Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7845–7849, October 1986
Genetics

Transformation of a human poliovirus receptor gene into mouse cells

(virus receptor/gene transfer/picornavirus)

CATHY MENDELSOHN*, BARBARA JOHNSON†, KATHRYN ANN LIONETTI†, PETER NOBIS‡, ECKARD WIMMER†, AND VINCENT R. RACANIELLO*

*Department of Microbiology, Columbia University College of Physicians & Surgeons, New York, NY 10032; †Department of Microbiology, School of Medicine, State University of New York, Stony Brook, NY 11794; and ‡Physiologisch-Chemisches Institut, University of Hamburg, 2000 Hamburg 13, Federal Republic of Germany

Communicated by Frank Lilly, July 7, 1986

ABSTRACT **The first step in poliovirus replication is binding of virus to a cellular receptor. Mouse L cells, which are resistant to poliovirus infection because they do not bear a poliovirus receptor, were transformed with HeLa cell (human) DNA to poliovirus sensitivity at a frequency of ≈1 in 50,000 transformants. Monoclonal antibody directed against the HeLa cell poliovirus receptor site was used in rosette assays to identify poliovirus-sensitive L-cell transformants in a background of L-cell tk$^+$ transformants. A cloned cell line, CM-1, was isolated that displayed a surface component recognized by the anti-poliovirus receptor antibody. CM-1 cells were susceptible to infection with all three poliovirus serotypes, and infection could be blocked by the antireceptor antibody. Poliovirus formed plaques in CM-1 and HeLa cells with equal efficiency. CM-1 and HeLa cells produced infectious poliovirus at a similar rate, although yield of virus in CM-1 cells was about 33% less than the yield in HeLa cells. These results suggest that DNA encoding the HeLa cell poliovirus receptor has been introduced into mouse cells, resulting in the expression of the receptor and susceptibility to poliovirus infection.**

Poliovirus is an icosahedral RNA-containing virus with a host range that is limited to primates and primate cell cultures. In the infected host, viral replication occurs predominantly in the intestinal mucosa, in certain lymphoid tissue, and in the central nervous system (1). A large body of evidence indicates that a cellular receptor is the major determining factor in cell and tissue susceptibility to poliovirus infection (reviewed in ref. 2). This conclusion is supported by the observation that bypassing the receptor binding step by transfection of cells with RNA permits one cycle of replication in many receptor-negative mammalian cell types (3). A complete understanding of poliovirus replication and pathogenesis therefore requires better knowledge of the structure, function, and expression of the viral receptor that plays an important role in cell and tissue tropism.

Early studies showed that tissues and cell types that are susceptible to poliovirus infection contain a membrane-associated activity that is capable of specifically binding poliovirus (4–6). Subsequent studies have shown that the virus binding activity, or receptor, is an integral membrane protein (7, 8). There are ≈3000 receptor sites on the HeLa cell membrane, but it is not known how many receptors comprise a binding site (9). The three poliovirus serotypes compete for a binding site that is distinct from that of other enteroviruses (10–13). Recently, a receptor protein from coxsackievirus B3, an enterovirus related to poliovirus, was purified from HeLa cells (14). Attempts to isolate and characterize the poliovirus receptor have not been successful, probably because there are so few receptors per cell and because so far it has not been possible to measure virus binding activity in the presence of detergents (7).

To circumvent the difficulties associated with receptor purification, we have decided to study the poliovirus receptor using a genetic approach. Our goal is to use DNA-mediated gene transfer to isolate a molecular clone of the cellular receptor gene, which will then be used to study receptor structure, function, and expression. Here we show that it is possible to transfer susceptibility to poliovirus infection from HeLa cells to poliovirus-resistant mouse L cells by DNA transformation. A mouse cell transformant has been isolated that is susceptible to multicycle infection with poliovirus. Infection of the mouse cell transformant can be blocked with several independently isolated monoclonal antibodies that are directed against the HeLa cell poliovirus receptor site. The simultaneous expression in mouse cell transformants of poliovirus susceptibility and the antigenic site recognized by the monoclonal antibodies suggests that the structural gene for the receptor has been transferred from HeLa to mouse cells. The poliovirus-sensitive mouse cell line will be a valuable reagent for the isolation of the cellular gene encoding the poliovirus receptor.

MATERIALS AND METHODS

Cells, Virus, and Antibodies. HeLa S3 cells were grown in suspension or monolayer as described (15). L tk$^-$ aprt$^-$ fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum, 100 μg of penicillin per ml, 100 μg of streptomycin per ml, 30 μg of bromodeoxyuridine per ml, and 50 μg of diaminopurine per ml. L tk$^-$ aprt$^-$ cells were subcultured in the same medium containing 25 μg of amphotericin B per ml and 20 μg of gentamicin per ml and without bromodeoxyuridine and diaminopurine 48 hr prior to DNA transformation. CM-1 cells and other poliovirus-sensitive primary transformants were grown in DMEM containing 10% fetal bovine serum (Hyclone, Logan, UT), penicillin, streptomycin, amphotericin B, and gentamicin at the above concentrations and 100 μM hypoxanthine/0.4 μM aminopterin/16 μM thymidine (HAT). Poliovirus strains used were type 1 Mahoney (15), type 2 Lansing (15), and type 3 Leon (16). Three different mouse monoclonal antibodies directed against the HeLa cell receptor for poliovirus were employed: D171 (17), M1, and M2 (M. Schmidt, K.A.L., J. R. Putnak, and E.W.; unpublished results). D171 and M2 are IgG1 antibodies, whereas M1 is an IgM antibody. Competition studies suggest that all of the receptor-specific monoclonal antibodies recognize the same antigenic site. Monoclonal antibody D171 was purified

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Abbreviations: moi, multiplicity of infection; pfu, plaque-forming units; kb, kilobase(s).

on protein A-Sepharose as recommended by the manufacturer (Pharmacia). Affinity-purified goat antibody directed against mouse IgG was purchased from Sigma.

DNA Transformation. L tk^- $aprt^-$ cells (5×10^5) that had been cultured free of drugs for 48 hr were seeded in 10-cm plastic cell culture plates 12 hr before use. A DNA-calcium phosphate coprecipitate consisting of 25 μg of high molecular weight HeLa cell DNA (18) and 1 μg of a plasmid containing the herpesvirus thymidine kinase gene (LS1 6/16; ref. 19) was prepared in a volume of 1 ml as described (20) and added to each plate of cells. After 16 hr of incubation at 37°C, the medium was replaced and incubation was continued for an additional 24 hr prior to addition of medium containing HAT. After 2 weeks under HAT selection, each plate contained ≈1000–5000 tk^+ colonies.

Virus Infections. Virus titers were determined by plaque assay on HeLa cell monolayers as described (15). To identify poliovirus-sensitive L-cell transformants, each plate of 1000–5000 transformants was split 1:2 and allowed to reach confluency. Monolayers were infected with poliovirus type 1 at a multiplicity of infection (moi) of 10 and, after adsorption for 45 min at 37°C, monolayers were washed three times with DMEM to remove residual virus and then covered with medium. Aliquots of medium were removed after 0 and 48 hr of incubation and assayed for the presence of infectious virus. One-step growth curves and yield experiments were performed by infecting monolayers of cells with poliovirus at a moi of 20. After adsorption the plates were washed three times, medium was added, and the cells were incubated at 37°C. Aliquots of the cell culture medium were removed at the indicated times for determination of virus titers.

For protection experiments, monolayers of cells in 96-well plates were treated with 10 μl of phosphate-buffered saline (PBS) containing 2% fetal bovine serum and 500, 50, and 5 μg of monoclonal antireceptor antibody or preimmune mouse serum per ml. After incubation at 37°C, 25 μl of virus (diluted in PBS containing 0.2% fetal bovine serum) was added to the wells for a moi of 5. Plates were incubated for 48 hr and then stained with crystal violet to visualize poliovirus-induced cytopathic effects (15).

Rosetting and Panning Procedures. Poliovirus receptor-positive transformants were visualized on plates of tk^+ transformants using an *in situ* rosette assay (21). Cell monolayers were treated with 1.25 μg of monoclonal antireceptor antibody D171 per ml for 1 hr at room temperature. Plates were washed with PBS and then human erythrocytes that had been coated with goat anti-mouse IgG antibody (22) were added to the monolayers as indicator. Cells expressing the poliovirus receptor became covered with erythrocytes and could be located and removed with cloning cylinders and grown into large cultures. These cultures were enriched for poliovirus receptor-positive cells by using a panning technique (23). Polystyrene Petri plates were coated with a solution of affinity-purified goat anti-mouse IgG antibody (10 μg/ml) by incubating overnight at 4°C in 0.05 M $NaHCO_3$ (pH 9.6). Plates were washed with PBS and then incubated for 2 hr at 37°C with 5 μg of monoclonal antibody D171 per ml. L-cell transformants were removed from cell culture plates with 0.5 mM Na_2EDTA and added to the coated Petri plates. After 15–30 min, poliovirus receptor-positive cells adhered very tightly to the surface of the plate, while receptor-negative cells did not adhere in the presence of EDTA and could be removed from the plate by washing 10–15 times with PBS. Cells were transferred to cell culture-treated plates 24–48 hr after panning.

Southern Hybridization. Genomic DNA was isolated from cultured cells (18), digested with *Eco*RI as recommended by New England Biolabs, and fractionated on 0.8% agarose gels containing 40 mM Tris acetate, 5 mM sodium acetate, and 2 mM Na_2 EDTA (pH 7.8). DNA was transferred from the gel to nitrocellulose filters (Schleicher & Schuell) according to the method of Southern (24). DNA bound to nitrocellulose filters was hybridized with ^{32}P-labeled RNA of the human *Alu* repeat Blur-8 (25) that was synthesized using SP6 polymerase (26). Conditions for hybridization of nitrocellulose filters with RNA probes were as described (26). After hybridization the filter was washed in 0.3 M NaCl/0.03 M sodium citrate, pH 7.0/0.1% $NaDodSO_4$, first at room temperature and then at 65°C.

RESULTS

DNA-Mediated Transfer of Susceptibility to Poliovirus Infection. Our strategy for obtaining a molecular clone of the poliovirus receptor is to employ DNA transformation to transfer susceptibility to poliovirus infection from HeLa cells to nonsusceptible mouse L cells. The human receptor gene might then be identified in a background of mouse DNA by virtue of its linkage to a human repetitive sequence element. Mouse L cells do not express poliovirus receptors (5, 6, 17) but are capable of supporting one round of poliovirus replication if the requirement for attachment is bypassed by transfecting the cells with naked viral RNA (27). Therefore, L cells that express a functional poliovirus receptor should become sensitive to poliovirus infection.

Experiments were initiated to determine whether it was possible to transfer susceptibility to poliovirus infection by DNA transformation. High molecular weight HeLa cell DNA was prepared and used to transform mouse L tk^- cells using *tk* (a cloned herpesvirus thymidine kinase gene) as the coselected marker. Transformed cells were placed under HAT selection and 14 days later tk^+ transformants were screened for sensitivity to poliovirus infection. To preserve the poliovirus-sensitive transformants, since poliovirus infection is lytic, each plate was trypsinized and divided into two populations. One plate was saved and the other was infected with poliovirus, and after 48 hr supernatants from the infected cells were assayed for poliovirus. Untransformed L cells did not produce virus, whereas in 1–2 of every 30 plates of transformed cells it was possible to detect production of new infectious poliovirus, indicating that some L cells had become susceptible to infection. The results of three independently performed transformations (Table 1) show that expression of sensitivity to poliovirus infection occurred at approximately the same frequency.

Isolation of a Poliovirus-Sensitive Mouse Cell Line. Once plates containing poliovirus-sensitive L-cell transformants were identified, poliovirus receptor-positive cells were isolated from sibling plates by using an *in situ* rosette assay. Monolayers of L tk^+ transformants were first treated with anti-poliovirus receptor antibody D171 and then with human erythrocytes that were coupled to goat anti-mouse IgG antibody. Receptor-bearing cells, visualized by phase-contrast microscopy as areas of the monolayer to which eryth-

Table 1. Frequency of DNA-mediated transformation of mouse L cells to poliovirus sensitivity

Transformation	Positive plates* total plates	PV-sensitive cells† tk^+ cells
1	1/28	1/42,000
2	2/28	1/60,000
3	2/28	1/65,000

*Plates of transformants containing poliovirus-sensitive cells. Virus yields were consistent with the presence of only one poliovirus-sensitive tk^+ transformant per original transformation plate.

†Calculated by assuming that each original plate of tk^+ transformants contained at most one poliovirus (PV)-sensitive colony out of 1000–5000.

rocytes attached, were recovered using cloning cylinders. However, since the cells were recovered from confluent monolayers, receptor-positive cells were always contaminated with receptor-negative cells. Receptor-positive cells seemed to grow much slower than other tk^+ transformants and had a very low plating efficiency, making single cell cloning difficult. A panning technique was therefore used to separate receptor-positive cells from other tk^+ transformants in these cloned populations. Polystyrene Petri plates were coated with goat antibody directed against mouse IgG, followed by mouse monoclonal antireceptor antibody D171. Approximately 10^3 cells isolated with a cloning cylinder were expanded to populations of about 10^6 cells. These cells were removed from culture plates with EDTA and transferred to the antibody-coated Petri plates. Within 30 min, a small number of cells adhered tightly to the Petri plate, while the majority of tk^+ transformants did not attach and could be removed by washing. By repeating the panning procedure several times and allowing cells to multiply between each enrichment, a population of cells was obtained that was nearly 100% rosette positive. From such an enriched population of receptor-positive cells it was possible to isolate several pure cell lines by single cell cloning. One of these cell lines, CM-1, was studied further.

Poliovirus Replication in CM-1 Cells. A series of experiments was performed to compare poliovirus replication in CM-1 cells and in HeLa cells. When CM-1 cells were infected with poliovirus type 1, the cells developed a typical poliovirus-induced cytopathic effect (Fig. 1). At 5½ hr after infection CM-1 cells began to show signs of altered morphology, and by 8 hr most of the cells had rounded up and pulled away from the plate. At 24 hr after infection nearly all of the cells had detached from the surface of the plate. Development of cytopathic effect in poliovirus-infected HeLa cells progressed at a similar rate (data not shown). The same results were obtained when several other poliovirus-sensitive primary L-cell transformants were infected with poliovirus (data not shown). In addition, the poliovirus-sensitive transformants were susceptible to infection with poliovirus types 2 and 3. Monolayers of L tk^- cells showed no evidence of cytopathic effect after infection, as expected.

To compare the rate of poliovirus production in CM-1 and HeLa cells, a one-step growth experiment was performed. Cells were infected with poliovirus type 1, and at hourly intervals after infection aliquots of medium were removed and assayed for infectious poliovirus. Although release of poliovirus from HeLa cells began at 2 hr after infection, release of virus from CM-1 cells was slightly delayed (Fig. 2). After 3 hr CM-1 cells began to release virus at the same rate as HeLa cells, although virus yield from CM-1 cells after 24 hr [524 plaque-forming units (pfu) per cell] was about 33% of the yield from HeLa cells (1600 pfu per cell). The virus titer of CM-1 and HeLa cell medium at 24 hr after infection was not changed by freezing and thawing, indicating that no virus remained cell-associated (data not shown). Therefore, the virus released after 24 hr represents the total virus produced. In four additional yield experiments, virus production in CM-1 cells was always 33% of the HeLa cell yield. These results show that CM-1 cells are susceptible to poliovirus infection, but there are slight differences in poliovirus replication in the mouse cells compared to HeLa cells.

To compare the plaquing efficiency of poliovirus in CM-1 and HeLa cells, monolayers were infected with virus, incubated under agar overlay, and stained for plaques 48 hr later. Poliovirus type 1 formed plaques on CM-1 monolayers with

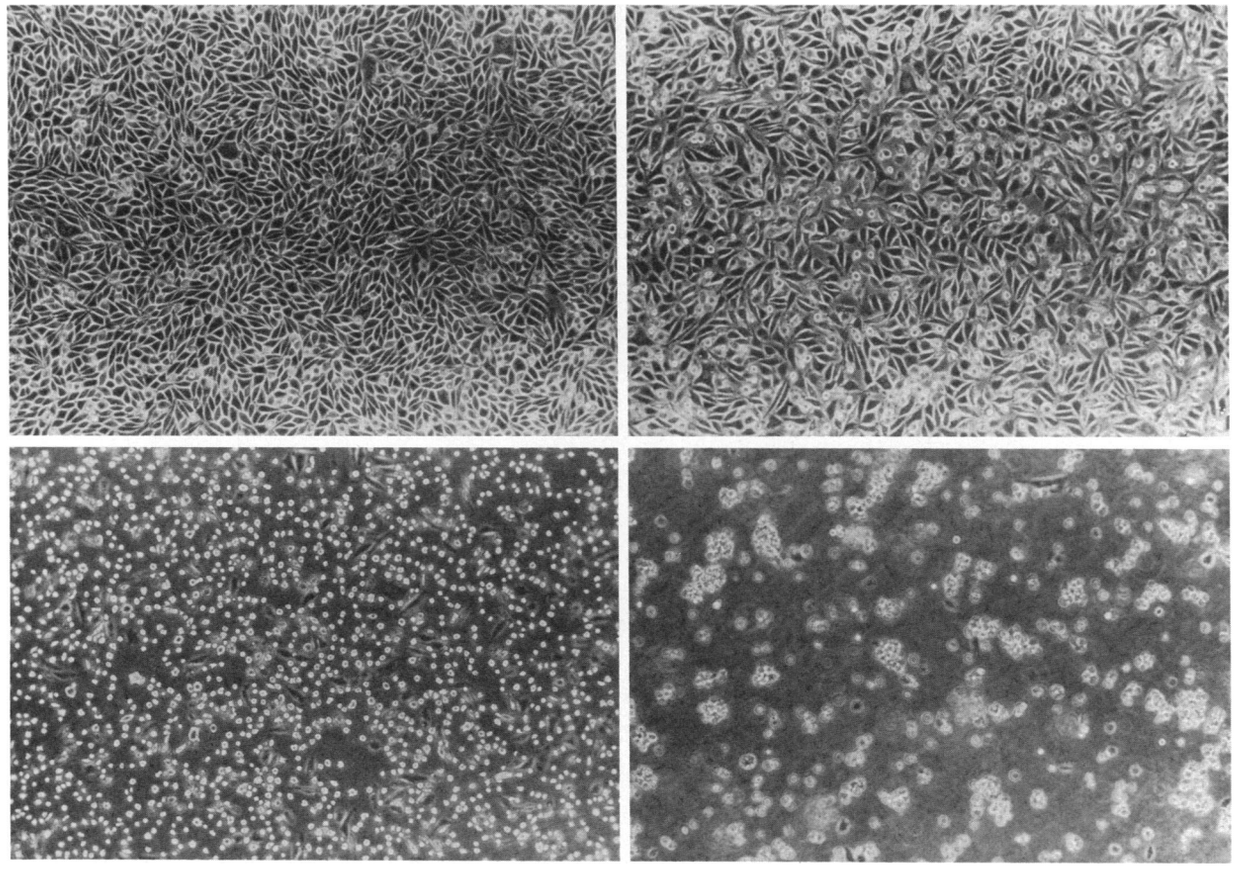

FIG. 1. Development of cytopathic effect in CM-1 cells infected with poliovirus type 1. Cells were infected with virus at a moi of 10, incubated at 37°C, and photographed through a phase-contrast microscope (×25) at indicated times. (*Upper Left*) Uninfected cells. (*Upper Right*) Five and one-half hours after infection. (*Lower Left*) Eight hours after infection. (*Lower Right*) Twenty-four hours after infection.

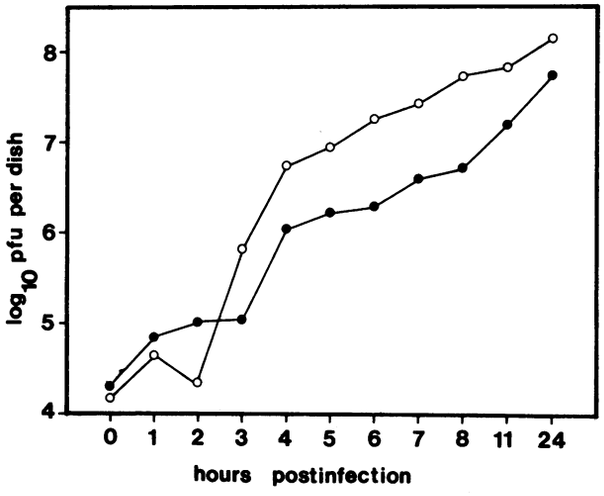

FIG. 2. Time course of release of poliovirus from CM-1 cells (●) and HeLa cells (○). Cells were infected with virus at a moi of 20 and incubated at 37°C, and at indicated times culture medium was assayed for infectious virus. Total pfu in the entire culture dish was calculated and is shown on the ordinate as $\log_{10}$ pfu.

efficiency and morphology similar to that in HeLa cells (data not shown).

It was important to determine whether the receptor recognized by poliovirus on CM-1 cells was similar to the HeLa cell receptor. Results of rosette assays indicated that the anti-HeLa cell poliovirus receptor antibody D171 reacted strongly with the surface of CM-1 cells but not L cells. To determine whether the reacting surface antigen on CM-1 transformants was a functional part of the poliovirus receptor, experiments were performed to determine if D171 could protect CM-1 cells from poliovirus infection. Monolayers of HeLa and CM-1 cells were incubated with the monoclonal antireceptor antibody D171 or normal mouse serum and then challenged separately with poliovirus type 1, 2, or 3. After 48 hr of incubation, the HeLa and CM-1 cells that had been treated with antibody D171 were fully protected from poliovirus infection by all three poliovirus serotypes at concentrations of 50 μg/ml and higher. Cells that had been incubated without antibody or with normal mouse serum were not protected from infection since cell monolayers were destroyed after 48 hr. Monoclonal anti-poliovirus receptor antibodies M1 and M2 also protected CM-1 cells from infection with all three poliovirus serotypes (data not shown).

Detection of Human Sequences in CM-1 Cells. Our strategy for cloning the poliovirus receptor gene depends on its proximity, in L-cell transformants, to a human repetitive sequence element. To determine the amount of DNA in CM-1 cells that is linked to the *Alu* repeat family, DNA from CM-1 cells was digested with *Eco*RI, fractionated on an agarose gel, and transferred to a nitrocellulose filter. DNA on the filter was hybridized with a labeled RNA prepared by SP6 transcription of the Blur-8 repeat (25). The *Alu* repeat sequence hybridized to at least seven size classes of *Eco*RI fragments in the genome of CM-1 cells, ranging in size from nearly 9.4 kilobases (kb) to <0.6 kb (Fig. 3). As expected, the *Alu* repeat did not hybridize to L tk⁻ cell DNA and hybridized strongly to HeLa cell DNA compared to CM-1 DNA. In other experiments, labeled L-cell DNA hybridized strongly with DNA of CM-1 and L cells, but there was no hybridization with HeLa cell DNA (data not shown). These results show that the genome of CM-1 primary transformants contains *Alu*-linked human DNAs. However, it is not known whether the *Alu* sequences are linked to the gene that confers poliovirus susceptibility to mouse cells.

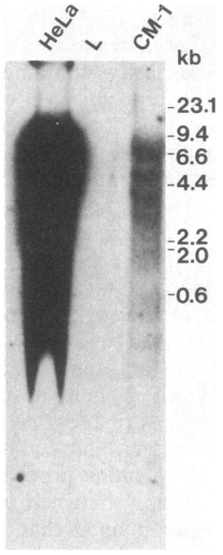

FIG. 3. Hybridization of genomic DNA with a human *Alu* repeat sequence. HeLa, L, and CM-1 cell genomic DNAs were cleaved with *Eco*RI, fractionated on an agarose gel, and transferred to a nitrocellulose filter. Immobilized DNAs were hybridized with ^{32}P-labeled RNA of the Blur-8 repeat. Molecular size markers used were generated by cleavage of bacteriophage λ DNA with *Hin*dIII.

DISCUSSION

We have shown that it is possible to transfer susceptibility to poliovirus infection from HeLa cells to mouse cells by DNA transformation. A cell line, CM-1, has been isolated that is susceptible to multicycle infection by all three poliovirus serotypes. By a number of criteria it appears that the gene for the HeLa cell poliovirus receptor has been transferred to L cells, resulting in expression of a poliovirus receptor at the cell membrane. The results of binding and infectivity studies with poliovirus and binding studies using three independently isolated monoclonal anti-poliovirus receptor antibodies indicate that L cells do not express a poliovirus receptor at the cell surface (refs. 5, 6, 17; unpublished results). However, CM-1 cells, which are L cells that have been transformed with HeLa cell DNA, display a surface molecule that reacts specifically with monoclonal antireceptor antibodies. CM-1 cells can be infected with poliovirus, and the infection can be blocked by treating cells with antireceptor antibody. These results suggest that CM-1 cells express a poliovirus receptor that is similar to the poliovirus receptor on HeLa cells.

There are several different possibilities for the structure of the poliovirus receptor and the arrangement of its gene(s) that are consistent with our ability to transfer susceptibility to poliovirus infection. The receptor might be composed of one polypeptide or more than one identical subunit. If the poliovirus receptor consisted of two different polypeptides, the corresponding genes would be cotransformed only if they were tightly linked (27). Alternatively, the poliovirus receptor might be synthesized as a single gene product, which is then cleaved to form different subunits, as was found for the insulin receptor (28).

We cannot rule out the possibility that transformation of L cells to poliovirus sensitivity results from expression of human sequences that are not related to the structural gene for the receptor. For example, DNA transformation might result in transfer of a gene encoding an enzyme that modifies a preexisting murine membrane protein so that it can serve as a poliovirus receptor. Since not only the poliovirion but also three monoclonal antibodies directed against the human poliovirus receptor react with CM-1 cells, these possibilities seem unlikely.

The host cell receptor clearly plays an important role in the initial steps of poliovirus replication, but its normal cellular function and its natural ligand are unknown. Poliovirus exhibits a characteristic tissue tropism in primates, where it appears to replicate only in motor neurons, certain lymphoid cells, Peyer patches, and intestinal mucosa (1). However,

establishment of cell cultures from other tissue types may lead to expression of the receptor. For example, primary monkey kidney cell cultures and the HeLa cell line (derived from a cervical carcinoma) are susceptible to poliovirus infection. Thus, it appears that the expression of the receptor is strictly controlled in differentiated cells. The molecular basis of this control would be of great interest to decipher.

The identity and cellular function of receptors for other RNA viruses are just beginning to emerge. For example, it has been suggested that the receptors for lactate dehydrogenase virus are Ia and Ie antigens (29), whereas the receptor for reovirus is structurally similar to the mammalian β-adrenergic receptor (30).

The ability of CM-1 cells to support multicycle poliovirus replication confirms the conclusion made many years ago by Holland *et al.* that the block to poliovirus replication in mouse cells is at the level of receptor (3). However, there are slight differences in the kinetics of virus release and the final yield of virus per cell compared to HeLa cells. These differences might be due to a cell population that is heterogeneous with respect to the yield of poliovirus per cell. Another possibility is that CM-1 cells produce less virus than HeLa cells due to differences in some of the host cell elements required for poliovirus replication. Possibly, the small differences between poliovirus replication in HeLa and CM-1 cells reflect subtle variations in some of these host factors and how efficiently they support poliovirus replication. A genetic approach to studying these host factors might employ CM-1 cells to isolate host range mutants of poliovirus that replicate in human cells but not in mouse cells.

It should be possible to isolate secondary poliovirus-sensitive L-cell transformants by transforming L cells with DNA prepared from CM-1 cells. This procedure will reduce the amount of nonessential human DNA in poliovirus-sensitive transformants. The poliovirus receptor gene may then be isolated from the background of mouse DNA in secondary transformants by virtue of its linkage to a human repetitive sequence element. A similar strategy has been used to isolate a variety of genes, including the human *RAS* oncogene (31) and the mouse ouabain-resistance gene (32). Isolation of a cloned receptor gene will enable us to analyze the structure of the receptor polypeptide, express the poliovirus receptor in different cell types, and study the individual steps by which poliovirus attaches to the receptor, a process that leads to uptake and uncoating. This information may be the basis for the development of additional antiviral drugs.

Note Added in Proof. Five independent poliovirus-sensitive L-cell secondary transformants have been isolated that share common human *Alu*-reactive DNA restriction fragments. These results strongly suggest that *Alu* sequences are linked to the gene that confers poliovirus susceptibility to mouse cells.

We thank Saul Silverstein for helpful suggestions during the course of this work and his interest in this project. This work was supported by Public Health Service Grants AI20017 (V.R.R.), AI15122 (E.W.), and CA28146 (E.W.) from the National Institutes of Health, by Grant RI-192/17-3-5 of the Deutsche Forschungsgemeinschaft (P.N.), and by Grant CCT84G115 to V.R.R. from the Chicago Community Trust/Searle Scholars Program. V.R.R. is an I. T. Hirschl Career Scientist.

1. Bodian, D. (1958) in *Viral and Rickettsial Infections of Man*, eds. Rivers, T. M. & Horsfall, F. L. (Lippincott, Philadelphia), pp. 479–498.
2. Crowell, R. L. & Landau, B. J. (1983) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum, New York), Vol. 18, pp. 1–42.
3. Holland, J. J., McLaren, J. C. & Syverton, J. T. (1959) *J. Exp. Med.* **110,** 65–80.
4. Bachtold, J. G., Bubel, H. C. & Gebhardt, L. P. (1957) *Virology* **4,** 582–589.
5. McLaren, L. C., Holland, J. J. & Syverton, J. T. (1959) *J. Exp. Med.* **109,** 475–485.
6. Holland, J. J. & McLaren, L. C. (1961) *J. Exp. Med.* **114,** 161–171.
7. Krah, D. L. & Crowell, R. L. (1982) *Virology* **118,** 148–156.
8. Krah, D. L. & Crowell, R. L. (1985) *J. Virol.* **53,** 867–870.
9. Lonberg-Holm, K. & Philipson, L. (1974) *Monogr. Virol.* **9,** 1–148.
10. Crowell, R. L. & Syverton, J. T. (1961) *J. Exp. Med.* **113,** 419–435.
11. Quersin-Thiry, L. & Nihoul, E. (1961) *Acta. Virol.* **5,** 283–293.
12. Crowell, R. L. (1963) *J. Bacteriol.* **86,** 517–526.
13. Crowell, R. L. (1966) *J. Bacteriol.* **91,** 198–204.
14. Mapoles, J. E., Krah, D. L. & Crowell, R. L. (1985) *J. Virol.* **55,** 560–566.
15. La Monica, N., Meriam, C. & Racaniello, V. R. (1986) *J. Virol.* **57,** 515–525.
16. Stanway, G., Hughes, P. J., Mountford, R. C., Reeve, P., Minor, P. D., Schild, G. C. & Almond, J. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1539–1543.
17. Nobis, P., Zibirre, R., Meyer, G., Kuhne, J., Warnecke, G. & Koch, G. (1985) *J. Gen. Virol.* **66,** 2563–2569.
18. Bollon, A. P. & Silverstein, S. (1982) in *Techniques in Somatic Cell Genetics*, ed. Shay, J. W. (Plenum, NY), pp. 415–429.
19. McKnight, S. L. & Kingsbury, R. (1982) *Science* **217,** 316–324.
20. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1373–1376.
21. Littman, D. E., Thomas, Y., Maddon, P. J., Chess, L. & Axel, R. (1985) *Cell* **40,** 237–246.
22. Goding, J. W. (1976) *J. Immunol. Methods* **10,** 61–66.
23. Mae, M. G., McHugh, L. L. & Rothstein, T. L. (1977) *J. Immunol. Methods* **15,** 47–56.
24. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
25. Jelinek, W. R., Toomey, T. P., Leinwand, L., Duncan, C. H., Biro, P. A., Choudary, P. V., Weissman, S. M. & Schmid, C. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1398–1402.
26. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12,** 7035–7056.
27. Kavathas, D. & Herzenberg, L. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 524–528.
28. Ullrich, A., Bell, J. R., Chen, E. Y., Herrera, R., Petruzzelli, L. M., Dull, T. J., Gray, A., Coussens, L., Liao, Y.-C., Tsubokawa, M., Mason, A., Seeburg, P. H., Grunfeld, C., Rosen, O. M. & Ramachandran, J. (1985) *Nature (London)* **313,** 750–762.
29. Inada, T. & Mims, C. A. (1984) *Nature (London)* **309,** 59–61.
30. Co, M. S., Gaulton, G. N., Tominaga, A., Homcy, C. J., Fields, B. N. & Greene, M. I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5315–5318.
31. Shih, C. & Weinberg, R. A. (1982) *Cell* **29,** 161–169.
32. Levenson, R., Racaniello, V., Albritton, L. & Housman, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1489–1493.