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A Chimeric Poliovirus/CD4 Receptor Confers Susceptibility to Poliovirus on Mouse Cells

HANS-CHRISTOPH SELINKA,†* ANDREE ZIBERT, AND ECKARD WIMMER

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

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The human poliovirus receptor consists of three extracellular immunoglobulinlike domains, a transmembrane domain, and an intracytoplasmic domain. The amino-terminal variable-type domain (V domain) of the human poliovirus receptor is necessary and sufficient for its function as a viral receptor (H.-C. Selinka, A. Zibert, and E. Wimmer, Proc. Natl. Acad. Sci. USA 88:3598–3602, 1991). In this paper, data are presented showing that transfer of the putative poliovirus receptor-binding domain to a truncated receptor for the human immunodeficiency virus results in a functional receptor for poliovirus. After expression in mouse cells, this chimeric protein confers susceptibility to poliovirus. Thus, unlike human immunodeficiency virus, poliovirus can enter mouse cells by way of a truncated CD4 receptor if the specific binding domain for poliovirus is provided.

Virus receptors are structures on the cell surface that mediate virus infection of the cell through attachment and internalization. A human poliovirus receptor (PVR) for all three serotypes of poliovirus has recently been identified by using a genetic approach (17, 18). PVR is a new member of the immunoglobulin superfamily with three extracellular domains in the order V-C2-C2, followed by a transmembrane region and a cytoplasmic tail. In the nonglycosylated form, PVR is a protein of 43 or 45 kDa, depending on the nature of the C terminus (18). After modification of some or all of its glycosylation sites, a glycoprotein of about 67 kDa could be demonstrated by Western blot (immunoblot) analysis of human tissues (4) and cultured cells (27), by expression in rabbit reticulocyte lysates, or by vaccinia virus expression in vivo (27). A similar protein of about 67 kDa was found in a baculovirus expression system (9). Recently, several deletion mutants of PVR have been described (5, 10, 22). Their properties suggest that the primary binding site for poliovirus resides in domain 1 (V domain) of PVR. We have mapped the major poliovirus-binding region to the N-terminal domain of PVR by means of a hybrid clone encoding a chimera of PVR and intercellular adhesion molecule 1 (ICAM-1) (22). In this chimeric PVRd1/ICAM receptor, the C2-type PVR domains (domains 2 and 3) were replaced by three C2-type immunoglobulinlike domains of the ICAM-1 molecule. Transfection of mouse cells with this hybrid PVRd1/ICAM receptor rendered these cells sensitive to poliovirus infection.

Immunoglobulin-related domains are described as being V- or C2-like, depending upon whether their pattern of β strands resembles variable (nine β strands) or constant (seven β strands) immunoglobulin domains (26). CD4, the human cell surface protein that serves as the receptor for the human immunodeficiency virus (HIV), is an immunoglobulinlike polypeptide with four extracellular domains. Determination of the crystal structure of the two N-terminal domains of CD4 has not completely resolved the controversy over the characteristics of the second and fourth domains of CD4 (3, 15, 24–26). Whereas domain 1 could be clearly identified as a V-like domain, domain 2 shows similarities to V- and C-like domains. However, much of the three-dimensional structure of domain 2 superimposes well on the first domain (25). To construct the PVR/CD4 chimera, we fused the N-terminal binding domain of PVR to domains 3 and 4 of CD4, since the V-like characteristic of domain 3 is undisputed and domain 4 can be considered a V domain. In this communication, we present evidence that the two C2-like domains (domains 3 and 4) of PVR can be replaced by immunoglobulin domains with a predominantly variable domainlike character. When expressed on mouse cells, this domain configuration (Fig. 1A), when attached to the transmembrane and cytoplasmic domains of CD4, resulted in a functional receptor for poliovirus.

Figure 1B outlines the organization of the three receptor constructs (PVRd1/CD4, PVR, and PVRd1) used in this study. Construction of expression plasmids pTMPVR, encoding the wild-type poliovirus receptor (PVR; 417 amino acids), and pTMd1, encoding a one-domain receptor (PVRd1; 260 amino acids), was described recently (22, 27). Plasmid pPVRd1/CD4, encoding a hybrid PVR/CD4 receptor of 398 amino acids, was constructed from pTMPVR and pSV7d, the latter containing CD4 cDNA (provided by Dan Littman). Plasmid pTMPVR contains two silent mutations at nucleotide 663 and nucleotide 946 harboring restriction sites for *Nhe*I and *Hpa*I at the C-terminal ends of domain 1 (*Nhe*I) and domain 2 (*Hpa*I), respectively. These mutations allowed us to join the immunoglobulinlike domains of PVR and CD4 at the desired boundaries. Plasmid pTMPVR was digested with *Nhe*I and *Hpa*I, and a *Nhe*I-*Hpa*I fragment of pSV7d was inserted, leading to an in-frame fusion of the coding sequence of domain 1 of PVR (amino acids 1 to 143) to the sequence coding for amino acids 214 to 468 of CD4 (Fig. 1A).

All constructs were expressed in the eukaryotic expression vector pTM1 (20), which is under the control of the bacteriophage T7 promoter, by transfection of mouse MOP8 cells through electroporation. Thirty minutes prior to transfection, the cells were infected with a recombinant vaccinia virus (vTF7-3 [7]) at a multiplicity of infection of 5 to provide T7 RNA polymerase in the cytoplasm. Flow cytometry of cells transfected with these recombinant receptors on a FACStar analyzer (Becton Dickinson) demonstrated cell

* Corresponding author.
† Present address: Abteilung Virology/Kandolf, Max-Planck Institut für Biochemie, D-8033 Martinsried, Germany.

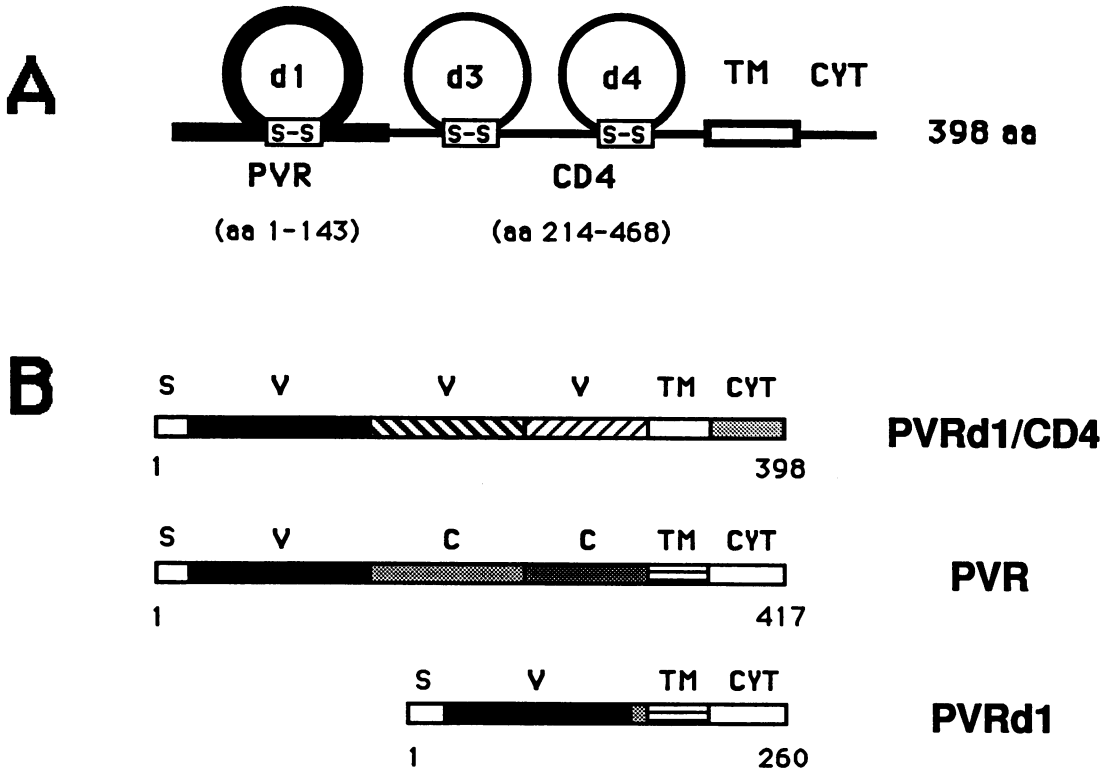

FIG. 1. Recombinant poliovirus receptors. (A) Schematic diagram of the chimeric PVR/CD4 receptor. The N-terminal binding domain of PVR (amino acids 1 to 143) was fused to a truncated CD4 receptor molecule (amino acids 214 to 468) to form a chimeric receptor with three extracellular domains. SS, disulfide bridges; aa, amino acids. (B) PVRd1/CD4 encodes a chimeric receptor with three V-like domains of PVR and CD4. In the native PVR the N-terminal V domain is followed by two C-like domains. PVRd1 is a receptor construct with only one extracellular V-like domain. S, signal peptide; TM, transmembrane domain; Cyt, cytoplasmic domain. The numbers refer to the number of amino acids.

surface expression of these molecules (Fig. 2). After being stained with poliovirus receptor-specific monoclonal antibody D171 (21), the expression of the putative receptor molecules was found to be proportional to the relative fluorescence intensity. All three receptor constructs were expressed in similar amounts on the surfaces of transfected cells. In order to determine the specific attachment of poliovirus to these cells, binding assays with ^{35}S-labeled poliovirus were conducted. As depicted in Fig. 3, a significant increase in binding of poliovirus was detected on receptor-transfected mouse cells. Therefore, fusion of the PVR binding domain to the CD4 molecule (PVRd1/CD4) maintained the overall structure of the binding site for poliovirus. Virus binding to both mutant receptors (PVRd1/CD4 and PVRd1), however, did not exceed 20 to 30% of binding to the wild-type receptor. As discussed below, domains 2 and 3 of PVR may improve presentation of the poliovirus-binding domain. We then addressed the question of whether the PVRd1/CD4 molecule is able to mediate uptake of poliovirus into cells. Poliovirus infections were carried out 12 to 16 h after transfection of receptor DNA, as described previously (22). As shown by one-step growth curves (Fig. 4), poliovirus replicated in PVRd1/CD4 receptor transfectants. The yield of infectious virus 4 h postinfection was significantly lower in cells expressing the chimeric PVRd1/CD4 receptor. However, later in infection, similar virus titers were recovered from both cell populations expressing the truncated receptors PVRd1 and PVRd1/CD4.

The observation that the PVR/CD4 chimeric receptor molecule, as well as the PVR/ICAM-1 chimera described before (22), is a functional viral receptor is interesting for several reasons. First, it confirms that the N-terminal domain of PVR is the virus-binding domain and that it is sufficient to confer receptor function for poliovirus infection (22). The N-terminal domains of the HIV receptor (CD4 [1, 11, 19]) and the rhinovirus receptor (ICAM-1 [12, 23]) are also virus-binding domains, and this may not be surprising since the outermost domain should be best accessible to virus attachment. Second, it shows that domains 2 and 3 of PVR can be replaced by V-like as well as C-like immunoglobulin-type domains without occluding receptor function. However, the data suggest that virus binding and uptake are more effective in the presence of the native domains of PVR. This observation indicates that specific sequences of the second domain, and possibly also of the third domain, are likely to influence structural features of the V domain, thereby augmenting the receptor function of PVR. Third, similar efficiencies in virus binding and uptake were obtained with recombinant receptors consisting of one (PVRd1), three (PVRd1/CD4), or four (PVRd1/ICAM) immunoglobulinlike domains. Therefore, the distance of the virus-binding domain from the cell surface did not improve efficiency, and thus spacing may not be a critical determinant for receptor function. Moreover, interactions with other cell surface molecules do not appear to be essential for virus uptake. Since the transmembrane regions of PVR, CD4, and ICAM-1

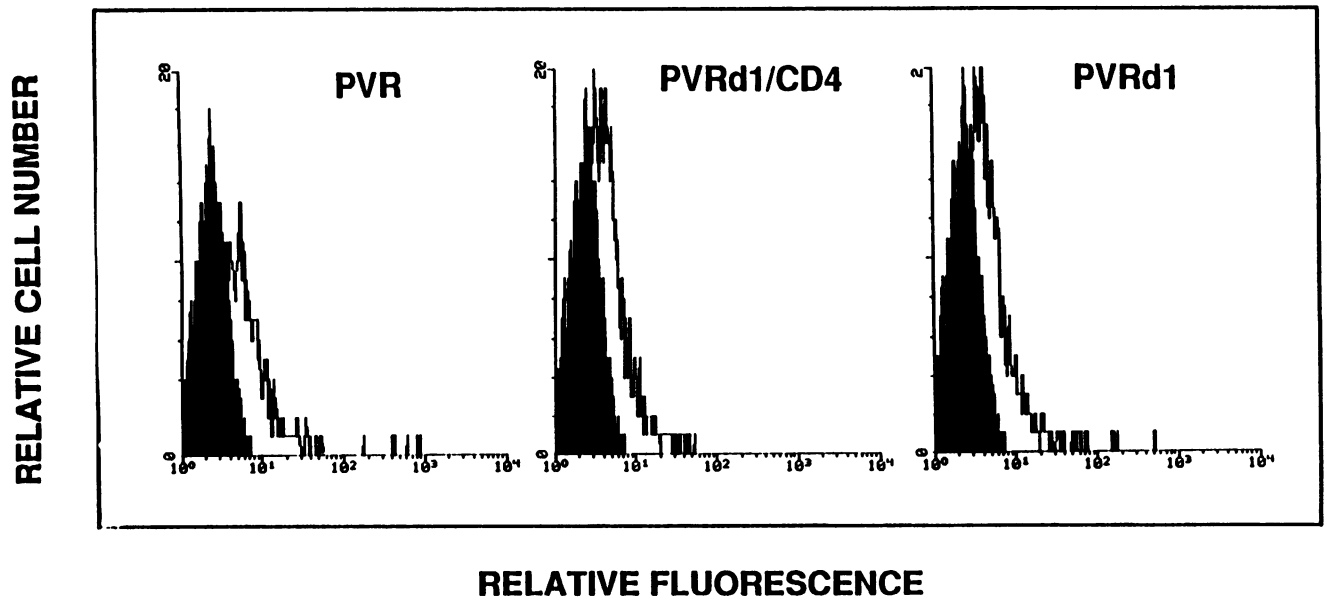

FIG. 2. Surface expression of poliovirus receptors in transiently transfected mouse MOP8 cells. The indicated transfectants were incubated with a poliovirus receptor-specific monoclonal antibody (D171), followed by labeling with a phycoerythrin-conjugated anti-mouse IgG antibody. Each fluorescence-activated cell sorter histogram represents an analysis of 10,000 viable cells of a representative transfection experiment. White areas represent fluorescence intensities of receptor-positive cells. Autofluorescence of vector-transfected (receptor-negative) cells is indicated by filled areas.

show considerable variation in their amino acid sequences, and since the respective cytoplasmic sequences of these proteins are very different, these regions may not be directly involved in the uptake of the poliovirion. Fourth, whereas poliovirus can infect mouse cells by way of a CD4-related pathway (Fig. 4), HIV can only bind to, but not infect, nonhuman cells that express the CD4 surface protein (2). This block to infection can be overcome by inducing membrane fusion by treatment with polyethylene glycol, an observation showing that the early steps of HIV infection, membrane fusion and penetration, cannot occur with $CD4^+$ nonhuman cells (2). The strategy for penetration and uncoat-

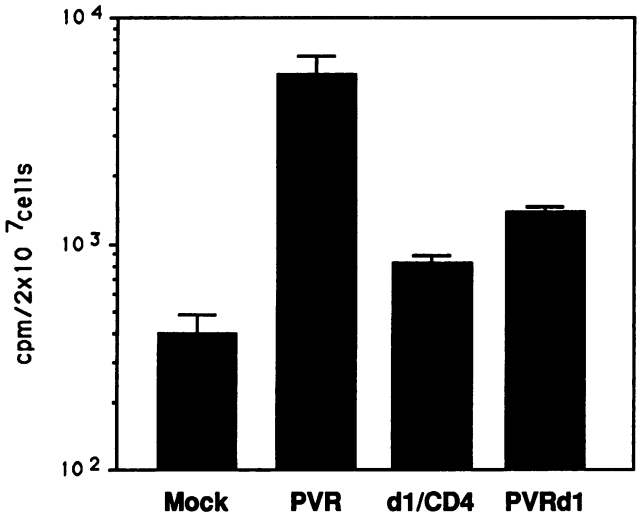

FIG. 3. Binding of poliovirus to mouse cells expressing wild-type or mutant poliovirus receptors. Monolayer cells, transfected with the indicated plasmid constructs, were incubated with ^{35}S-labeled poliovirus type 1 (4×10^4 cpm) for 2 h at 4°C. Cells were washed and lysed with 0.1 M NaOH–1% sodium dodecyl sulfate, and radioactivity was determined in Ecolume scintillation fluid. Data shown are means standard errors + SD of three experiments. Mock, mock-transfected cells.

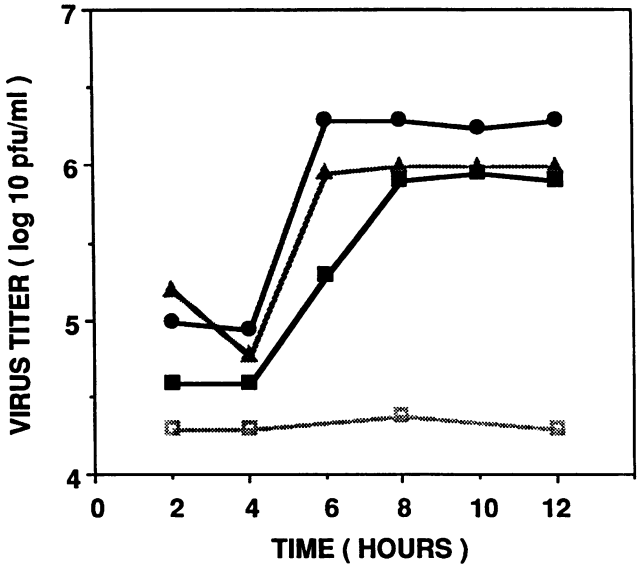

FIG. 4. Single-step growth curves of poliovirus type 1 (Mahoney) in mouse MOP8 cells transfected with recombinant poliovirus receptors. Virus was recovered at various times after infection, and the titer was determined by plaque assay on HeLa cells. ●, PVR; ■, PVRd1/CD4; ▲, PVRd1; □, pTM1 vector. Each datum point represents the mean value of three experiments.

ing of the naked poliovirus can be safely assumed to differ from that of the enveloped HIV, and that may be underscored by the data presented here.

The mechanism by which the poliovirus genome is released into the cytoplasm is poorly understood. The first step in uncoating is thought to be the receptor-mediated formation of A particles (particles lacking VP4) that, by means of their newly acquired hydrophobic properties, can interact with membranes (6, 13). Available evidence has been interpreted to mean that poliovirions, or A particles, enter cellular endosomes, whose ATP-dependent acidification is obligatory for infection (for a review, see reference 16). CD4 molecules undergo endocytosis (14), and thus it is conceivable that the PVRd1/CD4 chimeric receptor could transport poliovirus into endosomes via coated pits. On the other hand, the validity of the model of endocytosis for poliovirus uptake has been challenged recently (8). CD4-dependent HIV infection does not involve endocytosis (14). By analogy with HIV infection, it is possible that the function of the PVRd1/CD4 chimeric receptor is to position the virion in the vicinity of the plasma membrane and to convert it to an A particle that, in turn, will promptly interact with the membrane to initiate penetration. Such a mechanism, which bypasses endocytosis, could also apply to PVR-mediated poliovirus infection.

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