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Identification of a Consistent Pattern of Mutations in Neurovirulent Variants Derived from the Sabin Vaccine Strain of Poliovirus Type 2

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Complete nucleotide sequencing of the RNAs of two unrelated neurovirulent isolates of Sabin-related poliovirus type 2 revealed that two nucleotides and one amino acid (amino acid 143 in the major capsid protein VP1) consistently departed from the sequences of the nonneurovirulent poliovirus type 2 712 and Sabin vaccine strains. This pattern of mutation appeared to be a feature common to all neurovirulent variants of poliovirus type 2.

The attenuation of neurovirulence of a poliovirus strain can be operationally defined as the inability to replicate in neural cells. The nature of the restriction event has remained elusive, though it is possible to visualize at least three levels of cell-virus interaction susceptible of preventing in a tissue-type-specific manner the generation of viral progeny: the recognition of and interaction with specific cell receptors at the cell membrane may be impaired, the ability to undergo proper uncoating following internalization of the virion may be arrested, and the interaction of the viral RNA with a host factor(s) required for either efficient translation or transcription may be blocked. There is no reason to believe that these mechanisms of restriction are mutually exclusive.

Systematic studies conducted in several countries have established that a large proportion of the sporadic cases of acute spinal paralytic disease still observed are indeed caused by neurovirulent variants derived from the poliovirus type 2 Sabin vaccine strain (P2/Sabin) (11, 14, 32). Reversion to neurovirulence, therefore, although a rather rare event, seems not to be totally precluded (1, 6). Poliovirus, the etiological agent of poliomyelitis, is a picornavirus of the enterovirus group. Four peptides (60 copies each) form the naked, icosahedrical capsid of the virion, with the larger structural protein (VP1; apparent M_r = 34,000) directly involved in the formation of a deep valley, a kind of moat or canyon, that surrounds the 12 vertices on the fivefold symmetry axis (21).

The genomic RNA of poliovirus is a single-stranded molecule about 7,500 nucleotides in length of positive polarity, with a poly(A) stretch at the 3′ end and a small protein (VPg) covalently linked to the 5′-terminal UpUp.

In 1981 and again in 1983, poliovirus type 2 was isolated from the rectal swabs of two unrelated cases of acute spinal paralytic disease (17). They were characterized as neurovirulent P2/Sabin-derived variants. In an attempt to understand the molecular basis underlying the attenuation of neurovirulence of poliovirus, we have determined the complete sequence of the genomes of two P2/Sabin-like isolates obtained from cases of paralytic disease.

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Working stocks of viruses were grown in HEp-2 cells and purified from the clarified supernatants as described previously (6, 12, 31). Viral RNAs were phenol extracted and sequenced by the dideoxynucleotide chain termination procedure (26), using a panel of synthetic oligonucleotides as primers in reactions driven by reverse transcriptase. Each nucleotide position was established after at least two determinations and three independent readings. Sequences were entered and analyzed by the Digital VAX computer, using the programs of the GCG Wisconsin software package (3). The amino acid sequences were deduced by using the program TRANSLATE of the same software package.

Sequence analysis of strain P2/712 and isolates P2/VL and P2/GS. We determined the genomic sequence of the original strain P2/712 (ancestor of P2/Sabin) (25), finding two nucleotide differences that resulted in no amino acid change in comparison with the vaccine strain. These results were in agreement with similar observations recently reported (15, 24).

The neurovirulent isolates P2/VL and P2/GS, in contrast, departed considerably from either P2/712 or P2/Sabin: 17 and 26 genome differences, respectively. These changes were scattered throughout the genome, with no obvious pattern of clustering or preference for a particular domain (Table 1). There were no insertions or deletions, and the total genome length remained unchanged (7,439 nucleotides). These data are meaningless if considered separately, as they do not allow one to discriminate between changes representing mere individual variations of the P2/Sabin sequence from those that actually correlate with (and are responsible for) the neurovirulent phenotype. Consequently, the sequences of several unrelated isolates were systematically compared, and the genome positions in which at least one individual isolate conserved at that point the standard sequence of P2/Sabin despite its neurovirulent character were excluded from the list of mutations potentially responsible for neurovirulence. In doing so, we implicitly assumed that all type 2 neurovirulent revertants of poliovirus are the result of the same sequence change. However, the possibility must be entertained that reversion to neurovirulence can occur in different viruses by different genetic events and therefore by different mechanisms. With this caveat, we compared the

TABLE 1. Nucleotide and amino acid differences[a] among P2/Sabin, P2/VL, and P2/GS

Nucleotide position	P2/Sabin[b]	P2/VL	P2/GS	Amino acid position	P2/Sabin	P2/VL	P2/GS
301	C	C	G				
481	A	G	G				
576	C	T	C				
627	T	C	T				
690	T	C	T				
1080	C	C	T				
1317	T	T	C				
1348	T	T	C				
1629	C	T	C				
2285	A	A	C	173 Vp3	Asn	Asn	Thr
2547	T	T	C				
2745	T	T	C				
2789	G	G	A	103 Vp1	Arg	Arg	Lys
2908	A	G	G	143 Vp1	Ile	Val	Val
2992	A	A	G	171 Vp1	Asn	Asn	Asp
3157	G	G	C	226 Vp1	Asp	Asp	Hys
3291	C	C	T				
3435	C	C	T				
3706	G	T	G	107 2A	Asp	Tyr	Asp
3771	C	C	T				
3920	G	G	A	30 2B	Ser	Ser	Asn
3921	C	T	C				
4201	A	C	C	27 2C	Lys	Gln	Gln
4264	A	G	A	48 2C	Thr	Ala	Thr
4363	C	C	A				
4473	G	G	A				
4652	A	G	A	177 2C	Asp	Gly	Asp
4815	G	G	T				
5016	G	A	G				
5040	C	T	C				
5055	T	C	T				
5755	A	A	T	107 3C	Ser	Ser	Cys
6009	A	A	G				
6069	C	T	C				
6156	A	G	A				
6410	G	G	A	142 3D	Arg	Arg	Gln
6798	C	C	T				
6804	C	C	T				
7386	T	C	C				

[a] Boxed positions.
[b] From reference 24.

TABLE 2. Comparison of amino acid 143 of VP1 in different poliovirus type 2 strains

Strain	Amino acid[a]						
P2/Sabin[b]	TCA	AAC	TAC	ATT	GAT	GCA	AAT
P2/VL	TCA	AAC	TAC	GTT	GAT	GCA	AAT
P2/GS	TCA	AAC	TAC	GTT	GAT	GCA	AAT
P2/117[b]	TCA	AAC	TAC	GTT	GAT	GCA	AAT
P2/ME1	TCA	AAC	TAC	ACT	GAT	GCA	AAT
P2/ME2	TCA	AAC	TAC	AAT	GAT	GCA	AAT
P2/ME3	TCA	AAC	TAC	ACT	GAT	GCA	AAT
P2/Sabin	S[c]	N	Y	I	D	A	N
P2/VL	S	N	Y	V	D	A	N
P2/GS	S	N	Y	V	D	A	N
P2/117	S	N	Y	V	D	A	N
P2/ME1	S	N	Y	T	D	A	N
P2/ME2	S	N	Y	N	D	A	N
P2/ME3	S	N	Y	T	D	A	N

[a] Amino acids that differ among the strains are boxed.
[b] From reference 24.
[c] One-letter code.

complete genome sequences of isolates P2/VL and P2/GS, looking for a pattern of mutations associated with neurovirulence. This procedure allowed us to narrow down to four point mutations and two amino acid substitutions the minimal requirement for P2/Sabin to become neurovirulent.

The mutation of nucleotide 7386, in the 3′-terminal noncoding region (NCR) immediately adjacent to the poly(A) tract, occurred in an area of the genome likely to be involved in specific template recognition at initiation of negative-strand RNA synthesis (22a). Examination of the region of the genome coding for the nonstructural peptides (bases 3385 through 7368) showed that a single amino acid transition (Lys to Glu) characterized these two isolates: amino acid 27 of peptide 2C (Table 1). Nonetheless, the fact that nucleotide 7386 and amino acid 27 of the peptide 2C of the neurovirulent isolate reported by Pollard et al. (24) was identical with that of the nonneurovirulent P2/712 and P2/Sabin strains tends to exclude the direct involvement of this change in the neurovirulent phenotype of isolates P2/VL and P2/GS.

Identification of a consistent pattern of mutations in neurovirulent isolates of poliovirus type 2. A comparison of the sequences of the genomes of isolates P2/VL and P2/GS with each other and with those of the nonneurovirulent P2/712 and P2/Sabin strains revealed two consistent changes associated with the neurovirulent phenotype of these isolates: nucleotides 481 (in the 5′ NCR) and 2908, in the codon of amino acid 143 of VP1 (Table 1). Partial nucleotide sequencing of the genomes of P2/ME1, P2/ME2, and P2/ME3, three isolates from far-removed geographical areas (unpublished data), confirmed that the substitution observed in amino acid 143 of VP1 (Table 2) and mutation of nucleotide 481 in the 5′ NCR were consistently present in variants of poliovirus type 2 associated with paralytic disease.

Several years ago, Evans et al. (4) identified a C-to-U transition at position 472 consistently associated with the attenuation of neurovirulence of type 3 poliovirus, and ample evidence has accumulated since then indicating that a similar change at position 480 accompanies attenuation of neurovirulence of poliovirus type 1 (18). Unsurprisingly, the nonneurovirulent P2/712 and P2/Sabin strains differed from the neurovirulent isolates at position 481, the substitution in this case involving an A-to-G transition (15, 24). The association of so dramatic a phenotypic change with just a single point mutation in a region of the genome that all available evidence indicates as a noncoding one raises the question of the function(s) of the sequences preceding the open reading frame and their involvement in internal initiation of translation (2, 19, 20, 22, 23).

Conceivably, the reported mutations at bases 480, 481, and 472 in types 1, 2, and 3, respectively (4, 13, 18), by destabilizing locally the folding of the RNA may force it to adopt a configuration less suitable for the interaction with a host factor required for internal initiation of translation (27). In view of the recent identification of a cellular peptide (p52) that binds to poliovirus RNA in a 68-nucleotide stem-and-loop structure extending between positions 559 and 624 and most likely required to secure internal initiation of translation (10), it is tempting to speculate that differences in the ability of p52 of different tissues to tolerate an altered RNA configuration in this critical region may explain the inability of neural cells to support replication of the attenuated (but not wild-type) poliovirus. Conversely, the translation machinery of other cell types may be less stringent in its

TABLE 3. Comparison of amino acid sequences of polioviruses of all three serotypes through region 130 to 160 of VP1

Virus	Amino acid sequence[a]																													
	130										140									150										160
P2/Sabin[b]	F	D	M	E	F	T	F	V	V	T	S	N	Y	I	D	A	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I
P2/GS	F	D	M	E	F	T	F	V	V	T	S	N	Y	V	D	A	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I
P2/VL	F	D	M	E	F	T	F	V	V	T	S	N	Y	V	D	A	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I
P2/Lansing[c]	F	D	M	E	F	T	F	V	V	T	S	N	Y	T	D	A	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I
P1/Sabin[d]	F	D	M	E	F	T	F	V	V	T	A	N	F	T	E	T	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I
P3/Sabin[e]	F	D	M	E	F	T	F	V	V	T	A	N	F	T	N	A	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I
P1/Mahoney[f]	F	D	M	E	L	T	F	V	V	T	A	N	F	T	E	T	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I
P3/Leon 37[g]	F	D	M	E	F	T	F	V	V	T	A	N	F	T	N	A	N	N	G	H	A	L	N	Q	V	Y	Q	I	M	Y	I

[a] Amino acids that are identical among all strains are boxed; those that differ among P2 strains are underlined.
[b] From references 24 and 30.
[c] From reference 9.
[d] From reference 16.
[e] From reference 28.
[f] From reference 8.
[g] From reference 29.

requirements, accepting both the wild-type and the attenuated RNA folding.

The major capsid protein VP1 appeared to carry a significant alteration in one of the most conserved regions of this peptide: of six individual sequences of neurovirulent isolates so far examined, none carried isoleucine as amino acid 143 of VP1; valine, threonine, and asparagine were found, suggesting that the attenuation of neurovirulence in P2/712 and P2/Sabin may correlate with the presence of isoleucine in this variable domain of the D-E loop (Table 3). Given the rigid structural constraints imposed by the need for tertiary folding (7), it is not surprising that the entire domain presents a remarkable homology (virtually identity) in the three serotypes. This is even more evident if the regions immediately flanking the D-E loop are considered (Table 3). Within the loop itself, four amino acids (142 to 145, in the external surface of the virion) distinguish poliovirus type 2 from the other two serotypes (5).

Amino acid 143, isoleucine in the nonneurovirulent P2/712 and P2/Sabin strains, changed to valine (P2/VL, P2/GS, and P2/117), threonine (P2/ME1 and P2/ME3), or asparagine (P2/ME2) in the neurovirulent isolates (Table 2). We have been so far unable to find any neurovirulent strain of poliovirus type 2 carrying isoleucine at position 143 of VP1.

In this context, it is interesting that the mouse-adapted, neurovirulent P2/Lansing strain differs from the sequence of P2/Sabin at amino acid position 143 of VP1, where threonine has replaced isoleucine. This change constitutes the sole variation in an otherwise perfectly conserved domain (Table 3).

We still are uncertain about how the observed changes at position 143 of VP1 may alter the architecture of the neurovirulent virions, nor can we predict the functional consequences of these putative structural changes. It is not yet clear whether the transition of amino acid 143 to isoleucine results in a conformational change of the viral surface such as to prevent its interaction with neural (but not epithelial) cells. A direct analysis of the three-dimensional structures of virions carrying different amino acids at position 143 of VP1 will help to clarify this issue.

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