daniels., Turbide., Chow., Basile., Holmes, K.V., Beauchemin. N., 1994, Bgp2, a New Member of the Carcinoembryonic Antigen-Related Gene Family, Encodes an Alternative Receptor for Mouse Hepatitis Viruses, *J. Virol*, **64**:4525–37.

Schochetman G., Stevens, RH., Simpson, W., 1977, Presence of infectious polyadenylated RNA in the coronavirus avian infectious bronchitis virus, *Virology* **77**: 772–778.

Parish, C.R. 1994, The emergence and evolution of canine parvovirus- an example of recent host range mutation, *Semin. Virol.* **5**:121–132.

Williams, R.K., Jiang, G-S. and Holmes, K.V., 1991, Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins, *Proc. Natl. Acad. Sci. USA.*, **88**:5533–5536.

Yokomori, K., and Lai, M.M.C., 1992a, Mouse hepatitis virus utilizes two carcinoembryonic antigens as alternative receptors, *J. Virol.*, **66**:6149–6199.

Yokomori, K., and Lai, M.M.C., 1992b, The receptor for mouse hepatitis virus in the resistant mouse strain SJL is functional: Implications for the requirement of a second factor for viral infection, *J. Virol.* **66**:6931–6938.