Molecular Cloning of a Complementary DNA for a Membrane Cofactor Protein (MCP, CD46)/Measles Virus Receptor on Vero Cells and Its Functional Characterization

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We isolated a 1257-bp cDNA encoding a membrane cofactor protein (MCP, CD46)/measles virus (MV) receptor-like protein from a cDNA library of Vero cells, in which wild MV strains were established. Vero cells contain MCP mRNA splice products encoding different cytoplasmic tails like human cells. The deduced amino acid sequence of the cDNA was 86% identical to that of human MCP. Vero cell MCP expressed on CHO cells was recognized by monoclonal antibodies against human MCP, and served as a potent MV receptor. In addition, Vero MCP was as effective as human MCP in human factor I-mediated C3b cleavage. Thus, the high MV susceptibility of Vero cells can in part be attributed to an MCP-like molecule that is structurally and functionally similar to human MCP.

Key words cDNA cloning; complement; measles virus; membrane cofactor protein (MCP, CD46); Vero cells

Materials and Methods

Cells, Antibodies and Proteins CHO cells were obtained from American Type Culture Collection (ATCC). Vero cells, a modified Nagahata strain of MV, and a monoclonal antibody (mAb) against MV H protein (anti-MV H) were provided by Dr. Ueda (Osaka University, Japan). Monoclonal antibodies against human MCP, M75, M160, and M177, were produced in our laboratory, and other mAbs were as described. Glycosidases were obtained as follows: neuraminidase from Sigma, and O-Glycanase from Genzyme.

Preparation of a cDNA Library and Isolation of cDNA Clones Encoding MCP Homologue Poly(A)+ RNA was purified from Vero cells using a Fast Track mRNA Isolation Kit (Invitrogen). A cDNA library was constructed from 1 μg of mRNA according to the manufacturer’s instructions (ZAP Express cDNA Synthesis kit, Stratagene). Hybridization was performed at 40°C using [α-32P]dCTP-labeled cDNA of human MCP (ST5/CYT2 phenotype). Positive clones were excised from the phages in the form of the pBK-CMV phagemid, then the nucleotide sequences were determined with a sequencer (ABI 373A) in both directions by primer walking.

Reverse Transcriptase (RT)-PCR cDNA encoding the ST-CYT regions were obtained by RT-PCR using a GeneAmp RNA PCR kit (Perkin-Elmer Cetus). The sequence of the 5′ ST region-specific primer was TTACC-TCAACGGCAGGGCAAAAA, and that of the 3′ CYT primer was AAAGATGAACTAGCAACAGG. PCR (30 cycles) was performed by denaturation at 94°C for 1 min, annealing at 65°C for 2 min, and extension at 72°C for 3 min. The PCR product was subcloned into the pCR™II vector (Invitrogen) and sequenced.

Expression of cDNA of Vero MCP in CHO Cells CHO cells were transfected with MCP-Vero subcloned into the expression vector pCXN2, then selected in a medium containing 0.5 mg/ml of G418 (GIBCO BRL). The mean fluorescence shifts of CHO cells expressing MCPs were


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assessed by flow cytometry using anti-human MCP mAbs (Profile II). Preparation of solubilized MCP, treatment with glycosidases, and SDS–PAGE followed by immunoblotting were performed as described.9

**Determination of MV Infectivity** MV binding and MV-mediated syncytia formation were determined as described.9,17 Transfectants were infected with MV at 1.25 × 10⁵—5 plaque-forming units (pfu) per cell. After the cells were cultured for 49 h at 37 °C, the number of syncytia were counted under a microscope.

**Factor I-Cofactor Activity** Cofactor activity was determined by immunoblotting factor I-catalyzed C3b fragments. C3b (0.4 µg) and factor I (0.04 µg) purified from human plasma9 were incubated in a solution of 20 mM phosphate buffer (pH 6.5) for 3h at 37 °C with various amounts of solubilized MCPs. The samples were resolved by SDS–PAGE (7.5% acrylamide) under reducing conditions, blotted onto a PVDF (polyvinylidene difluoride) membrane (Millipore), and detected with rabbit anti-C3d polyclonal antibody and a peroxidase-labeled second antibody. The amount of the C3b fragment was quantified on a Macintosh computer (Power Macintosh 7100) using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available from the Internet by anonymous FTP from nih.gov or on floppy disk from the National Technical Information Service, Springfield, Virginia, part number PB95-500195GEI).

**RESULTS**

**cDNA Cloning** Full length human MCP (ST⁵/CYT2) cDNA⁹ was used as a probe for screening 4.9 × 10⁵ independent plaques from the cDNA library of Vero cells. One of 33 positive clones, named MCP-Vero, was excised from the phage in the form of a phagemid and was sequenced. Figure 1A shows that the clone contained, between the 5' and 3' non-coding region, an open reading frame encoding 369 amino acids, including a signal peptide of 34 amino acids, with an initiation methionine at its N-terminus. Hydropathy analysis suggested that the amino acid residues 281-302 constitute a transmembrane (TM) domain. Therefore, the carboxyl terminal intracellular domain is composed of 33 amino acid residues. The molecular mass of a mature protein estimated from the amino acid composition was 37412 Da. Moreover, there were six potential N-glycosylation sites (Asn-X-Ser/Thr; Asn⁹⁹, Asnⁱ⁰⁰, Asnⁱ⁷⁸, Asnⁱ⁷⁹, Asn²²⁹, and Asn²³⁵).

Sequence alignment in Fig. 1B indicates that the protein encoded by the Vero clone is highly homologous to that of human MCP, including the signal peptide (86%). The Vero protein consists of putative functional domains such as SCR2, ST⁵, TM, and CYT2, which are 88, 100, 75, and 91% identical to the corresponding domains of the human MCP, respectively. Northern blot of the RNA from Vero cells, using the [³²P]dCTP-labeled MCP-Vero clone as a probe, showed a main band of 3.3—3.6 kb (data not shown).

**Analysis of the ST-CYT Region** To search for the ST- and CYT-variants, these regions in the mRNA of Vero cells were amplified by RT-PCR. Two products of 370 and 460 bp were obtained (not shown) and subcloned into pCR11. Eight clones were isolated and sequenced. Although only one of the clones corresponded to the 370-bp product, its sequence was the same as that of the ST⁵/CYT2 region in MCP-Vero. On the other hand, seven other clones contained a TGA stop codon at the position indicated in Fig. 1C and encoded ST⁵ and a small CYT domain that had 94% amino acid identity with the CYT1 domain of human MCP. However, no splicing products with different ST domains were obtained.

**Expression of Vero MCP on CHO Cells** CHO cells were transfected with the cDNA of MCP-Vero and a CHO transfectant, #23-6, was selected to test whether the Vero MCP is responsible for the MV infection of Vero cells. Four mAbs (M75, M177, M160, and MH61), of which the epitopes are located in either the SCR2 or SCR3 of human MCP, bound to the CHO clone, #23-6. Figure 3A shows typical flow cytometric profiles of two CHO clones, the Vero-MCP transfectant, clone #23-6, and the human-MCP transfectant, clone #8. The levels of Vero MCP expressed on CHO clone, #23-6, were the same as those of human MCP on the CHO clone #8. However, only two (4-23SB and H316) of six mAb with epitopes located in the SCR1 of human MCP bound to #23-6 (not shown).

As shown in Fig. 2, SDS–PAGE of membrane fractions of the CHO clone #23-6 and Vero cells, followed by immunoblotting with the SCR2-reactive mAb M75, revealed a single 58 kDa band from the CHO clone #23-6, while two bands of 53 and 57 kDa were detected from the Vero cells. These MCPs were slightly larger than human MCP (ST⁵/CYT2 phenotype) expressed on CHO clone #2.¹⁸ The two bands of Vero cells as well as the 58 kDa band of the CHO clone #23-6 shifted to a single 49 kDa band after the cells were digested with O-Glycanase.

**Determination of MV Infectivity and C Regulatory Activity** The data in Table 1 show that Vero MCP serves as an MV receptor. The numbers of syncytia formed increased with the amount of MV source. The results were also supported by an MV binding assay (Fig. 3B). Thus, MV can infect Vero cells by using an MCP homologue as a receptor.

Vero MCP also acted as the cofactor in human factor I-mediated cleavage of human C3b, and its activity was comparable to that of human MCP (Table 1). No cofactor activity was detected in the normal CHO cell lysate (not shown). Thus, Vero MCP appears to satisfy the structural and functional criteria of MCP.

**DISCUSSION**

Here, we obtained cDNA and predicted the amino acid sequence of the non-human primate MCP/MV receptor. Our conclusions were based on the following structural characteristics. 1) The cDNA consisted of 1257 bp with a long open reading frame very closely matching the consensus from that for human MCP (93%). 2) The predicted amino acid sequence contains four SCRIs in which the four Cys residues are all conserved as in human MCP. 3) Like human MCP, Vero cells can express several isoforms of the MCP-like protein with different cyto-
Fig. 1. The Nucleotide and Deduced Amino Acid Sequences

(A) cDNA (MCP-Vero) sequence and predicted amino acid sequence of Vero MCP. The numbers at the right side show the positions of nucleotides, whereas the amino acid positions, numbered from the predicted first residue of a mature protein, are shown below. The hydrophobic TM domain is indicated by a bold underline. The potential N-glycosylation sites are marked with arrows. The asterisk denotes the stop codon. (B) Amino acid sequence alignment of Vero MCP with human MCP (ST-CYT2 phenotype). α-Amino acid identity between the MCPs is indicated by dashed lines and the conservative substitutions of amino acids are dotted. Each SCR domain boundary is shown by arrows, and boxed amino acid residues display the conserved domains which correspond to ST and CYT2. The potential N-linked glycosylation sites are underlined. (C) The nucleotide and deduced amino acid sequences of a cDNA (MCP-Vero CYT1) obtained by RT-PCR. The amino acid residues (upper) which correspond to the CYT1 domain of human MCP (lower) are boxed.
Table 1. Functional Analysis of Vero MCP Expressed on CHO Transfectants

<table>
<thead>
<tr>
<th>CHO cell expressing</th>
<th>MV infectivity/50MV (×10⁻¹ pfa)</th>
<th>Human factor I- cofactor activity/50cell lystate (×10⁴ units/50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero MCP</td>
<td>1250</td>
<td>5 0.5</td>
</tr>
<tr>
<td>Human MCP</td>
<td>250</td>
<td>4 0.0</td>
</tr>
<tr>
<td></td>
<td>50 25</td>
<td>0 0.5</td>
</tr>
<tr>
<td></td>
<td>39 18</td>
<td>2 0.0</td>
</tr>
</tbody>
</table>

Clones 48 and 5214 were positive controls for the assays of MV infectivity and cofactor activity, respectively. The activity is expressed as arbitrary amounts of cell lysate, which were determined by measuring the density of the 51 fragment of c3B on immunoblotting. No fragments were generated when this assay was performed with the normal CHO cell lystate or without the transfected lystate. The cofactor activity equivalent to one microliter of lystate obtained from the CHO clone 52 expressing human MCP was defined as one unit.

O-Glycanase (kDa)

![Glycanase gel](image)

Fig. 2. Immunoblots of MCPs on CHO Transfectants and Vero Cells
A CHO clone #2 expressing human MCP14 (lane 2 and 3), Vero cells (lane 4 and 5), and the CHO clone #23-6 expressing Vero MCP (lane 6 and 7) were solubilized. CHO cells having no MCP were used as the control (lane 1). The proteins were resolved by SDS PAGE (10% acrylamide) under non-reducing conditions. Total proteins from solubilized cells were incubated with 1+1 or without (--) O-Glycanase. Immunoblotting was performed using the M75 mAb to human MCP as the primary antibody.

Fig. 3. Flow Cytometric Profiles of Vero and Human MCP on CHO Cell Clones

(A) Expression of Vero and human MCP. Stable CHO transfectants of MCP (clone #23-6 and clone #8 expressing Vero and human MCP, respectively) were stained with anti-human MCP mAb M17. (B) Binding of MV to CHO cells. Cells were incubated with purified MV, washed and stained with anti-MV H mAb. Both cell-bound mAbs were detected with an FITC-labeled second antibody.

plasmic tails, which will have arisen secondarily to alternative splicing of the mRNA.

The homologies in the amino acid sequence of the SCR1, SCR2, SCR3, and SCR4 between Vero and human MCP were 89. 76, 92, and 93%, respectively. The SCR2, which is responsible, together with SCR1, for MV binding to human MCP, was the lowest in terms of the homology scores in comparison to human SCRs. However, MV was sufficiently infectious to the CHO cell clone expressing Vero MCP (Table 1). M75 and M177 mAbs, which recognize the SCR2 of human MCP, can bind Vero MCP expressed on the CHO clone (Figs. 2 and 3A) as well as Vero cells. These results suggested that the conserved amino acid sequences in the SCR2 of human and Vero MCP would be essential to MV binding and provide further information on the epitopes of these mAbs.

The molecular mass of Vero MCP was estimated to be 37 kDa from its amino acid sequence, but those of the mature proteins separated from CHO transfectants and Vero cells were estimated to be 53–58 kDa by Western blotting (Fig. 2). The values were similar to those reported by Mains and others. There were six potential N-linked glycosylation sites in the SCR domains and two putative O-glycosylation sites on the ST domain in Vero MCP (Fig. 1B). We estimated that, after O-Glycanase treatment, the Vero MCP on native cells and on CHO transfectants was 49 kDa (Fig. 2). This molecular mass was the same as that of the ST³/CYT2 phenotype of human MCP, suggesting that Vero and human MCP contain similar amounts of N-linked sugars, although Vero MCP includes twice as many potential N-glycosylation sites compared with human MCP. Structural diversity in the CYT regions of Vero MCP, like that of human MCP, was also revealed by RT-PCR, which showed that Vero MCP had a smaller CYT type, corresponding to human CYT1 (Fig. 1C). With regard to the ST domains, only the ST⁵ type was detected. These results suggested that non-human primates also express MCP, differing in the sizes of the CYT domains. The four C-terminal amino acids (FTSL) in CYT1 of human MCP, which determines the intracellular processing rate of an MCP precursor, were also preserved in Vero MCP. Moreover, the nuclear targeting sites (KKKGK or KKKGK) in human MCP were also preserved at the N-terminal sides of the two CYT domains of Vero MCP. These amino acid homologies indicated that Vero MCP is expressed through the same post-translational modification pathway as human MCP. However, the few phosphorylation sites (Thr residue) in the human CYT domains were not conserved in Vero MCP, suggesting that the signal transduction pathway coupled to Vero MCP is different from that to
human MCP.

Most of the RCA proteins serve as virus receptors, since
their SCRs exhibit high affinity for various viruses as well
as for complement C3b. In human MCP, SCR1 and
SCR2 contribute to MV binding, and SCR2—4 is essential
for C3b binding and C regulation. Here, we provided
evidence that Vero MCP is not only a structural, but also
a functional, homologue of human MCP. Vero MCP acts
as a cofactor for human factor I and inactivates human
C3b. It also functions as a major receptor for MV. The
protein confers fusion competence upon CHO cells,
which are otherwise non-permissive to MV. In addition,
blocking this protein on Vero cells with M75 and M177
(which block human MCP-mediated MV infection)
abolishes CPE formation. Thus, the two fundamental
functions of human MCP are conserved in Vero MCP.

Taken together, relevant MV strains of human origin
were established through the Vero cell MCP/MV receptor,
suggesting that the tropism of MV in primates could
parallel the expression of an MCP-like molecule.

Acknowledgments We are grateful to Drs. K. Toyo-
shima, H. Akedo, M. Matsumoto (Center for Adult
Diseases), H. Nishimura (Hokkaido University), and K.
Iwata for valuable discussion. This work was supported
in part by Grants-in-Aid from the Ministry of Education,
Science, Sports and Culture and the Ministry of Health
and Welfare, of Japan.

REFERENCES AND NOTES

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reported in this paper will appear in the DDBJ/EMBL-GenBank
databases with the accession numbers D63811 and D78368,
respectively.

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