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Nucleotide Sequence of a Neurovirulent Variant of the Type 2 Oral Poliovirus Vaccine

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Infectious cDNAs of the Sabin type 2 poliovirus vaccine virus and a vaccine-derived neurovirulent type 2 strain (P2/117) have been cloned in *Escherichia coli*. Nucleotide sequence analysis revealed that P2/117 differs from the vaccine strain by just 23 point mutations. Three occur in the 5′ noncoding region. The remainder result in a total of 5 coding changes located in VP1, VP4, 2B, and 3D. The likely role of these mutations in the evolution to neurovirulence is discussed.

Poliovirus is a member of the *Picornaviridae* family and exists as three distinct serotypes (19). The virus consists of a small icosahedral particle composed of 60 copies each of four proteins (VP1 to VP4). These surround a single-stranded polyadenylylated RNA genome of approximately 7,500 bases covalently linked to a small protein VPg at its 5′ terminus (19).

Poliomyelitis is controlled effectively in most of the developed world through the use of both live-attenuated and inactivated vaccines (20, 22). Most widely used is the oral poliovirus vaccine OPV (20), based on the attenuated strains developed by Sabin. Countries using this vaccine, however, still experience a low level of disease, at least some of which is caused by reversion to virulence of the vaccine viruses (1, 2).

The type 2 vaccine, P2/P712,Ch,2ab (Sabin 2), is known to cause disease in vaccinees and their contacts, though at a lower frequency than for the type 3 vaccine (1, 2). This is despite the fact that the original progenitor virus of the type 2 vaccine, P2/P712/56, was a naturally attenuated wild-type strain (21). To investigate the genetic basis of type 2 vaccine-associated disease, we have cloned the genomes and constructed infectious cDNAs of both the Sabin type 2 vaccine strain and a neurovirulent type 2 strain, P2/117, which was isolated from a vaccine-associated case of poliomyelitis (8). The close genetic relatedness of P2/117 to the Sabin 2 vaccine strain was indicated by its ribonuclease T_1 oligonucleotide fingerprint (8).

The Sabin vaccine strain of poliovirus, P2/P712,Ch,2ab (Sabin 2), and the neurovirulent virus P2/117 were propagated in Hep-2c cells as described previously (11). Virus was purified by sucrose gradient centrifugation, and virion RNA was extracted and cloned by using the RNA-cDNA hybrid cloning method (3, 25). Tet^r Amp^s colonies were screened by colony hyridization (6, 7) with nick-translated DNA from an infectious cDNA of the type 3 poliovirus, P3/Leon/37 (25). Plasmids isolated from positive colonies were characterized by restriction enzyme analysis to identify clones which together contained cDNA covering the entire genomes of P2/117 and Sabin 2. This enabled the construction of plasmids containing full-length cDNAs, designated p2/117 and p2/SAB2, respectively. T7 RNA polymerase promoters were engineered adjacent to the 5′ end of the viral genome to make p2/T7/117 and p2/T7/SAB2. Viruses were recovered from both p2/T7/117 and p2/T7/SAB2 after transfection of Hep-2c cells with T7 RNA transcripts (27). Serum neutralization assays (4) showed that both recovered viruses were serotype 2, and World Health Organization vaccine safety tests (29), using 4 animals per test, confirmed their neurovirulence phenotypes as summarized in Table 1. The virus recovered from p2/T7/SAB2 produced no signs of clinical paralysis in any of the animals and gave a low average lesion score of 0.27. This phenotype is typical of an attenuated poliovirus and is in good agreement with the stock Sabin 2 virus. Virus recovered from p2/T7/117, however, paralyzed all four animals tested and gave a mean histological lesion score of 1.76. This is also in agreement with the virus stock from which it was derived. The nucleotide sequence of the cDNA insert of pT7/117 was determined from M13mp18 or mp19 subclones by dideoxynucleotide sequence analysis with M13 universal primer or poliovirus-specific synthetic DNA primers. The genome was 7,439 nucleotides in length [excluding the poly(A) tract] and differed from the published Sabin 2 sequence of Toyoda et al. (26) at 42 positions (see Fig. 1). These included 34 point mutations and 4 paired insertion-deletions. However, direct sequence analysis of the Sabin 2 cDNA (14), p2/SAB2, showed that it differed at only 23 of these positions from the cloned P2/117 (Fig. 1). Moreover this result was confirmed by sequence analysis of viral RNA of the stocks of both P2/117 and Sabin 2. The concordance of the cDNA and RNA sequences also showed that our two cDNAs were not derived from minority variants within our virus stocks. We, therefore, conclude that the published sequence contains a number of errors or differences due to clonal variation, and we are confident that our P2/117 and Sabin 2 stocks differ by just 23 point mutations.

TABLE 1. Results of neurovirulence tests on Sabin 2 and P2/117 recovered from cloned cDNA and of the virus stocks from which the cDNAs were derived

Virus	No. with clinical symptoms/no. tested	Mean histological lesion score[a]
P2/117 stock	8/8	1.85
P2/117 cloned	4/4	1.76
Sabin 2 stock	0/4	0.50
Sabin 2 cloned	0/4	0.27

[a] Determined as previously described (30).

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Recently the nucleotide sequence of an attenuated type 2 poliovirus (P2/712) was published (13). The name of this strain suggests it to be the Sabin 2 progenitor; however, its passage history was not known and it is not clear whether it is the original progenitor strain or a descendant of Sabin 2. Of interest, however, was that 18 of the 19 differences identified here with the Sabin 2 sequence of Toyoda et al. (26) were the same, although the insertions or deletions were interpreted as adjacent point mutations (13). Two differences (at 3996 and 6435) between P2/712 and our Sabin 2 occur at third-base positions and do not affect coding. The P2/712 nucleotide sequence is, therefore, almost identical to that of Sabin 2 and substantiates our conclusion that P2/117 and Sabin 2 differ by only 23 point mutations.

Of the 23 verified mutations shown here to have occurred in P2/117, 3 are in the 5′ noncoding region of the genome. The remaining 20 mutations occur in the coding region and lead to five amino acid changes, one each in the capsid proteins VP4 and VP1, one in the nonstructural protein 2B, and two in the RNA polymerase 3D.

	position	Sabin publ	117 RNA cDNA	Sabin RNA cDNA	(diff)	Coding changes Sabin	117
	28	T	C	C			
	72	T	C	C			
5'NCR	309	G	A	A			
	355	T	C	C			
	437	T	C	T	437		
	481	A	G	A	481		
	685	A	T	A	685		
VP4	868	G	T	G	868	ALA	SER
	1137	C	T	C	1137		
	1176	G	A	G	1176		
VP2	1298	del T					
	1302	ins A					
	1670	T	C	C			
	1734	T	C	T	1734		
	1893	C	T	C	1893		
	1952	ins A					
VP3	1955	del A					
	1971	T	C	T	1971		
	2349	T	C	T	2349		
	2481	A	G	A	2481		
	2502	A	G	G			
	2567	del G					
VP1	2573	ins A					
	2908	A	G	A	2908	ILE	VAL
	3180	A	G	A	3180		
2A							
	3996	T	C	C			
2B	4076	A	G	A	4076	LYS	ARG
	4117	G	A	A			
	4147	G	A	A			
	4290	G	T	T			
	4383	C	T	C	4383		
2C	4449	T	C	T	4449		
	4827	T	C	T	4827		
	5043	T	C	T	5043		
3A							
3B							
	5565	T	C	T	5565		
	5802	del A					
3C	5806	ins A		G	5805	(A in 117)	
	5823	A	T	A	5823		
	6079	T	C	T	6079	TYR	HIS
	6098	A	G	A	6098	LYS	ARG
3D	6744	C	A	A			
	7329	G	A	A			
3'NCR							
poly A							

FIG. 1. Differences between P2/117 and the published sequence (first two columns) and the 23 differences verified by sequencing of cDNA and viral RNA (next two columns). The last two columns illustrate the coding changes resulting from the 23 point mutations. The poliovirus genome is shown diagramatically on the left of the diagram.

TABLE 2. Comparison of the genomic sequences of polioviruses of all three serotypes through the region 465 to 484[a]

Virus	Nucleotide sequence
P1/Sabin	UAA UCC CAA CCU CGG GGC AG
P2/Sabin	UAA UCC UAA CCA CGG AAC AG
P3/Sabin	UAA UUC UAA CCU CGG AGC AG
P1/Mahoney	UAA UCC CAA CCA CGG AGC AG
P2/Lansing	UAA UCC UAA CCA CGG AGC AG
P3/Leon	UAA UUC UAA CCA CGG AGC AG
P2/117	UAA UCC UAA CCA CGG AGC AG
Consensus	UAA UCC UAA CCA CGG AGC AG

[a] Numbering is according to the type 2 sequence. Differences from the consensus are underlined. Sequences were obtained from the following sources: P1/Sabin (13); P2/Sabin (26); P3/Sabin (23, 26); P1/Mahoney (9, 18); P2/Lansing (10); P3/Leon (25).

Mutations occurring in the 5′ noncoding region of the genome have been shown to have an effect on neurovirulence in poliovirus types 1 and 3 (16, 28). The A-to-G change at position 481 in P2/117 represents a mutation back to a consensus sequence for several polioviruses (Table 2). In common with the mutations in the type 1 and 3 vaccine strains at 480 and 472, respectively, this mutation has been shown to occur upon passage of the virus in the human gut (12). For vaccine strains of serotypes 1 and 3, the mutations at 480 and 472, respectively, have significant effects on the neurovirulence of these viruses (5, 16, 27). Recently new models for the secondary structure of the 5′ noncoding region of the poliovirus genome have been proposed (17, 22a) which suggest that mutations in this region which perturb secondary structure may have an effect on neurovirulence. However, the change at 481 in type 2 is not in a position directly involved in base pairing in this model, but its rapid selection in vivo suggests that it may be associated with an increase in neurovirulence. Interestingly the change at 437 restores base pairing in a stem-loop structure proposed in the new models for the region (17, 22a).

Of the five amino acid substitutions caused by the remaining 20 mutations, all appear to be conservative, and it is not obvious which, if any, are likely to be important in the acquisition of neurovirulence by P2/117. The infectious cDNAs described here will enable the construction of defined recombinants between P2/117 and Sabin 2 so that the effects of the various mutations can be examined directly. Such studies should contribute to a better understanding of neurovirulence determinants of polioviruses which, in turn, may lead to the construction of defined vaccine strains incapable of reversion to neurovirulence.

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