ASSESSMENT OF EFFICACY OF A LIVE ORAL POLIOVIRUS VACCINE FOR VIRULENT SABIN-LIKE POLIOVIRUS 1 STRAINS IN JAPAN

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Summary. – Virulent Sabin-like poliovirus (VSLP) was isolated from river and sewage waters between October 1993 and September 1995 in Toyama Prefecture, Japan (Yoshida *et al*., *Lancet* **356**, 1461–1463, 2000). In this study, to assess the possibility of an epidemic of poliomyelitis caused by a VSLP in Japan under the current vaccination policy of administration of live attenuated oral poliovirus vaccine (OPV), we determined titers of serum neutralizing antibodies to poliovirus 1 (PV-1) strains Sabin (vaccine strain), Mahoney (wild-type strain) and G4-12 (VSLP) in various groups of residents of Toyama Prefecture, Japan. The seropositivity and geometric mean neutralizing antibody titers against these strains in the individuals who obtained two doses of OPV were 99.1%, 94.5% and 95.5%, respectively, and 564, 186 and 194, respectively. Although the antibody titers to G4-12 were lower compared with those to Sabin, these results indicate that the OPV vaccination policy in Japan has been effective in preventing poliomyelitis caused by VSLPs. These results also suggest that (i) an epidemic of poliomyelitis caused by a VSLP has not occurred in Japan due to herd immunity, and (ii) the possibility of reemergence of VSLPs will be prevented if sufficient herd immunity is acquired immediately after completion of the OPV vaccination in accordance with the poliomyelitis eradication program.

Key words: poliovirus 1; poliomyelitis; oral poliovirus vaccine; inactivated poliovirus vaccine; virulent Sabin-like poliovirus

Introduction

Live OPV containing Sabin's attenuated strains of PV-1, PV-2 and PV-3, has been used as a major tool for worldwide eradication of poliomyelitis. It is usually given to an individual in three subsequent doses. It confers a high seropositive rate (seropositivity) against all three polioviruses (Lago *et al*., 1994; Ramsay *et al*., 1994; World Health Organization Collaborative Study Group on Oral and Inactivated Poliovirus Vaccines, 1995). Ninety-five or more percent of the recipients probably develop a life-long immunity to all three polioviruses after the vaccination (Recommendation of the Advisory Committee on Immunization Practices, 1997). Paralytic poliomyelitis was a common disease in Japan during the 1950s, as in many other countries. Following the introduction of the two-dose administration of OPV, which was imported from Canada and Soviet Union, to children of 3 months to 12 years of age in 1961–1963, the number of patients declined dramatically; before the use of OPV, 1,000–5,000 paralytic cases of poliomyelitis were reported annually (Takatsu *et al*., 1973; Shimojo, 1984). A two-dose administration of domestic OPV to infants of 3–48 months (in 1964–1994) and 3–90 months (since 1995 till now) of age at intervals longer than 6 weeks has been performed routinely since 1964. Till now, wild-type polioviruses were isolated from one patient with poliomyelitis in 1980, and from two patients

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Abbreviations: IPV = inactivated poliovirus vaccine; OPV = oral poliovirus vaccine; PV-1 = poliovirus 1; VDPV = vaccine-derived poliovirus; VSLP= virulent Sabin-like poliovirus

with non-acute flaccid paralysis in 1984 and 1993, respectively (Infectious Agents Surveillance Center, 1997). The two-dose administration of OPV therefore appears effective in preventing poliomyelitis caused by wild-type polioviruses in Japan.

The poliomyelitis eradication program of WHO is close to the final stage of replacing virulent wild-type polioviruses with OPV strains (World Health Organization, 2003a). Recently, epidemics of poliomyelitis by vaccine-derived polioviruses (VDPVs) have been reported worldwide (Kew *et al*., 2004). According to WHO criteria a VDPV is defined as a strain, provided there is more than a 1% drift in the VP1 capsid protein at nucleotide level (World Health Organization, 2003b; Kew *et al*., 2004). Fortunately, poliomyelitis cases caused by VDPVs have not been reported in Japan yet. However, a total of 78 VDPV or VSLP isolates (16 PV-1, 31 PV-2, and 31 PV-3) were obtained from river and waste waters sampled between October 1993 and September 1995. As a result of VP1 gene nucleotide sequence analysis of the isolates, their divergence from Sabin strains was less than 1.4% (Matsuura *et al*., 2000).

Furthermore, using PCR and restriction analysis (Chumakov *et al*., 1991, 1994), we have found virulent genotype in some isolates of PV-1 (Horie *et al*., 2002a), PV-2 (Yoshida *et al*., 2002) and PV-3 (Yoshida *et al*., 2000) In particular, one of the PV-1 VSLPs, G4-12, displayed a phenotype similar to that of wild-type Mahoney strain in terms of neurovirulence, temperature sensitivity, plaque-forming ability and neutralization by monoclonal antibodies (Horie *et al*., 2002b). Nevertheless, G4-12 was categorized as a VSLP because of its VP1 nucleotide divergence from that of the Sabin strain of 0.6% only. Earlier, we have suggested the possibility of existence of VSLPs in the community as a result of environmental surveillance (Yoshida *et al*., 2000; Horie *et al*., 2002a). We believe that the reason why an epidemic of poliomyelitis caused by a VSLP has not occurred in Japan is the herd immunity.

To confirm this idea, we determined neutralizing antibody titers against PV-1 strains Sabin, Mahoney and G4-12 in the sera of various groups of residents of Toyama Prefecture, Japan. In this way, we attempted to assess retrospectively the efficacy of the current poliovirus vaccination policy, namely the two-dose administration of OPV in preventing epidemics of poliomyelitis caused by VSLPs in Japan.

Materials and Methods

Viruses. The strains Sabin and Mahoney of PV-1 were obtained from the National Institute of Infectious Diseases, Tokyo, Japan. The G4-12 strain of PV-1 (VSLP) was originally isolated from a sewage disposal plant located downstream of the Oyabe River, Toyama Prefecture, Japan in 1993. Its divergence from the Sabin strain of PV-1, as determined for the VP1 sequence, was 0.6% at nucleotide and 1.0% at amino acid level (Matsuura *et al*., 2000).

Sera. A total of 244 serum samples from residents of Toyama Prefecture, Japan, of up to 76 years of age, collected in 1998, were tested. The vaccination history of individual groups of residents is shown in Table 1.

Neutralization test was performed in 96-well microtiter plates using Vero cells in standard manner (World Health Organization, 1995). Cytopathic effect was read on day 7 post infection. A sample with a neutralizing antibody titer ≥8 was regarded as positive. Geometric means of titers were used for comparison of different groups of individuals.

Results and Discussion

In the individuals who were born after the enforcement of the domestic OPV vaccination but were not vaccinated (8 sera), the seropositivity against the strains Sabin, Mahoney and G4-12 was in the range 50.0–62.5% (individual values 62.5%, 50.0% and 62.5%, respectively) (Table 1). In contrast, in the individuals of the same group (110 sera),

Table 1. Vaccination history and seropositivity against PV-1 strains Sabin, Mahoney and G4-12 of residents of Toyama Prefecture, Japan

Vaccination history (No. of OPV doses)		No. of serum samples	Seropositivity (%)		
			Sabin	G4-12	Mahoney
Residents born after 1964 (the introduction of OPV)[a]	Non-vaccinated	8	62.5	62.5	50.0
	Vaccinated[d] (1)	14	85.7	85.7	85.7
	Vaccinated[d] (1 or 2)	10	90.0	80.0	80.0
	Vaccinated[d] (2)	110	99.1	95.5	94.5
	Vaccinated[d] (unknown)	33	90.9	78.8	81.8
Residents born before 1964 (the introduction of OPV)[b]	0[c]	69	92.8	89.9	89.9

[a]Below 35 years of age in 1998.

[b]Over 35 years of age in 1998.

[c]A small number of individuals received imported OPV in 1961–1963.

[d]The OPV, prepared by the Japan Poliomyelitis Research Institute, Tokyo, Japan, contained $10^{6.0 \pm 0.5}$, $10^{5.0 \pm 0.5}$ and $10^{5.5 \pm 0.5}$ $TCID_{50}$ of Sabin strains of PV-1, PV-2 and PV-3, respectively, in one dose.

standard vaccination with two doses of OPV resulted in a 94.5–99.1% seropositivity (individual values 99.1%, 94.5% and 95.5%, respectively), and even the vaccination with one dose (14 sera) gave a 85.7% seropositivity. All individual values represent geometric means. These results validate the efficacy of OPV vaccination.

In the individuals who were born before the enforcement of the domestic OPV vaccination and, as a rule, were not vaccinated (69 sera), the seropositivity was 89.9–92.8% (individual values 92.8%, 89.9% and 89.9%, respectively). This relatively high seropositivity, close to that found in the vaccinated group, may be explained by natural infection, as there were still occurring epidemics of poliomyelitis caused by poor public health conditions at that time in Japan.

Neutralizing antibody titers (geometric means) against the strains Sabin, Mahoney and G4-12 of the individuals vaccinated with two doses of OPV as compared with non-vaccinated individuals were significantly higher, namely neutralizing antibody titers for these strains for vaccinated individuals were 564, 186 and 194, respectively; corresponding titers for non-vaccinated individuals were 181, 74.0 and 62.5, respectively (Fig. 1). Within the group of vaccinated individuals, the antibody titers against Mahoney and G4-12 did not differ but were lower than those against Sabin. Even though they were lower, they were still sufficiently high (about 180) to prevent poliomyelitis caused by those virulent viruses (Mahoney and G4-12). These results indicate that the Japan's OPV vaccination policy has been effective in preventing poliomyelitis caused not only by wild-type poliovirus, but also by a VSLP. Although a similar investigation of the situation with PV-2 and PV-3 VSLPs is necessary, the results with PV-1 VSLPs strongly suggest that the absence of an epidemic of poliomyelitis caused by a VSLP in Japan is due to the herd immunity.

Serum neutralizing antibody titers of all 244 individuals regardless of their grouping, presented as pairwise strain comparisons, are plotted in Fig. 2. The titers against Mahoney were much lower than those against Sabin and their correlation was low (r = 0.223). Similarly, he titers against G4-12 were lower than those against Sabin; however, they did not correlate at all (r = 0.124). On the other hand, the titers against G4-12 were very similar to those against Mahoney and their correlation was high (r = 0.933). Previously, we found that G4-12, in reacting with various monoclonal antibodies, had properties similar to Mahoney (Horie *et al*., 2002b). The results of this study support the view that both viruses are antigenically quite similar.

In this study, we presented the concept of VSLP for the first time. A VSLP, like the G4-12 strain, with altered antigenicity and neurovirulence has existed in the environment though its divergence from the vaccine Sabin strain, based on VP1 gene, was less than 1%. VDPV/VSLP would not be eliminated from the environment as long as

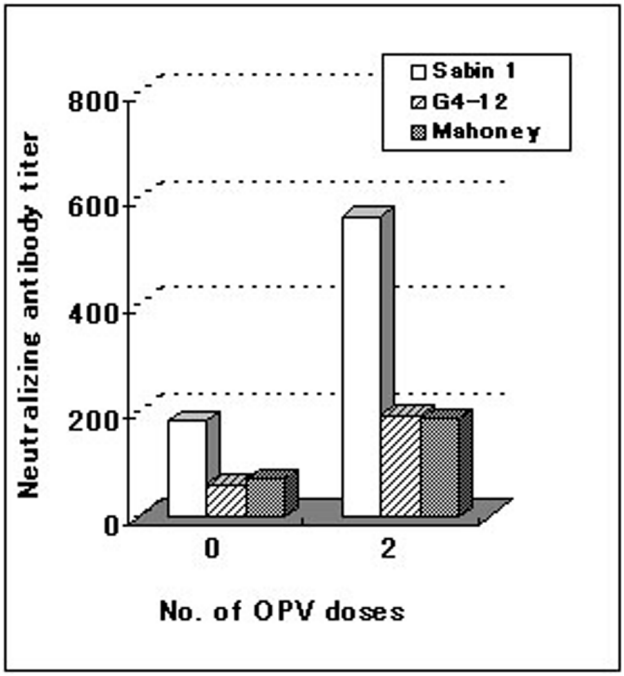

Fig. 1

Serum neutralizing antibody titers against PV-1 strains Sabin, Mahoney and G4-12 in vaccinated and non-vaccinated residents of Toyama Prefecture, Japan

Geometric means of titers in vaccinated (2 doses of OPV) and non-vaccinated individuals (no OPV dose) are indicated. Titers of $\geq 2^{10}$ and $<2^2$ were used to calculate in regard as 2^{10} and 2^2, respectively.

the use of OPV is continued. Introduction of an inactivated poliovirus vaccine (IPV) would be effective in eliminating the abovementioned viruses. After replacing OPV with IPV, VDPV/VSLP would probably remain in the environment for short time. These viruses might exist in the environment for a longer time due to immunodeficient patients excreting them for periods of approximately ten years (Kew *et al.*, 1998). Moreover, mutations in the excreted viruses may accumulate (Kew *et al*., 1998; Bellmunt *et al*., 1999; Martin *et al*., 2000). It is unclear whether IPV would be effective against VDPV/VSLP, which incurs mutations in neutralizing antigenic sites. In fact, an outbreak of poliomyelitis caused by virulent variant of PV-3, comprising 9 paralytic cases and 1 non-paralytic case, occurred in Finland where IPV had been used between August 1984 and January 1985 (Hovi *et al*., 1986). In particular, 8 of these cases had been vaccinated with 3–5 doses of IPV. Although the epidemic strain was not a VDPV/VSLP, its antigenic properties were slightly different from those of the Saukett strain, the PV-3 of IPV. The epidemics were obstructed by OPV administration. It is known that OPV induces a wider range of neutralizing antibodies compared to IPV, as the

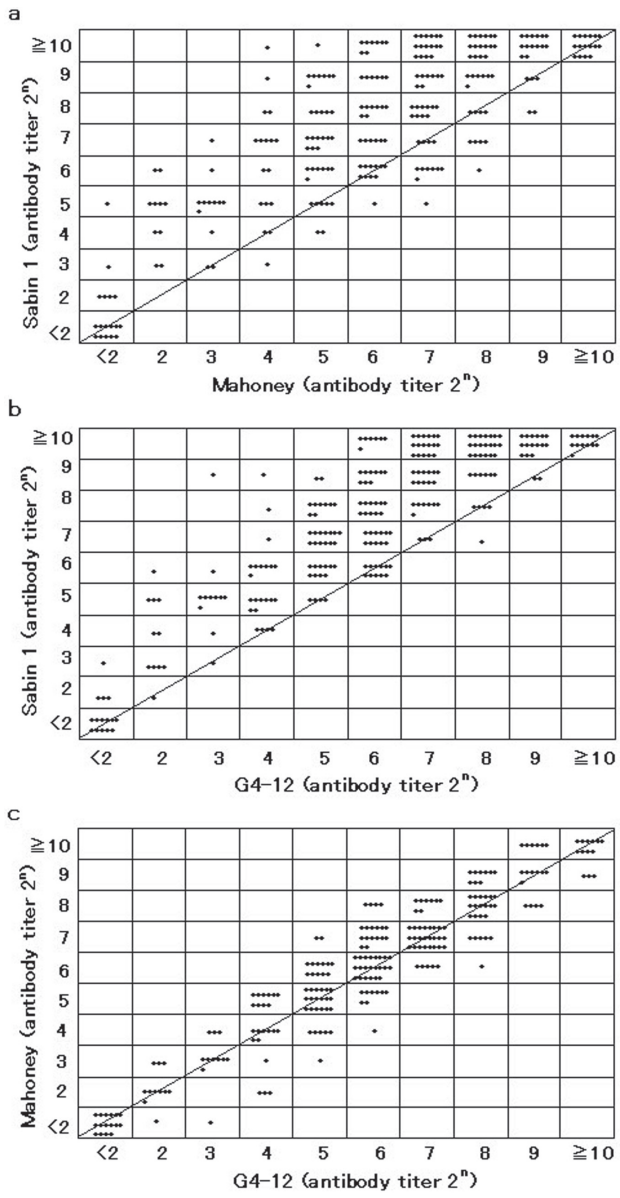

Fig. 2

Pairwise strain comparisons of serum neutralizing antibody titers for residents of Toyama Prefecture, Japan

Plots of neutralizing antibody titers for the strain pairs Sabin/Mahoney (a), Sabin/G4-12 (b), and Mahoney/G4-12 (c) for all 244 serum samples regardless of their grouping is shown in Table 1 are indicated.

vaccination with OPV is closer to the natural infection with poliovirus.

In eliminating VDPV/VSLP by replacement of OPV with IPV, it would be extremely important to continue for certain time period the investigation of the polioviruses in the form of surveillance of the environment and the population as well. Our results strongly suggest that the reason why an epidemic of poliomyelitis has not occurred in Japan is the herd immunity despite the persistence of VSLPs in the environment and/or population. They also suggest the possibility that a reemergence of VSLPs would be prevented if sufficiently strong herd immunity is acquired immediately after ending the OPV vaccination in accordance with the poliomyelitis eradication program.

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References

Bellmunt A, May G, Zell R, Pring-Akerblom P, Verhagen W, Heim A (1999): Evolution of poliovirus type 1 during 5.5 years of prolonged enteral replication in an immunodeficient patient. *Virology* **265**, 178–184.

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, 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.

Horie H, Yoshida H, Matsuura K, Miyazawa M, Ota Y, Nakayama T, Doi Y, Hashizume S (2002a): Neurovirulence of type 1 polioviruses isolated from sewage in Japan. *Appl. Environ. Microbiol.* **68**, 138–142.

Horie H, Yoshida H, Matsuura K, Miyazawa M, Wakabayashi K, Nomoto A, Hashizume S (2002b): Isolation of vaccine-derived type 1 polioviruses displaying similar properties to virulent wild strain Mahoney from sewage in Japan. *J. Med. Virol.* **68**, 445–451.

Hovi T, Cantell K, Houvilainen A, Kinnunen E, Kuronen T, Lapinleimu K, Poyry T, Roivainen M, Salama N, Stenvik M, Silander A, Thoden CJ, Salminen S, Weckstrom P (1986): Outbreak of paralytic poliomyelitis in Finland: widespread circulation of antigenically altered poliovirus type 3 in a vaccinated population. *Lancet* **1**, 1427–1432.

Infectious Agents Surveillance Center (1997): Poliomyelitis, Japan, 1962–1995. *Infect. Agents Surveill. Rep*. **18**, 1–2.

Kew OM, Sutter RW, Nottay BK, McDonough MJ, Prevots DR, Quick L, Pallansch MA (1998): Prolonged replication of a type 1 vaccine-derived poliovirus in an immunodeficient patient. *J. Clin. Microbiol*. **36**, 2893–2899.

Kew OM, Wright P, Agol VI, Delpeyroux F, Shimizu H, Nathanson N, Pallansch MA (2004): Circulating vaccine-derived polioviruses: current state of knowledge. *Bull. W.H.O.* **82**, 16–23.

Lago PM, Bravo JR, Andrus JK, Comellas MM, Galindo MA, Quadros CA, Bell E (1994): Lessons from Cuba: mass campaign administration of trivalent oral poliovirus vaccine and seroprevalence of poliovirus neutralizing antibodies. *Bull. W.H.O.* **72**, 221–225.

Martin J, Dunn G, Hull R, Patel V, Minor PD (2000): Evolution of the Sabin strain of type 3 poliovirus in an immunodeficient patient during the entire 637-day period of virus excretion. *J. Virol.* **74**, 3001–3010.

Matsuura K, Ishikura M, Yoshida H, Nakayama T, Hasegawa S, Ando S, Horie H, Miyamura T, Kitamura T (2000): Assessment of poliovirus eradication in Japan: genomic analysis of the polioviruses isolated from the river water and the sewage in Toyama Prefecture. *Appl. Environ. Microbiol.* **66**, 5087–5091.

Ramsay M, Begg N, Gandhi J, Brown D (1994): Antibody response and viral excretion after live polio vaccine or a combined schedule of live and inactivated polio vaccines. *Pediatr. Infect. Dis. J.* **13**, 1117–1121.

Recommendation of the Advisory Committee on Immunization Practices (ACIP) (1997): Poliomyelitis prevention in the United States: introduction of a sequential vaccination schedule of inactivated poliovirus vaccine followed by oral poliovirus vaccine. *M.M.W.R.* **46** (RR-3), 1–25.

Shimojo H (1984): Poliomyelitis Control in Japan. *Rev. Infect. Dis.* **6** (Suppl. 2), S427–430.

Takatsu T, Tagaya I, Hirayama M (1973): Poliomyelitis in Japan during the period 1962–68 after the introduction of mass vaccination with Sabin vaccine. *Bull. W.H.O.* **49**, 129–137.

World Health Organization (1995): Manual of laboratory methods for potency testing of vaccines used in the WHO Expanded Programme on Immunization. WHO publication No. WHO/BLG/95.1. World Health Organization, Geneva.

World Health Organization Collaborative Study Group on Oral and Inactivated Poliovirus Vaccines (1995): Factors affecting the immunogenicity of oral poliovirus vaccine: a prospective evaluation in Brazil and the Gambia. *J. Infect. Dis.* **171**, 1097–1106.

World Health Organization (2003a): *Global polio eradication initiative strategic plan 2004–2008*. World Health Organization, Geneva.

World Health Organization (2003b): Laboratory surveillance for wild and vaccine-derived polioviruses, January 2002–June 2003. *Wkly. Epidemiol. Rec*. **78**, 341–348.

Yoshida H, Horie H, Matsuura K, Miyamura T (2000): Characterization of polioviruses isolated from sewage and river water in Japan. *Lancet* **356**, 1461–1463.

Yoshida H, Horie H, Matsuura K, Kitamura T, Hashizume S, Miyamura T (2002): Prevalence of vaccine-derived polioviruses in the environment. *J. Gen. Virol.* **83**, 1107–1111.