

## Construction of a Poliovirus Type 1/Type 2 Antigenic Hybrid by Manipulation of Neutralization Antigenic Site II

ANDREW D. MURDIN\* AND ECKARD WIMMER

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

Received 16 June 1989/Accepted 5 September 1989

**There are three serotypes of poliovirus, poliovirus type 1 (PV-1), PV-2, and PV-3. These viruses each display four distinct neutralization antigenic sites, designated N-AgI, N-AgII, N-AgIIIa, and N-AgIIIb. It has been demonstrated previously that part of N-AgI can be replaced with heterogeneous amino acid sequences, resulting in hybrid viruses expressing heterogeneous antigenic determinants. To study whether hybrid viruses could be constructed by modifying another antigenic site, a part of N-AgII (amino acids 158 to 173 of VP2) of PV-1(Mahoney) was replaced with the equivalent sequence from PV-2(Lansing). The resulting hybrid was viable and expressed both PV-1 and PV-2 antigenic determinants. When inoculated into rabbits, the hybrid induced neutralizing antibodies against both PV-1 and PV-2, showing that amino acids 158 to 173 of VP2 are able to function as an antigenic site independent of the rest of N-AgII. Manipulation of N-AgII represents a useful alternative method for the production of hybrid polioviruses.**

Poliovirus is the causative agent of poliomyelitis, an acute disease of the central nervous system which results in paralysis and sometimes death. Although two excellent vaccines are available (21, 22), the disease remains a serious health problem worldwide. The total eradication of circulating poliovirus by the year 2000 is a goal of international health organizations (World Health Organization, Resolution of the 41st World Health Assembly [WHA 41.28], 13 May 1988). However, this requires continuing research on the physical and biological properties of the virion, its route of infection, and the immune response to infection.

Poliovirus is an enterovirus, a member of the family *Picornaviridae*. Picornaviruses are small, icosahedral, non-enveloped viruses containing a positive-sense, single-stranded RNA genome. The capsid is composed of 60 copies each of four capsid proteins, VP1, VP2, VP3, and VP4 (2, 12). The genomes of representatives of all three serotypes of poliovirus have been sequenced and found to be closely related (9, 10, 18, 20, 23, 24), and the crystal structure of poliovirus has been determined (6). The structural core of the capsid proteins VP1, VP2, and VP3 is an eight-stranded, antiparallel beta barrel. A total of 180 of these, 60 from each protein, form a shell surrounding the genomic RNA. The fourth capsid protein, VP4, is located inside this shell. The surface features of the virus are formed by the loops, of various lengths, linking the beta strands within the beta barrels of VP1, VP2, or VP3.

Poliovirus occurs as three antigenically distinct serotypes, defined by the inability of a virus of one serotype to elicit neutralizing antibodies against viruses of the other two serotypes. The antigenic structure of poliovirus is essentially identical for all serotypes. The virion displays four distinct neutralization antigenic sites (N-Ags), specific sites to which neutralizing antibody can bind (7, 8, 13, 17, 19, 26).

These sites map largely to the protruding surface loops of VP1, VP2, and VP3 (Table 1). N-AgI includes a linear antigenic site located on the particularly well-exposed surface loop linking beta strands B and C, the BC loop, of VP1. That is, the BC loop alone, whether in its native state, denatured, or presented as a peptide, can bind neutralizing

monoclonal antibodies. N-AgI also includes residues from the DE loop of VP1 and, at least in poliovirus type 3(Sabin) [PV-3(S)], residues from the EF and GH loops. N-AgII is also discontinuous in nature, being composed of amino acids from VP1 and VP2. N-AgIII is two independent sites, IIIA and IIIB. N-AgIIIA includes amino acids of the beta turn preceding the beta B strand of VP3 and from the beta B strand itself, while N-AgIIIB includes amino acids from the BC loops of VP2 and VP3. Also described as N-AgIIIA in the PV-3(S) strain are amino acids 1287 to 1290 (we use the four-digit nomenclature for the amino acids, in which the first digit denotes the capsid protein and the remaining three digits denote the position of the amino acid within the protein; thus, amino acids 1287 to 1290 are amino acids 287 to 290 of VP1). Note that N-AgI and N-AgII may also be subdivided but that the divisions are less distinct than those in N-AgIII.

There is some evidence to suggest that a part of N-AgII, amino acids 2164 to 2172, can function as an independent

TABLE 1. Antigenic structure of poliovirus<sup>a</sup>

Antigenic site	Location	
	Structure	Amino acids
N-AgIA	BC loop of VP1	1097-1101
N-AgIB	BC loop of VP1	1099-1101
	DE loop of VP1	1142, 1144, and 1147
N-AgIIA	EF loop of VP2	2164-2170
N-AgIIB	GH loop of VP1	1221-1226
	EF loop of VP2	1221-1226
	C terminus of VP2	2270
N-AgIIIA	Beta turn preceding beta B strand	3058-3060
	Beta strand of VP3	3071-3073
N-AgIIIB	BC loop of VP2	2072
	BC loop of VP3	3076-3079

<sup>a</sup> Although described here in detail for PV-1, the general locations of the neutralization antigenic sites are common to all three serotypes. For additional details of rare mutations, see the text.

\* Corresponding author.

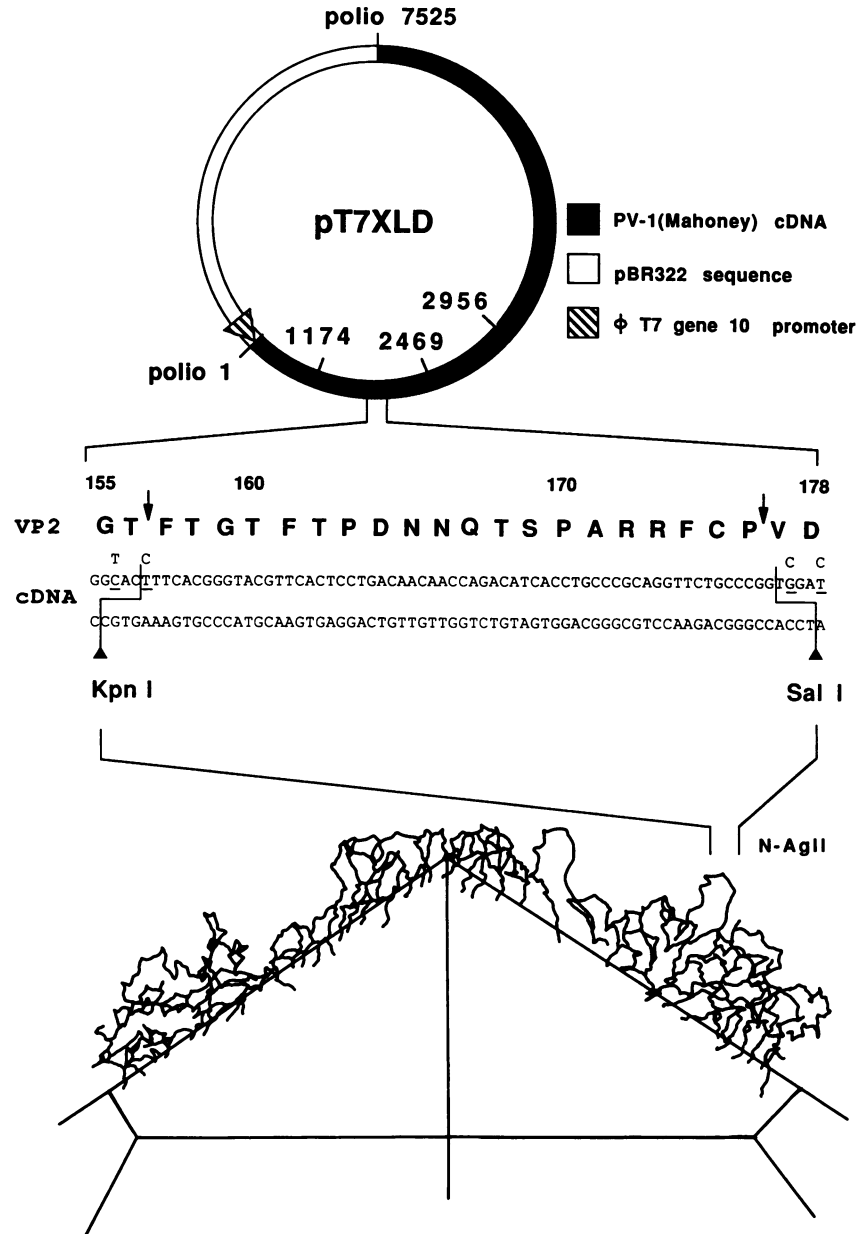


FIG. 1. Strategy for construction of a poliovirus type 1/type 2 hybrid at N-AgII. Mutations induced in the positive strand (underlined bases) of a subclone (bases 1174 to 2956) of PV-1(M) cDNA in M13mp10 form a mutagenesis cartridge bounded by unique restriction sites in double-stranded cDNA. N-AgII may be manipulated by replacing the sequence within the cartridge with synthetic oligonucleotides. Hybrid virus is produced by transferring part of the subclone, bases 1174 to 2469, into full-length poliovirus cDNA in the transcription vector pT7XLD and preparing infectious RNA transcripts as described in the text. The part of N-AgII encoded by the sequence within the mutagenesis cartridge lies on a large surface loop in VP2 (6).

antigenic site in a fashion similar to that of the BC loop of VP1. Thus Emini et al. (4) found that a synthetic peptide copying amino acids 2164 to 2175 of PV-1(Mahoney) [PV-1(M)] was immunogenic in rabbits, and Page et al. (19) have described monoclonal antibodies which differentiate this part of N-AgII from the rest of N-AgII in PV-1(S). Here we describe results which strongly support the hypothesis that the EF loop of VP2 carries an independent linear antigenic site.

A number of groups have described the construction of antigenic hybrids of poliovirus in which part of N-AgI, of the BC loop of VP1, of PV-1(M) (11, 15, 16) or PV-1(S) (1) has

been replaced with the equivalent antigenic site from type 2 or 3 viruses or with other sequences. Such hybrids are viable and possess antigenic and biological characteristics associated with both the parent virus and the exchanged antigenic site. In order to analyze the linear nature of the antigenic site formed by the EF loop of VP2, including amino acids 2164 to 2172, we decided to examine the possibility of constructing hybrids in this region, too. We have prepared a hybrid virus in which this part of N-AgII of PV-1(M) has been exchanged with the equivalent sequence from PV-2(Lansing) [PV-2(L)]. This article describes the antigenic and biological characteristics of this hybrid, on the basis of which we propose that

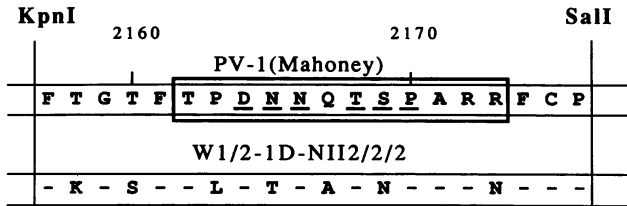


FIG. 2. Amino acid sequence of PV-1(M) and of the hybrid, NII2/2/2, within the N-AgII mutagenesis cartridge. This region lies on the EF loop of VP2. Dashes indicate residues identical in the two viruses. Residues underlined in the PV-1(M) sequence show the sites of neutralization escape mutations in PV-1(S) (19). Boxed residues correspond to the immunogenic peptide described by Emini et al. (4).

amino acids on the EF loop of VP2 form an independent linear antigenic site designated N-AgIIA (Table 1).

**MATERIALS AND METHODS**

**Construction of hybrid virus.** The hybrid was constructed by using the mutagenesis cartridge strategy developed by Murray et al. (16). A fragment of PV-1(M) cDNA (bases 1174 to 2956) was transferred into M13mp10. In this vector, a mutagenesis cartridge (Fig. 1) was constructed by mismatched-oligonucleotide site-directed mutagenesis (27). The mutated fragment was then transferred to pBS- (Bluescribe; Stratagene, La Jolla, Calif.), and in this vector the sequence within the cartridge was replaced by synthetic oligonucleotides coding for the equivalent region from PV-2(L). This resulted in the alteration of seven amino acids between amino acids 2158 and 2173 (Fig. 2). Bases 1174 to 2469 were returned to a full-length cDNA clone of PV-1(M), pT7XLD, derived from pT7PV1-5 (25) (Fig. 1). Monolayer HeLa cells were transfected with RNA transcripts of the mutant cDNA (25), and hybrid virus was harvested after complete cytopathic effect (2 to 4 days). This virus was used as a seed to prepare a working stock of virus. The identity of the virus was confirmed by sequencing the cDNA and virion RNA through the mutagenesis cartridge region.

**Immunizations.** Approximately  $2 \times 10^8$  PFU of CsCl-purified virus in 0.1 ml of water was inoculated into the peripopliteal lymph nodes of two New Zealand White rab-

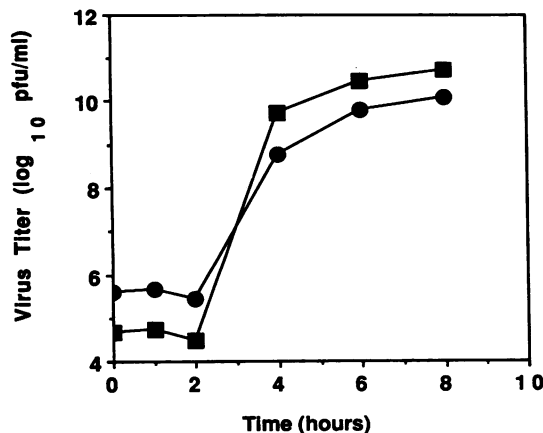


FIG. 3. Single-step growth curves of PV-1(M) (■) and the hybrid virus NII2/2/2 (●). Virus was grown and recovered as described previously (16) and then titered by plaque assay.

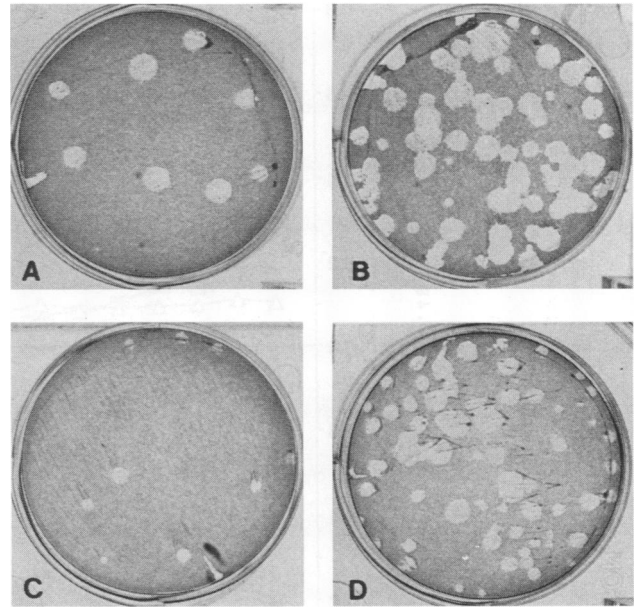


FIG. 4. Plaque phenotypes of PV-1(M) (A and B) or NII2/2/2 (C and D). Plaques were obtained during a plaque assay, as described in the text. Panels B and D show results obtained with a 10-fold greater concentration of virus than that in panels A and C. The infected HeLa cell monolayers were grown in six-well cell culture plates with 3.5-cm-diameter wells.

bits. At the same time a further  $2 \times 10^8$  PFU in Freund complete adjuvant was injected subcutaneously. Similar doses in Freund incomplete adjuvant were administered subcutaneously at 14-day intervals. The animals were bled at intervals, and the sera were tested for reactivity with PV-1(M) or PV-2(L).

**Assays.** Plaque assays were performed essentially as described by Emini et al. (3). Briefly, virus stocks were diluted in serial 1:10 steps, and then 0.1 ml of each dilution was used to infect confluent HeLa cell monolayers growing in six-well plates (Falcon 3046). The cell monolayers were stained 48 h after infection.

Serial dilutions of sera were titered by their ability to neutralize 100 50% tissue culture infective doses of poliovirus, by the method of Golding et al. (5).

Enzyme-linked immunosorbent assays (ELISAs) were performed by standard techniques. Microdilution plates (Immunoplate I; Nunc, Roskilde, Denmark) were coated with  $2 \times 10^8$  PFU of virus per well. Trapped virus was probed with rabbit antisera which were in turn probed with horseradish peroxidase-conjugated swine anti-rabbit immunoglobulin (P399; Dako Corp.). Coating was carried out overnight at 4°C with the antigen in a carbonate buffer of pH 9.6. Dilutions of antisera or conjugate were made in 0.3 M NaCl-1% Tween 20-5% calf serum in a phosphate buffer of pH 7.6. Plates were washed three times with 0.3 M NaCl-0.05% Tween 20 in phosphate buffer, pH 7.6, between each stage of the assay. The substrate for the peroxidase was o-phenylenediamine with H<sub>2</sub>O<sub>2</sub>, and the reaction was stopped with 25% H<sub>2</sub>SO<sub>4</sub> after 5 min. The A<sub>492</sub> was determined by using a Titertek Multiskan plate reader.

**RESULTS**

**Growth characteristics.** Transfection of HeLa cells with RNA transcripts of the hybrid cDNA resulted in cytopathic

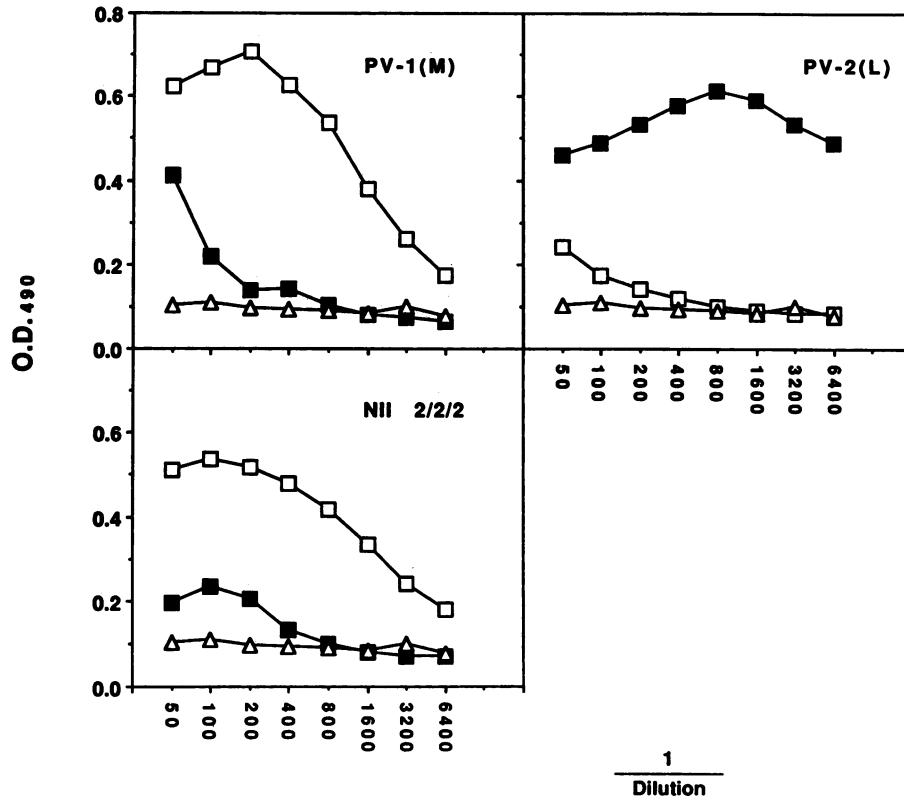


FIG. 5. ELISA showing reactivity of antisera to PV-1(M) (□) and PV-2(L) (■) with the NII2/2/2 hybrid virus, PV-1(M), or PV-2(L). A reagent blank (△) is also shown. Serial dilutions of sera were assayed as described in the text. The extent to which the sera cross-react is indicated by the relative areas under the titration curves. The ratios of these areas are as follows: anti-PV-1 versus PV-1 to anti-PV-1 versus PV-2, 8.3:1; anti-PV-2 versus PV-1 to anti-PV-2 versus PV-2, 1:14.9; anti-NII2/2/2 versus PV-1 to anti-NII2/2/2 versus PV-2, 6.4:1. Ratios closer to 1:1 indicate greater cross-reactivity.

effect and the production of a viable hybrid poliovirus, which we have designated W1/2-1B-NII2/2/2. We have arbitrarily divided the amino acid sequence of PV-1(M) between amino acids 157 and 176 of VP2 into three segments which we plan to exchange, independently of each other, with the corresponding sequences of PV-2(L). Because the hybrid described here contains the entire PV-2(L) sequence in this part of N-AgII, its designation includes the term NII2/2/2, by which we shall refer to the virus.

The growth efficiency of hybrid NII2/2/2 was impaired in comparison with that of wild-type PV-1(M). In a single-step growth cycle, the yield of infectious NII2/2/2 was typically only 10 to 30% of the yield of PV-1(M) (Fig. 3). Furthermore, the hybrid possessed a small-plaque phenotype (Fig. 4), with plaques approximately 50 to 60% of the diameter of wild-type plaques.

**Antigenicity and immunogenicity.** Hybrid virus NII2/2/2 was tested to determine whether it expressed any PV-2

TABLE 2. Neutralizing activity of rabbit antisera to PV-1(M), PV-2(L), or the NII2/2/2 hybrid

Serum (immunizing antigen) <sup>a</sup>	Activity <sup>b</sup> against virus:		
	PV-1(M)	PV-2(L)	NII2/2/2
Rb659 [PV-1(M)] HI	4,096 (10,298.5)	(<2)	>2,048
Rb359 [PV-2(L)] HI	(<2)	>16,384	2 (4.9)
Rb541 (NII2/2/2) PB 42 day <sup>c</sup>	(<4) >65,536	(<4) 8 (22.6)	(<4) 16,384 (77,936.9)
Rb542 (NII2/2/2) PB 42 day <sup>c</sup>	(<4) 8,192 (16,384.0)	(<4) 256 (781.4)	(<4) 16,384 (40,064.5)

<sup>a</sup> HI, Hyperimmune; PB, prebleed.

<sup>b</sup> Reciprocal dilution of serum giving 100% endpoint or, parentheses, 50% endpoint in a neutralization assay against 100 50% tissue culture infective doses of virus.

<sup>c</sup> Sera taken 42 days after initial vaccination.

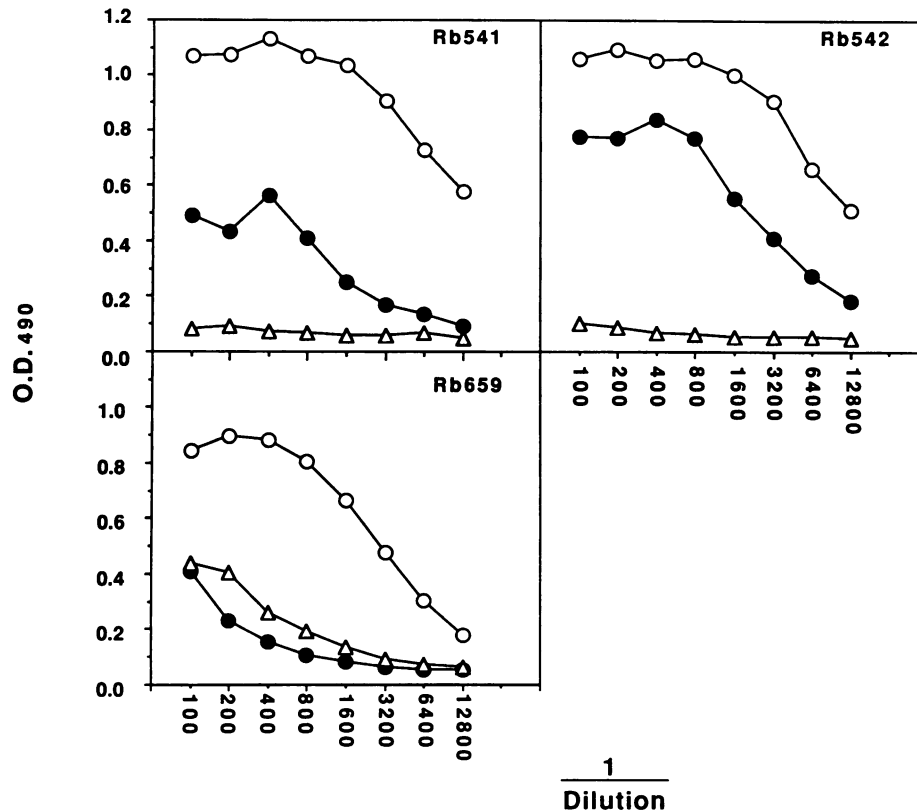


FIG. 6. ELISA showing reactivity of antisera to the NII2/2/2 hybrid virus (rabbits [Rb] 541 and 542) or PV-1(M) (rabbit 659) with PV-1(M) (○) or PV-2(L) (●). Prebleed and/or nonimmune serum (△) is also indicated. Serial dilutions of sera were assayed as described in the text.

antigenic determinants and whether it could induce antibodies to PV-2 in rabbits.

NII2/2/2 was recognized in an ELISA and efficiently neutralized by antiserum to PV-1(M) (Fig. 5; Table 2). This is the expected result, since the hybrid should express unaltered N-AgI, N-AgIIIA, and N-AgIIIB with PV-1 specificity. Significantly, the hybrid was also recognized, albeit weakly, by antisera to PV-2(L). When probed in an ELISA with antiserum to the two wild-type viruses, NII2/2/2 was more cross-reactive, as shown by the relative areas under the titration curves for each antiserum, than were either PV-1(M) or PV-2(L) (Fig. 5).

Poliovirus bound to a solid surface, as in this assay, may become more cross-reactive, and it could be argued that this accounts for the result. However, we think it unlikely that the enhanced cross-reactivity of NII2/2/2 is solely due to this effect, since all viruses were assayed under identical conditions. Furthermore, in a neutralization assay, NII2/2/2 was neutralized by antisera to PV-1(M) and PV-2(L) (Table 2). The antiserum to PV-2 neutralized the hybrid only weakly, but the effect was reproducible. This result must have been due to the presence of a PV-2(L) antigenic determinant, since the antiserum to PV-2(L) did not neutralize wild-type PV-1(M) (Table 2).

Conclusive proof that NII2/2/2 expressed a type 2 antigenic determinant was obtained when the virus was inoculated into rabbits. Antisera taken from inoculated rabbits recognized both PV-1(M) and PV-2(L) in an ELISA (Fig. 6) and were able to neutralize both of these viruses (Table 2). Interestingly, antisera to NII2/2/2 neutralized PV-2(L) considerably better than antiserum to PV-2(L) neutralized the

hybrid, despite the fact that anti-PV-2(L) had a higher titer against the homologous virus.

## DISCUSSION

We have constructed a mutagenesis cartridge which allows us to manipulate part of N-AgII, amino acids 2157 to 2176, of PV-1(M). Replacing amino acids 2158 to 2173 with the PV-2(L) equivalents resulted in a hybrid virus expressing both PV-1 and PV-2 antigenic determinants. Previous studies by a number of groups (1, 11, 15, 16) have shown that a part of N-AgI, the BC loop of VP1, can be successfully manipulated to yield antigenic hybrid viruses. To our knowledge, this is the first report of the use of N-AgII in the construction of a viable and immunogenic poliovirus hybrid.

It is of interest that the sequence replaced constitutes only part of N-AgII; the full site includes amino acids from VP1 and VP2 in addition (Table 1). On the basis of the results of Emimi et al. (4) and Page et al. (19), it appeared that the EF loop of VP2 was a neutralization antigenic determinant in its own right, able to function independently of the rest of N-AgII. The results presented here show clearly that this loop does indeed carry an independent linear antigenic site. Accordingly, we propose that this site be designated N-AgIIA and that the other antigenic determinants within N-AgII be designated N-AgIIB (Table 1). By analogy, the BC loop of VP1 should then be designated N-AgIA.

Manipulation of N-AgIIA was detrimental to virus growth but no more so than manipulation of N-AgIA. The NII2/2/2 virus grew at least as well as the type 1/type 3 N-AgIA hybrid described by Murray et al. (16), although not as well

as the type 1/type 2 N-AgIA hybrid described by Murray et al. (15). Both of these hybrids exhibited a small-plaque phenotype (Murray et al., unpublished observations), in common with the NII2/2/2 hybrid.

The relatively weak recognition and neutralization of NII2/2/2 by rabbit antiserum to PV-2(L) imply that the EF loop of VP2 is not recognized as a major antigenic determinant on PV-2(L) by the rabbit immune system. However, it is clearly recognized sufficiently well on NII2/2/2 to stimulate significant titers of loop-specific neutralizing antibody. This appears to be a situation similar to that reported previously (14), in which a hybrid expressing part of the PV-2(L) N-AgI on a PV-1(M) capsid was not neutralized by antiserum to PV-2 but could elicit PV-2-neutralizing antibodies in rabbits. We interpret this to mean that the hybrids and the wild-type viruses stimulate different sets of loop-specific antibodies. The full significance of this finding is unclear, but some comments can be made. First, it is not sufficient to screen hybrids by assessing the extent to which they are neutralized by antisera raised against the donor of the inserted antigenic site [in this case PV-2(L)], since a poor result in such a test does not mean that the hybrid will not induce antibodies that bind to and neutralize the donor virus. Second, this result implies either that the inserted sequence is expressed differently on the hybrid than on PV-2(L), even though the sequence occupies an equivalent location on the capsid, or that, in rabbits, N-AgIIA of PV-2(L) is relatively immunorecessive compared with N-AgIIA of PV-1(M). Whichever is true, the result serves as a useful reminder of the constraints affecting the expression of heterogeneous antigenic sites on hybrid viruses.

The ability to successfully manipulate a second antigenic site on poliovirus is potentially useful, for several reasons. The N-AgI cartridge described by Murray et al. (16) spans 16 amino acids, but 8 of these lie within beta sheet B, part of the structural core of VP1, rather than within the BC loop comprising N-AgIA. The N-AgII cartridge described here spans 20 amino acids, all of which are included in the largest surface loop on the virus, amino acids 2127 to 2185 (6). A number of hybrids have been constructed in this laboratory which are antigenically inert; for instance, a part of N-AgII of PV-2(L) was placed in N-AgI of PV-1(M), and a viable hybrid was obtained which did not display any PV-2 antigenic characteristics. One interpretation of this result is that the structural constraints imposed by the N-AgI site are unfavorable to the inserted sequence. The N-AgII site offers a new site, subject to different structural constraints, which may be more favorable for some sequences.

The use of the two sites in conjunction offers the possibility of constructing a trivalent hybrid poliovirus expressing type 1, 2, and 3 antigenic sites or, more exotically, rhinovirus, hepatitis A virus, and poliovirus antigenic sites, for example. We hope to explore fully the uses of this new mutagenesis cartridge.

#### ACKNOWLEDGMENTS

This work was supported by grant 88P00009 from the World Health Organization and by Public Health Service grants AI 15122 and CA 28146 from the National Institutes of Health to E.W.

We acknowledge Michael G. Murray for much helpful advice and Atsuko Kameda and Christopher Helmke for technical assistance, artwork, and photography.

#### LITERATURE CITED

- Burke, K. L., G. Dunn, M. Ferguson, P. D. Minor, and J. W. Almond. 1988. Antigen chimaerae of poliovirus as potential new

- vaccines. *Nature (London)* 332:81-82.
- Cooper, P. D., V. I. Agol, H. L. Bachrach, F. Brown, Y. Ghendon, A. J. Gibbs, J. H. Gillespie, K. Lonberg-Holm, B. Mandel, J. L. Melnick, S. B. Mohanty, R. L. Povey, R. R. Rueckert, F. L. Schaffer, and D. A. J. Tyrrell. 1978. Picornaviridae: second report. *Intervirology* 10:165-180.
- Emini, E. A., B. A. Jameson, and E. Wimmer. 1983. Priming for and induction of anti-poliovirus neutralising antibodies by synthetic peptides. *Nature (London)* 304:699-703.
- Emini, E. A., B. A. Jameson, and E. Wimmer. 1984. Identification of a new neutralization antigenic site on poliovirus coat protein VP2. *J. Virol.* 52:719-721.
- Golding, S. M., R. S. Hedger, P. Talbot, and J. Watson. 1976. Radial immunodiffusion and serum neutralisation techniques for the assay of antibodies to SVD. *Res. Vet. Sci.* 20:142-147.
- Hogle, J. M., M. Chow, and D. J. Filman. 1985. Three dimensional structure of poliovirus at 2.9A resolution. *Science* 229:1358-1365.
- Hogle, J. M., M. Chow, and D. J. Filman. 1987. The three dimensional structure of poliovirus: implications for assembly and immune recognition, p. 79-92. *In* M. A. Brinton and R. R. Rueckert (ed.), *Positive strand RNA viruses*. Alan R. Liss, Inc., New York.
- Jameson, B. A., J. Bonin, E. Wimmer, and O. Kew. 1985. Natural variants of the Sabin type 1 vaccine strain of poliovirus and correlation with a poliovirus neutralization site. *Virology* 143:337-341.
- Kitamura, N., B. L. Semler, P. G. Rothberg, G. R. Larsen, C. J. Adler, A. J. Dorner, E. A. Emini, R. Hanecak, J. J. Lee, S. van der Werf, C. W. Anderson, and E. Wimmer. 1981. Primary structure, gene organization and polypeptide expression of poliovirus RNA. *Nature (London)* 291:547-553.
- La Monica, N., C. Meriam, and V. R. Racaniello. 1986. Mapping of sequences required for mouse neurovirulence of poliovirus type 2 Lansing. *J. Virol.* 57:515-525.
- Martin, A., C. Wychowski, T. Couderc, R. Crainic, J. Hogle, and M. Girard. 1988. Engineering a type 2 antigenic site on a type 1 capsid results in a chimeric virus which is neurovirulent for mice. *EMBO J.* 7:2839-2847.
- Melnick, J. L. 1985. Enteroviruses: polioviruses, coxsackie viruses, echoviruses and the newer enteroviruses, p. 739-794. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, J. Melnick, B. Roizman, R. Shope (ed.), *Virology*. Raven Press, New York.
- Minor, P. D., D. M. A. Evans, M. Ferguson, G. C. Schild, J. W. Almond, and G. Stanway. 1987. Molecular basis of antigenicity of poliovirus, p. 539-553. *In* M. A. Brinton and R. R. Rueckert (ed.), *Positive strand RNA viruses*. Alan R. Liss, Inc., New York.
- Murray, M. G., J. Bradley, A. D. Murdin, H.-C. Selinka, E. Wimmer, M. Arita, N. Kawamura, A. Nomoto, E. G. Moss, and V. R. Racaniello. 1989. Hybrid polioviruses as potential vaccines and as tools to study biological function, p. 415-422. *In* R. A. Lerner, H. Ginsberg, R. M. Chanock, and F. Brown (ed.), *Vaccines '89*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Murray, M. G., J. Bradley, X.-F. Yang, E. Wimmer, E. G. Moss, and V. R. Racaniello. 1988. Poliovirus host range is determined by a short amino acid sequence in neutralization antigenic site I. *Science* 241:213-215.
- Murray, M. G., R. J. Kuhn, M. Arita, N. Kawamura, A. Nomoto, and E. Wimmer. 1988. Poliovirus type 1/type 3 antigenic hybrid virus constructed *in vitro* elicits type 1 and type 3 neutralizing antibodies in rabbits and monkeys. *Proc. Natl. Acad. Sci. USA* 85:3203-3207.
- Murray, M. G., and E. Wimmer. 1988. Chemical synthesis of poliovirus peptides and neutralizing antibody responses, p. 129-140. *In* E. Kurstak, R. G. Marusyk, F. A. Murphy, and M. H. V. Van Regenmortel (ed.), *Applied virology research*, vol. 1. Plenum Publishing Corp., New York.
- Nomoto, A., T. Omata, H. Toyoda, S. Kuge, H. Horie, Y. Kataoka, Y. Genba, Y. Nakano, and N. Imura. 1982. Complete nucleotide sequence of the attenuated poliovirus Sabin 1 strain genome. *Proc. Natl. Acad. Sci. USA* 79:5793-5797.

19. **Page, G. S., A. G. Mosser, J. M. Hogle, D. J. Filman, R. R. Rueckert, and M. Chow.** 1988. Three-dimensional structure of poliovirus serotype 1 neutralizing determinants. *J. Virol.* **62**: 1781-1794.
20. **Racaniello, V. R., and D. Baltimore.** 1981. Molecular cloning of poliovirus cDNA and determination of the complete nucleotide sequence of the viral genome. *Proc. Natl. Acad. Sci. USA* **78**:4887-4891.
21. **Sabin, A. B.** 1985. Oral poliovirus vaccine: history of its development and use and current challenge to eliminate poliomyelitis from the world. *J. Infect. Dis.* **151**:420-436.
22. **Salk, J., and D. Salk.** 1987. Control of influenza and poliomyelitis with killed virus vaccines. *Science* **195**:834-847.
23. **Stanway, G., A. J. Cann, R. Hauptmann, P. Hughes, L. D. Clark, R. C. Mountford, P. D. Minor, G. C. Schild, and J. W. Almond.** 1983. The nucleotide sequence of poliovirus type 3 Leon 12A, 43B; comparison with poliovirus type 3. *Nucleic Acids Res.* **11**:5629-5643.
24. **Toyoda, H., M. Kohara, Y. Kataoka, T. Suganuma, T. Omata, N. Imura, and A. Nomoto.** 1984. Complete nucleotide sequences of all three poliovirus serotype genomes. *J. Mol. Biol.* **174**: 561-585.
25. **van der Werf, S., J. Bradley, E. Wimmer, F. W. Studier, and J. J. Dunn.** 1986. Synthesis of infectious poliovirus RNA by purified T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA* **83**:2330-2334.
26. **Wiegers, K., H. Uhlig, and R. Dernick.** 1989. N-AgIB of poliovirus type 1: a discontinuous epitope formed by two loops of VP1 comprising residues 96-104 and 141-152. *Virology* **170**: 583-586.
27. **Zoller, M. J., and M. Smith.** 1984. Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template. *DNA* **3**:479-488.