MOLECULAR EVOLUTION OF ORAL POLIOVIRUS VACCINE STRAINS DURING MULTIPLICATION IN HUMANS AND POSSIBLE IMPLICATIONS FOR GLOBAL ERADICATION OF POLIOVIRUS

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Summary. – The oral poliovirus vaccine (OPV) has been effectively used in the control of poliomyelitis and in the eradication of wild polioviruses. Although there are many advantages in using attenuated OPV strains in the campaign to eradicate poliomyelitis, several studies have demonstrated that there are some disadvantages such as (a) excretion by vaccines of OPV-derived polioviruses with genomic modifications known to increase the neurovirulence, (b) appearance of vaccine-associated paralytic poliomyelitis (VAPP) and other adverse effects in vaccinees, (c) occurrence of persistent infections caused by OPV-derived strains in immunodeficient patients with VAPP, (d) transmission of OPV-derived polioviruses to susceptible individuals which develop VAPP, and (e) detection of OPV-derived polioviruses in the environment, which could be a source of infection for humans in the future. Different studies indicate that it is important to consider the possibility of persistent infections and excretion of OPV-derived polioviruses for long periods by humans, and also the survival in the environment of OPV-derived polioviruses excreted by humans, which could be transmitted and circulate in a non-immune population after stopping poliovirus vaccination. The findings reported here may have important implications for global poliomyelitis eradication initiative and indicate that surveillance of OPV-derived strains will also be important in the final step of eradication of poliomyelitis from the planet.

Key words: poliovirus; poliomyelitis; oral poliovirus vaccine, poliomyelitis eradication, vaccine-derived polioviruses, mutation, recombination, deletion

Introduction

OPV is a major component of the World Health Organization's campaign for global eradication of poliomyelitis and has dramatically reduced the number of poliomyelitis cases caused by wild polioviruses (Dowdle *et al.*, 1999). This vaccine has also been very important in the control of transmission and circulation of wild poliovirus strains. Polioviruses 1, 2 and 3, three different species of the *Enterovirus* genus, the *Picornaviridae* family (Wimmer *et al.*, 1993) are the causative agents of poliomyelitis. These viruses consist of icosahedric particles composed of 60 copies of each of four capsid proteins, VP1, VP2, VP3 and VP4, surrounding the viral genome, which is a single-stranded RNA of positive polarity of about 7500 nucleotides (Wimmer *et al.*, 1993). The RNA molecule contains a 5'-non-coding region (5'-NCR) of about 740 nucleotides, a single open reading frame (ORF) coding for structural and non-structural proteins, and a 3'-non-coding region (3'-NCR) of about 70 nucleotides followed by a poly(A)-tract. These viruses infect humans by oral route and their primary multiplication site is the digestive tract. As a result, polioviruses can be isolated from nasopharyngeal secretions

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Abbreviations: CNS = central nervous system; 3Dpol = 3D polymerase; 3'-NCR = 3'-non-coding region; 5'-NCR = 5'-non coding region; OPV = oral poliovirus vaccine; ORF = open reading frame; VAPP = vaccine-associated paralytic poliomyelitis

and routinely from stool. Polioviruses may induce paralysis by infecting and destroying motoric neuron cells. During the replication of polioviruses in humans, antigenic changes and genomic modifications such as mutations (substitutions, deletions, and insertions) and recombinations occur (Wimmer *et al.*, 1993, Agol, 1997). The capacity of polioviruses to be transmitted depends on virus properties such as minimum infective dose, multiplication capacity, amount and duration of virus excretion in oropharyngeal fluid and feces, and survival in the environment (Kimman *et al.*, 1999). Transmisson of polioviruses is also determined by host immunity, hygiene, sanitation, and frequency and nature of social contacts.

OPV is composed of three live attenuated poliovirus vaccine strains (Sabin 1, 2 and 3) which multiplicate in the gastrointestinal tract of vaccinees, inducing local intestinal, as well as long-lived systemic immunity. Despite the advantages in using the live attenuated OPV strains in the eradication of wild strains, there are some disadvantages such as the occurrence of poliomyelitis and other adverse effects in vaccinees (Gutierrez and Abzug, 1990; Friedrich, 1997; Firus and Sick, 1998; Burgess and McIntyre, 1999; Chitsike and Furth, 1999; D'Souza *et al.*, 1999; Yoneyama *et al.*, 1999). Another concern associated with OPV is the observation of genomic modifications and an increase in the virulence of the vaccine strains during multiplication in humans (Kew *et al.*, 1981; Evans *et al.*, 1985; Contreras *et al.*, 1992). As OPV-derived strains are excreted in nasopharyngeal secretions and stool after vaccination, this vaccine is also a source of dissemination of polioviruses, which may be transmitted to susceptible individuals and cause poliomyelitis (Morse *et al.*, 1966; Balduzzi and Glasgow, 1967; Swanson *et al.*, 1967; Cesario *et al.*, 1969; Basilico and Bernat, 1978; Bateman *et al.*, 1987). OPV-derived polioviruses detected in aerosol from waste water plants could potentially also be a source of virus contamination for humans (Muscillo *et al.*, 1997). The fecal contamination by OPV-derived polioviruses of natural waters (Divizia *et al.*, 1999; Muscillo *et al.*, 1999), with inadequate or no treatment, might also allow the viruses to disseminate and contaminate large areas. Although the possibility may be remote, the excretion of vaccine-derived polioviruses by humans in high latitudes and altitudes could potentially contamite the environment, allowing the viruses to survive frozen and to become in the future a source of infection for humans. OPV-derived polioviruses have been isolated from the central nervous system (CNS), blood, nasopharynx, urine and stool of patients with VAPP and other neurological complications (Fiore *et al.*, 1987; Gutierez and Abzug, 1990; Georgescu *et al.*, 1994, 1997a; Friedrich *et al.*, 1995a; David and Doyle, 1997; Yeung *et al.*, 1997; Ehrengut, 1998), from stool of healthy contacts of OPV-associated cases (Friedrich *et al.*, 1995a; Diamanti *et al.*, 1998), from stool of healthy vaccinees (Macadam *et al.*, 1989, 1991; Buonagurio *et al.*, 1999), and from environmental samples (Divizia *et al.*, 1999; Muscillo *et al.*, 1997, 1999). The potential of transmission of OPV-derived strains with genomic modifications known to increase neurovirulence was confirmed in one of these studies (Friedrich *et al.*, 1995a, 1996a). Different studies have described the isolation of OPV-derived mutant polioviruses for long periods from stool excreted by immunodeficient patients with VAPP (Yoneyama *et al.*, 1982; Kew *et al.*, 1998; Bellmunt *et al.*, 1999). With the approaching eradication of wild poliovirus strains, it has been suggested (Friedrich, 1997; Kimman *et al.*, 1999) that (a) excretion by humans of OPV-derived polioviruses in nasopharyngeal secretions, urine and stool and (b) contamination of the environment, which might be source of infection for humans, could have possible implications for eradication of OPV-derived polioviruses and must be better evaluated before a decision is made to stop poliovirus vaccination.

Molecular analysis of OPV-derived polioviruses isolated from patients with persistent paralytic poliomyelitis

Characterization of OPV-derived polioviruses isolated from vaccinated or non-vaccinated patients with VAPP has revealed genomic and antigenic modifications, and also an increase in neurovirulence. Analysis of P1/Sabin-derived polioviruses isolated from stool excreted by patients with VAPP has detected mutations, antigenic changes and in some cases genomic recombination (Otelea *et al.*, 1993; Guillot *et al.*, 1994; Li *et al.*, 1996; Georgescu *et al.*, 1997a). Inoculation of P1/Sabin-derived strains with genomic modifications, isolated from patients with VAPP, in transgenic mice expressing the human poliovirus receptor has demonstrated an increase in the neurovirulence (Li *et al.*, 1996; Georgescu *et al.*, 1997a). Mutations detected in P1/Sabin-derived polioviruses have been observed in the 5'-NCR, the capsid proteins coding region, the non-structural proteins coding region, and the 3'-NCR (Otelea *et al.*, 1993; Li *et al.*, 1996; Georgescu *et al.*, 1997a). Among the mutations known to increase the neurovirulence, a reverse mutation at nt 480 (G to A) and a second-site suppressor mutation at nt 525 (U to C) in the 5'-NCR have been detected in most of P1/Sabin-derived polioviruses isolated from stool of patients with VAPP (Otelea *et al.*, 1993; Guillot *et al.*, 1994; Li *et al.*, 1996; Friedrich *et al.*, 1996b, Georgescu *et al.*, 1997a). Other reverse mutations associated with an increase in the neurovirulence were also detected in the P1/Sabin-derived polioviruses isolated from stool of VAPP cases (Otelea *et al.*, 1993; Li *et al.*, 1996; Georgescu *et al.*, 1997a). These mutations are located in the 5'-NCR at nt 189 (U to C), the capsid proteins coding region at nt 935 (U to G) (VP4), nt 2438 (A to U) (VP3), nt 2749 (A to G) and nt

2795 (A to G) (VP1), the viral 3D polymerase ($3D^{pol}$) coding region at nt 6203 (C to U), and the 3'-NCR at nt 7441 (G to A). These mutations in the capsid proteins coding region and the $3D^{pol}$ coding region caused amino acid substitutions. Among the P1/Sabin-derived polioviruses with mutations associated with an increase in neurovirulence at nt 480, 2438, 2795, and 6203, isolated from stool of patients with VAPP, two were characterized as recombinants (vaccine in VP1/non-vaccine in $3D^{pol}$) with the $3D^{pol}$ coding region probably derived from wild poliovirus strains (Li *et al.*, 1996). Analysis of two P1/Sabin-derived strains isolated from CNS of two patients with VAPP also detected the nt 480 (G to A) mutation in the isolate from one patient (Otelea *et al.*, 1993), while the isolate from the other patient maintained nt 480 (G) but mutated at nt 1885 (U to C) in VP3 (Georgescu *et al.*, 1997a).

Characterization of P2/Sabin-derived polioviruses isolated from CNS and stool excreted by patients with VAPP has demonstrated genomic and antigenic changes and an increase in the neurovirulence (Yoneyama *et al.*, 1982; Fiore *et al.*, 1987; Pollard *et al.*, 1989; Equestre *et al.*, 1991; Lipskaya *et al.*, 1991; Macadam *et al.*, 1991, 1993; Georgescu *et al.*, 1994, 1997b; Friedrich *et al.*, 1995a, 1996a). Mutations have been identified in the 5'-NCR, the capsid proteins coding region, the non-structural proteins coding region, and the 3'-NCR (Pollard *et al.*, 1989; Equestre *et al.*, 1991). Among the mutations detected in the P2/Sabin-derived polioviruses, two mutations important for reversion to neurovirulence were detected in most of the viruses isolated from stool or CNS of patients with VAPP. One mutation was located at nt 481 (A to G) in the 5'-NCR, while the other at nt 2908 or 2909 in the codon of aa 143 in VP1, leading to substitution of Ile by Val, Ser, Thr or Asn (Pollard *et al.*, 1989; Equestre *et al.*, 1991; Muzychenko *et al.*, 1991; Macadam *et al.*, 1991, 1993; Guillot *et al.*, 1994, Friedrich *et al.*, 1995a; Georgescu *et al.*, 1994; 1997b; Diamanti *et al.*, 1998). A mutation at nt 398 (U to C) in the 5'-NCR, suspected to have some role in increasing the neurovirulence, has also been frequently observed in the P2/Sabin-derived viruses isolated from stool or CNS of VAPP cases (Macadam *et al.*, 1991; Muzychenko *et al.*, 1991; Friedrich *et al.*, 1995a; Georgescu *et al.*, 1997b; Diamanti *et al.*, 1998). Mutations at other nucleotide positions have also been identified in the coding and non-coding regions of P2/Sabin-derived polioviruses isolated from stool or CNS of VAPP cases (Pollard *et al.* 1989; Equestre *et al.*, 1991; Macadam *et al.*, 1991; Friedrich *et al.*, 1995a; Diamanti *et al.*, 1998). OPV-derived recombinant poliovirus 2 isolates with mutations at nt 481 in the 5'-NCR and nt 2908 or 2909 in VP1 have also been frequently obtained from stool or CNS of patients with VAPP (Georgescu *et al.*, 1994; Friedrich *et al.*, 1995a; 1996a). Most of the poliovirus 2 recombinants analyzed maintained the 5'-NCR and the capsid proteins coding region from the Sabin 2 strain, while in the 3'-terminal half of the genome it was observed that the segments coding for non-structural proteins and the 3'-NCR were derived from the Sabin 1 and/or Sabin 3 strains, or possibly from multiple-mutated OPV-derived strains, wild strains or other non-poliovirus enteroviruses (Lipskaya *et al.*, 1991; Furione *et al.*, 1993; Georgescu *et al.*, 1994; 1997b; Friedrich *et al.*, 1996a). In one of the VAPP cases analyzed, an OPV-derived poliovirus 2 recombinant strain with a mutation at nt 2909 (U to C) and aa 143 (Ile to Thr) in VP1 and with a part of the 3'-terminal half derived from the Sabin 1 strain was also isolated from nasopharyngeal secretions (Georgescu *et al.*, 1997b). Analysis of OPV-derived recombinants with genomic sequences coding for $3D^{pol}$ derived from the Sabin 1 strain also detected mutations at nt 6203 (C to U) and aa 73 (His to Tyr) and at nt 7441 (G to A) (Georgescu *et al.*, 1994, 1997a,b; Friedrich 1996a). Analysis of one of the OPV-derived poliovirus 2 recombi-nants isolated from stool and CNS of a patient with VAPP demonstrated that the isolate had a tripartite genome organization with most of the capsid proteins coding region and a part of the 2A protease region derived from the Sabin 2 strain, while the 5'-NCR, almost the entire non-structural proteins coding region, and the 3'-NCR were probably derived from wild strains (Georgescu *et al.*, 1995a). This tripartite recombinant OPV-derived poliovirus 2 strain had a mutation at nt 2909 (U to C) in the capsid proteins coding region derived from the Sabin 2 strain, leading to the aa 143 substitution (Ile to Thr) in VP1 (Georgescu *et al.*, 1995a). It was suggested that nucleotide and/or amino acid substitu-tions caused by replacement of a segment of the genome of one strain by a segment from other strain by recombination could also increase the neurovirulence or have some replicative advantage for OPV-derived isolates.

Characterization of P3/Sabin-derived polioviruses isolated from CNS or stool of patients with VAPP has shown mutations (substitutions and deletions), antigenic changes, recombinations and an increase in the neurovirulence (Cann *et al.*, 1984; Evans *et al.*, 1985; Macadam *et al.*, 1989; Minor *et al.*, 1989; Furione *et al.*, 1993; Georgescu *et al.*, 1994; Driesel *et al.*, 1995). Two reverse mutations important to reversion to neurovirulence of the P3/Sabin strain were identified at nt 472 (U to C) in the 5'-NCR and nt 2034 (U to C) and aa 91 (Phe to Ser) in VP3 (Evans *et al.*, 1985; Macadam *et al.*, 1989). The mutation at nt 472 in the 5'-NCR was detected in all the P3/Sabin-derived polioviruses isolated from stool or CNS of patients with VAPP, while the mutation at nt 2034 in VP3 was also detected in several of these isolates (Cann *et al.*, 1984; Evans *et al.*, 1985; Macadam *et al.*, 1989; Georgescu *et al.*, 1994, Driesel *et al.*, 1995; Friedrich *et al.*, 1995b; Diamanti *et al.*, 1998). Although many isolates have not mutated at nt 2034 in VP3, they did so in other regions of the capsid that could increase

neurovirulence and suppress the absence of the mutation at nt 2034 (Macadam *et al.*, 1989; Minor *et al.*, 1989; Driesel *et al.*, 1995). Mutations at other positions have also been detected in the coding and non-coding regions of the genome of P3/Sabin-derived strains isolated from stool or CNS of patients with VAPP (Cann *et al.*, 1984; Driesel *et al.*, 1995). P3/Sabin-derived recombinant strains with mutations at nt 472, nt 2034 and/or other positons in the capsid coding region have also been frequently isolated from stool or CNS of patients with VAPP (Macadam *et al.*, 1989; Georgescu *et al.*, 1994; Driesel *et al.*, 1995). Analysis of the genome of these recombinants has demonstrated that they maintained the 5'-NCR and the capsid proteins coding region of the Sabin 3 strain, while in the 3'-terminal half of the genome the segments coding for non-structural proteins and the 3'-NCR were derived from the Sabin 1 and/or Sabin 2 strains, or possibly from multiple-mutated OPV-derived strains, wild strains or other non-poliovirus enteroviruses (Macadam *et al.*, 1989; Furione *et al.*, 1993; Georgescu *et al.*, 1994, Driesel *et al.*, 1995). Analysis of P3/Sabin-derived polioviruses isolated from CNS or stool of VAPP cases with mutations at nt 472 and in the capsid proteins coding region also detected deletions in the 5'-NCR and/or in the non-structural proteins coding region (Driesel *et al.*, 1995). One isolate with deletions in the non-structural proteins coding region had the Arg 455 deletion in $3D^{pol}$, while the other isolate had the same Arg 455 deletion in $3D^{pol}$ and a Thr 18 deletion in 2B protein (Driesel *et al.*, 1995).

Molecular characterization of OVP-derived polioviruses isolated from patients with transient paralysis

Analysis of some OPV-derived polioviruses isolated from stool of patients with transient paralysis associated with OPV also identified genomic modifications and antigenic changes. Analysis of P2/Sabin-derived polioviruses isolated from stool of two patients with transient paralysis demonstrated that they had mutations important for reversion to neurovirulence: one isolate had a mutation at nt 481 (A to G) while the other mutated at nt 2908 (A to G) and aa 143 (Ile to Val) in VP1 (Friedrich *et al.*, 1995a). In a recent study, a P1/Sabin-derived poliovirus isolate displaying aberrant phenotypic and genetic features was obtained from stool of a patient with transient paralysis (Mulders *et al.*, 1999). The patient had probably received the standard OPV as infant and had not been repeatedly vaccinated prior to the onset of disease. Characterization of this isolate demonstrated (1) mutations leading to amino acid substitutions in the regions encoding three major neutralizing antigenic sites (N-Ag I, IIb and IIIb) in VP1, VP2, and VP3, respectively, and also (2) existence of a hexonucleotide deletion in VP1, which gave rise to a two-amino acid deletion in the BC loop of neutralizing antigenic site I. In spite of these antigenic changes, the isolate was readily serotyped as poliovirus 1 and the standard vaccination protected against this aberrant virus. Although this virus had not reverted at nt 480 (G), a mutation at nt 476 (U to A) in the 5'-NCR and other positions were also observed.

Genomic characterization of OPV-derived polioviruses isolated from patients with other neurologic complications

OPV has also rarely been associated with other neurologic complications (Gutierrez and Abzug, 1990; Friedrich, 1997; Yeung *et al.*, 1997; Ehrengut, 1998; Yoneyama *et al.*, 1999), and OPV-derived polioviruses were also isolated from different sites/materials of these patients such as nasopharynx, stool and CNS. Partial nucleotide sequencing of some of these isolates also demonstrated mutations known to increase the neurovirulence. In one of these studies (Friedrich *et al.*, 1995c), genomic characterization of P1/Sabin-derived polioviruses isolated from stool of four patients with Guillain-Barré syndrome demonstrated a mutation at nt 480 (G to A) and/or nt 525 (U to C) in the 5'-NCR in three of four isolates analyzed, a mutation known to participate in reversion to neurovirulence. One of the isolates with a mutation at nt 480 (G to A) in the 5'-NCR had also a mutation (known to increase neurovirulence) at nt 6203 (C to U) and aa 73 (His to Tyr) in $3D^{pol}$ (Friedrich *et al.*, 1995c). Analysis of two P1/Sabin-derived poliovirus isolates from stool of patients with facial paralysis demonstrated a mutation at nt 480 (G to A) in the 5'-NCR of one isolate, while the other isolate had a mutation at nt 525 (U to C) in the 5'-NCR (Friedrich *et al.*, 1995c). In another study (Friedrich *et al.*, 1995b), a reverse mutation at nt 472 (U to C) in the 5'-NCR known to increase neurovirulence was also detected in P3/Sabin-derived poliovirus isolates from stool of a patient with Guillain-Barré syndrome and from stool of a patient with facial paralysis. These studies demonstrate that genomic modifications known to increase neurovirulence were also detected in OPV-derived polioviruses isolated from stool of patients with other neurologic complications associated with OPV. In many of the patients with Guillain-Barré syndrome and transverse myelitis, in which OPV-derived polioviruses were isolated from stool, the last vaccine dose was given months or years before the onset of the disease, suggesting a persistent infection or transmission of OPV viruses to the patients (Friedrich *et al.*, 1995c; Friedrich, 1997).

Persistent infections caused by polioviruses

Several studies *in vitro* have demonstrated the capacity of poliovirus strains, including the attenuated vaccine strains to cause persistent infection in cells of neural or non-neural origin (Lloyd *et al.*, 1993; Pavio *et al.*, 1996; Colbere-

Garapin *et al.*, 1998). Analysis of P1/Sabin-derived poliovirus mutants selected in persistently infected cells detected mutations at nt 525 (U to C) in the 5'-NCR, in the capsid coding region and also elsewhere (Pelletier *et al.*, 1991, 1998; Borzakian *et al.*, 1993; Colbere-Garapin *et al.*, 1998). Different studies have also demonstrated the potential of OPV strains to cause persistent infection in humans. In one of these studies it was reported that an immunodeficient patient with VAPP excreted P2/Sabin-derived polioviruses during a 3.5-years period (Yoneyama *et al.*, 1982). Analysis of the P2/Sabin-derived polioviruses excreted in stool of this patient demonstrated the occurrence of genomic and antigenic modifications (Yoneyama *et al.*, 1982). Another study of P1/Sabin-derived polioviruses isolated from stool collected over an 189-day period from another immunodefient patient with VAPP demonstrated a prolonged multiplication of the virus (Kew *et al.*, 1998). In this study, the extent and rate of nucleotide divergence of sequences of the P1/Sabin vaccine strain were consistent with prolonged replication of the vaccine-derived polioviruses in the patient, and suggested that the infection with the virus began about 9 years earlier (Kew *et al.*, 1998).

Paralytic poliomyelitis is sometimes followed, after decades of clinical stability, by new symptoms collectively known as the post-polio syndrome. This has raised the question of possible poliovirus persistence in post-polio patients (Colbere-Garapin *et al.*, 1998). Viral RNA or its sequences have been detected in the CNS of patients with post-polio syndrome, suggesting that polioviruses might cause a persistent infection in humans (Leon-Monzon and Dalakas, 1995; Leparc-Goffart *et al.*, 1996; Julien *et al.*, 1999). The isolation of OPV-derived polioviruses from stool of immunodeficient patients with VAPP and the detection of poliovirus genome sequences in CNS of patients with post-polio syndrome indicate that chronic infection of even a small number of individuals with OPV-derived (or wild) polioviruses and excretion of these viruses might represent a potential reservoir of polioviruses and might have implications in the effort to eradicate poliovirus from the planet. The potential of polioviruses to cause persistent infection in humans should be more thoroughly studied before a decision is made to stop vaccination against poliomyelitis.

Molecular analysis of OPV-derived polioviruses isolated from healthy vaccinees

Molecular characterization of OPV-derived isolates from stool of healthy vaccinees has also demonstrated genomic modifications, antigenic changes and an increase in the neurovirulence (Evans *et al.*, 1985; Minor *et al.*, 1986; Macadam *et al.*, 1989). Mutations known to increase neurovirulence observed in OPV-derived strains isolated from patients with VAPP were also detected in those obtained from healthy vaccinees. Analysis of the 5'-NCR of P1/Sabin-derived polioviruses isolated from stool of healthy vaccinees has detected a mutation at nt 480 (G to A) or a second-site suppressor mutation at nt 525 (U to C), which are known to increase neurovirulence (Minor and Dunn, 1988; Dunn *et al.*, 1990; Muzychenko *et al.*, 1991; Ogra *et al.*, 1991; Abraham *et al.*, 1993; Guillot *et al.*, 1994; Mallet *et al.*, 1997; Chezzi *et al.*, 1998). Analysis of P2/Sabin-derived polioviruses isolated from stool of healthy vaccinees also detected mutations important for reversion to neurovirulence at nt 481 (A to G) and aa 143 in VP1 leading to the substitution of Ile by other amino acids (Minor and Dunn, 1988; Dunn *et al.*, 1990; Macadam *et al.*, 1991, 1993; Ogra *et al.*, 1991; Abraham *et al.*, 1993; Guillot *et al.*, 1994; Mallet *et al.*, 1997; Chezzi *et al.*, 1998). A mutation at nt 472 (U to C) and aa 91 (Phe to Ser) in VP3 and/or suppressor mutations known to increase the neurovirulence have also been observed in P3/Sabin-derived polioviruses isolated from stool of healthy vaccinees (Evans *et al.*, 1985; Minor and Dunn, 1988; Macadam *et al.*, 1989; Dunn *et al.*, 1990; Ogra *et al.*, 1991; Tatem *et al.*, 1991; Contreras *et al.*, 1992; Abraham *et al.*, 1993; Driesel *et al.*, 1995; Mallet *et al.*, 1997; Chezzi *et al.*, 1998). Analysis of P3/Sabin-derived strains isolated from stool of healthy vaccinees also detected recombinant genomes (Minor *et al.*, 1986; Cammack *et al.*, 1988; Macadam *et al.*, 1989; Tatem *et al.*, 1991; Driesel *et al.*, 1995). Nucleotide deletions in the 5'-NCR were also detected in a P3/Sabin-derived recombinant poliovirus strain with mutations at nt 472 in the 5'-NCR and in the coding region, isolated from a healthy vaccinee (Driesel *et al.*, 1995). A recent study (Reimerink *et al.*, 1999) of seven P3/Sabin-derived poliovirus strains isolated from stool of healthy adoptive children, taken after their arrival to the Netherlands, detected antigenic changes and also mutations important for reversion to neurovirulence at nt 472 in the 5'-NCR in all the isolates, and a mutation at nt 2034 in VP3 in four of seven isolates analyzed. Despite the immunological aberrations in these P3/Sabin-derived poliovirus strains imported to the Netherlands, sera from vaccinated persons efficiently neutralized the mutants. By inoculation of these strains into transgenic mice which express the human poliovirus receptor, one was identified as highly neurovirulent, three as intermediate, and three as attenuated (Reimerink *et al.*, 1999). This study suggested that these strains with antigenic and genomic modifications are a potential risk to the unvaccinated but not the vaccinated population.

Molecular analysis of OPV-derived polioviruses isolated from the environment

The polioviruses present in the environment might potentially be a source of infection for humans. Contamina-

ted aerosols, drinking water and food are some of the means of enteric virus transmission to humans. River or sea water contaminated with these viruses could also be a potential source of infection for bathers. Characterization of OPV-derived polioviruses isolated from environmental samples has also revealed genomic modifications such as mutation and recombination. Characterization (Muscillo *et al.*, 1997) of poliovirus 3 isolates from an aerosol generated by a waste water plant identified the P3/Sabin strain as the ancestor of the virus and demonstrated mutations at several nucleotides, including nt 472 (U to C) in the 5'-NCR, which is known to increase neurovirulence. Analysis of polioviruses isolated from a river (Divizia *et al.*, 1999) identified one of them as P2/Sabin-like, while another was identified as a recombinant poliovirus (P2/Sabin-like with wild type poliovirus 1), in the 5'-NCR and VP1, respectively. Both isolates had a mutation (known to increase neurovirulence) at nt 481 (A to G) in the 5'-NCR. Analysis of P3/Sabin-derived polioviruses isolated from water collected from a river estuary and from two closely situated bathing places in the Adriatic sea detected a mutation at nt 472 (U to C) in three samples (Muscillo *et al.*, 1999). These studies showed that OPV-derived mutant and recombinant poliovirus strains can also be isolated from environmental specimens, demonstrating the potential of infection for humans. The presence of such strains demonstrates that the monitoring of OPV-derived polioviruses in the environment is also important in the eradication of poliovirus from the planet.

Transmission of OPV-derived strains

Transmission of OPV-derived strains from person to person has been observed (Friedrich, 1997). In many cases, transmission of OPV-derived strains from healthy vaccinees to their susceptible contacts was associated with VAPP (Morse *et al.*, 1966; Balduzzi and Glasgow, 1967; Swanson *et al.*, 1967; Basilico and Bernat, 1978; Bateman et al 1987). In one study, transmission of OPV-derived mutant and recombinant strains was confirmed by partial nucleotide sequencing of the 5'-NCR, VP1 and $3D^{pol}$ regions of OPV-derived poliovirus 2 isolates from stool of patients with VAPP and their healthy contacts (Friedrich *et al.*, 1995a; 1996a). Analysis of these genomic regions enabled to identify the same nucleotide sequences and the same mutations in these recombinants. These recombinants had parts of their 5'-NCR and VP1 region from the Sabin 2 strain (Friedrich *et al.*, 1995a), while their $3D^{pol}$ region was from non-vaccine strains (Friedrich *et al.*, 1996a). One Sabin 2-derived recombinant strain with mutations at nt 398 (U to C) and nt 481 (A to G) in the 5'-NCR, nt 2909 (U to A) and aa 143 (Ile to Asn) in VP1 and with the $3D^{pol}$ sequence derived from a non-vaccine strain was isolated from stool of a vaccinated patient with VAPP and from stool of one of his healthy contacts (Friedrich *et al.*, 1995a; 1996a). In another case of VAPP, Sabin 2-derived recombinant polioviruses with the same nucleotide sequences were isolated from a patient with VAPP and his three healthy contacts (Friedrich *et al.*, 1995a; 1996a). Although the patient with VAPP was not vaccinated, available data showed that at least one of the healthy contacts was vaccinated 81 days before the onset of the motoric deficiency in the patient with VAPP. Analysis of these Sabin 2-derived recombinant polioviruses isolated from stool of this patient with VAPP and his three healthy contacts demonstrated that they all had a mutation at nt 398 (U to C) and nt 481 (A to G) in the 5'-NCR, nt 2909 (U to A) and aa 143 (Ile to Asn) in VP1 (Friedrich *et al.*, 1995a), and that their $3D^{pol}$ region was from non-vaccine strains (Friedrich *et al.*, 1996a). Analysis of other Sabin 2 vaccine-derived polioviruses isolated from stool of patients with VAPP and their healthy contacts (Diamanti *et al.*, 1998) also demonstrated mutations at nt 398 (U to C), nt 481 (A to G), and other positions in the 5'-NCR, and also at aa 143 in VP1 leading to substitution of Ile by other amino acids. Thus, the analysis of OPV-derived polioviruses isolated from patients with VAPP and their healthy contacts has confirmed the transmission of OPV-derived polioviruses with genomic modifications known to increase the neurovirulence (Friedrich *et al.*, 1995a, 1996a). The studies reported here have also demonstrated the potential of circulation of these strains in non-immune populations and the occurrence of diseases associated with OPV.

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