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Virus Research, 31 (1994) 49–56

Virus Research

VIRUS 00952

Nucleotide sequence comparison of the membrane protein genes of three enterotropic strains of mouse hepatitis virus

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(Received 6 August 1993; revision received 8 September 1993; accepted 13 September 1993)

Summary

The nucleotide sequences of the membrane (M) protein genes and their deduced amino acid sequences of three enterotropic strains of the coronavirus mouse hepatitis virus (MHV) -Y, -RI and -DVIM were determined and compared with the previously reported sequences of two respiratory MHV strains -A59 and -JHM. The five MHV strains shared extensive nucleotide (95.2–99.0%) as well as amino acid homology (95.6–98.7%). A variable region, including a 15 nucleotide deletion unique to MHV-RI, could be identified at the 5′-terminus of the gene. This region of the M protein may be immunogenic and may contribute to the antigenic diversity of the MHV strains. Sequence relationships between the strains showed no correspondence with the primary cell tropism. This may suggest that evolution of enterotropism was not a single occurrence among different MHV strains. No sequence unique to either tropism group could be identified, indicating that the M protein of MHV probably has no part in the determination of MHV tissue tropism.

Nucleotide sequence; Membrane protein; MHV; Coronavirus; Enterotropic; (Mouse)

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SSDI 0168-1702(93)E0073-7

Coronaviridae are an important virus family infecting many mammalian and avian species, including humans. In most species they cause either respiratory or enteric disease. Mouse hepatitis virus (MHV), the coronavirus of mice, is a common, highly infectious and mutable group of viruses consisting of a number of antigenically different strains. Like coronaviruses of other species, these strains can be divided according to their primary tissue tropism into respiratory and enterotropic groups (Barthold, 1986). Respiratory strains of MHV initially replicate in the upper respiratory tract, then disseminate to multiple other organs including liver and brain, causing hepatitis, encephalitis as well as many other forms of disease (Compton et al., 1993). Enterotropic strains of MHV replicate in the intestinal mucosa and only rarely spread to other tissues. Neonatal enteritis is the predominant symptom caused by enterotropic MHV strains (Compton et al., 1993). Primary cell tropism of the different coronavirus strains is still poorly understood, since a morphological structure of the virion has yet to be identified that is responsible for enterotropism (Compton et al., 1993). So far the only means to differentiate between respiratory and enterotropic coronaviruses is pathology.

MHV offers an excellent opportunity to study the conditions responsible for the primary tissue tropism of coronaviruses. Within its antigenic group, MHV is considered the prototype coronavirus; most of the research on the molecular biology and the replication of coronaviruses has been done with MHV (Lai, 1990). The mouse also is naturally infected with coronavirus strains of both types of tropism. MHV contains a single-stranded, positive-sense RNA genome and three or four structural proteins, depending on the strain (Compton et al., 1993). We can speculate that the tissue tropism of MHV is most likely determined by its structural proteins. We may therefore be able to demonstrate the differences between the tropism groups on the genomic level by comparing the nucleotide sequence of the structural proteins of respiratory and enterotropic strains of MHV. The molecular biology of respiratory MHV has been extensively studied since all prototype strains of MHV belong to this group. On the other hand, little information on the molecular biology of enterotropic strains of MHV is available. This study reports the nucleotide sequence of the coding region of the membrane (M) protein of three different enterotropic strains of MHV and compares it with the previously published corresponding sequence of two respiratory strains.

MHV-Y was originally isolated from an infant mouse with typhlocolitis (Barthold et al., 1982) by passage in NCTC-1469 cells and MHV-RI from a nude mouse (Barthold et al., 1985) by passage in CMT-93 cells. Both virus strains were propagated by infant mouse passage. DVIM was provided by Kathryn V. Holmes (Uniformed Services University of the Health Sciences, Bethesda, MD) and passaged in NCTC-1469 cells. Stock virus of all three MHV strains consisted of 10% infant mouse intestinal homogenates.

Total RNA was isolated from intestinal homogenates by sodium dodecyl sulfate treatment, phenol extraction and ethanol precipitation and first-strand cDNA was synthesized by reverse transcription using oligo(dT) as primer (Homberger et al., 1991). The M genes of the three virus strains were amplified by polymerase chain reaction (PCR) as described (Homberger et al., 1991). The primers flanking the M

gene region which were used in the PCR reaction (5′-AATACTTTG-GTGCTGTCC-3′ and 5′-AAGTGGTCTTCTTGAGG-3′) were synthesized based on sequence information from MHV-A59 (Susan R. Weiss, personal communication) (Microsynth AG, Windisch, Switzerland). PCR fragments were blunt-ended by removing 3′ overhangs with Klenow fragment of DNA polymerase I, phosphorylated by T4 polynucleotide kinase and ligated into the *Sma*I site of pUC 18 by T4 DNA ligase using a cloning kit (Pharmacia, Dubendorf, Switzerland). Three different clones were constructed for each virus strain, each clone originating from a separate PCR reaction.

Double-stranded plasmid DNA was first denatured with sodium hydroxide and then sequenced according to the Sanger dideoxy-mediated chain-termination method (Sambrook et al., 1989). Sequencing reaction was performed using Sequenase 2.0 (United States Biochemical, Lucerne, Switzerland) and ^{35}S-labelled dATP (Amersham, Zurich, Switzerland) according to the manufacturer's protocol.

Using internal primers (Homberger, 1991), as well as the PCR primers mentioned above, three independent clones of the M gene for each of the three virus strains were sequenced at least once in both directions. Comparing the three independent clones of each strain among themselves, it was found that all MHV-Y clones and all -DVIM clones were identical. One of the MHV-RI clones had one nucleotide difference when compared with the other two (data not shown). The M gene sequence of MHV-RI in Fig. 1 reflects the results of the two identical clones.

The M gene nucleotide sequences of the enterotropic strains MHV-Y, MHV-RI and -DVIM were compared with the previously reported sequences of the respiratory strains MHV-A59 (Armstrong et al., 1984) and MHV-JHM (Pfleiderer et al., 1986) (Fig. 1). The most prominent difference was a 15-base deletion at the 5′ end of the M gene of MHV-RI. This deletion cannot be found in any other MHV strain, either respiratory or enterotropic. All other variations between the strains were only nucleotide substitutions, no frameshifts or nonsense mutations were observed. In the following homology comparison, the deletion in MHV-RI was counted as a single mutation. The greatest homology of 99.0% was found between the strains MHV-Y and MHV-RI (7 nucleotide differences). MHV-A59 also seemed to be closely related to these strains (11 and 12 nucleotide differences) (Table 1). MHV-JHM (96.9–96.2% homology, 21–26 nucleotide differences) and DVIM (96.2–95.2% homology, 26–33 nucleotide differences) differed more significantly from MHV-Y, -RI and -A59. Between MHV-JHM and -DVIM 28 nucleotide differences (95.9% homology) were found (Table 1). Comparison of the deduced amino acid (aa) sequences of the five MHV strains (Fig. 2) indicated that MHV-Y and MHV-RI are the closest (3 aa differences) and MHV-JHM and -DVIM (10 aa differences) the most distantly related strains (Table 1). Many of the amino acid changes were located between residues 5 and 22 from the N-terminus (Fig. 2).

Coronaviruses produce a nested set of mRNAs which all have the same 3′ ends (Lai, 1990). Each mRNA starts with a common leader sequence originating from the 5′ end of the genome which binds to a conserved sequence (CTAATC-CAAAC) immediately upstream of each gene (Budzilowicz et al., 1985). In all

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MHV-Y     GTGAGACCGC CCCCGTTAGA GGTGGATGAT ATAATAATCC AAACATTATG AGTAGTACCA CTCAGGCCCC  70
MHV-RI                                                                          ----  66
DVIM                                                                         CA       70
MHV-A59   A      T      TA  GT              -C                           T             70
MHV-JHM   oooooooooo oooooooooo oooooooooo oooC                                         70

MHV-Y     AGAGCCCGTC TATCAATGGA CGGCCGACGA GGCAGTTCGA TTCCTTAAGG AATGGAACTT CTCGTTGGGC  140
MHV-RI    ---------- -                   C          A                          C       125
DVIM      TC    GA                                  A                                  140
MHV-A59                                             A                                  140
MHV-JHM     G          C           A             C    A                                140

MHV-Y     ATTATACTAC TCTTTATTAC TATCATACTA CAGTTCGGTT ACACGAGCCG TAGCATGTTT ATTTATGTTG  210
MHV-RI                                                                        C        195
DVIM                                                                          C        210
MHV-A59                                                                                210
MHV-JHM                                                                       C        210

MHV-Y     TGAAAATGAT AATCTTGTGG TTAATGTGGC CACTGACTAT TGTTTTGTGT GTTTTCAATT GCGTCTATGC  280
MHV-RI                                                                                 265
DVIM                     T                                       A                G    280
MHV-A59                                                          A                G    280
MHV-JHM                                          T               A G              G    280

MHV-Y     GCTAAATAAT GTGTACCTTG GATTTTCTAT AGTGTTTACT ATAGTGTCCA TTGTAATGTG GATTATGTAT  350
MHV-RI                     T                                                           335
DVIM                       T                                       T                   350
MHV-A59                    T                                            C              350
MHV-JHM                    T                                  G                        350

MHV-Y     TTTGTTAATA GCATCAGGTT GTTTATCAGG ACTGGTAGCT GGTGGAGCTT CAACCCTGAA ACAAACAACC  420
MHV-RI                                                                                 405
DVIM                                                                   C               420
MHV-A59                     A                                          C               420
MHV-JHM                                                                C               420

MHV-Y     TTATGTGTAT AGATATGAAA GGTACCGTGT ATGTTAGACC GATTATTGAG GATTACCATA CACTAACAGC  490
MHV-RI                                               C                                 475
DVIM       A     C                     T             C                                 490
MHV-A59                                              C                                 490
MHV-JHM    A     C                                   C                                 490

MHV-Y     CACTATTATT CGTGGCCACC TCTACATGCA AGGTGTTAAG CTAGGCACCG GTTTCTCTTT GTCTGATTTG  560
MHV-RI                                                                                 545
DVIM            C  C                  T                      T         C     G         560
MHV-A59                                                                         C      560
MHV-JHM                             T     T                                     C      560

MHV-Y     CCCGCTTATG TTACAGTTGC TAAGGTGTCA CACCTTTGCA CTTATAAGCG CGCATTCTTA GATAAGGTAG  630
MHV-RI                                                                                 615
DVIM        T               A           T  G                           TC              630
MHV-A59                                                                          C     630
MHV-JHM     T               A           T  C                      T              C     630

MHV-Y     ACGGTGTTAG CGGTTTTGCT GTTTATGTGA AGTCCAAGGT CGGAAATTAC CGACTGCCCT CAAACAAACC  700
MHV-RI                                                                            T    685
DVIM                                                    T   C                     T    700
MHV-A59                                                                                700
MHV-JHM                                                                                700

MHV-Y     GAGTGGCGCG GACACCGCAT TGTTGAGAAT CTAATCTAAA CTTTAAGGAT GTCTTTTGTT             750
MHV-RI                                                                                 735
DVIM                                                                                   750
MHV-A59                                                                                750
MHV-JHM                         T                                                      750
```

TABLE 1

Nucleotide and amino acid sequence homology between the membrane protein genes, the deduced membrane proteins of three enterotropic strains (MHV-Y, MHV-RI and -DVIM) and two respiratory strains (MHV-A59 and MHV-JHM) of MHV. The deletion in MHV-RI was counted as a single mutation.

MHV strain	Nucleotide differences (homology %)	Amino acid differences (homology %)
Y-RI	7 (99.0)	3 (98.7)
A59-Y	11 (98.4)	3 (98.7)
A59-RI	12 (98.2)	4 (98.2)
JHM-RI	24 (96.5)	7 (96.9)
JHM-A59	21 (96.9)	7 (96.9)
JHM-Y	26 (96.2)	7 (96.9)
DVIM-RI	26 (96.2)	6 (97.3)
DVIM-A59	32 (95.3)	6 (97.3)
DVIM-Y	33 (95.2)	7 (96.9)
JHM-DVIM	32 (95.3)	10 (95.6)

three enterotropic strains of MHV this leader binding site could be identified for the mRNA 6 (M protein), where it was slightly modified by the substitution of the initial C by an A, as well as for the mRNA 7 (nucleoprotein), where it was found, as in the other MHV strains, to overlap with the 3′ end of the encoding region of M protein and its termination codon (Fig. 1). The short intergenic region between the M protein and the nucleoprotein was identical for all MHV strains.

All MHV strains contain at least three different structural proteins, the nucleoprotein (N), the membrane protein (M) and the spike protein (S); hemagglutinin/esterase (HE) is found in only a few strains and then mostly in an inactive form (Compton et al., 1993). Of these proteins, the M protein is the most antigenically conserved among different MHV strains (Fleming et al., 1983). The high degree of sequence homology found in this study confirms these previous observations. While most changes in the deduced amino acid sequences of the membrane protein of the three enterotropic strains of MHV were conservative, a variable region was found toward the N-terminal of the protein, which included the five amino acid deletion unique to MHV-RI. This variable region is somewhat acidic

Fig. 1. Nucleotide sequence comparison of the membrane (M) protein genes of three enterotropic strains (MHV-Y, MHV-RI, -DVIM) and two respiratory strains of MHV (MHV-A59, MHV-JHM). The initiation and termination codons of the M gene are underlined and the initiation codon of the nucleoprotein gene is marked by double lines. The leader sequence binding sites of both the M and the nucleoprotein gene are shown by dotted lines. Nucleotide changes that result in amino acid changes are in bold print. Dashes are deleted nucleotides. The 5′ non-encoding sequence of MHV-JHM beyond the leader sequence binding site (○○○) was not available for comparison, since this information originated from an mRNA 6 clone (Pfleiderer et al., 1986).

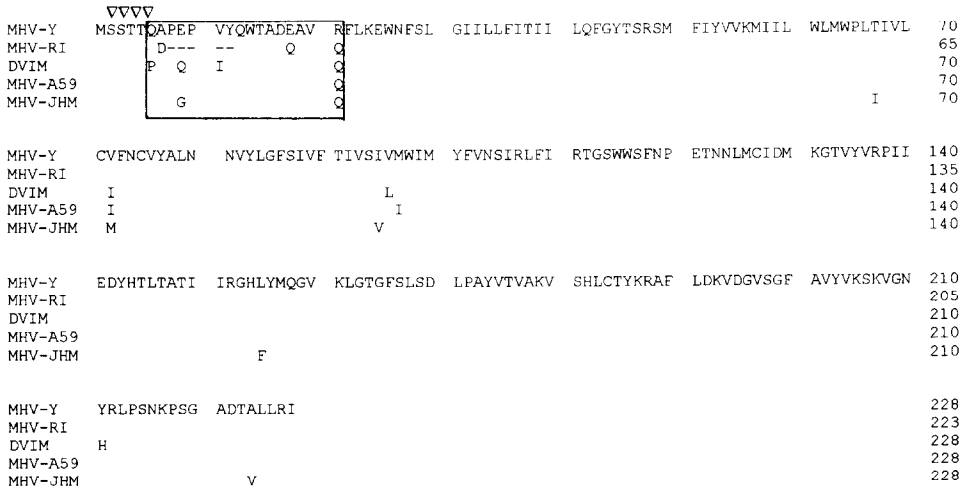

Fig. 2. Comparison of the deduced amino acid sequences of the membrane (M) proteins of three enterotropic strains (MHV-Y, MHV-RI, DVIM) and two respiratory strains (MHV-A59, MHV-JHM) of MHV. The proposed O-glycosylation sites at the N-terminal are marked (∇). The variable region containing the deletion of MHV-RI is boxed.

and hydrophilic. Since it is also located within the most exposed part of the M protein as proposed by Pfleiderer et al. (1986) it may represent an important epitope on the M protein contributing to the antigenic variability of the different strains of MHV. This region is known to show high variation among different isolates of infectious bronchitis virus, the coronavirus of chicken (Cavanagh and Davis, 1988) and it is believed to determine the ability to induce interferon production in transmissible gastroenteritis virus, an enterotropic coronavirus of the swine (Laude et al., 1992). Recent, yet unpublished findings suggest that certain amino acid changes in this variable region are correlated with neurovirulence in MHV-2/A59 recombinants (Susan R. Weiss, personal communication). The proposed O-glycosylation site (Pfleiderer et al., 1986) between the variable region and the N-terminus of the deduced protein was found to be conserved in all strains sequenced (Fig. 2).

Genetic heterogeneity of MHV strains with different pathogenicity and geographically different locations of isolation had previously been demonstrated using oligonucleotide fingerprinting (Lai et al., 1983). By comparing the nucleotide sequence of the M genes of different MHV strains, the present study was able to confirm these findings. MHV-Y, MHV-RI and MHV-A59 were shown to be relatively closely related, suggesting that they were recently derived from a common ancestor. MHV-JHM and -DVIM, on the other hand, apparently represent more distant side branches of the MHV family tree. This genetic relatedness between the strains does not correspond with either the tropism of the strain or the location of the original isolation. Respiratory MHV-A59 is much closer to enterotropic strains Y and RI than to respiratory strain JHM, whereas the strains

Y, RI and JHM were all originally isolated in the New England area (Barthold et al., 1982, 1985; Cheever et al., 1949). This suggests that the change in tissue tropism was not a single occurrence in the genesis of the different MHV strains, but may have happened at many points in time, thus creating antigenically and genomically closely related strains with distinctly different pathology. This would also explain the report of two antigenically indistinguishable MHV strains (MHV-/CDCS and MHV-S) of which one was enterotropic, whereas the other belonged to the respiratory group (Barthold, 1986).

No sequence unique to either tropism group could be identified. The findings of this study indicate that the M protein of MHV likely has no part in the determination of the tissue tropism of the respective virus strain. It may, however, independently of the tropism, reflect the phylogenetic relationship between different strains of MHV. The part of the virion which is responsible for the tropism of the strain is therefore located in either the nucleoprotein or even more likely in the spike protein, if indeed it is determined by one of the structural proteins at all.

Acknowledgements

This work was supported in part by a grant from the "Stiftung für wissenschaftliche Forschung" of the University of Zurich, Switzerland. I thank Drs. Martin Schwyzer, Stephen W. Barthold and Susan R. Compton for their help with the manuscript. The technical assistance of Kirsten Rappold is appreciated.

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