# GENOMIC MODIFICATIONS IN SABIN VACCINE STRAINS ISOLATED FROM VACCINATION-ASSOCIATED CASES, HEALTHY CONTACTS AND HEALTHY VACCINEES

F. FRIEDRICH

Departamento de Virologia, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brazil 4365, 21040-360 Rio de Janeiro, RJ, Brazil

*Received March 11, 1996*

**Summary.** – The three attenuated strains developed by A.B. Sabin have been effectively used as an oral live poliovirus vaccine (OPV) to control poliomyelitis in many countries. Although rarely, vaccination-associated paralytic poliomyelitis (VAPP) cases occur with the type 2 and 3 strains, and less frequently with the type 1 strain. The greater number of attenuating mutations in the P1/Sabin strain is probably reflected in the higher safety of this strain in comparison to type 2 and 3 strains. For the P1/Sabin strain, many attenuating mutations were already identified in the 5'-non-coding region (5'NCR), in the capsid proteins coding region, in the 3Dpol coding region, and the 3'-non-coding region (3'NCR). For the P2/Sabin and P3/Sabin strains, one mutation in 5'NCR and another in the capsid proteins coding region have been demonstrated to be important determinants of attenuation, although it has been suggested that other mutations may also have some effect, though minor. Although reverting mutations in attenuating determinants, supressor mutations, mutations in antigenic sites and genomic recombination have been observed in strains isolated from VAPP cases, the observation of similar genomic modifications in strains isolated from healthy contacts and from healthy vaccinees has supported the view that host factors are also involved in the establishment of the disease. Reverting mutations at nucleotides (nt) 480 (G->A), 481 (A->G) and 472 (U->C) for the P1/Sabin, P2/Sabin and P3/Sabin strains, respectively, have been detected in almost all strains isolated from VAPP cases and also from healthy vaccinees. Although the Sabin vaccine strains have been implicated in rare VAPP cases, recent studies have suggested that the vaccine strains could also trigger the Guillain-Barré syndrome (GBS), transverse myelitis (TM) and facial paralysis.

**Key words:** poliovirus; Sabin vaccine strains; genome modifications; mutations; recombination; neurovirulence; attenuation; vaccination-associated cases

## Introduction

Polioviruses are members of the *enterovirus* genus (Melnick, 1993), belonging to the *Picornaviridae* family and consist of an icosahedrical particle composed of 60 copies of capsid proteins VP1, VP2, VP3 and VP4 (Hogle *et al.*, 1985), surrounding a single-stranded positive-sense RNA genome of approximately 7500 nt (Kitamura *et al.*, 1981). The viral RNA contains a 5'NCR of about 740 nt with a terminally linked protein (viral genomic protein, VPg), preceding a single open reading frame (ORF) coding for the structural and nonstructural proteins, and terminates in a 3'NCR of about 70 nt followed by a poly(A)-sequence (Kitamura *et al.*, 1981; Wimmer and Nomoto, 1993). Polioviruses are grouped on the basis of the antigenicity of the capsid into three serotypes designated 1, 2, and 3. These viruses are the causative agents of poliomyelitis, a paralytic and sometimes fatal disease of humans (Almond, 1987; Racaniello, 1988).

**Abbreviations:** GBS = Guillain-Barré syndrome; NCR = non-coding region; OPV = oral poliovirus vaccine; ORF = open reading frame; VAPP = vaccination-associated paralytic poliomyelitis; VPg = viral genomic protein; TM = transverse myelitis

The attenuated poliovirus strains (P1/Sabin, P2/Sabin, and P3/Sabin) developed by A.B. Sabin (Almond, 1987; Racaniello, 1988; Horaud, 1993) were obtained by passaging wild type isolates in monkey tissue *in vitro* and *in vivo* under a variety of conditions which differed for each of the three serotypes. These attenuated strains have been effectively used as OPV to control poliomyelitis in many countries. Although rarely, VAPP cases occur with the type 2 and 3 strains, and even less frequently with the type 1 strain (Basilico and Bernat, 1978; Kew *et al.*, 1981; WHO, 1982; Minor, 1982; Fiore *et al.*, 1987; Nkowane *et al.*, 1987; Strebel *et al.*, 1992, 1994; Fillipis *et al.*, 1994; Groom *et al.*, 1994; Friedrich *et al.*, 1996a). Molecular studies have demonstrated mutations in the genome of Sabin vaccine-derived strains isolated from VAPP cases (Cann *et al.*, 1984; Evans *et al.*, 1985; Pollard *et al.*, 1989; Macadam *et al.*, 1989, 1991a; Equestre *et al.*, 1991; Muzychenko *et al.*, 1991; Otelea *et al.*, 1993; Georgescu *et al.*, 1994; Friedrich *et al.*, 1995b,c; Driesel *et al.*, 1995).

A comparison of the nucleotide sequence of the attenuated vaccine strains, vaccine neurovirulent revertants and wild neurovirulent ancestors of the vaccine strains has led to identification of nucleotide differences in these strains and possible mutations involved in attenuation and reversion to neurovirulence. Poliovirus can be recovered from cloned cDNA copies of the genome (Racaniello and Baltimore, 1981a; Racaniello, 1993). This approach has permitted the construction of recombinants between neurovirulent and attenuated strains, and the use of site-directed mutagenesis in the identification of mutations involved in attenuation and reversion to neurovirulence. Knowledge of the molecular basis of attenuation and reversion towards neurovirulence of the Sabin strains (Almond, 1987; Racaniello, 1988; Ehrenfeld, 1992; Minor 1992, 1993; Minor *et al.*, 1993; Racaniello *et al.*, 1993; Macadam *et al.*, 1994b) may allow rational improvement of vaccines (WHO, 1990; Ghendon, 1993; Agol, 1993) and vaccine production methods (Chumakov *et al.*, 1994). The latters may provide alternative models for vaccine safety tests on transgenic mice (Ren *et al.*, 1990; Koike *et al.*, 1991, 1993; Abe *et al.*, 1995a,b) and/or molecular approaches (Chumakov *et al.*, 1991, 1992, 1993; Fenyves, 1993; Svitkin *et al.*, 1993; Rezapkin *et al.*, 1994, 1995; Taffs *et al.*, 1995), and may avoid costly safety testing of vaccine batches in primates.

*Molecular basis of attenuation and reversion to neurovirulence of the P1/Sabin strain*

The attenuated P1/Sabin strain (P1/LSc, 2ab) was derived from a wild neurovirulent strain, P1/Mahoney/41, isolated from stool of a healthy child (Almond, 1987; Racaniello, 1988). Comparison of the complete nucleotide sequence of the attenuated P1/Sabin strain (Nomoto *et al.*, 1982; Toyoda *et al.*, 1984) with that of its neurovirulent ancestor, P1/Mahoney strain (Kitamura *et al.*, 1981; Racaniello and Baltimore, 1981b) demonstrated that these two strains differ by more than 50 mutations. Neurovirulence tests in monkeys of recombinants between these two strains demonstrated that the mutations important for the attenuation of the P1/Sabin strain are scattered throughout the entire genome (Omata *et al.*, 1986), with an important attenuating mutation A->G at nt 480 of 5'NCR (Kawamura *et al.*, 1989). The analysis of P1/Sabin neurovirulent revertants selected at high temperatures suggested that an U->C mutation at nt 525 of 5'NCR, which has been suggested to supress the attenuating G at nt 480, a G->A mutation at nt 7441 of 3'NCR, and a C->U mutation at nt 6203 of the 3Dpol coding region, leading to a His->Tyr substitution at aa 73 of the viral RNA polymerase, are important for reversion of this strain to neurovirulence in monkeys (Christodoulou *et al.*, 1990). This study also suggested that mutations at nt 2438 (VP3), 2741 (VP1) and 2795 (VP1), leading to amino acid substitutions in these capsid proteins, could also have some effect on the reversion to neurovirulence. The use of type 1/type 2 mouse-adapted chimeric polioviruses in the study of determinants of poliovirus type 1 neurovirulence, strongly suggested that a C at nt 6203 of the 3Dpol coding region, in the codon for His at aa 73 in the viral RNA polymerase, was an attenuating determinant of the P1/Sabin strain, although its effect was weaker than that of a G at nt 480 of 5'NCR (Martin *et al.*, 1991; Tardy-Panit *et al.*, 1993).

Neurovirulence tests of recombinants between the virulent P1/Mahoney strain and the attenuated P1/Sabin strain in transgenic mice carring the human poliovirus receptor, suggested an important contribution of nt 480 to the expression of neurovirulence or attenuation (Horie *et al.*, 1994). This study also suggested the participation of mutations at nt 189 (C->U) of 5'NCR, nt 21 (U->C) of 5'NCR, and/or nt 935 (G->U) of VP4 in the attenuation of the P1/Sabin strain. Other neurovirulence tests (Bouchard *et al.*, 1995) in transgenic mice expressing the human poliovirus receptor of recombinants between the P1/Sabin and P1/Mahoney strains, and of site-directed mutants, identified additional attenuating mutations in the P1/Sabin strain at nt 935 (also identified in the previous study), 2438, 2795 and 2879. The attenuating G->U mutation at nt 935 led to an Ala->Ser substitution at aa 65 of VP4, the U->A mutation at nt 2438 to a Leu->Met substitution at aa 225 of VP3, the G->A mutation at nt 2795 to an Ala->Thr substitution at aa 106 of VP1, while the C->U mutation at nt 2879 to a Leu->Phe substitution at aa 134 of VP1.

Thus, these molecular studies have suggested that an A->G mutation at nt 480 of 5'NCR of the P1/Sabin strain has an important contribution to the attenuation, while an U->C mutation at nt 6203, resulting in an amino acid sub-

stitution in the viral RNA polymerase, contributes to attenuation to a lesser extent. To assess the contributions of these two mutations (McGoldrick *et al.*, 1995) for virus attenuation in monkeys, site-directed mutagenesis generating mutants of the P1/Sabin and P1/Mahoney strains at nt 480 and 6203 was performed, indicating that (in contrast to previous findings) G at nt 480 had only a slight effect on the attenuation, while C at nt 6203 had even a lesser one, confirming that attenuating mutations are located elsewhere in the genome of the P1/Sabin strain.

Other studies demonstrated that vaccine lots of the P1/Sabin strain with an increased portion of G->A (nt 480) and U->C (nt 525) revertants presented a higher neurovirulence (Rezapkin *et al.*, 1994). In still another study, cultured human neuroblastoma cells SK-N-MC were found to be highly resistant to the attenuated P1/Sabin strain, while they produced relatively good yields of the neurovirulent P1/Mahoney strain (Agol *et al.*, 1989). Experiments with intratypic poliovirus recombinants suggested that the major genomic determinants limiting the replication of the attenuated P1/Sabin strain in neuroblastoma cells are located in the 5'-half of the viral RNA, even though the 3'-half also appeared to contribute somewhat to this phenotype.

It is known that nt 480 pairs with nt 525 in a predicted secondary structure of 5'NCR (Pilipenko *et al.*, 1989; Skinner *et al.*, 1989; Agol, 1991; Muzychenko *et al.*, 1991; Hellen *et al.*, 1994). The attenuating G at nt 480 of 5'NCR of the P1/Sabin strain resulted in the alteration of the A-U base pair (nt 480-525) found in the neurovirulent wild P1/Mahoney strain (ancestor of the P1/Sabin strain) to the G-U base pair (nt 480-525) found in the P1/Sabin vaccine strain, suggesting that the attenuated phenotype is partially associated with a weakening of a base pairing in a highly conserved structure (Christodoulou *et al.*, 1990; Muzychenko *et al.*, 1991; Agol, 1991; Macadam *et al.*, 1991a, 1994b; Minor, 1992, 1993; Minor *et al.*, 1993; Tardy-Panit *et al.*, 1993). The alteration of the secondary structure of 5'NCR, caused by the attenuating G at nt 480, could alter the interaction of the viral RNA with one or more host factors required for the initiation of translation of poliovirus RNA (Svitkin *et al.*, 1988; Agol, 1991), leading to the observed deficiency in translation (Svitkin *et al.*, 1985; Muzychenko *et al.*, 1991) associated with an A->G attenuating mutation at nt 480 (Kawamura *et al.*, 1989). The G->A reverting mutation at nt 480 or the U->C supressor mutation at nt 525 have been suggested to revert this process. The Tyr->His attenuating substituition at aa 73 of the viral RNA polymerase could act by decreasing the efficiency of the synthesis of viral RNA (Christodoulou *et al.*, 1990; Tardy-Panit *et al.*, 1993). The G at nt 7441 of 3'NCR could alter its secondary structure or the affinity of the host factor to viral RNA or both, inhibiting the initiation of replication (Christodoulou *et al.*, 1990).

*Genomic modifications in type 1 polioviruses isolated from VAPP cases and healthy vaccinees*

The greater number of attenuating mutations in the P1/Sabin strain has been proposed as an explanation for the higher degree of safety of this strain in comparison to the P2/Sabin and P3/Sabin strains. As the number of poliovirus type 1 VAPP cases was low, the genomic modifications occuring in these strains have therefore been studied less. A molecular study involving partial nucleotide sequencing of P1/Sabin-derived strains isolated from five VAPP cases (four from stool and one from CNS) demonstrated a G->A reverse mutation at nt 480 in all these strains (Otelea *et al.*, 1993; see also Guillot *et al.*, 1994). In one of these strains isolated from stool, many additional reverting mutations at nt 2749 (VP1), 6141 and 6203 (3Dpol), 7441 (3'NCR), and several new mutations were observed (Otelea *et al.*, 1993). Thus, since all five P1/Sabin-derived strains isolated previously from VAPP cases mutated at nt 480 (Otelea *et al.*, 1993), it is interesting to note that in a recent study, a P1/Sabin-derived strain without mutations at nt 480 and 525, and also without mutations at nt 476, 2741, 2795, 6203 and 7441, was isolated from CNS of a VAPP case (Georgescu *et al.*, 1994). In another recent study, a P1/Sabin-derived strain isolated from stool of a VAPP case was analysed and no mutations were observed at nt 480, 525 and 6203 (Friedrich *et al.*, 1996a). Although this strain was isolated from stool and was not necessarily the etiological agent of the disease, the possibility that this strain also invaded CNS of the patient and caused the disease cannot be ruled out. These studies strenghten the hypothesis that some P1/Sabin-derived strains without mutations at nt 480, 525 and 6203 replicate efficiently in the human gut (Friedrich *et al.*, 1995a, 1996a), reach CNS of the patient (Georgescu *et al.*, 1994) and cause paralysis. The mutations at nt 480 (Minor and Dunn, 1988; Dunn *et al.*, 1990; Muzychenko *et al.*, 1991; Ogra *et al.*, 1991; Abraham *et al.*, 1993; Guillot *et al.*, 1994) and 525 (Muzychenko *et al.*, 1991) were also observed in P1/Sabin-derived strains after replication in the gut of healthy vaccinees (see also Minor 1992; Minor *et al.*, 1993). In another study, although no G->A reverting mutation at nt 480 was observed in the P1/Sabin strain after passage in the intestinal tract of healthy infants, an U->A mutation at nt 476 was observed, and it was suggested that this mutation could also play a role in reversion to neurovirulence (Contreras *et al.*, 1992). This mutation at nt 476 converts an U-U mismatch at nt 476-528 to an A-U pair in a secondary structure of 5'NCR in a P1/Sabin-derived strains (Macadam *et al.*, 1994b).

Recent analysis of strains isolated from VAPP cases showed that His at position 73 (C at nt 6203) and Ile at position 362 (U at nt 7071) of the viral RNA polymerase, and G at nt 7441 of 3'NCR are selected against *in vivo*, although

**Table 1. Characteristics of various P1/Sabin-derived poliovirus strains**

| Strain | 5'NCR | | 3Dpol | | Source |
|---|---|---|---|---|---|
| | nt 480 | nt 525 | codon | aa 73 | |
| P1/Sabin | G | U | CAC | His | |
| P1/Mahoney | **A** | U | **U**AC | **Tyr** | |
| P1/PG | **A** | U | CAC | His | VAPP |
| P1/IG | **A** | U | CAC | His | VAPP |
| P1/NIR | **A** | U | CAC | His | VAPP |
| P1/BA | **A** | U | CAC | His | VAPP |
| P1/AF | **A** | U | **U**AC | **Tyr** | VAPP |
| P1/3-IVc | G | U | CAC | His | VAPP |
| P1/18876 | G | U | CAC | His | VAPP |
| P1/2360 | G | U | CAC | His | GBS |
| P1/2938 | **A** | U | CAC | His | GBS |
| P1/8879 | G/**A** | U/**C** | CAC | His | GBS |
| P1/7404 | **A** | U | **U**AC | **Tyr** | GBS |
| P1/5838 | **A** | U | CAC | His | FP |
| P1/2746 | G | **C** | CAC | His | FP |
| P1/2933 | G | U | CAC | His | ND |

Data in bold indicate differences from the original P1/Sabin strain. FP = facial paralysis; ND = neuroviral disease.

Ile-362 appeared to be more stable than either His-73 or G-7441 (Georgescu *et al.*, 1995b). Although genomic recombination has been frequently observed in type 2 and type 3 Sabin-derived strains isolated from VAPP cases, none of the type 1 isolates from VAPP cases studied were found to be recombinants (Furione *et al.*, 1993; Otelea *et al.*, 1993; Georgescu *et al.*, 1994).

The characteristics of various P1/Sabin-derived strains are summarized in Table 1.

*Molecular basis of attenuation and reversion to neurovirulence of the P2/Sabin strain*

The attenuated P2/Sabin strain (P2/ P712, Ch, 2ab) was derived from the wild P2/P712/56 strain, isolated from stool of a healthy child (Almond, 1987; Racaniello, 1988). Although the complete nucleotide sequence of the P2/Sabin strain is known (Toyoda *et al.*, 1984; Pollard *et al.*, 1989), the complete nucleotide sequence of its ancestor was not yet determined (Muzychenko *et al.*, 1991). The construction of recombinants between the neurovirulent mouse-adapted P2/Lansing strain and an attenuated P2/P712 strain (a closely related strain to P2/Sabin), and the use of site-directed mutagenesis have demonstrated that A at nt 481 of 5'NCR and Ile at nt 143 of the capsid protein VP1 are the major determinants of attenuation of this P2/P712 strain for normal mice and for transgenic mice expressing the human poliovirus receptor (Moss *et al.*, 1989; Ren *et al.*, 1991). The construction of recombinants between the attenuated P2/Sabin strain and the neurovirulent vaccine revertant P2/117, isolated from a VAPP case, and the use of site-directed mutagenesis demonstrated that A at nt 481 and Ile at nt 143 of VP1 are also important determinants of attenuation of the P2/Sabin strain in primates (Macadam *et al.*, 1991a, 1993). Although mutations at these two positions are important for attenuation and reversion of the P2/Sabin strain towards neurovirulence, mutations at nt 398 and 437 of 5'NCR and at other nt positions could also have some effect, even though minor (Ren *et al.*, 1991; Macadam *et al.*, 1991a, 1993).

Molecular studies have suggested that A at nt 481 and U at nt 398 could act by destabilizing the secondary and/or tertiary structure of 5'NCR of the P2/Sabin strain (Muzychenko *et al.*, 1991; Agol, 1991; Macadam *et al.*, 1991a, 1992, 1993, 1994b; Minor, 1992, 1993; Minor *et al.*, 1993), altering its interaction with host factors (Svitkin *et al.*, 1988; Agol, 1991) necessary for translation initiation, leading to an unefficient translation of the viral RNA (Muzychenko *et al.*, 1991), and partially decreasing its replication and neurovirulence. It is still unclear whether Ile-143 of VP1 of the P2/Sabin strain causes a conformational change of the viral surface such as to prevent its interaction with neural but not epithelial cells (Equestre *et al.*, 1991; Macadam *et al.*, 1993).

*Genomic modifications in type 2 polioviruses isolated from VAPP cases, healthy contacts and healthy vaccinees*

Reverting mutations in attenuating determinants of the P2/Sabin strain have been observed in strains isolated from VAPP cases. An A->G mutation at nt 481 of 5'NCR and mutations in the codon for aa 143 of VP1, leading to the substitution of the attenuating Ile by Val, Thr, Asn or Ser, were observed previously in all strains isolated from stool and/or CNS od VAPP cases (Pollard *et al.*, 1989; Equestre *et al.*, 1991; Muzychenko *et al.*, 1991; Macadam *et al.*, 1991a, 1993; Minor *et al.*, 1993; Guillot *et al.*, 1994; Georgescu *et al.*, 1994, 1995a). The only exception in one of these studies (Georgecu *et al.*, 1994) was a strain isolated from CNS of a VAPP case that mutated at nt 481 but maintained the attenuating 143-Ile of VP1. In our recent study (Friedrich *et al.*, 1995b), although most of the strains isolated from stool of VAPP cases mutated at nt 481, and at aa 143 of VP1, some strains did not and maintained the original sequence, suggesting that reverting mutations in these attenuating determinants are not essential to the establishment of the disease. An U->C mutation at nt 398 of 5'NCR was also frequently observed in P2/Sabin-derived isolates from VAPP cases (Muzychenko *et al.*, 1991; Macadam *et al.*, 1991a; Friedrich *et al.*, 1995b), while an U->C mutation at nt 500 of 5'NCR was also observed in strains isolated from two VAPP cases (Macadam *et al.*, 1991a; Friedrich *et al.*, 1995b). Mutations at nt 4201 of peptide 2C and at nt 7386 of 3'NCR were also observed in two strains isolated from VAPP cases (Equestre *et al.*, 1991).

An A->G mutation at nt 481 observed in the P2/Sabin-derived strains isolated from VAPP cases was also observed in the strains isolated from stool of healthy contacts of VAPP cases (Friedrich *et al.,* 1995b) and in the strains isolated from stool of healthy vaccinees (Minor and Dunn, 1988; Dunn *et al.,* 1990; Macadam *et al.,* 1991a; Ogra *et al.,* 1991; Abraham *et al.,* 1993; Minor, 1992; Minor *et al.,* 1993). As there is a strong selection against A at this nt position during replication of the virus in the human gut, it is interesting that some P2/Sabin-derived strains isolated from stool of VAPP cases maintained A at nt 481 (Friedrich *et al.,* 1995b). One strain isolated from stool of a VAPP case, collected 138 days after the administration of a single dose of vaccine, maintained A at nt 481 and Ile at nt 143 of VP1. It is possible that one or more mutations in the viral genome could suppress the attenuating effect of 481- A. It also suggests that strains maintaining this attenuating determinant could replicate efficiently in the human gut, reaching CNS and causing paralysis in certain cases.

The substitution of Ile-143 of VP1 by other amino acids observed in P2/Sabin-derived strains isolated from VAPP cases, was also found in strains isolated from stool of healthy contacts of VAPP cases (Friedrich *et al.,* 1995b) and in strains isolated from stool of healthy vaccinees after replication in the gut (Minor, 1992; Minor *et al.,* 1993; Macadam *et al.,* 1993), also demonstrating a selection against Ile-143 of VP1. The latter was conserved in about half of the isolates from healthy vaccinees (Macadam *et al.,* 1993), but was substituted by other amino acids in most isolates from VAPP cases. As some isolates from VAPP cases and from healthy vaccinees maintained Ile-143 of VP1 (Georgescu *et al.,* 1994; Friedrich *et al.,* 1995b), the possibility cannot be excluded that supressor mutations occured in the capsid proteins of these isolates. These studies suggest that selection pressure against A-481 is stronger than that against Ile-143 of VP1 (Macadam *et al.,* 1993).

The frequent isolation of P2/Sabin-derived poliovirus recombinants from VAPP cases has suggested that genomic recombination could also increase the neurovirulence of

**Table 2. Comparison of aa 69 and 113 of the viral RNA polymerase of various poliovirus strains**

| Strain | RNA polymerase | | | |
|---|---|---|---|---|
| | codon | aa 69 | codon | aa 113 |
| P2/Sabin | GAU | Asp | ACC | Thr |
| P2/15517 | GAG | **Glu** | AGU | **Ser** |
| P2/15561 | GAG | **Glu** | AGU | **Ser** |
| P2/15562 | GAG | **Glu** | AGU | **Ser** |
| P2/15815 | GAG | **Glu** | AGU | **Ser** |
| P2/1400 | GAA | **Glu** | AGU | **Ser** |
| P2/1402 | GAA | **Glu** | AGU | **Ser** |
| P2/1397 | GAA | **Glu** | UCC | **Ser** |
| P2/Lansing | GAA | **Glu** | AGU | **Ser** |
| P1/Sabin | GAA | **Glu** | UCC | **Ser** |
| P1/Mahoney | GAA | **Glu** | UCC | **Ser** |
| P3/Sabin | GAG | **Glu** | UCU | **Ser** |
| P3/Leon/37 | GAG | **Glu** | UCU | **Ser** |
| P3/23127 | GAA | **Glu** | AGC | **Ser** |

Data in bold indicate differences from the original P2/Sabin strain.

**Table 3. Characteristics of various P2/Sabin-derived poliovirus strains**

| Strain | 5'NCR | | VP1 | | Source |
|---|---|---|---|---|---|
| | nt 3 98 | nt 481 | codon | aa 143 | |
| P2/Sabin | U | A | AUU | Ile | |
| P2/712[a] | U | A | AUU | Ile | |
| P2/712[b] | **C** | **G** | – | – | |
| P2/117 | U | **G** | **GUU** | **Val** | VAPP |
| P2/144 | **C** | **G** | ACU | **Thr** | VAPP |
| P2/154 | **C** | **G** | **GUU** | **Val** | VAPP |
| P2/188 | **C** | **G** | ACU | **Thr** | VAPP |
| P2/v1256 | **C** | **G** | AAU | **Asn** | VAPP |
| P2/147 | U | **G** | AAU | **Asn** | VAPP |
| P2/155 | U | **G** | AGU | **Ser** | VAPP |
| P2/HW | U | **G** | AAU | **Asn** | VAPP |
| P2/VL | U | **G** | **GUU** | **Val** | VAPP |
| P2/GS | U | **G** | **GUU** | **Val** | VAPP |
| P2/2-Is | – | **G** | ACU | **Thr** | VAPP |
| P2/2-Ic | – | **G** | **ACC** | **Thr** | VAPP |
| P2/2-IIs | – | **G** | ACU | **Thr** | VAPP |
| P2/2-IIc | – | **G** | ACU | **Thr** | VAPP |
| P2/2-IIIs$^{I}$ | – | **G** | **GUU** | **Val** | VAPP |
| P2/2-IIIs$^{II}$ | – | **G** | ACU | **Thr** | VAPP |
| P2/2-IIIs$^{III}$ | – | **G** | ACU | **Thr** | VAPP |
| P2/2-IIIc$^{II}$ | – | **G** | AUU | Ile | VAPP |
| P2/2-IVs$^{I}$ | – | **G** | AAU | **Asn** | VAPP |
| P2/2-IVs$^{II}$ | – | **G** | ACU | **Thr** | VAPP |
| P2/2-IVc | – | **G** | ACU | **Thr** | VAPP |
| P2/15517 | **C** | **G** | AAU | **Asn** | VAPP |
| P2/15561 | **C** | **G** | AAU | **Asn** | hc |
| P2/15562 | **C** | **G** | AAU | **Asn** | hc |
| P2/15815 | **C** | **G** | AAU | **Asn** | hc |
| P2/1400 | **C** | **G** | AAU | **Asn** | VAPP |
| P2/1401 | **C** | **G** | AAU | **Asn** | hc |
| P2/1402 | **C** | **G** | AAU | **Asn** | hc |
| P2/1397 | **C** | **G** | ACU | **Thr** | VAPP |
| P2/1398 | **C** | **G** | ACU | **Thr** | hc |
| P2/2645 | **C** | **G** | AGU | **Ser** | VAPP |
| P2/1006 | **U/C** | **G** | ACU | **Thr** | VAPP |
| P2/13121 | U | **G** | AAU | **Asn** | VAPP |
| P2/10989 | U | **G** | **GUU** | **Val** | VAPP |
| P2/1851 | U | **G** | AUU | Ile | VAPP |
| P2/998 | U | **A>G** | AUU | Ile | VAPP |
| P2/7790 | U | A | AUU | Ile | VAPP |
| P2/2735 | U | A | **GUU** | **Val** | sp |
| P2/2975 | U | A | AUU | Ile | sp |
| P2/2864 | U | **G** | AUU | Ile | sp |
| P2/1281 | U | **G** | ACU | **Thr** | hc |

(-) = data not available; hc = healthy contact; sp = suspected poliomyelitis. For the rest of legend see Table 1.

[a] The passage history of this strain closely, related to P2/Sabin strain, ist not known.

[b] Predecessor of the P2/Sabin strain .

these strains or have some advantage in replication in the gut and in the CNS of the patients (Lipskaya *et al.*, 1991; Furione *et al.*, 1993; Georgescu *et al.*, 1994, 1995a,b; Friedrich *et al.*, 1996c). Both the vaccine/vaccine and vaccine/non-vaccine recombinants were detected. P2/Sabin-derived recombinant strains have also been isolated from healthy contacts of VAPP cases (Friedrich *et al.*, 1996c). A comparison of part of the amino acid sequence of the viral RNA polymerase of the P2/Sabin strain with the predicted one of P2/Sabin-derived recombinants isolated from VAPP cases and healthy contacts (Friedrich *et al.*, 1996c) demonstrated that an Asp->Glu substitution at aa 69 was observed in most of these isolates, while a Thr->Ser substitution at aa 113 was observed in all these isolates (Table 2). Both the P1/Sabin and P3/Sabin strains (Friedrich *et al.*, 1996c) and also other wild strains (La Morica *et al.*, 1986; Hughes *et al.*, 1986), have Glu at aa 69 and Ser at aa 113. Although the nucleotide sequence of the ancestor of the P2/Sabin strain is not known, it cannot be ruled out that Asp-69 and Thr-113 of the viral RNA polymerase play a role in attenuation, even though minor.

Mutations in antigenic sites of the capsid proteins of the P2/Sabin-derived strains isolated from VAPP cases (Fiore *et al.*, 1987) were also observed, and could act as an escape mechanism from the immune response. Thus, these molecular studies have demonstrated that many genomic modifications, some of them alredy known as able to increase the neurovirulence, can occur in the P2/Sabin strain after replication in humans.

The characteristics of various P2/Sabin-derived strains are summarized in Table 3.

*Molecular basis of attenuation and reversion to neurovirulence of the P3/Sabin strain*

The P3/Sabin strain (P3/Leon $12a_1$,b) was derived from the wild P3/Leon/37 strain, isolated from the brain and spinal cord of a victim of a fatal case of paralytic poliomyelitis (Almond, 1987; Racaniello, 1988). Comparison of the complete nucleotide sequence of the attenuated P3/Sabin strain (Stanway *et al.*, 1983; Toyoda *et al.*, 1984; Weeks-Levy *et al.*, 1991) with that of its neurovirulent ancestor, P3/Leon/37 strain (Stanway *et al.*, 1984) demonstrated that these two strains differ by several mutations. By constructing recombinant viruses between the attenuated P3/Sabin strain and the neurovirulent P3/Leon/37 strain, and by testing their neurovirulence in monkeys, two major mutations important for the attenuation of the P3/Sabin were found: a C->U mutation at nt 472 of 5'NCR, and a C->U mutation at nt 2034 leading to a Ser->Phe substitution at aa 91 of the capsid protein VP3 (Westrop *et al.*, 1989). Recent evidence has suggested that an U->C mutation at nt 2493, leading to an Ile->Thr substitution at aa 6 of the capsid protein VP1, may also be involved in the attenuation (Weeks-Levy *et al.*, 1991; Tatem *et al.*, 1992; Mento *et al.*, 1993). It was demonstrated (La Monica *et al.*, 1987) that recombinant viruses having C at nt 472 are neurovirulent for mice, while those with an U at this nt position are attenuated. When viral recombinants were grown in human neuroblastoma cell line SH-SY5Y (La Monica and Racaniello, 1989), recombinants having U-472 replicated to approximately 10-fold-lower titers than did neurovirulent viruses with a C at this position, although the viruses replicated equally well in Hela cells. It has also been shown (Chumakov *et al.*, 1991) that OPV type 3 containing C at nt 472 is rapidly selected during serial passages in African green monkey kidney cells that are used to produce the vaccine, and vaccine lots that had failed in the intraspinal monkey neurovirulence test contained a higher proportion of C at nt 472 than all other lots that had passed this test.

The nt 472 and 537 of 5'NCR pair in a predicted secondary structure (Pilipenko *et al.*, 1989; Skinner *et al.*, 1989; Agol, 1991). The attenuating U at nt 472 of 5'NCR of the P3/Sabin strain resulted in the alteration of a C-G base pair (nt 472-537) found in the neurovirulent wild strain P3/Leon/37 (ancestor of the P3/Sabin strain) to a U-G base pair (nt 472-537) found in the P3/Sabin vaccine strain, suggesting that the attenuated phenotype is partially associated with a weakening of a base pairing in a highly conserved structure (Agol, 1991; Macadam *et al.*, 1992, 1994b; Minor, 1992, 1993; Minor *et al.*, 1993). The alteration of the secondary structure of 5'NCR caused by the attenuating U at nt 472 could alter the interaction of viral RNA with one or more host factors required for the initiation of translation of poliovirus RNA (Agol, 1991; Svitkin *et al.*, 1988), leading to the observed deficiency in translation (Svitkin *et al.*, 1985, 1990) and decrease in neurovirulence associated with this C->U (nt 472) mutation (Westrop *et al.*, 1989). The attenuating Phe-91 of the capsid protein VP3 inhibits virion assembly, specifically at the promoter-to-pentamer step (Macadam *et al.*, 1991b). The location of aa 91 of VP3 and of a number of candidate supressor mutations in the atomic structure of the virion suggested that it might act by destabilization of the particle and that supressor mutations may function by stabilizing specific interfaces (Minor *et al.*, 1989).

*Genomic modifications in type 3 polioviruses isolated from VAPP cases and healthy vaccinees*

The attenuating U at nt 472 was observed to revert to C in all P3/Sabin-derived strains isolated from stool and/or CNS from VAPP cases, but Phe-91 of VP3 was maintained in most cases (Cann *et al.*, 1984; Almond *et al.*, 1985; Evans

*et al.*, 1985; Macadam *et al.*, 1989; Minor *et al.*, 1989, 1993; Georgescu *et. al.*, 1994; Friedrich *et al.*, 1995c; Driesel *et al.*, 1995). It was suggested that second site-mutations in the capsid proteins are able to suppress the attenuating effect of Phe 91 of VP3 (Macadam *et al.*, 1989; Minor *et al.*, 1989; Driesel *et al.*, 1995). An additional mutation at aa 54 of VP1, leading to the substitution of Ala by Thr or Val, was also frequently found in isolates from VAPP cases (Minor *et al.*, 1989; Macadam *et al.*, 1989). A G->A mutation at nt 143 of 5'NCR occured in several strains isolated from VAPP cases (Driesel *et al.*, 1995). An U->C mutation at nt 472 found in strains isolated from VAPP cases was also observed in strains isolated from stool of healthy vaccinees, demonstrating a strong selection against U at this position in virus replication (Evans *et al.*, 1985; Minor and Dunn, 1988; Dunn *et al.*, 1990; Macadam *et al.*, 1989; Ogra *et al.*, 1991; Tatem *et al.*, 1991; Contreras *et al.*, 1992; Minor, 1992; Abraham *et al.*, 1993; Minor *et al.*, 1993; Driesel *et al.*, 1995). A Phe->Ser mutation at aa 91 of V P3 or supressor mutations in the capsid proteins were also observed in the strains isolated from healthy vaccinees (Macadam *et al.*, 1989; see also Contreras *et al.*, 1992). Although strains with C instead of U at nt 472 apparently grow more efficiently in the nervous system of primates, an isolate recovered from the brain of a monkey inoculated intraspinaly with the P3/Sabin strain (with U-472) was identified, which replicated efficiently in monkey nervous tissue and maintained the attenuating U-472 (Tatem *et al.*, 1991). Since there is a strong selection against an U at nt 472 in the replication of P3/Sabin strain in the human gut (and also in CNS), it is very interesting that a strain isolated from stool of a transverse myelitis (TM) case, collected 136 days after the administration of a single dose of vaccine, maintained U-472 (Friedrich *et al.*, 1995c). Although the strain was isolated from stool and might not be necessarily the etiological agent of the disease, we assume that viruses maintaining U-472 could also replicate efficiently in the human gut in certain cases.

Genomic recombination has also been observed in P3/Sabin-derived strains isolated from VAPP cases (Macadam *et al.*, 1989; Furione *et al.*, 1993; Georgescu *et al.*, 1994, 1995b; Driesel *et al.*, 1995) and healthy vaccinees (Minor *et al.*, 1986; Cammack *et al.*, 1988; Macadam *et al.*, 1989; Tatem *et al.*, 1991; Minor, 1992; Minor *et al.*, 1993; Driesel *et al.*, 1995). Also mutations in antigenic sites were observed in P3/Sabin-derived strains isolated from VAPP cases and healthy vaccinees (Minor *et al.*, 1986, 1989; Macadam *et al.*, 1989). These studies demonstrated that many genomic modifications, some of them known to increase the neurovirulence, also occur in the P3/Sabin strain after replication in humans.

The characteristics of various P3/Sabin-derived strains are summarized in Table 4.

**Table 4. Characteristics of various P3/Sabin-derived poliovirus strains**

| Source | Isolate | Mutation at nt 472 | Antigenic change protein/aa position/mutation | Possible supressor protein/aa position/mutation |
|---|---|---|---|---|
| VAPP | P3/106 | U->C | | $VP_3$ 178 Gln->Leu |
| VAPP | P3/115 | U->C | $VP_2$ 166 Val->Ala | $VP_3$ 178 Gln->Leu |
| VAPP | P3/116 | U->C | | $VP_3$ 91 Phe->Ser |
| | | | | $VP_1$ 54 Ala->Thr |
| VAPP | P3/119 | U->C | | $VP_2$ 200 Arg->Lys |
| | | | | $VP_2$ 215 Leu->Met |
| | | | | $VP_1$ 54 Ala->Val |
| VAPP | P3/122 | U->C | | $VP_2$ 265 Val->Ala |
| | | | | $VP_3$ 108 Thr->Ala |
| VAPP | P3/131 | U->C | $VP_2$ 164 Asn->Asp | $VP_1$ 54 Ala->Val |
| | | | $VP_3$ 77 Asp->Asn | |
| VAPP | P3/132 | U->C | | $VP_1$ 34 Ala->Val |
| | | | | $VP_3$ 175 Thr->Ala |
| VAPP | P3/146 | U->C | | $VP_1$ 54 Ala->Val |
| | | | | $VP_2$ 215 Leu->Met |
| VAPP | P3/156 | U->C | $VP_3$ 77 Asp->Asn | $VP_1$ 54 Ala->Thr |
| | | | | $VP_3$ 91 Phe->Ser |
| | | | $VP_1$ 286 Arg->Lys | $VP_2$ 146 Asn->Lys |
| VAPP | P3/158 | U->C | | $VP_1$ 54 Ala->Val |
| | | | | $VP_2$ 215 Leu->Met |
| | | | | $VP_1$ 263 Lys->Thr |
| VAPP | P3/161 | U->C | $VP_3$ 77 Asp->Asn | $VP_3$ 91 Phe->Ser |
| | | | | $VP_1$ 54 Ala->Thr |
| VAPP | P3/382 | U->C | | $VP_1$ 54 Ala->Thr |
| | | | | $VP_1$ 263 Lys->Ile |
| VAPP | P3/7574 | U->C | $VP_2$ 165 Ala->Thr | $VP_3$ 108 Thr->Ala |
| hv | P3/KT2-28 | U->C | $VP_2$ 165 Ala->Thr | $VP_3$ 91 Phe->Ser |
| | | | $VP_3$ 77 Asp->Asn | |
| hv | P3/DM66 | U->C | $VP_2$ 165 Ala->Thr | $VP_2$ 18 Leu->Ile |
| | | | $VP_3$ 77 Asp->Asn | |
| hv | P3/EM47 | U->C | $VP_2$ 165 Ala->Thr | $VP_4$ 34 Lys->Arg |
| | | | | $VP_3$ 108 Thr->Ala |
| | | | | $VP_1$ 54 Ala->Val |
| | | | | $VP_1$ 105 Met->Thr |
| hv | P3/NH-15 | U->C | | $VP_3$ 91 Phe->Ser |

hv = healthy vaccinee. For the rest of legend see Tables 1,2.

*Factors involved in the establishment of paralytic poliomyelitis*

As reverting mutations in 5'NCR at nt 480 for the P1/Sabin strain, at nt 481 for the P2/Sabin strain and at nt 472 for the P3/Sabin strain have been observed in almost all isolates from stool and/or CNS of VAPP cases, it was suggested that reverting mutations at these positions are important for the establishment of the disease (Evans *et al.*, 1985; Pollard *et al.*, 1989; Equestre *et al.*, 1991; Macadam *et al.*, 1989, 1991a, 1993; Muzychenko *et al.*, 1991; Otelea

*et al.*, 1993; Georgescu *et al.*, 1994; Friedrich *et al.*, 1995b,c). The recent analysis of Sabin vaccine-derived strains isolated from VAPP cases without mutations at these positions suggested that the abovementioned mutations are not essential for the establishment of poliomyelitis (Georgecu *et al.* 1994; Friedrich *et al.*, 1995a,b,c, 1996a). One possibility is that other mutations are able to suppress the attenuatting effect of G-480, A-481 and U-472 for the P1/Sabin, P2/Sabin and P3/Sabin strains respectively, and that they could include a mutation(s) occuring directly in 5'NCR or in the region coding for some viral factor interacting with 5'NCR. Another possibility is that mutations in the 2A protease coding region, suggested to be involved in cap-independent translation (Minor *et al.*, 1993; Macadam *et al.*, 1994a), are able to suppress the attenuating effect of G-480, A-481 and U-472, for the three Sabin strains, respectively. As host factor(s) is (are) involved in interactions with 5'NCR (Svitkin *et al.*, 1988; Meerovitch *et al.*, 1989; Agol, 1991), if not supressing mutations in the viral RNA exist for attenuating determinants in 5'NCR in certain isolates from VAPP cases, it cannot be excluded that certain biochemical characteristics of the host factor(s), interacting with 5'NCR of the viral RNA, could select isolates maintaining important attenuating determinants in 5'NCR, supressing the effect of attenuating mutations in 5'NCR.

Although mutations increasing the yield and neurovirulence of the Sabin strains are important for the establishment of the disease (Macadam *et al.*, 1989, 1991a, 1993; Otelea *et al.*, 1993), the frequent isolation of poliovirus recombinants from VAPP cases (Macadam *et al.*, 1989; Lipskaya *et al.*, 1991; Furione *et al.*, 1993; Georgescu *et al.*, 1994, 1995a,b; Driesel *et al.*, 1995; Friedrich *et al.*, 1996c) has suggested that recombination may also increase the neurovirulence of the Sabin strains, while mutations in antigenic sites (Fiore *et al.*, 1987; Macadam *et al.*, 1989; Minor *et al.*, 1989) could act as an escape mechanism from the immune response. The isolation of the Sabin-derived neurovirulent isolates with similar genomic modifications from healthy contacts of VAPP cases (Friedrich *et al.*, 1995b, 1996c) and from healthy vaccinees (Macadam *et al.*, 1989, 1991a, 1993) has supported the view that host factors are also important in the establishment of poliomyelitis. They could be involved in the replication of the virus in human cells through interaction with 5'NCR (Agol, 1991). As host factors could be regarded also immuno-deficiencies (WHO, 1982; Nkowane *et al.*, 1987; Groom *et al.*, 1994; Zuckerman *et al.*, 1994) such as heritable immunedeficiencies (Lederman and Winkelstein, 1985), immunedeficiencies caused by protein/calorie malnutrition (Arya, 1994), deficiency in vitamin A (Arya, 1994) or HIV infection (Arya, 1994; Wyatt, 1994, Ion-Nedelcu *et al.*, 1994). Intramuscular injections within 30 days after exposure to OPV through vaccine or contact with a recent vaccinee might also be a risk factor for VAPP cases (Strebel *et al.*, 1995). Other host factors and other pathological conditions could also be involved. The constant genomic characterization of Sabin-derived strains isolated from VAPP cases and healthy individuals might help to elucidate the complex interactions of host and virus factors that are important for the establishment of the disease.

*Sabin-related poliovirus vaccine-related strains isolated from cases of GBS, transverse myelitis and facial paralysis*

The cause of GBS and TM, demyelinating diseases of CNS, is unknown, although GBS (Leneman, 1966; Ropper, 1992; Stratton *et al.*, 1994; Bolton, 1995) and TM (Holt *et al.*, 1976; Whittle and Roberton, 1977; Barak and Schwartz, 1988; Vahidy *et al.*, 1989; D'Costa *et al.*, 1990; Dodson, 1990; Heller *et al.*, 1990; Miles *et al.*, 1993, Caldas *et al.*, 1994; Graber *et al.*, 1994; Hill *et al.*, 1994) are frequently preceded by viral infections and vaccinations. A significantly increased incidence of GBS cases were observed in Finland in 1985 during and soon after a mass vaccination campaign against poliomyelitis with OPV (Kinnunen *et al.*, 1989; Uhari *et al.*, 1989). In Finland, immunization against poliomyelitis is routinaly performed with inactivated poliovirus vaccine, and OPV was used in an one-time- campaign in 1985. Also A.B. Sabin himself developed GBS after working for many years with enteroviruses (Melnick and Horaud, 1993).

The isolation of Sabin-derived poliovirus vaccine strains in Brazil from cases of GBS, TM and facial paralysis has suggested that this paralysis could also be rarely caused or triggered by poliovirus infection (Friedrich *et al.*, 1995a,c,d, 1996b). In these studies, the isolation of Sabin poliovirus vaccine-related strains in all the cases analysed, where the last vaccine dose was mostly given months or years before the establishment of the disease, has suggested a persistent infection or transmission of these strains to the patients. The isolation of Sabin poliovirus vaccine-related strains from cases of GBS, TM and facial paralysis several days or weeks after the onset of motor deficiency demonstrated a temporal association between the disease and the isolated viruses. The analysis of four isolates from stool of GBS cases at nt 480, 525 and 6203, demonstrated a G->A mutation at nt 480 and a C->U mutation at nt 6203 in the first strain, a G->A mutation at nt 480 in the second strain, a G->A mutation at nt 480 and an U->C mutation at nt 525 in the third strain (demonstrating the presence of at least two subpopulations), while the fourth strain did not mutate either at nt 480, 525 or 6203 (Friedrich *et al.*, 1995a). Interestingly, in three out of four of these GBS cases, where mutations in the isolates were observed, the last vaccine dose was given several months or years before the onset of the disease. The mutation at nt 480 or 525 were also observed in strains isolated from facial paralysis cases (Friedrich *et al.*, 1995a). In another study (Friedrich *et al.*, 1995c), a P3/Sabin-derived

strain isolated from stool, collected 17 days after a single OPV administration, from a patient presenting GBS also presented a mutation at nt 472. Still another P3/Sabin-derived strain isolated from stool, collected more than four months after a single OPV dose from a patient presenting TM, maintained the attenuating U-472 of 5'NCR (Friedrich *et al.*, 1995c).

The capacity of Sabin-derived poliovirus strains to cause a persistent infection in human neuroblastoma cells has been demonstrated (Colbere-Garapin *et al.*, 1989). The nucleotide sequence analysis of the P1/Sabin-derived strains revealed mutations at nt 525 and elsewhere (Pelletier *et al.*, 1991; Borzakian *et al.*, 1993). Other studies have demonstrated the capacity of Theiler's virus, also a member of the *Picornaviridae* family, to cause a persistent infection leading to demyelination of CNS in mice (Jarousse *et al.*, 1994; Yauch and Kim, 1994; Johnston *et al.*, 1995; Michiels *et al.*, 1995). Although the Sabin poliovirus vaccine-derived strains isolated from cases of GBS and TM in Brazil may not be the etiological agents of the disease, these studies support the idea that infections caused by these strains could also trigger demyelination of CNS of humans in certain cases (Friedrich *et al.*, 1995a,c,d, 1996b).

## Conclusions

Many studies have demonstrated that most of the rare VAPP cases are related to poliovirus type 2 and 3 strains, and more rarely to type 1 strain. As more mutations are involved in the attenuation of the P1/Sabin strain in comparison with the other two vaccine strains, this property has been suggested to be an important factor for the greater safety of this strain. Although many genomic modifications have been detected in Sabin vaccine strains isolated from VAPP cases, similar genomic modifications have been found in strains isolated from healthy contacts and healthy vaccinees, supporting the view that host factors are also involved in the establishment of poliomyelitis. The observation of isolates from VAPP cases without reverting mutations in important attenuating determinants has led us to assume that reverting mutations may not be essential for the establishment of the disease. It is likely that other mutations could increase the neurovirulence or participate in some way in the establishment of the disease. The isolation of Sabin poliovirus vaccine-derived strains from GBS, TM and facial paralysis cases has suggested that in general the vaccine strains could also trigger these diseases in rare cases and not just poliomyelitis.

**Acknowledgements.** I thank Dr. H.G. Schatzmayr, the head of the Department of Virology, and Dr. E.E. Da Silva, the head of the Enterovirus Laboratory of the Department of Virology, both from Instituto Oswaldo Cruz, for supporting my studies on polioviruses.

## References

Abe S, Ota Y, Koike S, Kurata T, Horie H, Nomura T, Hashizume S, Nomoto A (1995a): Neurovirulence test for oral live poliovaccines using poliovirus-sensitive transgenic mice. *Virology* **206**, 1075–1083

Abe S, Ota Y, Doi Y, Nomoto A, Nomura T, Chumakov KM, Hashizume S (1995b): Studies on neurovirulence in poliovirus-sensitive transgenic mice and cynomolgus monkeys for the different temperature-sensitive viruses derived from the Sabin type 3 virus. *Virology* **210**, 160–166.

Abraham R, Minor P, Dunn G, Modlin JF, Ogra PL (1993): Shedding of virulent poliovirus revertants during immunization with oral poliovirus vaccine after prior immunization with inactivated polio vaccine. *J. Infect. Dis.* **168**, 1105–1109.

Agol VI, Drozdov SG, Ivannikova TA, Kolesnikova MS, Korolev MB, Tolskaya EA (1989): Restricted growth of attenuated poliovirus strains in cultured cells of a human neuroblastoma. *J. Virol.* **63**, 4034–4038.

Agol VI (1991): The 5'-untranslated region of picornaviral genomes. *Adv. Virus Res.* **40**, 103–180.

Agol VI (1993): Genetic stability and instability of the *cis*-acting control element of the 5' untranslated region of the poliovirus RNA. *Dev. Biol. Stand.* **78**, 11–16.

Almond JW, Westrop GD, Cann AJ, Stanway G, Evans DMA, Minor PD, Schild, GC (1985): Attenuation and reversion to neurovirulence of the Sabin poliovirus type 3 vaccine. In Lener RA, Chanock RM, Brown F (Ed.): *Vaccines* **85**. New York, Cold Spring Harbor Laboratory, pp. 271–277.

Almond JW (1987): The attenuation of poliovirus neurovirulence. *Ann. Rev. Microbiol.* **41**, 153–180.

Arya SC (1994): Vaccine-associated poliomyelitis. *Lancet* **343**, 610–611.

Barak Y, Schwartz JF (1988): Acute transverse myelitis associated with Echo type 5 infection. *Amer. J. Dis. Child.* **142**, 128.

Basilico FC, Bernat JL (1978): Vaccine-associated poliomyelitis in a contact. *JAMA* **239**, 2275.

Bolton CF (1995): The changing concepts of Guillain-Barré syndrome. *New Engl. J. Med.* **333**, 1415–1417.

Borzakian S, Pelletier I, Calvez V, Colbere-Garapin F (1993): Precise missense and silent point mutations are fixed in the genomes of poliovirus mutants from persistently infected cells. *J. Virol.* **67**, 2914–2917.

Bouchard MJ, Lam DH, Racaniello VR (1995): Determinants of attenuation and temperature sensitivity in the type 1 poliovirus Sabin vaccine. *J. Virol.* **69**, 4972–4978.

Caldas C, Bernicker E, Nogare AD, Luby JP (1994): Transverse myelitis associated with Epstein-Barr virus infection. *Amer. J. Med. Sci.* **307**, 45–48.

Cammack N, Phillips A, Dunn G, Patel V, Minor PD (1988): Intertypic genomic rearrangements of poliovirus strains in vaccinees. *Virology* **167**, 507–514.

Cann AJ, Stanway G, Hughes PJ, Minor PD, Evans DMA, Schild GC, Almond JW (1984): Reversion to neurovirulence of the live-attenuated Sabin type 3 oral poliovirus vaccine. *Nucleic Acids Res.* **12**, 7787–7792.

Christodoulou C, Colbere-Garapin F, Macadam A, Taffs LF, Marsden S, Minor P, Horaud F (1990): Mapping of mutations associated with neurovirulence in monkeys infected with Sabin 1 poliovirus revertants selected at high temperature. *J. Virol.* **64**, 4922–4929.

Chumakov KM, Powers LB, Noonan KE, Roninson IB, Levenbook IS (1991): Correlation between amount of virus with altered nucleotide sequence and the monkey test for acceptability of oral poliovirus vaccine. *Proc. Natl. Acad. Sci. USA* **88**, 199–203.

Chumakov KM, Norwood LP, Parker ML, Dragunsky EM, Ran Y, Levenbook IS (1992): RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence. *J. Virology* **66**, 966–970.

Chumakov K, Norwood L, Parker M, Dragunsky E, Taffs R, Ran Y, Ridge J, Levenbook I (1993): Assesment of the viral RNA sequence heterogeneity for control of OPV neurovirulence. *Dev. Biol. Stand.* **78**, 79–89.

Chumakov KM, Dragunsky EM, Norwood LP, Douthitt MP, Ran Y, Taffs RE, Ridge J, Levenbook IS (1994): Consistent selection of mutations in the 5'-untranslated region of oral poliovirus vaccine upon passaging in vitro. *J. Med. Virol.* **42**, 79–85.

Colbere-Garapin F, Christodoulou C, Crainic R, Pelletier I (1989): Persistent poliovirus infection of human neuroblastoma cells. *Proc. Natl. Acad. Sci. USA* **86**, 7590–7594.

Contreras G, Dimock K, Furesz J, Gardell C, Hazlett D, Karpinski K, McCorkle G, Wu L (1992): Genetic characterization of Sabin types 1 and 3 poliovaccine virus following serial passage in the human intestinal tract. *Biologicals* **20**, 15–26.

D'Costa DF, Cooper A, Pye IF (1990): Transverse myelitis following cholera, typhoid and polio vaccination. *J. Roy. Soc. Med.* **83**, 653.

Dodson D (1990): Transverse myelitis and spastic paraparesis in a patient with HIV infection. *New Engl. J. Med.* **322**, 1322.

Driesel G, Diedrich S, Künkel U, Schreier E (1995): Vaccine-associated cases of poliomyelitis over a 30 year period in East Germany. *Europ. J. Epidemiol.* **11**, 1–8.

Dunn G, Begg NT, Cammack N, Minor PD (1990): Virus excretion and mutation by infants following primary vaccination with live oral poliovaccine from two sources. *J. Med. Virol.* **32**, 92–95.

Ehrenfeld E (1992): International workshop: 'Poliovirus attenuation: molecular mechanisms and practical aspects'. *Biologicals* **20**, 167–169.

Evans DMA, Dunn G, Minor PD, Schild GC, Cann AJ, Stanway G, Almond JW, Currey K, Maizel JrJV (1995): Increased neurovirulence associated with a single nucleotide change in a noncoding region of the Sabin type 3 poliovaccine genome. *Nature* **314**, 548–550.

Equestre M, Genovese D, Cavaliere F, Fiore L, Santoro R, Perez Bercoff R (1991): Identification of a consistent pattern of mutations in neurovirulent variants derived from the Sabin vaccine strain of poliovirus type 2. *J. Virol.* **65**, 2707–2710.

Fenyves A (1993): The potencial use of viral genomic markers in estimating the neurovirulence of oral poliomyelitis vaccine (OPV): Regulatory aspects. *Dev. Biol. Stand.* **78**, 157–159.

Filippis AMB, Schatzmayr HG, Ferreira FC, Chagas SAR, Costa MC, Santos AP, Da Silva EE (1994): Intratypic differentiation of polioviruses isolated from suspected cases of poliomyelitis in Brazil during the period of 1990 to 1993. *Mem. Inst. Oswaldo Cruz* **89**, 513–518.

Fiore L, Pierangeli A, Lombardi F, Santoro R, Crainic R, Venuti A, Perez Bercoff R (1987): Antigenic and biochemical characterization of poliovirus type 2 isolated from two cases of paralytic disease. *Intervirology* **27**, 196–204.

Friedrich F, Filippis AMB, Ferreira FC, Schatzmayr HG, Da Silva EE (1995a): Genomic characterization of type 1 Sabin-related polioviruses isolated in Brazil. *Acta Virol.* **39**, 23–29.

Friedrich F, Filippis AMB, Ferreira FC, Schatzmayr HG, Da Silva EE (1995b): Genomic characterization of type 2 polioviruses isolated from vaccine-associated cases in Brazil. *Brazil. J. Med. Biol. Res.* **28**, 733–742.

Friedrich F, Filippis AMB, Ferreira FC, Schatzmayr HG, Da Silva EE (1995c): Genomic characterization of type 3 polioviruses isolated from vaccine-associated poliomyelitis cases in Brazil. *Brazil. J. Med. Biol. Res.* **28**, 195-200.

Friedrich F, Filippis AMB, Schatzmayr HG (1995d): Sabin-related poliovirus vaccine strains isolated from transverse myelitis cases in Brazil. *Rev. Inst. Med. Trop S. Paulo* **37**, 543–545.

Friedrich F, Filippis AMB, Ferreira FC, Oliveira MJC, Schatzmayr HG, Da Silva EE (1996a): Poliovirus type 1 isolated from a vaccine-associated case of paralytic poliomyelitis in Brazil. *Brazil. J. Med. Biol. Res.* **29**, 15–18.

Friedrich F, Filippis AMB, Schatzmayr HG (1996b): Temporal association between the isolation of Sabin-related poliovirus vaccine strains and the Guillain-Barré syndrome. *Rev. Inst. Med. Trop. S. Paulo* **38**, 55–58.

Friedrich F, Da Silva EE, Schatzmayr HG (1996c): Type 2 poliovirus recombinants isolated from vaccine-associated cases and from healthy contacts in Brazil. *Acta Virol.* **40**, 27–33.

Furione M, Guillot S, Otelea D, Balanant J, Candrea A, Crainic R (1993): Poliovirus with natural recombinant genomes isolated from vaccine-associated paralytic poliomyelitis. *Virology* **196**, 199–208.

Georgescu MM, Delpeyroux F, Tardy-Panit M, Balanant J, Combiescu M, Combiescu AA, Guillot S, Crainic R (1994): High diversity of poliovirus strains isolated from the central nervous system from patients with vaccine-associated paralytic poliomyelitis. *J. Virol.* **68**, 8089–8101.

Georgescu MM, Delpeyroux F, Crainic R (1995a): Tripartite genome organization of a natural type 2 vaccine/nonvaccine recombinant poliovirus. *J. Gen. Virol.* **76**, 2343–2348.

Georgescu MM, Tardy-Panit M, Guillot S, Crainic R, Delpeyroux F (1995b): Mapping of mutations contributing to the temperature sensitivity of the Sabin 1 vaccine strain of poliovirus. *J. Virol.* **69**, 5278–5286.

Ghendon Y (1993): WHO Recommendation on potencial use of new poliomyelitis vaccines. *Dev. Biol. Stand.* **78**, 133–139.

Graber D, Fossoud C, Grouteau E, Gayet-Mengelle C, Carriere JP (1994): Acute transverse myelitis and coxsackie $A_9$ virus infection. *Pediat. Infect. Dis. J.* **13**, 77.

Groom SN, Clewley J, Litton PA, Brown DW (1994): Vaccine-associated poliomyelitis. *Lancet* **343**, 609–610.

Guillot S, Otelea D, Delpeyroux F, Crainic R (1994): Point mutations involved in the attenuation/neurovirulence alternation in type 1 and 2 oral polio vaccine strains detected by site-specific polymerase chain reaction. *Vaccine* **12**, 503–507.

Hellen CUT, Pestova TV, Litterst M, Wimmer E (1994): The cellular polypeptide p57 (pyrimidine tract-binding protein) binds to multiple sites in the poliovirus 5' untranslated region. *J. Virol.* **68**, 941–950.

Heller HM, Carnevale NT, Steigbigel RT (1990): Varicella zoster virus transverse myelitis without cutaneous rash. *Amer. J. Med.* **88**, 550–551.

Hill AE, Hicks EM, Coyle PV (1994): Human herpes virus 6 and central nervous system complications. *Develop. Med. Child. Neurol.* **36**, 651–652

Hogle JM, Chow M, Filman DJ (1985): Three-dimensional structure of poliovirus at 2.9 Å resolution. *Science* **229**, 1358–1365.

Holt S, Hudgins D, Krishnan KR, Critchley EMR (1976): Diffuse myelitis associated wiht rubella vaccination. *Brit. Med. J.* **30**, 1037–1038.

Horaud F (1993): Albert B. Sabin and the development of oral poliovaccine. *Biologicals* **21**, 311–316.

Horie H, Koike S, Kurata T, Sato-Yoshida Y, Ise I, Ota Y, Abe S, Hioki K, Kato H, Taya C, Nomura T, Hashizume S, Yonekawa H, Nomoto A (1994): Transgenic mice carring the human poliovirus receptor: new animal model for study of poliovirus neurovirulence. *J. Virol.* **68**, 681–688.

Hughes PJ, Evans DMA, Minor PD, Schild GC, Almond JW, Stanway G (1986): The nucleotide sequence of a type 3 poliovirus isolated during a recent outbreak of poliomyelitis in Finland. *J. Gen. Virol.* **67**, 2093–2102.

Ion-Nedelcu N, Dobrescu A, Strebel PM, Sutter RW (1994): Vaccine-associated paralytic poliomyelitis and HIV infection. *Lancet* **343**, 51–52.

Jarousse N, Grant RA, Hogle JM, Zhang L, Senkowski A, Roos RP, Michiels T, Brahic M, McAllister A (1994): A single amino acid change determines persistence of a chimeric Theiler's virus. *J. Virol.* **68**, 3364–3368

Johnston ICD, Usherwood EJ, Nash AA, Brown TDK (1995): Theiler's murine encephalomyelitis virus 3D RNA polymerase: its expression in the CNS and the specific immune response generated in persistently infected mice. *J. Gen. Virol.* **76**, 2765–2778.

Kawamura N, Kohara M, Abe S, Komatsu T, Tago K, Arita M, Nomoto A (1989): Determinants in the 5' noncoding region of poliovirus Sabin 1 RNA that influence the attenuation phenotype. *J. Virol.* **63**, 1302–1309.

Kew OM, Nottay BK, Hatch MH, Nakano JH, Obijeski JF (1981): Multiple genetic changes can occur in the oral poliovaccines upon replication in humans. *J. Gen. Virol.* **56**, 337–347.

Kinnunen E, Färkkilä M, Hovi T, Juntunen J, Weckström P (1989): Incidence of Guillain-Barré syndrome during a nationwide oral poliovirus vaccine campaign. *Neurology* **39**, 1034–1036.

Kitamura N, Semler BL, Rothberg PG, Larsen GR, Adler CJ, Dorner AJ, Emini EA, Hanecak R, Lee JJ, Van Der Werf S, Anderson CW, Wimmer E (1981): Primary structure, gene organization and polypeptide expression of poliovirus RNA. *Nature* **291**, 547–553.

Koike S, Taya C, Kurata T, Abe S, Ise I, Yonekawa H, Nomoto A (1991): Transgenic mice susceptible to poliovirus. *Proc. Natl. Acad. Sci. USA* **88**, 951–955.

Koike S, Horie H, Sato Y, Ise I, Taya C, Nomura T, Yoshioka I, Yonekawa H, Nomoto A (1993): Poliovirus-sensitive transgenic mice as a new animal model. *Dev. Biol. Stand.* **78**, 101–107.

Hughes PJ, Evans, DMA., Minor, PD, Schild, GC, Almond, GW, and Sanway, G. (1986): The nucleotide sequence of a type 3 poliovirus isolated during a recent outbreak of poliomyelitis in Finland. *J. Gen. Virol.* **67**, 2093–2102.

La Monica N, Meriam C, Racaniello VR (1986): Mapping of sequences required for mouse neurovirulence of poliovirus type 2 Lansing. *J. Virol.* **57**, 515–525.

La Monica N, Almond JW, Racaniello VR (1987): A mouse model for poliovirus neurovirulence identifies mutations that attenuate the virus for humans. *J. Virol.* **61**, 2917–2920.

La Monica N, Racaniello VR (1989): Differences in replication of attenuated and neurovirulent polioviruses in human neuroblastoma cell line SH-SY5Y. *J. Virol.* **63**, 2357–2360.

Lederman HM, Winkelstein JA (1985): X-Linked Agammaglobulinemia: an analysis of 96 patients. *Medicine* **64**, 145–156.

Leneman F (1966): The Guillain-Barré syndrome. *Arch. Intern. Med.* **118**, 139–144.

Lipskaya GY, Muzychenko AR, Kutitova OK, Maslova SV, Equestre M, Drozdov SG, Perez Bercoff R, Agol VI (1991): Frequent isolation of intertypic poliovirus recombinants with serotype 2 specificity from vaccine-associated polio cases. *J. Med. Virol.* **35**, 290–296.

Macadam AJ, Arnold C, Howlett J, John A, Marsden S, Taffs F, Reeve P, Hamada N, Wareham K, Almond J, Cammack N, Minor PD (1989): Reversion of the attenuated and temperature-sensitive phenotypes of the Sabin type 3 strain of poliovirus in vaccinees. *Virology* **172**, 408–414.

Macadam AJ, Pollard SR, Ferguson G, Dunn G, Skuce R, Almond JW, Minor PD (1991a): The 5'noncoding region of the type 2 poliovirus vaccine strain contains determinants of attenuation and temperature sensitivity. *Virology* **181**, 451–458.

Macadam AJ, Ferguson G, Arnold C, Minor PD (1991b): An assembly defect as a result of an attenuating mutation in the capsid proteins of the poliovirus type 3 vaccine strain. *J. Virology* **65**, 5225–5231.

Macadam AJ, Ferguson G, Burlison J, Stone D, Skuce R, Almond JW, Minor PD (1992): Correlation of RNA secondary structure and attenuation of Sabin vaccine strains of poliovirus in tissue culture. *Virology* **189**, 415–422.

Macadam AJ, Pollard SR, Ferguson G, Skuce R, Wood D, Almond JW, Minor PD (1993): Genetic Basis of attenuation of the Sabin type 2 vaccine strain of poliovirus in primates. *Virology* **192**, 18–26.

Macadam AJ, Ferguson G, Fleming T, Stone DM, Almond JW, Minor PD (1994a): Role for poliovirus protease 2A in cap independent translation. *EMBO J.* **13**, 924–927.

Macadam AJ, Stone DM, Almond JW, Minor PD (1994b): The 5' noncoding region and virulence of poliovirus vaccine strains. *Trends Microbiol.* **2**, 449–454.

Martin A, Benichou D, Courdec T, Hogle JM, Wychowski C, Van der Werf S, Girard M (1991): Use of type 1/type 2 chimeric polioviruses to study determinants of poliovirus type 1 neurovirulence in a mouse model. *Virology* **180**, 648–658.

McGoldrick A, Macadam AJ, Dunn G, Rowe A, Burlison J, Minor PD, Meredith J, Evans DJ, Almond JW (1995): Role of mutations G-480 and C-6203 in the attenuation phenotype of Sabin type 1 poliovirus. *J. Virol.* 69, 7601-7605.

Meerovitch K, Pelletier J, Sonenberg N (1989): A cellular protein that binds to the 5'-noncoding region of poliovirus RNA: implications for internal translation initiation. *Genes Dev.* **3**, 1026–1034.

Melnick JL (1993): The discovery of the Enteroviruses and the classification of poliovirus among them. *Biologicals* **21**, 305–309.

Melnick JL, Horaud F (1993): Albert B. Sabin. *Biologicals* **21**, 299–303.

Mento SJ, Weeks-Levy C, Tatem JM, Gorgacz EJ, Waterfield WF (1993): Significance of a newly identified attenuating mutation in Sabin 3 oral poliovirus vaccine. *Dev. Biol. Stand.* **78**, 93–100.

Michiels T, Jarousse N, Brahic M (1995): Analysis of the leader and capsid coding regions of persistent and neurovirulent strains of Theiler's virus. *Virology* **214**, 550–558.

Miles C, Hoffman W, Lai CW, Freeman JW (1993): Cytomegalovirus- associated transverse myelitis. *Neurology* **43**, 2143–2145.

Minor PD (1982): Characterization of strains of type 3 poliovirus by oligonucleotide mapping. *J. Gen. Virol.* **59**, 307–317.

Minor PD, John A, Ferguson M, Icenogle JP (1986): Antigenic and molecular evolution of the vaccine strain of type 3 poliovirus during the period of excretation by a primary vaccinee. *J. Gen. Virol.* **67**, 693–706.

Minor PD, Dunn G (1988): The effect of sequences in the 5' noncoding region on the replication of polioviruses in the human gut. *J. Gen. Virol.* **69**, 1091–1096.

Minor PD, Dunn G, Evans DMA, Magrath DI, John A, Howlett J, Phillips A, Westrop G, Wareham K, Almond JW, Hogle JM (1989): The temperature sensitivity of the Sabin type 3 vaccine strain of poliovirus: molecular and structural effects of a mutation in the capsid protein VP3. *J. Gen. Virol.* **70**, 1117–1123.

Minor PD (1992): The molecular biology of poliovaccines. *J. Gen. Virol.* **73**, 3065–3077.

Minor PD (1993): Attenuation and reversion of the Sabin vaccine strains of poliovirus. *Dev. Biol. Stand.* **78**, 17–26.

Minor PD, Macadam AJ, Stone DM, Almond JW (1993): Genetic basis of attenuation of the Sabin oral poliovirus vaccines. *Biologicals* **21**, 357–363.

Moss EG, O'Neil RE, Racaniello VR (1989): Mapping of attenuating sequences of an avirulent poliovirus type 2 strain. *J. Virol.* **63**, 1884–1890.

Muzychenko AR, Lipskaya GY, Maslova SV, Svitkin YV, Pilipenko EV, Nottay BK, Kew OM, Agol VI (1991): Coupled mutations in the 5'- untranslated region of the Sabin poliovirus strains during in vivo passages: structural and functional implications. *Virus Res.* **21**, 111–122.

Nkowane BM, Wassilak SGF, Orenstein WA, Bart KJ, Schonberger LB, Hinman AR, Kew OM (1987): Vaccine-associated paralytic poliomyelitis in the United States: 1973 through 1984. *JAMA* **257**, 1335–1340.

Nomoto A, Omata T, Toyoda H, Kuge S, Horie H, Kataoka Y, Genba Y, Nakano Y, Imura N (1982): Complete nucleotide sequence of the attenuated poliovirus Sabin 1 strain genome. *Proc. Natl. Acad. Sci. USA* **79**, 5793–5797.

Ogra PL, Faden HS, Abraham R, Duffy LC, Sun M, Minor PD (1991): Effect of prior immunity on the shedding of virulent revertant virus in faeces after oral immunization with live attenuated poliovirus vaccines. *J. Infect. Dis.* **164**, 191–194.

Omata T, Kohara M, Kuge S, Komatsu T, Abe S, Semler BL, Kameda A, Itoh H, Arita M, Wimmer E, Nomoto A (1986): Genetic analysis of the attenuation phenotype of poliovirus type 1. *J. Virol.* **58**, 348–358.

Otelea D, Guillot S, Furione M, Combiescu AA, Balanant J, Candrea A, Crainic R (1993): Genomic modifications in naturally occuring neurovirulent revertants of Sabin 1 polioviruses. *Dev. Biol. Stand.* **78**, 33–38.

Pelletier I, Couderc T, Borzakian S, Wyckoff E, Crainic R, Ehrenfeld E, Colbere-Garapin F (1991): Characterization of persistent poliovirus mutants selected in human neuroblastoma cells. *Virology* **180**, 729–737.

Pilipenko EV, Blinov VM, Romanova LI, Sinyakov AN, Maslova SV, Agol VI (1989): Conserved structural domains in the 5'-untranslated region of picornaviral genomes: an analysis of the segment controlling translation and neurovirulence. *Virology* **168**, 201–209.

Pollard SR, Dunn G, Cammack N, Minor PD, Almond JW (1989): Nucleotide sequence of a neurovirulent variant of the type 2 oral poliovirus vaccine. *J. Virol.* **63**, 4949–4951.

Racaniello VR, Baltimore D (1981a): Cloned poliovirus complementary DNA is infectious in mammalian cells. *Science*, **214**, 916–919.

Racaniello VR, Baltimore D (1981b): Molecular cloning of poliovirus cDNA and determination of the complete nucleotide sequence of the viral genome. *Proc. Natl. Acad. Sci.* USA **78**, 4887–4891.

Racaniello VR (1988): Poliovirus neurovirulence. *Adv. Virus Res.* **34**, 217–246.

Racaniello VR, Ren R, Bouchard M (1993): Poliovirus attenuation and pathogenesis in a transgenic mouse model for poliomyelitis. *Dev. Biol. Stand.* **78**, 109–116.

Racaniello VR (1993): Infectious cDNA, cell receptors and transgenic mice in the study of Sabin's poliovirus vaccines. *Biologicals* **21**, 365–369.

Ren R, Costantini F, Gorgacz EJ, Lee JJ Racaniello VR (1990): Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. *Cell* **63**, 353–362.

Ren R, Moss EG, Racaniello VR (1991): Identification of two determinants that attenuate vaccine-related type 2 poliovirus. *J. Virol.* **65**, 1377–1382.

Rezapkin GV, Chumakov KM, Lu Z, Ran Y, Dragunsky EM, Levenbook IS (1994): Microevolution of Sabin 1 strain *in vitro* and genetic stability of oral poliovirus vaccine. *Virology* **202**, 370–378.

Rezapkin GV, Norwood LP, Taffs RE, Dragunsky EM, Levenbook IS, Chumakov KM (1995): Microevolution of type 3 Sabin strain of poliovirus in cell cultures and its implications for oral poliovirus vaccine quality control. *Virology* **211**, 377–384.

Ropper AH (1992): The Guillain-Barré syndrome. *New Engl. J. Med* **326**, 1130–1136.

Skinner MA, Racaniello VR, Dunn G, Cooper J, Minor PD, Almond JW (1989): New model for the secondary structure of the 5' non-coding RNA of poliovirus is supported by biochemical and genetic data that also show that RNA secondary structure is important in neurovirulence. *J. Mol. Biol.* **207**, 379–392.

Stanway G, Cann AJ, Hauptmann R, Hughes P, Clarke LD, Mountford RC, Minor PD, Schild GC, Almond JW (1983): The nucleotide sequence of poliovirus type 3 Leon $12a_1b$: comparison with poliovirus type 1. *Nucleic Acids Res.* **11**, 5629–5643.

Stanway G, Hughes PJ, Mountford RC, Reeve P, Minor PD, Schild GC, Almond JW (1984): Comparison of the complete nucleotide sequences of the genomes of the neurovirulent poliovirus P3/Leon/37 and its attenuated Sabin vaccine derivative P3/Leon $12a_1b$. *Proc. Natl. Acad. Sci. USA* **81**, 1539–1543.

Stratton KR, Howe CJ, Johnston RB (1994): Adverse events associated with childhood vaccines other than pertussis and rubella. *JAMA* **271**, 1602–1605.

Strebel PM, Sutter RW, Cochi SL, Biellik RJ, Brink EW, Kew OM, Pallansch MA, Orenstein WA, Hinman AR (1992): Epidemiology of poliomyelitis in the United States one decade after the last report case of indigenous wild virus-associated disease. *Clin. Infect. Dis.* **14**, 568–579.

Strebel PM, Aubert-Combiescu A, Ion-Nedelcu N, Biberi-Moroeanu S, Combiescu M, Sutter RW, Kew OM, Pallansch MA, Patriarca PA, Cochi SL (1994): Paralytic poliomyelitis in Romania, 1984-1992: Evidence for a high risk of vaccine-associated disease and reintroduction of wild- virus infection. *Am. J. Epidemiol.* **140**, 1111–1124.

Strebel PM, Ion-Nedelcu N, Baughman AL, Sutter RW, Cochi SL (1995): Intramuscular injections within 30 days of immunization with oral poliovirus vaccine - a risk factor for vaccine-associated paralytic poliomyelitis. *N. Engl. J. Med.* **332**, 500–506.

Svitkin YV, Maslova SV, Agol VI (1985): The genomes of attenuated and virulent poliovirus strains differ in their *in vitro* translation efficiencies. *Virology* **147**, 243–252.

Svitkin YV, Pestova TV, Maslova SV, Agol VI (1988): Point mutations modify the response of poliovirus RNA to a translation initiation factor: a comparison of neurovirulent and attenuated strains. *Virology* **166**, 394–404.

Svitkin YV, Cammack N, Minor PD, Almond JW (1990): Translation deficiency of the Sabin type 3 poliovirus genome: association with an attenuating mutation C472->U. *Virology* **175**, 103–109.

Svitkin YV, Alpatova GA, Lipskaya GA, Maslova SV, Agol VI, Kew O, Meerovitch K, Sonenberg N (1993): Towards development of an *in vitro* translation test for poliovirus neurovirulence. *Dev. Biol. Stand.* **78**, 27–32.

Taffs RE, Chumakov KM, Rezapkin GV, Lu Z, Douthitt M, Dragunsky EM, Levenbook IS (1995): Genetic stability and mutant selection in Sabin 2 strain of oral poliovirus vaccine grown under different cell culture conditions. *Virology* **209**, 366–373.

Tardy-Panit M, Blondel B, Martin A, Tekaia F, Horaud F, Delpeyroux F (1993): A mutation in the RNA polymerase of poliovirus type 1 contributes to attenuattion in mice. *J. Virol.* **67**, 4630–4638.

Tatem JM, Weeks-Levy C, Mento SJ, DiMichele SJ, Georgiu A, Waterfield WF, Sheip B, Costalas C, Davies T, Ritchey MB, Cano FR (1991): Oral poliovirus vaccine in the United States: molecular characterization of Sabin type 3 after replication in the gut of vaccinees. *J. Med. Virol.* **35**, 101–109.

Tatem JM, Weeks-Levy C, Georgiu A, DiMichele SJ, Gorgacz EJ, Racaniello VR, Cano FR, Mento SJ (1992): A mutation present in the amino terminus of Sabin 3 poliovirus VP1 protein is attenuating. *J. Virol.* **66**, 3194–3197.

Toyoda H, Kohara M, Kataoka Y, Suganuma T, Omata T, Imura N, Nomoto A (1984): Complete nucleotide sequences of all three poliovirus serotype genomes. Implication for genetic relationship, gene function and antigenic determinants. *J. Mol. Biol.* **174**, 561–585.

Uhari M, Rantala H, Niemelä M (1989): Cluster of childhood Guillain- Barré cases after an oral poliovaccine campaign. *Lancet* **2**, 440–441.

Vahidy F, Kazmi K, Rab SM (1989): Transverse myelitis and hepatites in varicella. *J. Trop. Med. Hyg.* **92**, 295–296.

Weeks-Levy C, Tatem JM, DiMichele SJ, Waterfield W, Georgiu AF, Mento SJ (1991): Identification and characterization of a new base substitution in the vaccine strain of Sabin 3 poliovirus. *Virology* **185**, 934–937. - Erratum (1992): *Virology* **187**, 845.

Westrop GD, Wareham KA, Evans DMA, Dunn G, Minor PD, Magrath DI, Taffs F, Marsden S, Skinner MA, Schild GC, Almond JW (1989): Genetic basis of attenuation of the Sabin type 3 oral poliovirus vaccine. *J. Virol.* **63**, 1338–1344.

Wimmer E, Nomoto A (1993): Molecular biology and cell-free synthesis of poliovirus. *Biologicals* **21**, 349–356.

Whittle E, Roberton NRC (1977): Transverse myelitis after diphtheria, tetanus, and polio immunization. *Brit. Med. J.* **1**, 1450.

WHO (1982): The relation between acute persisting spinal paralysis and poliomyelitis vaccine - results of a ten-year enquiry. *Bull. WHO* **60**, 231–242.

WHO (1990): Potencial use of new poliomyelitis vaccines: Memorandum from a WHO meeting. *Bull. WHO* **68**, 545–548.

Wyatt HV (1994): Vaccine-associated poliomyelitis. *Lancet* **343**, 610.

Yauch RL, Kim BS (1994): A predominant viral epitope recognized by T cells from the periphery and demyelinating lesions of SJL/J mice infected with Theiler's virus is located within $VP1_{233-244}$. *J. Immunol.* **153**, 4508–4519.

Zuckerman MA, Brink NS, Kyi M, Tedder RS (1994): Exposure of immunocompromised individuals to health-care workers immunized with oral poliovaccine. *Lancet* **343**, 985–986.