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Reversion to neurovirulence of the live-attenuated Sabin type 3 oral poliovirus vaccine

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ABSTRACT

The complete nucleotide sequence has been determined of a strain of poliovirus type 3, P3/119, isolated from the central nervous system of a victim of fatal vaccine-associated poliomyelitis. Comparison of this sequence with those obtained previously for the Sabin type 3 vaccine, P3/Leon 12a$_1$b and its neurovirulent progenitor, P3/Leon/37, reveals that these three strains are on a direct geneaological lineage and therefore that P3/119 is a *bona fide* revertant of the vaccine. P3/119 differs in sequence from its attenuated vaccine parent at just seven positions. Only one of these differences, a mutation from U to C at position 472 in the presumed non-coding region of the genome, is a back mutation to the wild type sequence. Of the six other differences, three give rise to coding changes in virus structural proteins, two are silent changes in the major open reading frame of the genome and one affects the 3'-terminus just prior to the poly A tract. These differences indicate that there are three possible types of molecular change which could, singly or collectively, result in attenuation and reversion to neurovirulence of the Sabin type 3 vaccine.

INTRODUCTION

Polioviruses are members of the enterovirus group of the family picornaviridae. The three distinct serotypes, 1, 2 and 3, cause an identical disease and are very similar in structure and composition. Each serotype possesses a single-stranded, messenger-sense RNA genome of approximately 7500 nucleotides enclosed in a 27nm diameter icosahedral capsid composed of 60 copies each of four virus polypeptides, VP1-VP4 (for a review see ref 1).

Over the past twenty years, the disease of poliomyelitis has been successfully controlled in many areas of the world, through the use of the live-attenuated oral poliovirus vaccines developed by Dr. Albert Sabin[2]. Although countries using these vaccines have experienced a dramatic fall in the incidence of poliomyelitis, a low level of the disease has persisted[3,4]. These occasional cases are often associated temporally with vaccination and the viruses isolated from them (mainly serotypes 2 and 3) are frequently designated "vaccine-like" on the the basis of T_1 oligonucleotide fingerprints and serology[4,5,6]. Because this association is circumstantial,

some investigators have been reluctant to accept that the vaccines are responsible for this low level of disease[7,8], pointing out that human passage frequently alters genetic markers and that some wild strains have the same characteristics as vaccine strains.

This paper discusses the complete nucleotide sequence of a neurovirulent candidate revertant of the serotype 3 Sabin vaccine. Comparison of this sequence with those obtained previously for the Sabin type 3 vaccine, P3/Leon $12a_1b$ (published in full in ref. 9) and its neurovirulent progenitor, P3/Leon/37 (published in full in ref. 10), provides conclusive evidence that the Sabin vaccine strains may revert to a neurovirulent phenotype and allows a reappraisal of the mutations likely to be involved in attenuation of the vaccine. A precise understanding of the molecular basis of these events may suggest ways in which Sabin's vaccines could be modified to improve their safety.

MATERIALS AND METHODS

Poliovirus cDNA. The preparation of a plasmid containing cDNA corresponding to the complete genome of P3/119, using the RNA.cDNA hybrid method, has been described previously[11,12]. This plasmid, pOLIO 119, was used as a source of cDNA for nucleotide sequence analysis.

Nucleotide sequence determination. pOLIO 119 (10µg) was digested with *Pst* I, and the two poliovirus-specific fragments were isolated. These were circularized by ligation and then sheared by sonication[13]. The random fragments thus generated were end-repaired using the Klenow fragment of *E. coli* DNA polymerase I and fractionated on a 1.5% agarose gel. DNA of size 500-1000 base pairs was electroeluted, sub-cloned into *Sma* I digested M13 mp8 and sequenced by the dideoxynucleotide method[14,15]

RESULTS AND DISCUSSION

P3/119 was selected as a possible revertant because of its isolation history and its close genetic similarity to the Sabin type 3 vaccine[6]. The strain was plaque-purified prior to molecular cloning in order to reduce the risk that individual cDNA clones might be derived from an unrepresentative sub-population of the original virus stock[16]. Both the original isolate and a plaque-purified derivative of it, designated #643, were found to be highly neurovirulent in vaccine safety tests[16]. After cloning the genome of #643 in *E. coli* using an efficient RNA.cDNA hybrid method previously described[11], overlapping sub-genomic cDNAs were used to construct a

complete copy of the virus genome, referred to as pOLIO 119[12]. The nucleotide sequence of the complete cDNA was determined using the shotgun dideoxynucleotide method[13,14,15]. To ensure accuracy, all regions of the cDNA were sequenced at least twice. At each position where a nucleotide sequence difference was detected between P3/119 and the vaccine strain, P3/Leon 12a$_1$b, or its parent, P3/Leon/37, the sequence was checked in both orientations and in some cases also in independent cDNA clones. Extensive sequence analysis in our laboratories both from cDNA and directly from RNA suggests that errors due to the infidelity of reverse transcription and/or repair during hybrid cloning are extremely rare.

Excluding the poly(A) tract, the genome of P3/119 was found to be 7429 nucleotides in length, lacking two nucleotides at the 3′ terminus when compared to that of P3/Leon/37[10] and three when compared to that of P3/Leon 12a$_1$b[9]. The seven nucleotide differences, of which three result in amino acid changes between P3/119 and the Sabin vaccine, P3/Leon 12a$_1$b, are shown in figure 1, together with the ten nucleotide and three amino acid differences between the vaccine strain and its parent, P3/Leon/37, as reported previously[10]. With the exception of the 3′ termini, the differences observed between the three strains were single base substitutions widely distributed throughout the genomes. The P3/119 sequence was vaccine-

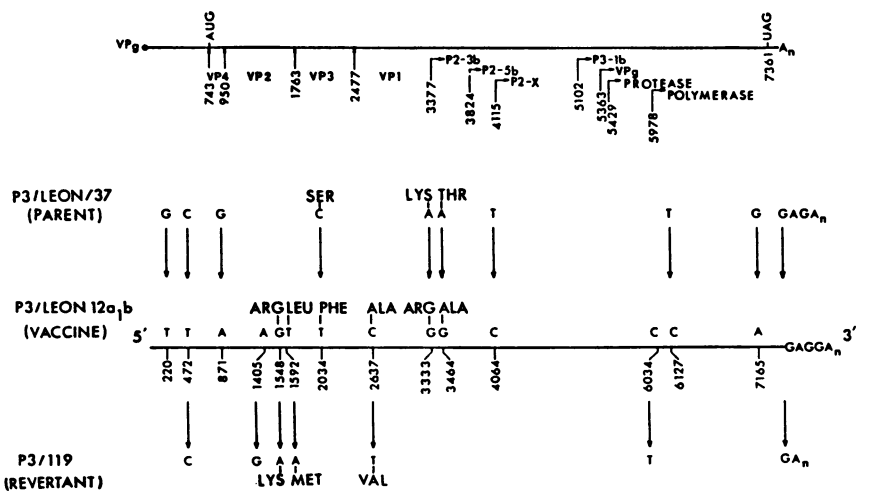

Figure 1. Nucleotide and predicted amino acid sequence differences between poliovirus type 3 strains, P3/Leon/37 (parent), P3/Leon 12a$_1$b (Sabin vaccine) and P3/119 (neurovirulent vaccine revertant). The complete sequences of P3/Leon/37 and P3/Leon 12a$_1$b are presented in refs. 10 and 9 respectively.

like at eight of the ten positions at which there were differences between the vaccine strain and its parent, P3/Leon/37. This illustrates that the three strains are on a direct geneaological lineage and provides the strongest possible evidence that P3/119 is a *bona fide* revertant of the Sabin type 3 vaccine. Although this had been suspected on the basis of T_1 oligonucleotide fingerprinting[6], the possibility that P3/119 and other such isolates were P3/Leon/37-like wild-type strains, could not previously be ruled out[7,8].

The availability of the complete nucleotide sequence of a vaccine revertant also allows the likely contribution to attenuation of the mutations observed in the vaccine[10] to be reconsidered. Thus, the mutations in the vaccine virus at positions 220, 871, 4064, 6127 and 7165 are unlikely to be significant, since none of these affect coding and furthermore, do not revert in P3/119. Likewise, the silent nucleotide substitutions in P3/119 at positions 1405 and 6034 are unlikely to play a role in reversion. The coding change at position 3464 in the vaccine strain which results in a threonine to alanine substitution in non-structural protein P2-3b, is also unlikely to be relevant to attenuation. This is suggested by the observation that there are no amino acid changes in the non-structural proteins of P3/119, in which, apart from a silent mutation at 6034, this region of the genome is entirely vaccine-like.

There remain three possible genetic bases for the attenuated phenotype of the Sabin type 3 vaccine. The first of these involves position 472. Of all the base changes detected between the three strains, only position 472 involved direct back mutation. This 5'-non-coding nucleotide is a cytosine in P3/Leon/37 and P3/119, but a uridine in the vaccine strain. The presence of uridine at position 472 in the attenuated strain was confirmed by direct sequencing of the RNA of the original P3/Leon $12a_1b$ virus from which the plaque-purified isolate was derived[16]. Uridine was also detected at position 472 in two preparations of commercial Sabin type 3 vaccine and in independent sequencing studies on P3/Leon $12a_1b$[17]. The nucleotide at this position is the only one which correlates directly with virulence, being uridine in the attenuated strain and cytidine in both virulent strains. Furthermore, sequencing studies of other type 3 poliovirus strains suggest that the nucleotide at position 472 has a profound effect on the ability of the virus to grow in the human intestinal tract and may have important implications for the secondary structure of the RNA (unpublished).

A second possible mechanism of attenuation is by amino acid

substitution in the region of the genome coding for the capsid proteins. In this region there are only two sequence differences between P3/Leon/37 and the vaccine strain which give rise to amino acid changes (serine to phenylalanine in VP3 at nucleotide position 2034 and lysine to arginine in VP1 at nucleotide position 3333)[10] and neither of these revert in P3/119. Thus, if these changes influence neurovirulence, their attenuating effect(s) must be suppressed, most likely by one or more of the three mutations causing amino acid substitutions in revertant P3/119, i.e. arginine to lysine and leucine to methionine in VP2 at nucleotide positions 1548 and 1592 respectively and alanine to valine in VP1 at nucleotide 2637 (although supression by the silent mutations canot be formally eliminated). On the basis of predicted effect on secondary structure, we have argued previously that the serine to phenylalanine substitution in VP3 is the one most likely to have a major effect on virulence[10], however we cannot exclude a contribution from the lysine-arginine change in VP1.

The remaining possible genetic basis of attenuation is mutation at the 3′ terminus of the genome adjacent to the poly A tract. The sequences from position 7428 of the three strains are; $GGAG(A)_n$ in P3/Leon/37, $GGAGG(A)_n$ in the vaccine and $GG(A)_n$ in P3/119. The function of this highly conserved region of the genome[11,17] is unknown but it is conceivable that such differences could influence virulence by, for example, an effect on polymerase binding and/or the initiation of replication. It is noteworthy however, that 3′ terminal sequence variation has been observed in different stocks of a neurovirulent type 1 strain, P1/Mahoney[18,19], without any reported effects on neurovirulence.

Further studies, including partial sequencing of other vaccine revertants and the construction of recombinants via infectious cDNAs[20] between the strains discussed above, are in progress. These will enable the effects of the three types of change identified here to be assessed individually and should provide a clearer understanding of the molecular basis of attenuation and reversion to neurovirulence. Such studies, together with the confirmation presented here, that reversion does occur, should provide new impetus for the development of completely safe poliovirus vaccines.

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