JOURNAL OF VIROLOGY, Dec. 1992, p. 7368–7373
0022-538X/92/127368-06$02.00/0

Vol. 66, No. 12

N Glycosylation of the Virus Binding Domain Is Not Essential for Function of the Human Poliovirus Receptor

ANDREE ZIBERT†* AND ECKARD WIMMER

Department of Microbiology, State University of New York at Stony Brook, Stony Brook, New York 11794-8621

Received 4 June 1992/Accepted 20 August 1992

The human poliovirus receptor (hPVR) is a glycoprotein with three immunoglobulin-like extracellular domains, of which the N-terminal domain (V-type domain) is necessary and sufficient for virus binding and uptake. The effect of N glycosylation of the V domain of hPVR on binding and entry of poliovirus was studied. Stable mouse L-cell lines were generated that express PVR-specific cDNA. One of the cell lines expressed a mutant of hPVR, in which both asparagine residues of the two N-glycosylation sites of the V domain were changed to aspartate (N105D) and serine (N120S), respectively. In the second mutant cell line, the portion of the cDNA encoding the V domain of hPVR was substituted by the homologous sequence of the recently isolated PVR cDNA from monkey cells. This V domain naturally lacks both N glycosylation sites and encodes D105 and S120 at the respective positions of the open reading frame. Absence of N glycosylation at these sites was demonstrated by in vitro translation of the two mutant coding sequences in the presence of microsomal membranes. Both PVR mutant cell lines were capable of poliovirus binding and replication. However, binding of anti-PVR monoclonal antibody D171 and protection from viral replication by this antibody were observed only with the glycosylation mutant carrying the human V domain. In contrast, infection of the cell line expressing the monkey-human hybrid receptor was not blocked even though monkey cells are fully protected by monoclonal antibody D171. The data suggest that N glycosylation of the V domain of hPVR is not essential for viral replication in human tissues and that differential glycosylation of hPVR at these sites is likely not a determinant of viral tissue tropism. Furthermore, the virus binding site and the epitope recognized by monoclonal antibody D171 do not appear to overlap.

Attachment of poliovirus to its specific receptor on host cells is a prerequisite for viral infection and pathogenesis. The availability of cDNAs encoding the receptor for poliovirus from human cells (hPVR) (11, 18) and from primate cells (13) has allowed further detailed studies of the early events in infection and could ultimately lead to an understanding of the role of PVR in mediating viral tissue tropism. Surprisingly, hPVR-specific mRNAs have been detected in human tissues that were originally not considered sites of viral replication and tissue destruction (18). Moreover, Northern (RNA) blot analyses as well as serological studies indicated a heterogeneity of hPVR-related signals, with respect to the size of both mRNA (18) and polypeptides (6, 36). This suggested that posttranscriptional or posttranslational modifications yield different species of receptor molecules.

hPVR is a polypeptide belonging to the immunoglobulin (Ig) superfamily (18), with three extracellular Ig-like domains in the order V-C2-C2 (33), a transmembrane region, and a short cytoplasmic tail. The first 143 amino acids of the coding sequence that include the N-terminal V domain of hPVR were demonstrated to be necessary and sufficient for binding and uptake of virus, when fused directly to the transmembrane region of PVR and expressed in receptor-negative mammalian cells (7, 12, 28). The two C2-type domains and the cytoplasmic portion of hPVR seemed to contribute only marginally to viral receptor activity and could be deleted (7, 12, 28) or even replaced by unrelated sequences from Ig-like domains of ICAM-1 (intercellular adhesion molecule 1) and CD4 (28, 29) or IgG1 (7). The N-terminal 143 amino acids of hPVR also carry the epitope that is recognized by anti-PVR monoclonal antibody (MAb) D171 (12, 28). In addition to its specificity to bind to all poliovirus-susceptible cells tested so far, MAb D171 efficiently blocked poliovirus replication in permissive cells by competing for the virus binding site, a property originally used for selection of this antibody (21).

Earlier studies suggested that hPVR is a glycoprotein. Lonberg-Holm (15) showed that incubation of susceptible cells with concanavalin A reduced the binding of poliovirus type 2, while Tomassini et al. (31) found that wheat germ lectin almost completely inhibited receptor-mediated binding of poliovirus type 1 (PV1). These experiments implied that the carbohydrate moieties are near the site of, or even involved in, virus binding.

On the basis of the sequence of hPVR-specific mRNA, hPVR has eight potential N-glycosylation sites, of which two occur in the first domain (11, 18). Overexpression of hPVR in insect (10) or mouse (34) cells or synthesis of receptor polypeptides by in vitro translation of hPVR-specific mRNA in the presence of dog pancreas membranes (34) confirmed glycosylation of the protein. The extent and chemical composition of carbohydrates of hPVR have not been studied. Available evidence suggests, however, that both N-glycosylation sites of the hPVR V domain are glycosylated (35).

Tissue-specific N glycosylation of a protein can account for differences in biological function of this protein (26). It seemed possible that differential glycosylation of hPVR

* Corresponding author.
† Present address: Institut für Virologie, Universitätsklinikum Essen, W-4300 Essen, Germany.

could determine whether a human tissue would be susceptible to poliovirus infection. We therefore addressed the question whether N glycosylation of the hPVR virus binding V domain is required for virus binding at all. Our studies were aided by the observation that cDNA clones of the PVR homolog derived from monkey cells (13) predict an amino acid sequence for the V domain which lacks the two N-glycosylation sites.

MATERIALS AND METHODS

Construction of plasmids. Oligodeoxynucleotides 5'-GCG AGGCGTCCCGGAGCTCCG-3' and 5'-GGTGTAGGAGC CTTCATCC-3' were used simultaneously for site-directed mutagenesis (14) of the single-strand template derived from pTMPVR (34) that contains the coding region of H20A cDNA (18). The introduced mutations were analyzed by determination of the nucleotide sequence, and the 375-bp *Spe*I-*Nhe*I fragment was reinserted into pTMPVR to generate pTMhPVR^{d1DS}. Oligodeoxynucleotides 5'-GCGCTACT AGTGCTGTCCTGG-3' and 5'-GGGGCTTGGCTAGCACT CGG-3' were used for a polymerase chain reaction with *Taq* polymerase (Cetus), using pSV2AGMα2 (13) as a template. The *Spe*I- and *Nhe*I-digested polymerase chain reaction product was inserted into the correspondingly digested pTMPVR, to generate pTMhPVR^{d1mPVR}. Determination of the nucleotide sequence confirmed the replacement of amino acids 18 to 143 of the hPVR by homologous sequences of the monkey cell-derived PVR (mPVR) (13). The 1,998-bp *Hin*dIII-*Sca*I fragments of both plasmids and of pTMPVR were each inserted into the vector APtag-1 (5), which was digested with *Hin*dIII and *Hpa*I. The plasmids generated were termed pAPhPVR^{d1DS}, pAPhPVR^{d1mPVR}, and pAPhPVR, respectively.

Generation of cell lines. Plasmids pAPhPVR^{d1DS}, pAPh PVR^{d1mPVR}, and pAPhPVR (2 μg) were transfected into mouse Ltk$^-$ cells by using a Cell-Porator (Bethesda Research Laboratories). Plasmid pLNCX (0.1 μg) expressing the thymidine kinase resistance gene (*tk*) was coelectroporated. At 30 h posttransfection, HAT (100 μg of hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine) (GIBCO) was added to the medium (Dulbecco's modified Eagle's medium, 10% fetal calf serum). Eight thymidine kinase-resistant cell colonies were isolated, propagated, and tested for binding of [^{35}S]methionine-labeled PV1 and replication of PV1. The selected cell lines were termed LhPVR^{d1DS}, LhPVR^{d1mPVR}, and LhPVR, respectively.

In vitro translation. Plasmids pTMhPVR^{d1DS}, pTMh PVR^{d1mPVR}, and pTMPVR were linearized with *Sca*I before in vitro transcription with T7 RNA polymerase (kindly provided by J. J. Dunn, Brookhaven Laboratory) as described previously (34). Cell-free reticulocyte lysate and dog pancreas microsomes were used for in vitro translation as described by the manufacturer (Promega).

Virus binding assay. PV1-infected cells were incubated with [^{35}S]methionine, and labeled poliovirus was purified by sucrose gradients (35). Incubation of stable cell lines (5×10^6 cells) with labeled poliovirus (5×10^6 PFU) was performed as described in the figures legends.

Flow cytometry. MAb D171 (100 μg/ml) was incubated 1:100 with the transfected cell lines (10^7 cells) in 100 μl of phosphate-buffered saline (PBS)–1% bovine serum albumin for 1 h at room temperature (RT). After the cells were washed in PBS (three times), a fluorescein 5-isothiocyanate-conjugated secondary antibody (1:100; Bethesda Research Laboratories) was incubated in a volume of 100 μl for 1 h at RT. The washed (two times in PBS) cells were analyzed on a FACStar analyzer (Becton Dickinson) (28).

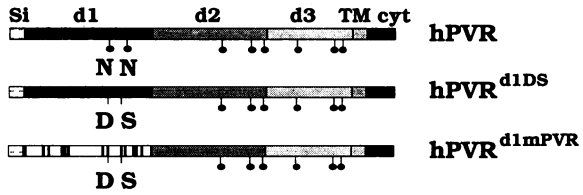

FIG. 1. Schematic overview of hPVR (wild type) and the glycosylation mutants hPVR^{d1DS} and hPVR^{d1mPVR}. The potential N-glycosylation sites at Asn (N) were changed to Asp (D) and Ser (S) at amino acids 105 and 120 of hPVR, respectively. In mutant hPVR^{d1mPVR}, the first domain of hPVR (amino acids 18 to 143) was replaced by homologous monkey sequences that also have the D and S residues. Vertical lines in hPVR^{d1mPVR} refer to the 13 amino acid differences compared with hPVR. Abbreviations and symbols: Si, signal sequence; d1 to d3, extracellular Ig-like domains; TM, transmembrane region; cyt, cytoplasmic tail; lollipops, potential N-glycosylation sites.

Plaque assays of poliovirus-infected cell lines. The transformed cell lines (10^7 cells) were incubated with PV1 (multiplicity of infection of 10) in PBS for 30 min at RT, washed five times in PBS, and incubated at 37°C for the indicated times. Cells were lysed in the culture medium by freeze-thawing, and poliovirus titers were determined on HeLa cell monolayer cultures (35).

RESULTS

Construction of mutant cDNA clones and in vitro glycosylation. The V domain (domain 1, referred to as d1) of the hPVR has two potential N-glycosylation sites, N105-A-S and N120-Y-T, while the other six glycosylation sites reside in domains 2 and 3 (18). The corresponding residues in the mPVR are D-A-S and S-Y-T (13). Accordingly, we changed the two asparagine residues at positions 105 and 120 of the hPVR to aspartate and serine, respectively. This hPVR mutant, referred to as hPVR^{d1DS}, therefore has only six glycosylation sites (Fig. 1). In a second construct, domain 1 of hPVR (amino acids 18 to 143) was replaced by homologous sequences of domain 1 derived from mPVR, thereby also reducing the total N-glycosylation sites in this hybrid receptor to six. The chimeric monkey-human PVR mutant is referred to as hPVR^{d1mPVR}. The replaced portion of the receptor has, in addition to the N-to-D and N-to-S replacements, 11 more amino acid changes (13). hPVR was used throughout this study as a positive control.

To test the integrity of the open reading frame of our constructs and to determine the effect of the mutations on N glycosylation, we placed the open reading frames downstream of the IRES element of encephalomyocarditis virus, as recently described (34). The IRES element ensures efficient translation in vitro and in vivo, and a bacteriophage T7 promoter placed upstream of the IRES element allows transcription with T7 RNA polymerase (9).

Synthetic mRNAs of the respective constructs were generated by in vitro transcription and translated in rabbit reticulocyte lysate in the presence or absence of dog pancreas microsomes. As can been seen in Fig. 2, neither mRNA significantly stimulated protein synthesis in the absence of microsomes (lanes 1, 3, and 5), an unexplained observation made previously (34). Nevertheless, weak bands in lanes 1 and 3 correspond to a molecular mass of 45

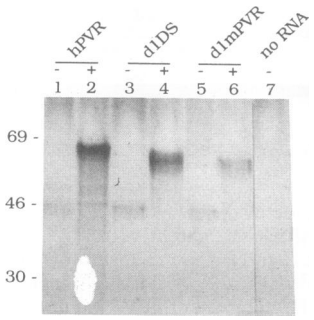

FIG. '. In vitro translation of PVR-specific RNA derived from constructs depicted in Fig. 1. The respective open reading frames were placed into plasmids pTMPVR, pTMhPVR^{d1DS}, and pTMhPVR^{d1mPVR}, which are suitable for in vitro transcription and translation. RNA (0.2 μg) was incubated with reticulocyte lysate (30 μl) in the presence (+) or absence (−) of dog pancreas microsomes. After 60 min of incubation with [^{35}S]methionine at 30°C, proteins were subjected to sodium dodecyl sulfate-12.5% polyacrylamide gel electrophoresis. RNA was derived from pTMPVR (lanes 1 and 2), pTMhPVR^{d1DS} (lanes 3 and 4), and pTMhPVR^{d1mPVR} (lanes 5 and 6). In lane 7, no RNA was added. Numbers on left show size in kilodaltons.

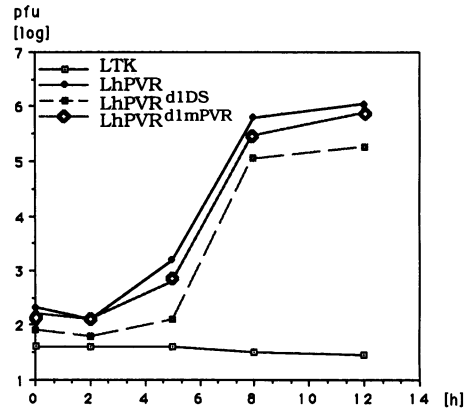

FIG. 3. Replication of poliovirus in transformed cell lines. About 10^7 cells of the cell line LhPVR, the glycosylation mutants LhPVR^{d1DS} and LhPVR^{d1mPVR}, or LTK cells (negative control) were incubated with PV1 (multiplicity of infection of 10) for 30 min at RT. Cells were then washed five times with PBS and incubated for the indicated hours at 37°C. Lysates of these incubations were prepared by freeze-thawing and plaqued on HeLa cell monolayer cultures. Numbers of plaques (PFU) are given as the mean of two experiments.

kDa, which is the expected value for the unmodified polypeptide chain (34). The corresponding band of hPVR^{d1mPVR} migrated slightly faster (Fig. 2, lane 5), which is probably due to the multiple amino acid differences in the monkey-derived portion. Addition of dog pancreas microsomes to the translation mixture led to the previously detected product of 67 kDa (34) with wild-type hPVR mRNA (Fig. 2, lane 2). The corresponding products of the mutant mRNAs that lack the N-glycosylation sites in the V domain migrated faster and had estimated molecular masses of 62 kDa (lane 4) and 61 kDa (lane 6), respectively. Immunoprecipitation of these products with hPVR-specific polyclonal antiserum 625 (34) confirmed the identity of these products (data not shown). The appearance of products with the reduced molecular weights of the PVR mutants and the previously observed in vitro glycosylation of the hPVR V domain (35) strongly suggest that mutation of the two asparagine residues abolishes glycosylation of the V domain of hPVR.

Replication of poliovirus in PVR N glycosylation mutant cell lines. The mutant PVR cDNAs were then tested for their ability to express a functional PVR. By insertion of the mutant cDNAs into a mammalian expression vector (5), the ORFs were driven by the control elements of the Moloney murine leukemia virus long terminal repeat. Stable cell lines derived after transfection of these vectors were established in mouse Ltk$^-$ cells. The three transformed cell lines, termed LhPVR, LhPVR^{d1DS}, and LhPVR^{d1mPVR}, were infected with poliovirus, and virus yields were assayed at various times after infection. As can be seen in Fig. 3, all three transformants gave rise to an increase of about 3 to 4 logs of PFU compared with receptor-negative cells (LTK). The wild-type LhPVR and the hybrid receptor LhPVR^{d1mPVR} cell lines reproducibly yielded higher levels of poliovirus than LhPVR^{d1DS}. The onset of poliovirus replication was delayed in LhPVR^{d1DS} as judged by two experiments, indicating that some of the early events (penetration and uptake) may be impaired in this cell line.

Binding of poliovirus and MAb D171. The poliovirus yields observed in the above experiments do not reveal the amount of PVR expressed in these cell lines. Also, the reduced viral yield with LhPVR^{d1DS} could be the result of a low expression of PVR. Detection of PVR-related proteins with available antibodies failed, most likely due to low levels of PVR expression. We therefore tested the three cell lines for their ability to bind virus and the receptor-specific MAb D171 (21).

Studies of poliovirus attachment at 4°C (Fig. 4A) showed that both the wild-type receptor (LhPVR)- and hybrid receptor (LhPVR^{d1mPVR})-expressing cell lines bound similar amounts of virus. The LhPVR^{d1DS} cell line, on the other hand, showed a slightly reduced binding (70%) that may correspond to the lower residual poliovirus yield at time zero postinfection observed in the above experiments (Fig. 3).

Using flow cytometry, we asked whether the N-glycosylation mutants can bind MAb D171. Previous studies showed that binding of MAb D171 to cells covaried in all cases with susceptibility to poliovirus infection (21). Moreover, the V domain of PVR alone was sufficient for binding MAb D171, which proved that poliovirus and MAb D171 bind to the same domain (12, 28, 29). Whether the D171 epitope and the virus binding site are identical, however, remained an open question. Figure 4B shows that LhPVR^{d1DS} cells bind MAb D171, although to a lesser extent than the wild-type LhPVR cells. In contrast, the cells expressing the human-monkey hybrid receptor LhPVR^{d1mPVR} did not bind MAb D171.

Blocking infection of PVR N glycosylation mutant cell lines with MAb D171. To date, binding of MAb D171 and its ability to protect cells from infection by poliovirus strictly correlate (21). If MAb D171 is unable to bind to LhPVR^{d1mPVR} cells (Fig. 4B), it should also fail to block infection of the cells with poliovirus. As expected, viral replication was completely abolished in LhPVR and LhPVR^{d1DS} cells at higher concentrations of MAb D171 (Fig. 5). In contrast, infection of LhPVR^{d1mPVR} was unaffected by the antibody. The concentration of MAb D171 used in these experiments was sufficient for blocking infection of monkey cells, and even higher concentrations of the antibody did not yield protection from poliovirus infection in LhPVR^{d1mPVR} cells (data not shown). Together with the results of virus binding (Fig. 4A), these data show that the

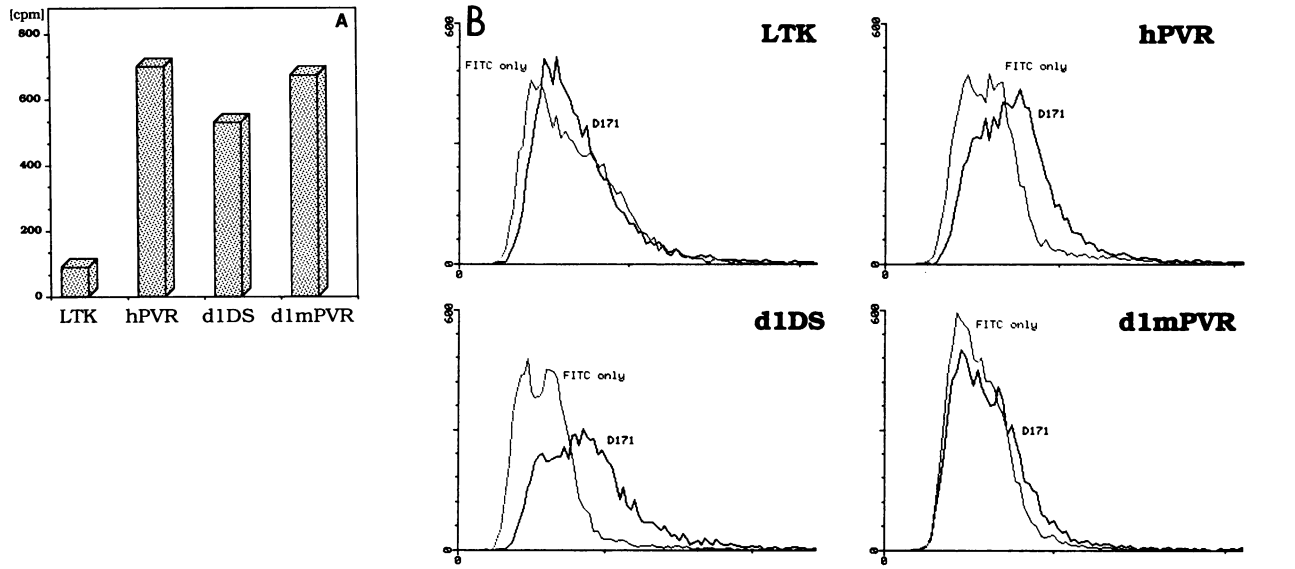

FIG. 4. Binding of ^{35}S-labeled poliovirus and MAb D171. (A) Poliovirus (5×10^6 PFU) was incubated with 5×10^6 transformed cells for 60 min at 4°C. Unbound virus was removed by washing with PBS (five times), after which the cells were collected and resuspended in Ecolume scintillation fluid (3.5 ml). Radioactivity (counts per minute) was determined in a Beckman scintillation counter (10 min). Data shown are means of five experiments. (B) Flow cytometry of transformands. The indicated cell lines (10^7 cells) were preincubated with PBS (FITC only) or MAb D171 (D171), washed, exposed to a fluorescein-conjugated secondary antibody, and analyzed by cytofluorometry. A fluorescence histogram is shown in which relative fluorescence (x axis) is plotted versus relative cell number (y axis).

epitope of MAb D171 and the poliovirus binding site are distinct entities.

DISCUSSION

The experiments presented here demonstrate that N glycosylation of the virus binding domain (V domain) of the hPVR is not a prerequisite for viral binding and uptake. An unglycosylated polypeptide comprising just domain 1 may thus be a candidate for solving the three-dimensional structure of PVR. Earlier reports that showed a reduction of poliovirus attachment by treatment of cells with sugar-specific agents (15, 31) could have been caused by direct steric hindrance of hPVR or by hindrance via unrelated glycoproteins that form complexes with hPVR.

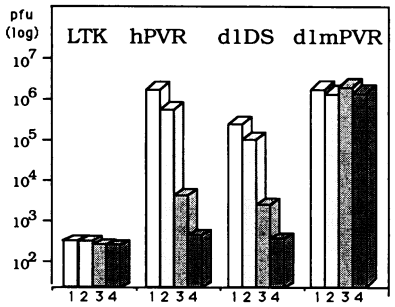

FIG. 5. Blocking of viral replication by MAb D171. Transformands (10^7 cells) were preincubated with preimmune serum (columns 1) or with serial dilutions of MAb D171 (100 μg/ml) of 1:50,000 (columns 2), 1:5,000 (columns 3), or 1:500 (columns 4). Preincubation was in PBS for 2 h at RT. PV1(Mahoney) was added (multiplicity of infection of 10) for 30 min, unbound virus was washed (five times with PBS), and cells were further cultured in medium for 14 h at 37°C. Cell lysates were prepared by freeze-thawing and plaqued on HeLa cell monolayers. Titers (PFU) shown are the mean of three experiments.

N-linked sugars have been shown to be involved in the interaction of a variety of viruses and their cellular receptors. Essential carbohydrate moieties may reside either on viral envelope proteins or on cellular receptor molecules. Examples for the former group are vesicular stomatitis virus (16), polyomavirus (8), and influenza A virus and Sendai virus (22). Moreover, tissue tropism of viral infection may be associated with glycosylation, as was reported for the HA1 protein of influenza A virus (2) and the E2 protein of Sindbis virus (3). On the other hand, glycosylation appears to be less important for viral receptors that are members of the Ig gene superfamily, although these molecules are, of course, N glycosylated (33). For example, CD4, the receptor for human immunodeficiency virus, does not contain N-glycosylation sites in its N-terminal virus binding domain, and bacterially synthesized soluble CD4 is fully functional in competing for the CD4 receptor on cells (32). Similarly, intercellular adhesion molecule 1 (ICAM-1), the receptor used by the major group of human rhinoviruses, does not harbor N-glycosylation sites in the virus binding N-terminal domain, and mutation of the two N-glycosylation sites from the second domain does not affect binding of rhinovirus (17). hPVR is therefore an exception in that its virus binding domain is glycosylated. Recently, it has been suggested that glycosylation of the receptor for mouse hepatitis virus, also a glycoprotein receptor belonging to the Ig gene superfamily (4), is involved in virus binding (23).

MAb D171 (21) is the best of all characterized antibodies that interfere with poliovirus attachment (11, 19, 30). Available evidence has shown that MAb D171 binds to domain 1 of PVR (12, 28, 29) and competes for binding with poliovirus (21). The data obtained with the human-monkey hybrid receptor cell line LhPVR^{d1mPVR} suggest that the epitope recognized by MAb D171 differs from the poliovirus binding

site. However, whereas these sites can be functionally separated, they may overlap extensively, and a subtle distortion of the molecule may have different effects with respect to the binding of the two ligands. The effect of the adjacent C2-like domains for the folding of the V domain may thus be underscored. A detailed mapping of the epitopes recognized by MAbs and the virus binding site has not been established for PVR. For other viral receptors, the epitopes recognized by antibodies that block viral infection and the respective virus binding sites have been mapped by extensive mutagenesis of the receptor. In viral receptors like ICAM-1 (17, 27) and CD4 (20, 24), those sites were closely related, whereas they were separated in the receptor used by the Epstein-Barr virus (1).

The reduced binding of MAb D171 to the LhPVR^{d1DS} cell line might be an indication of the sensitivity of the epitope to a slight distortion of the polypeptide folding introduced by the mutation. Indeed, MAb D171 has been of no use to immunoprecipitate or isolate the hPVR (18), and it has been difficult, if not impossible, to use MAb D171 in Western blots (immunoblots) to visualize the receptor, even from cells overexpressing PVR (36). Apparently, the epitope of MAb D171 is exquisitely sensitive to denaturation; hence, small changes in the amino acid sequence of the protein appear to alter this epitope.

N glycosylation of PVR could contribute to its yet unknown biological role, a phenomenon reported for differentially glycosylated forms of the insulin receptor (25). For its function as PVR, the absence of N-glycosylation sites in the V domain of the mPVR (13) already suggested that this posttranslational modification is not essential for the viral receptor activity. Although this issue was directly approached for hPVR in the studies presented here, it cannot be excluded that (hyper-) glycosylation of the two C-terminal domains may impair binding of poliovirus in those tissues that are not sites of poliovirus infection. Further studies of the posttranslational modification of PVR in tissues may shed light on factors regulating the tissue tropism of poliovirus and may also elucidate the physiological function of PVR.

ACKNOWLEDGMENTS

We are indebted to S. Koike and A. Nomoto for providing the cDNA clone pSV2AGMα2 prior to publication. We thank M. Freistadt for support in tissue culture techniques and helpful discussion, J. Simone for excellent flow cytometry, and G. Bernhardt for critical review of the manuscript.

This research was supported by Public Health Service grants AI-15122 and CA-28146 to E.W. A.Z. was supported in part by the Deutsche Forschungsgemeinschaft (DFG) and Deutscher Akademischer Austauschdienst (DAAD).

REFERENCES

1. **Carel, J.-C., B. L. Myones, B. Frazier, and V. M. Holers.** 1990. Structural requirements for C3d,g/Epstein-Barr virus receptor (CR2/CD21) ligand binding, internalization, and viral infection. J. Biol. Chem. **265**:12293–12299.
2. **Deom, C. M., A. J. Caton, and I. T. Schulze.** 1986. Host cell-mediated selection of a mutant influenza A virus that has lost a complex oligosaccharide from the tip of the hemagglutinin. Proc. Natl. Acad. Sci. USA **83**:3771–3775.
3. **Durbin, R. K., and V. Stollar.** 1986. Sequence analysis of the E2 gene of a hyperglycosylated, host restricted mutant of Sindbis virus and estimation of mutation rate from frequency of revertants. Virology **154**:135–143.
4. **Dveksler, G. S., M. N. Pensiero, C. B. Cardellichio, R. K. Williams, G.-S. Jiang, K. V. Holmes, and C. W. Dieffenbach.** 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.
5. **Flanagan, J. G., and P. Leder.** 1990. The kit ligand: a cell surface molecule altered in steel mutant fibroblasts. Cell **63**:185–194.
6. **Freistadt, M. S., G. Kaplan, and V. R. Racaniello.** 1990. Heterogeneous expression of poliovirus receptor-related proteins in human cells and tissues. Mol. Cell. Biol. **10**:5700–5706.
7. **Freistadt, M. S., and V. R. Racaniello.** 1991. Mutational analysis of the cellular receptor for poliovirus. J. Virol. **65**:3873–3876.
8. **Fried, H., L. Cahan, and J. C. Paulson.** 1981. Polyoma virus recognizes specific sialyloligosaccharide receptors on host cells. Virology **109**:188–192.
9. **Jang, S. K., T. Pestova, C. U. T. Hellen, G. W. Witherell, and E. Wimmer.** 1990. Cap-independent translation of picornavirus RNAs: structure and function of the internal ribosomal entry site. Enzyme **44**:292–309.
10. **Kaplan, G., M. S. Freistadt, and V. R. Racaniello.** 1990. Neutralization of poliovirus by cell receptors expressed in insect cells. J. Virol. **64**:4697–4702.
11. **Koike, S., H. Horie, I. Ise, A. Okitsu, M. Yoshida, N. Iizuka, K. Takeuchi, T. Takegami, and A. Nomoto.** 1990. The poliovirus receptor protein is produced both as membrane-bound and secreted forms. EMBO J. **9**:3217–3224.
12. **Koike, S., I. Ise, and A. Nomoto.** 1991. Functional domains of the poliovirus receptor. Proc. Natl. Acad. Sci. USA **88**:4104–4108.
13. **Koike, S., I. Ise, Y. Sato, H. Yonekawa, O. Gotoh, and A. Nomoto.** A second gene for poliovirus receptor of African green monkey that has no putative N-glycosylation site in the functional N-terminal immunoglobulin-like domain. Submitted for publication.
14. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
15. **Lonberg-Holm, K.** 1975. The effects of concanavalin A on the early events of infection by rhinovirus type 2 and poliovirus type 2. J. Gen. Virol. **28**:313–327.
16. **Mastromarino, P., C. Conti, P. Goldoni, B. Hauttecoeur, and N. Orsi.** 1987. Characterization of membrane components of the erythrocyte involved in vesicular stomatitis virus attachment and fusion at acidic pH. J. Gen. Virol. **68**:2359–2369.
17. **McClelland, A., J. DeBear, S. C. Yost, A. M. Meyer, C. W. Marlor, and J. M. Greve.** 1991. Identification of monoclonal antibody epitopes and critical residues for rhinovirus binding in domain 1 of intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA **88**:7993–7997.
18. **Mendelsohn, C. L., E. Wimmer, and V. R. Racaniello.** 1989. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell **56**:855–865.
19. **Minor, P. D., P. A. Pipkin, D. Hockley, G. C. Schild, and J. W. Almond.** 1984. Monoclonal antibodies which block cellular receptors of poliovirus. Virus Res. **1**:203–212.
20. **Mizukami, T., T. R. Fuerst, E. A. Berger, and B. Moss.** 1988. Binding region for human immunodeficiency virus (HIV) and epitopes for HIV-blocking monoclonal antibodies of the CD4 molecule defined by site-directed mutagenesis. Proc. Natl. Acad. Sci. USA **85**:9273–9277.
21. **Nobis, P., R. Zibirre, G. Meyer, J. Kühne, G. Warnecke, and G. Koch.** 1985. Production of a monoclonal antibody against an epitope on HeLa cells that is the functional poliovirus binding site. J. Gen. Virol. **66**:2563–2569.
22. **Paulson, J. C.** 1985. Interactions of animal viruses with cell surface receptors, p. 131–219. *In* P. M. Conn (ed.), The receptors, vol. 2. Academic Press, Inc., Orlando, Fla.
23. **Pensiero, M. N., G. S. Dveksler, C. B. Cardellichio, G.-S. Jiang, P. E. Elia, C. W. Dieffenbach, and K. V. Holmes.** 1992. Binding of the coronavirus mouse hepatitis virus A59 to its receptor expressed from a recombinant vaccinia virus depends on posttranslational processing of the receptor glycoprotein. J. Virol. **66**:4028–4039.
24. **Peterson, A., and B. Seed.** 1988. Genetic analysis of monoclonal

antibody and HIV binding sites on the human lymphocyte antigen CD4. Cell **54**:65–67.

25. **Podskalny, J. M., D. G. Rouiller, G. Grunberger, R. C. Baxter, A. McElduff, and P. Gorden.** 1986. Glycosylation defects alter insulin but not insulin-like growth factor I binding to Chinese hamster ovary cells. J. Biol. Chem. **261**:14076–14081.
26. **Rademacher, T. W., R. B. Parekh, and R. A. Dwek.** 1988. Glycobiology. Annu. Rev. Biochem. **57**:785–838.
27. **Register, R. B., C. R. Uncapher, A. M. Naylor, D. W. Lineberger, and R. J. Colonno.** 1991. Human-murine chimeras of ICAM-1 identify amino acid residues critical for rhinovirus and antibody binding. J. Virol. **65**:6589–6596.
28. **Selinka, H.-C., A. Zibert, and E. Wimmer.** 1992. A chimeric poliovirus/CD4-receptor confers susceptibility to poliovirus on mouse cells. J. Virol. **66**:2523–2526.
29. **Selinka, H. C., A. Zibert, and E. Wimmer.** 1991. Poliovirus can enter and infect mammalian cells by way of an intercellular adhesion molecule 1 pathway. Proc. Natl. Acad. Sci. USA **88**:3598–3602.
30. **Shepley, M. P., B. Sherry, and H. L. Weiner.** 1988. Monoclonal antibody identification of a 100-kDa membrane protein in HeLa cells and human spinal cord involved in poliovirus attachment. Proc. Natl. Acad. Sci. USA **85**:7743–7747.
31. **Tomassini, J. E., T. R. Maxson, and R. J. Colonno.** 1989. Biochemical characterization of a glycoprotein required for rhinovirus attachment. J. Biol. Chem. **264**:1656–1662.
32. **Traunecker, A., W. Lücke, and K. Karjalainen.** 1988. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. Nature (London) **331**:84–86.
33. **Williams, A. F., and A. N. Barclay.** 1988. The immunoglobulin superfamily. Domains for cell surface recognition. Annu. Rev. Immunol. **6**:381–405.
34. **Zibert, A., H. C. Selinka, S. O. Elroy, B. Moss, and E. Wimmer.** 1991. Vaccinia virus-mediated expression and identification of the human poliovirus receptor. Virology **182**:250–259.
35. **Zibert, A., H. C. Selinka, S. O. Elroy, and E. Wimmer.** 1992. The two N-terminal domains of the poliovirus receptor are sufficient for neutralization of virus. Virus Res. **25**:51–61.
36. **Zibert, A., H. C. Selinka, and E. Wimmer.** Unpublished data.