TABLE 1. Susceptibility to poliovirus infection

Inoculation route	Mouse strain[a]	No. of mice dead/no. inoculated at inoculum dose (log_{10} PFU/mouse):										LD_{50} (log_{10})
		−1	0	1	2	3	4	5	6	7	8	
Intracerebral	PVR-tg	0/6	1/6	1/6	1/6	4/6	5/6	6/6				2.5
	PVR-tg/*Ifnar* KO	0/6	1/6	3/6	6/6	6/6						0.8
Intravenous	PVR-tg			0/6	1/6	1/6	4/6	3/6	6/6			5.0
	PVR-tg/*Ifnar* KO		0/6	0/6	5/6	6/6						1.7
Intraperitoneal	PVR-tg						0/6	1/6	3/6	5/6	5/6	>6.2
	PVR-tg/*Ifnar* KO		0/6	3/6	5/6	6/6						1.2

[a] tg, transgenic; KO, knockout.

ach, and muscle at high levels, in the spleen and kidneys at intermediate levels, and in the liver at low levels (data not shown). The PVR-transgenic mice were susceptible to poliovirus infection via the intracerebral, intraperitoneal, and intravenous routes. The infected mice developed a paralytic disease resembling human poliomyelitis. Unlike humans, however, PVR-transgenic mice were not highly susceptible to oral infection with poliovirus. We performed subsequent experiments with this transgenic mouse model.

Mice lacking the alpha/beta IFN response become highly susceptible to several virus infections (26). We compared the susceptibility of PVR-transgenic mice with that of PVR-transgenic/*Ifnar* knockout mice to poliovirus infection via intracerebral, intravenous, and intraperitoneal inoculations. The mice showed paralysis and died within 2 weeks p.i. in a dose-dependent manner following inoculation via all routes. This suggested that the mice died mainly due to poliovirus infection in the central nervous system irrespective of the inoculation route. The results and the LD_{50} values are shown in Table 1. The PVR-transgenic/*Ifnar* knockout mice were more sensitive to fatal poliovirus infection than the PVR-transgenic mice by any infection route. The LD_{50} value for PVR-transgenic/*Ifnar* knockout mice inoculated intracerebrally was 50-fold lower than that for PVR-transgenic mice. Notably, PVR-transgenic/*Ifnar* knockout mice became 3,000- and >20,000-fold more sensitive to poliovirus via intravenous and intraperitoneal inoculation, respectively. Furthermore, 10^6 PFU of poliovirus inoculated orally caused paralysis in 50% of the PVR-transgenic/*Ifnar* knockout mice (data not shown). On the contrary, PVR-transgenic mice with the disrupted IFN-γ gene (42) did not exhibit a significant increase in susceptibility to poliovirus infection (data not shown). The results indicate that alpha/beta IFN strongly influences poliovirus pathogenesis.

Distribution of poliovirus infection in PVR-transgenic/*Ifnar* knockout mice. Increased susceptibility to poliovirus by peripheral infection routes suggested that the poliovirus replicated efficiently in nonneural tissues. We compared poliovirus titers in the various tissues of nontransgenic C57BL/6 mice, PVR-transgenic mice, and PVR-transgenic/*Ifnar* knockout mice intravenously inoculated with 2×10^7 PFU of poliovirus. After intravenous inoculation, poliovirus was immediately delivered to all tissues, including the central nervous system, independently of the presence of PVR (47). Most of the PVR-transgenic mice and PVR-transgenic/*Ifnar* knockout mice developed paralysis by day 3 or 4 p.i., while the nontransgenic mice did not.

Figure 1 shows the virus load of various tissues on day 3 p.i. The virus titers in the brain and spinal cord of PVR-transgenic mice were much higher than those recovered from the same tissues of nontransgenic mice. The virus titer in the pancreas of PVR-transgenic mice was also higher than that of nontransgenic mice but not as high as the titers in the neural tissues of transgenic mice. The viral load of most of the other tissues of PVR-transgenic mice was slightly higher than that recovered from nontransgenic mice. These results suggest that poliovirus replicated efficiently in the central nervous system and less efficiently in the pancreas and did not replicate or replicated to only a slight degree in other nonneural tissues of PVR-transgenic mice. On the contrary, in PVR-transgenic/*Ifnar* knockout mice, poliovirus titers in all of the tissues examined were very high (Fig. 1), suggesting that the virus can replicate in nonneural tissues if alpha/beta IFN signaling is disrupted.

Poliovirus replication site in the visceral tissues of PVR-transgenic/*Ifnar* knockout mice. We investigated the localiza-

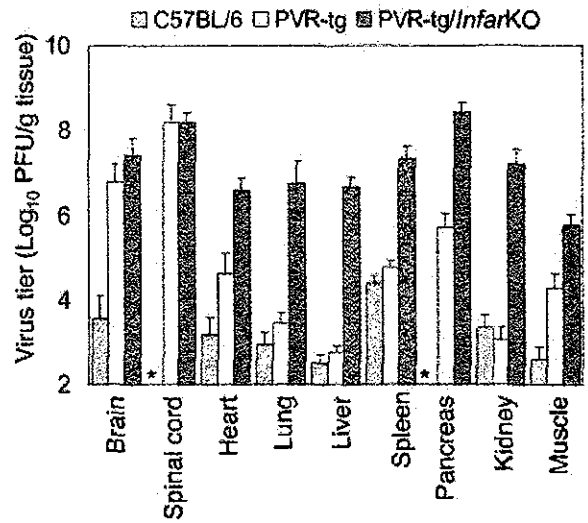

FIG. 1. Comparison of poliovirus titers in tissues of nontransgenic, PVR-transgenic, and PVR-transgenic/*Ifnar* knockout mice. The mice were inoculated intravenously with 2×10^7 PFU of poliovirus type 1 Mahoney strain. The tissues of nontransgenic mice (hatched bars), PVR-transgenic mice (open bars), and PVR-transgenic/*Ifnar* knockout mice (solid bars) were separated on day 3 p.i., and virus titers were determined by a plaque assay. The values represent the mean virus titer + standard deviation of six mice. The asterisk indicates that the values were below the limit of detection (2.0 log_{10} PFU/g).

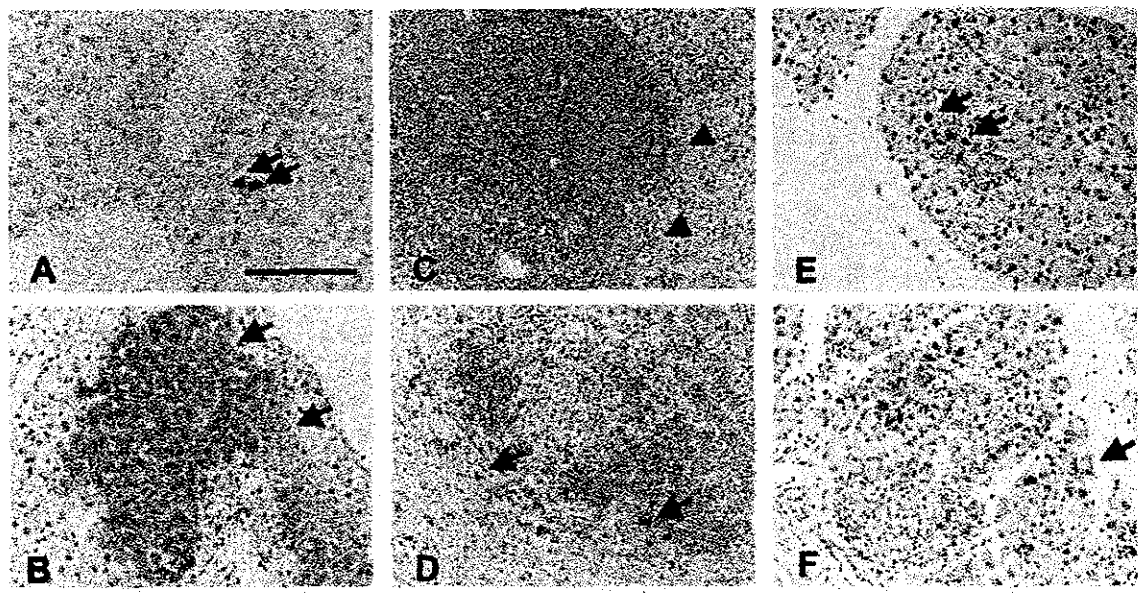

FIG. 2. Immunohistochemical detection of poliovirus antigen in infected mice. Poliovirus antigens were detected in PVR-transgenic (A, C, and E) and PVR-transgenic/*Ifnar* knockout (B, D, and E) mice with a rabbit polyclonal antibody recognizing the poliovirus capsid antigen. The mice were intravenously inoculated with 2×10^7 PFU of poliovirus. (A) Liver of the PVR-transgenic mice on day 1 p.i. Poliovirus antigen-positive cells, indicated by arrows, were focally observed with slight cellular infiltration around the infected cell. (B) Liver of PVR-transgenic/*Ifnar* knockout mice on day 1 p.i. Hepatic cells positive for poliovirus were observed in a zonal pattern. (C) Spleen of PVR-transgenic mice on day 1 p.i. A few very weakly stained cells are observed in the marginal zone, indicated by arrowheads. (D) Spleen of PVR-transgenic/*Ifnar* knockout mice on day 1 p.i. Many poliovirus antigen-positive large cells are localized in the marginal zone. The cells were identified as macrophages on the basis of the detection of CD11. (E) Pancreas of PVR-transgenic mice on day 3 p.i. A small cluster of cells positive for poliovirus antigen was observed in the lobulus in association with a slight inflammatory reaction. The poliovirus antigen was observed constantly in all mice. (F) Pancreas of PVR-transgenic/*Ifnar* knockout mice on day 3 p.i. Numerous acinar cells positive for the poliovirus antigen were distributed in many lobuli of the pancreas. Only a few poliovirus antigen-positive cells were observed in Langerhans' islets in the bottom left. (A) Bar, 125 μm.

tion of the poliovirus antigen and corresponding pathological changes to determine the virus replication sites. In the liver of PVR-transgenic mice, cells positive for the poliovirus antigen were detected occasionally, after careful observation. The antigens were found sporadically as a single cell or as a group of a few poliovirus antigen-positive cells with cellular damage in the liver on day 1 p.i., but were rarely detected after day 2 p.i. Inflammatory cell infiltration was also observed around the infected cells (Fig. 2A). Strong poliovirus antigen staining was not detected in the spleen, but a few very weakly stained cells were observed in the marginal zone (Fig. 2C). In the pancreas, groups of a few poliovirus antigen-positive cells were sporadically observed on day 1 p.i., with the number increasing slightly on day 3 p.i. (Fig. 2E). Although the infected area in the pancreas was not large, the antigens were always detected in all the mice. This observation is consistent with a higher poliovirus titer in the pancreas than in the other visceral tissues (Fig. 1).

In contrast, in PVR-transgenic/*Ifnar* knockout mice, zonal areas or clusters of poliovirus antigen-positive cells were observed in the liver on day 1 p.i. (Fig. 2B). These infected cells were identified morphologically as hepatocytes. The number of poliovirus antigen-positive cells decreased on day 3 p.i., but the inflammatory infiltrate became more evident. In the spleen, poliovirus antigen was detected mainly in large mononuclear cells in the marginal zone (Fig. 2D). These large mononuclear cells were positive for CD11 in serial sections and were shown to be macrophages (data not shown). In the pancreas, massive infection was observed in acinar cells (Fig. 2F).

The destruction of hepatocytes and pancreatic acinar cells was also confirmed by biochemical examination. Serum ALT and amylase levels were measured in intravenously inoculated mice on day 3 p.i. ALT values increased markedly in the PVR-transgenic/*Ifnar* knockout mice compared to the nontransgenic mice ($P < 0.05$, t test), whereas there was only a slight increase in the PVR-transgenic mice (Fig. 3A). This result indicates destruction

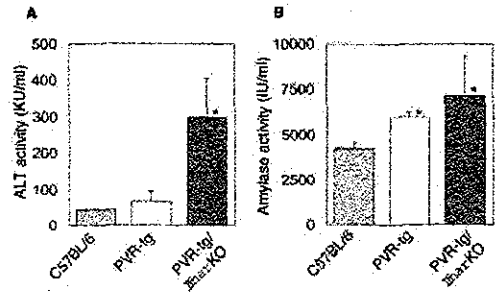

FIG. 3. Serum ALT and amylase activities in infected mice. The mice were inoculated intravenously with 2×10^7 PFU of poliovirus. The sera of nontransgenic (hatched bars), PVR-transgenic (open bars), and PVR-transgenic/*Ifnar* knockout (solid bars) mice were collected on day 3 p.i., and their ALT activity (A) and amylase activity (B) were determined. The mean values plus standard deviation of four mice are shown. The asterisks indicate that the values are significantly higher than those observed in the nontransgenic C57BL/6 mice ($P < 0.05$, t test).

of hepatocytes in the PVR-transgenic/*Ifnar* knockout mice. The amylase activity values in the serum of PVR-transgenic mice were 1.3- to 1.5-fold (mean, 1.4-fold) higher than that of the nontransgenic mice ($P < 0.05$, t test), while those of PVR-transgenic/*Ifnar* knockout mice were 1.2- to 2.3-fold higher (mean, 1.7-fold) than that of nontransgenic mice ($P < 0.05$, t test) (Fig. 3B). The results indicated acinar cell destruction in the pancreas and/or salivary glands in PVR-transgenic and PVR-transgenic/*Ifnar* knockout mice. These observations further indicate that the loss of alpha/beta IFN signaling apparently alters the tissue tropism. The hepatocytes, acinar cells, and macrophages in the spleen became potentially permissive for poliovirus infection, indicating that they express host factors required to support poliovirus replication.

With PVR-transgenic/*Ifnar* knockout mice, the virus antigens were not clearly detected in the kidneys, heart, and lungs of poliovirus-inoculated mice. Similar experiments were performed with another PVR-transgenic mouse strain, PVRtg25 (46). In this mouse strain, *PVR* mRNA expression levels are higher than those of PVRtg21. We further crossed this strain with *Ifnar* knockout mice and examined the susceptibility of PVRtg25/*Ifnar* knockout mice to poliovirus. All of the inoculated mice became moribund with jaundice on day 1 p.i. Immunohistological examination revealed that viral antigen-positive cells were detected in the liver, spleen, pancreas, kidneys, and heart (data not shown). Poliovirus infection in the liver was associated with massive necrosis of the parenchymal cells, which was correlated with liver failure. This confirmed that poliovirus can also replicate in the kidneys and heart when IFN signaling is disrupted.

Role of IFN in poliovirus spreading in the body. The above data indicate that the IFN response is particularly effective in restricting virus replication in the visceral tissues. Viremia occurred as a consequence of virus multiplication in extraneural sites. It is possible that the IFN response influences virus titer and, accordingly, that IFN also contributes in decreasing the incidence of paralytic and fatal poliovirus infection by lowering the chance of poliovirus entry into the central nervous system. To demonstrate this, we compared the virus titers in PVR-transgenic and PVR-transgenic/*Ifnar* knockout mice. We determined virus titers in the plasma of PVR-transgenic and PVR-transgenic/*Ifnar* knockout mice infected intraperitoneally with poliovirus (10^3 PFU). This dose was employed because it is below the LD_{50} of the PVR-transgenic and above that of the PVR-transgenic/*Ifnar* knockout mice (see Table 1).

Expectedly, a very high titer (10^8 to 10^9 PFU/ml) of poliovirus was detected on day 3 p.i. in the PVR-transgenic/*Ifnar* knockout mice, resulting in the death of the mice at day 4 p.i. In contrast, less than 10^3 PFU of poliovirus/ml was detected in the PVR-transgenic mice between days 2 and 5 p.i., and it was no longer detected at day 7 p.i. (Fig. 4). These results suggest that some cells, if not all, that express PVR can act as reservoirs of poliovirus during the progression of the disease in the viremic phase. However, only a small proportion of these cells produce poliovirus with a low efficiency because of the inhibitory effect of IFN, and high-titer viremia is prevented in animals with a normal IFN system. We consider that virus replication sites during the viremic phase have not been histologically identified because poliovirus replication levels are normally low in these cells in PVR-transgenic mice, monkeys, and humans.

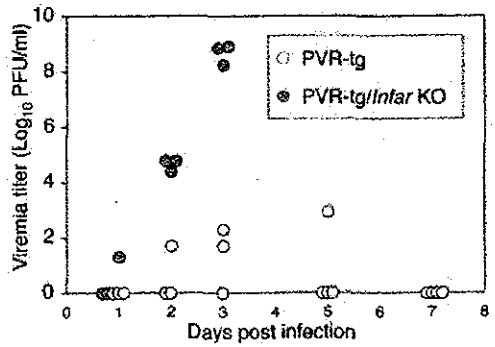

FIG. 4. Viremia in PVR-transgenic mice and PVR-transgenic/*Ifnar* knockout mice. Poliovirus (10^3 PFU) was inoculated intraperitoneally. Plasma from three or four infected mice was collected at the indicated day p.i., after which the virus titer in the plasma was determined. Open circles, PVR-transgenic mice; solid circles, PVR-transgenic/*Ifnar* knockout mice. Note that the virus titer in the PVR-transgenic/*Ifnar* knockout mice is very high. The data for PVR-transgenic/*Ifnar* knockout mice on days 5 and 7 p.i. were not available because all mice died on the fourth day after inoculation.

Expression of IFN-β and ISGs in the host. The susceptibility to poliovirus varied among the tissues. We therefore determined if the expression profiles of genes that confer an antiviral state in the IFN response were different between target and nontarget tissues. We determined the expression of mRNAs for IFN-β and ISGs with a quantitative real-time reverse transcription-PCR technique (Fig. 5). Since IFN-β is the first alpha/beta IFN induced after virus infection (36, 39, 41) and picornaviruses are known to be sensitive to OAS (7, 8) and protein kinase R (25), we focused on expression of IFN-β, OAS, and protein kinase R mRNAs. Of the ten genes that are similar to human OAS in the mouse genome, OAS1a, OAS1g, OAS2, OAS3, and OASL2 were shown to synthesize 2′-5′oligoadenylate (16).

We first determined the expression levels of IFN-β, OAS, and protein kinase R mRNAs in the noninfected PVR-transgenic mice. Very little expression of IFN-β mRNA was observed in all tissues of the noninfected mice (Fig. 5A). The expression levels of the mRNAs for OAS1a, OAS1g, OAS2, OAS3, OASL2, and protein kinase R are shown in Fig. 5B to G (open bars). The expression level of each ISG mRNA and the tissue distribution profiles were different. However, in general, they were expressed in the nontarget tissues more abundantly than in the target tissues of noninfected PVR-transgenic mice. No ISG was expressed at high levels in the central nervous system, an observation consistent with previous reports (1, 22, 30).

We then determined the changes in the expression levels of IFN-β and ISG mRNAs in the infected mice (Fig. 5A to G). On day 1 p.i., IFN-β mRNA expression in the spleen was observed at very high levels. Low-level IFN-β expression was observed in the heart, lungs, liver, kidneys, and muscle. No significant increase of IFN-β mRNA was observed in the brain and spinal cord on day 1 p.i. (Fig. 5A, gray solid bars). On day 3 p.i., IFN-β mRNA levels in the heart, lungs, liver, kidneys, and muscle decreased to nearly basal levels. In contrast, they increased to very high levels in the brain and spinal cord (Fig. 5A, solid black bars). Thus, the IFN-β mRNA expression pro-

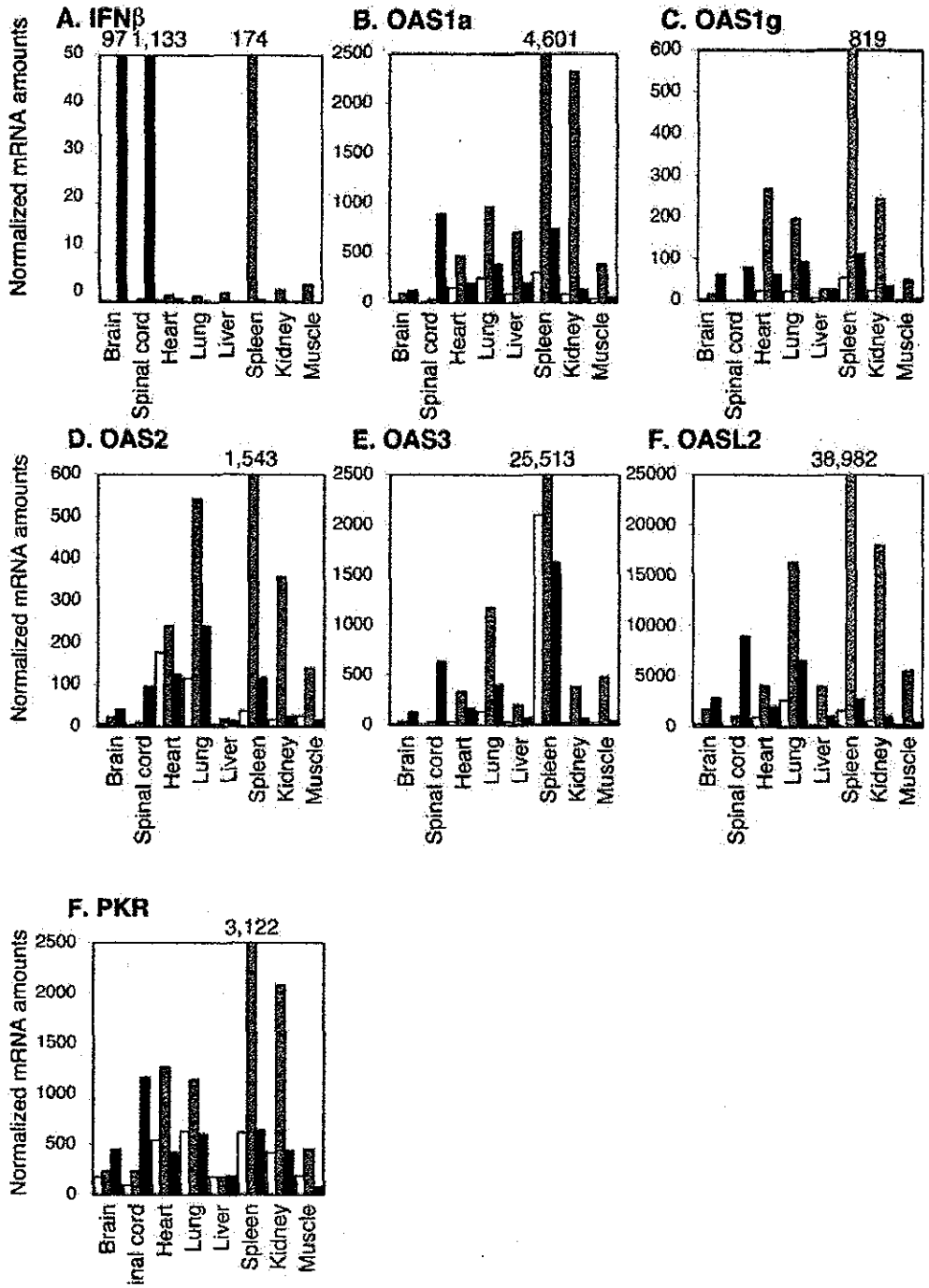

FIG. 5. Expression of IFN-β and ISGs in PVR-transgenic mice. The expression levels of IFN-β and ISG mRNAs in noninfected PVR-transgenic mice and PVR-transgenic mice infected intravenously with poliovirus (2×10^7 PFU) were determined by real-time quantitative PCR. The amounts of IFN-β (A), OAS1a (B), OAS1g (C), OAS2 (D), OAS3 (E), OASL2 (F), and protein kinase R (G) mRNAs normalized to 10^7 copies of 18S rRNA are shown. Open bars, gray solid bars, and black solid bars indicate the results for noninfected mice, infected mice at 1 day p.i., and infected mice at 3 days p.i., respectively. The mean values for three to six mice are indicated. The numbers above each figure indicate values that could not be represented within the figures. Note that the open bars in A are not visible because IFN-β mRNA expression in the noninfected mice was very low.

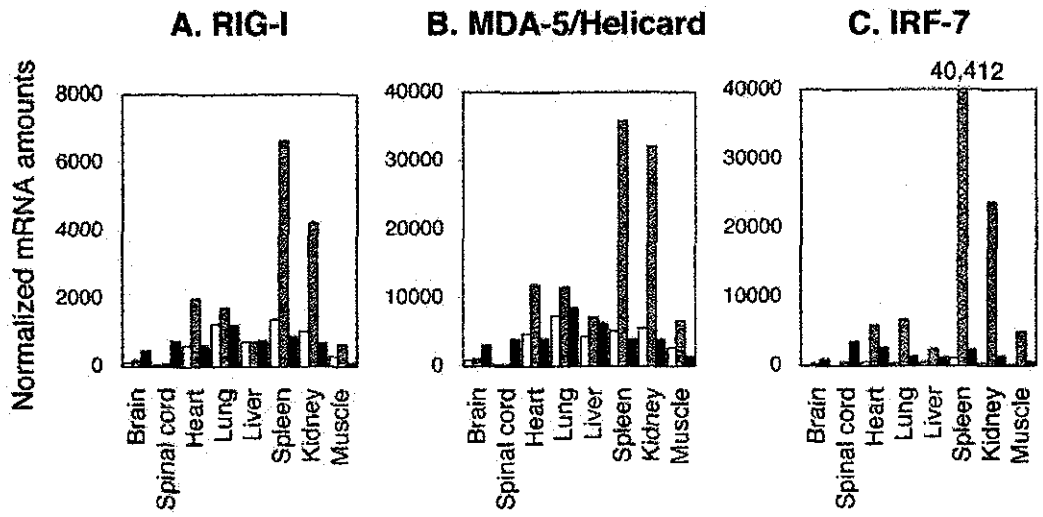

FIG. 6. Expression of RIG-I, helicard, and IRF-7 mRNAs in PVR-transgenic mice. The expression levels of RIG-I, helicard, and IRF-7 mRNAs of noninfected PVR-transgenic mice and PVR-transgenic mice *infected intravenously* with poliovirus (2×10^7 PFU) were determined by real-time quantitative PCR. The mean values for three mice are indicated. The amounts of RIG-I (A), helicard (B), and IRF-7 (C) mRNAs were determined. Open bars, gray solid bars, and black solid bars indicate the results of noninfected mice, infected mice at 1 day p.i., and infected mice at 3 days p.i., respectively. The amounts of mRNA per 10^7 copies of 18S rRNA are shown.

files are different between target tissues and nontarget tissues in poliovirus-infected mice.

The expression levels of ISG mRNAs also changed after poliovirus infection, consistent with the change in IFN-β mRNA levels (Fig. 5B to G, gray and solid black bars). The ISG expression pattern was clearly different between the target and nontarget tissues. ISG mRNAs increased in most of the visceral tissues on day 1 p.i. The expression level of ISG mRNAs increased most efficiently in the spleen. The levels of ISG mRNAs were relatively high in the heart, lungs, liver, kidneys, and skeletal muscle. In these tissues, the viral load was low (Fig. 1), and the poliovirus antigen was not detected. This indicates that ISG induction in these tissues was sufficient to inhibit poliovirus replication. The expression levels of ISG mRNAs in these tissues decreased on day 3 p.i., with a corresponding decrease in IFN-β mRNA levels. In the brain and spinal cord, however, significant induction of ISG mRNAs was not observed on day 1 p.i. The induction became evident only on day 3 p.i., when poliovirus destroyed a large number of neurons. We also noticed that the ratio of ISG and IFN-β mRNAs (ISG/IFN-β mRNA) in the brain and spinal cord on day 3 p.i. was much lower than that in nontarget tissues on day 1 p.i. (Fig. 5A to G). This indicates that the IFN response did occur in neurons in the brain and spinal cord but was not sufficient and failed to inhibit viral growth in the early phase of infection.

Expression of RIG-I, MDA-5/helicard, and IRF-7 in target and nontarget tissues. The data suggests that neurons in the target tissues failed to respond sufficiently to poliovirus infection. It is possible that there is a difference in the expression mechanism of the IFN response. We proceeded to examine a regulatory factor required for IFN response. Yoneyama et al. recently found that RIG-I functioned as a detector of intracellular double-stranded RNA. The cells that express this gene at high levels in vitro can induce IFN in an accelerated fashion and can survive against encephalomyocarditis virus and vesicular stomatitis virus infection (48). MDA-5/helicard is another caspase recruitment domain (CARD)-containing helicase, which is implicated as having a function similar to that of RIG-I (48). Both RIG-I and MDA-5/helicard are inducible by IFNs (17, 48).

Figures 6A and B show the changes in the RIG-I and MDA-5/helicard levels, respectively. Like that of other ISGs, the expression of these genes is low in the brain and spinal cord but high in nontarget tissues in the noninfected mice. The response of these genes after poliovirus infection is similar to that of other ISGs. They were induced at high levels in the nontarget tissues on day 1 p.i. but not in the target tissues. Thus, the nontarget tissues that expressed RIG-I and MDA-5/helicard at high levels may have an advantage in inducing IFN-β soon after poliovirus infection. We also examined the expression of IRF-7, another regulatory factor involved in the activation of IFN-α genes. IRF-7 thus is important to amplify the IFN response (37). The expression profile of IRF-7 was also similar to those of other ISGs (Fig. 6C).

Protection of mice from poliovirus infection by poly(I:C) treatment. The preceding data suggest that neurons in the brain and spinal cord were highly susceptible to poliovirus because expression levels of ISGs, including OASs and RIG-I, were low in the noninfected state. If this is the case, pretreatment to induce the antiviral state in the central nervous system would increase the survival rate of poliovirus-infected mice. Hence, treatment with poly(I:C) is expected to induce IFNs and ISGs and establish an antiviral state.

Poly(I:C) (200 μg) was administered intracerebrally to PVR-transgenic mice, and on the next day, RNA was prepared from the brain and spinal cord. The levels of OAS1a and RIG-I were determined by real-time quantitative PCR. As expected, expression of the mRNAs for OAS1a and RIG-I was elevated to high levels by poly(I:C) (Fig. 7A and B). PVR-transgenic mice treated with poly(I:C) or mock treated were challenged with poliovirus

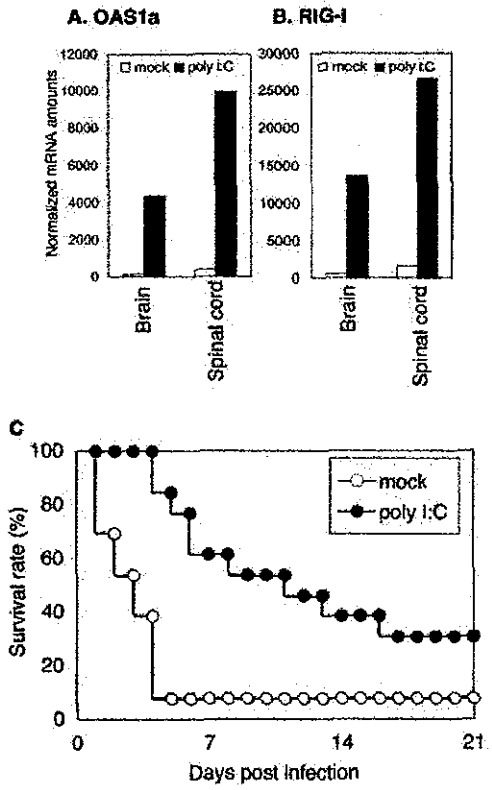

FIG. 7. Induction of mRNAs for OAS1a (A) and RIG-I (B) after poly(I:C) treatment. PVR-transgenic mice was administered poly(I:C) (solid bars) or mock treated (open bars). RNA was prepared from the mice 1 day after administration. The amounts of RNA were determined by real-time quantitative PCR. (C) Survival of infected mice. PVR-transgenic mice administered poly(I:C) (solid circles) or mock treated (open circles) (13 mice each) were challenged intracerebrally with 10^4 PFU of poliovirus. Mice were observed for 3 weeks. The survival rate of poly(I:C)-treated mice was significantly higher than that of mock-treated mice ($P < 0.05$, log-rank test).

(10^4 PFU) by the same route. The survival of the mice is shown in Fig. 7C. Mock-treated PVR-transgenic mice died at 2 to 6 days p.i., with a survival rate of 7.7%. After poly(I:C) treatment, mice died at 4 to 16 days p.i., increasing the survival rate to 30.8%. The clinical symptoms observed in the poly(I:C)-treated PVR-transgenic mice were almost the same as those observed in mock-treated mice, suggesting the same pathology. The results indicate that the poly(I:C)-treated mice survived longer than mock-treated mice, with the survival rate of poly(I:C)-treated mice being higher than that of mock-treated mice ($P < 0.05$, log rank test). These data suggest that the antiviral state induced by treatment with poly(I:C) was effective in preventing poliovirus replication in the central nervous system.

DISCUSSION

IFN system is a host factor that inhibits poliovirus replication. Picornaviruses are sensitive to IFNs. However, little is known about the role of alpha/beta IFN in the pathogenesis of poliovirus in vivo. We have shown the importance of the IFN response in poliovirus infection in vivo with a transgenic mouse expressing human PVR. In the PVR-transgenic mice, poliovirus replicates and produces severe lesions in the brain and spinal cord, while other tissues did not show severe pathological changes.

It would be reasonable to assume that some host factors required for poliovirus replication are lacking in the nontarget cells and tissues. PVR was thought to be such a determinant (13). However, previous studies revealed that many nontarget tissues expressed PVR (18, 21, 24, 31, 32). It is therefore impossible to explain poliovirus tissue tropism solely by the presence of PVR. Gromeier et al. (11) and Yanagiya et al. (46) proposed a hypothetical mechanism called the internal ribosome entry site (IRES)-dependent mechanism to explain the tissue tropism of viruses. The IRES controls the efficiency of protein synthesis in some viruses. Chimeric viruses containing the IRES of human rhinovirus and hepatitis C virus instead of the poliovirus IRES do not replicate in the central nervous system. Therefore, the poliovirus IRES confers the ability to replicate in neurons on the chimeric virus, while the IRESs of human rhinovirus and hepatitis C virus do not. It is possible that the poliovirus IRES, particularly in virulent strains, is designed to exhibit full activity in neurons. In this hypothesis, host factors related to poliovirus IRES function should be restricted to neurons. However, poliovirus can replicate in cultured cells of nonneural origin. This hypothesis does not completely explain why poliovirus does not replicate efficiently in nontarget tissues in vivo.

It is also possible to assume that some host factors that inhibit poliovirus replication are present in the nontarget tissues. The liver, spleen, and pancreas were spared severe poliovirus infection only when the IFN system was functional. These data demonstrate that the IFN system is one of the major factors that confers resistance against poliovirus infection. Limitation of poliovirus tissue tropism is achieved by inhibition of poliovirus replication by the IFN system in nontarget tissues. An altered tissue distribution of viral replication was observed in mice deficient in the *Ifnar* gene or in mice deficient in the signal transducer and activator of transcription 1 (*Stat-1*) gene infected with viruses other than poliovirus (9, 10, 28, 34, 44). In these animals, virus replication was observed in tissues that were normally considered nontarget tissues. Therefore, it is a general principle that the tissue tropism of viruses is determined, at least in part, by an IFN-dependent mechanism.

Unequal IFN response selectively inhibits poliovirus replication in nontarget tissues. In cultured cells, encephalomyocarditis virus replication occurs rapidly within 6 h, and the infected cells are usually destroyed by lytic replication of virus before they produce IFNs. However, constitutive expression of OAS or protein kinase R (7, 8, 25), which are effectors of antiviral activity, and expression of RIG-I (48), a regulator of IFN induction, inhibited viral replication. It is very likely that the same mechanism operates during poliovirus infection.

We determined the expression of ISGs in the tissues. The distribution of ISG mRNAs in the noninfected mice was not equal among tissues. They were expressed in the nontarget tissues more abundantly than in the target tissues (Fig. 5). These existing ISG products may help restrict virus replication and spread in the nontarget tissues during the initiation of infection in vivo. In PVR-transgenic/*Ifnar* knockout mice, the

expression levels of mRNAs for ISGs were greatly reduced. The mRNAs for OASs were detected only in the intestine and thymus (data not shown). This is also consistent with the result of Ueda et al. (43), which showed that OAS expression in most tissues was reduced in p48 (IRF-9)-deficient mice. This result suggests that ISG expression in most of the tissues, even in the noninfected state, is mainly dependent on the IFNAR-dependent pathway. It also suggests that these tissues were continuously exposed to IFN stimulation at low levels (43). Visceral tissues such as the intestine and lungs are continuously at risk of exposure to pathogens. These tissues may be programmed to respond readily to viral infection. Alternatively, they are constantly stimulated by nonpathogenic microorganisms present in the body and thus are already primed.

Furthermore, the IFN response after poliovirus infection was also different among tissues. High-level response was observed in the spleen but was not observed in the spinal cord. This suggests that the IFN response profile may differ depending on the cells and tissues. Since RIG-I and MDA-5/helicard are also IFN inducible (17, 48), they also existed more abundantly in the nontarget tissues (Fig. 6), like other ISGs (Fig. 5). Some of the important regulators of the IFN response, such as IRF-7 and IRF-9, are also IFN inducible. The cells that are primed even at low levels of IFNs may be equipped with all the machinery necessary for the IFN response. Thus, the nontarget tissues may be ready to respond to viral infection. Unequal distribution of the regulators of the IFN response is again consistent with the idea that poliovirus replication is selectively inhibited in nontarget tissues.

On the contrary, neurons in the brainstem and spinal cord could not induce a sufficient antiviral state after poliovirus infection. However, pretreatment with poly(I:C) increased the survival of PVR-transgenic mice against poliovirus challenge (Fig. 7). This suggests that neurons also became resistant to poliovirus infection as long as they were treated. It is therefore possible that the status of ISG expression in the early phase of infection is critical in determining the fate of infected cells and an unequal IFN response may be one of the reasons for the differential susceptibility of cells and tissues to poliovirus. Although the IFN response was not equal in PVR-transgenic mice, both the basal expression of ISGs and induction of ISGs after poliovirus infection are equally null in PVR-transgenic/*Ifnar* knockout mice. Without these unequal protective responses, replication of poliovirus was observed in PVR-transgenic/*Ifnar* knockout mice in the nontarget tissues as well.

Incidence of paralytic disease is influenced by the IFN response. In a natural poliovirus infection, less than 1% of infected individuals develop paralytic disease, and virus clearance occurs in most persons with asymptomatic or mild infections (4, 23, 35). Viremia is observed only transiently in experimentally infected chimpanzees and monkeys, with titers of less than 10^5 tissue culture infective doses per ml with virulent strains (3, 5). Viremia was not observed in a chimpanzee and a human volunteer administered attenuated vaccine strains (35). Thus, viremia titers seem to correlate with central nervous system invasion in primates.

Our data showed that viral replication in visceral tissues is inhibited by the IFN response in PVR-transgenic mice. Poliovirus can enter the central nervous system, penetrating the blood-brain barrier. This pathway is considered the main pathway of poliovirus entry into the central nervous system in the PVR-transgenic mice after poliovirus infection via peripheral routes (47). Inhibition thus results in reduction of the virus titer in the blood and reduction of the chance of virus entry into the central nervous system. In contrast, PVR-transgenic/*Ifnar* knockout mice showed viremia with a very high titer and a high incidence of paralytic disease. It is therefore possible to speculate that the low incidence of paralytic poliomyelitis in humans is also a result of inhibition of poliovirus replication in nonneural tissues by the host IFN response, although we have no experimental evidence on humans. Paralytic poliomyelitis may occur when the alpha/beta IFN response does not work sufficiently in patients with certain conditions. Individuals who have a defect(s) in a gene(s) that contributes to the IFN response would be more susceptible to paralytic poliomyelitis.

Conclusion. The tissue tropism and pathogenesis of viruses are determined by a combination of several factors. In the case of poliovirus infection, poliovirus replication sites are primarily determined by the presence of the receptor, with the capture and entry of the virus into the cells supported by the PVR. Cells expressing PVR at high levels may be favored for poliovirus infection (20). Thus, the tropism of poliovirus may be dependent on the amount of PVR. After virus entry into cells, efficient replication of poliovirus may be dependent on the milieu of infected cells. If the environment is optimal for RNA and viral protein synthesis, a large number of viral particles will be produced per cell. If antiviral activities, such as the IFN response, are sufficiently high, virus replication will be inhibited. Thus, the fate of infected cells is determined by the balance of the replicating capacity of poliovirus and the antiviral activity of the host. Visceral tissues will then fail to serve as a massive factory of poliovirus, and the chance of viral entry into the central nervous system is greatly reduced.

If the virus enters the central nervous system, virulent poliovirus strains can replicate in neurons, where the antiviral defense is not sufficiently ready, and the patient develops paralytic disease. Therefore, the innate antiviral defense is an important determinant of tissue tropism and pathogenicity of poliovirus. It is of interest to investigate if the alpha/beta IFN response also contributes to selective poliovirus infection in the motor neurons in the central nervous system or to infection in the gastrointestinal tract. These questions will be elucidated in future studies. In the case of other viruses, situations such as distribution of the receptor molecule, replication capacity in each tissue, and resistance to the IFN system may differ from those of poliovirus. It therefore seems likely that each virus displays a distinct disease pattern unique to that particular virus.

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Persistence of oral polio vaccine virus after its removal from the immunisation schedule in New Zealand

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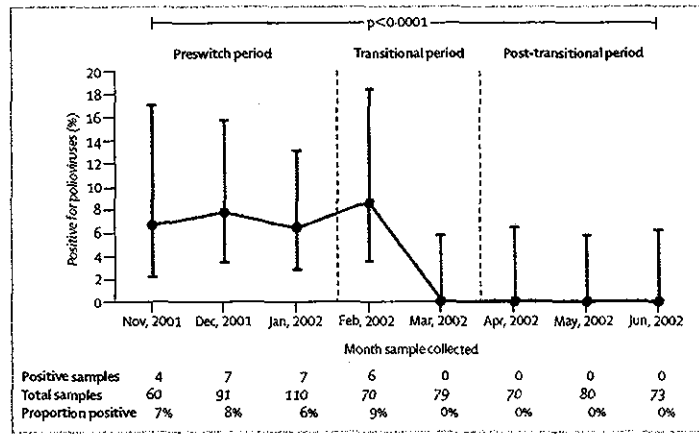
On Feb 1, 2002, inactivated poliomyelitis vaccines replaced live-attenuated oral poliovirus vaccine (OPV) in New Zealand's immunisation schedule, allowing systematic monitoring of OPV virus circulation. Findings of paediatric-inpatient surveillance indicate that 7% of children excreted polioviruses before this switch, but none did so 1 month afterwards. Acute flaccid paralysis and enterovirus surveillance detected poliovirus only once after the transition. Environmental surveillance identified polioviruses in sewage samples until May, 2002, after which they were detected infrequently. Intratypic differentiation and sequencing showed that all polioviruses were Sabin-like. Multiple surveillance methods hence showed that OPV strains did not persist for extended periods after a vaccine switch in a developed country with a temperate climate. Sequence homology with Sabin vaccine parent strains indicated that polioviruses detected more than 4 months after the switch were of recent origin, consistent with importation from OPV-using countries.

The global eradication of wildtype poliomyelitis by mass immunisation campaigns with live-attenuated oral poliovirus vaccine (OPV) is imminent, despite outbreaks of this disease in Nigeria in 2004–05 and persisting small reservoirs in Africa and Asia (http://www.polioeradication.org). A priority is to develop strategies of when and how to stop OPV immunisation once poliomyelitis is eradicated. However, whether vaccine virus transmission is sustained after withdrawal of OPV from immunisation schedules remains unknown. This question is important, since persistent circulation of OPV viruses increases the risk of reversion to fully neurovirulent vaccine-derived poliovirus strains in unvaccinated populations.[1]

After OPV vaccination, poliovirus is excreted by healthy children for 2–3 months and its persistence in populations is limited.[1] Reports[2] from several developing countries though indicate that circulating neurovirulent vaccine-derived poliovirus strains can be sustained for extended periods and cause poliomyelitis when population immunity is low. Since 1961, New Zealand has maintained OPV coverage of about 85%. However, after two instances of vaccine-associated paralytic poliomyelitis, inactivated poliovirus vaccine (IPV) replaced OPV in the infant immunisation schedule on Feb 1, 2002. This change provided an opportunity to monitor the persistence of OPV strains excreted by the last cohorts of children immunised with OPV. We did systematic population-based surveillance for OPV virus circulation and evolution before, during, and after the OPV/IPV switch with combined paediatric-inpatient, acute flaccid paralysis, enterovirus laboratory, and environmental surveillance systems. Based on Cuba's experience of annual mass immunisation campaigns, we postulated that polioviruses would be isolated during the preswitch period and then decline over a 2-month transitional period, after which no further polioviruses would be isolated.[3]

The Wellington Ethics Committee approved this study on behalf of Auckland and Waikato Ethics Committees. All patients or their parents or guardians provided written consent. We surveyed paediatric inpatients for 8 months (3 months before, 2 months during, and 3 months after the OPV/IPV switch) at three hospitals in Auckland, Hamilton, and Wellington. To detect a decline in OPV strain prevalence from 4·5% (previous national enterovirus surveillance data) to 0·5% (80% power, 95% significance, two-tailed test of difference in proportions), we approached every month 35 children (younger than age 15 years) consecutively admitted to each of the three hospitals with expectation of 80% participation.

Of 861 patients recruited, 633 (74%) provided stool samples for testing. The results of paediatric-inpatient surveillance indicate that vaccine viruses disappeared quickly after the switch (figure 1). During the preswitch and transition periods, we isolated polioviruses from 18



	Nov, 2001	Dec, 2001	Jan, 2002	Feb, 2002	Mar, 2002	Apr, 2002	May, 2002	Jun, 2002
Positive samples	4	7	7	6	0	0	0	0
Total samples	60	91	110	70	79	70	80	73
Proportion positive	7%	8%	6%	9%	0%	0%	0%	0%

Figure 1: Poliovirus prevalence (95% CI) between November, 2001, and June, 2002 by paediatric inpatient surveillance

of 261 (7%, 95% CI 4·1–10·7) and six of 149 children (4%, 1·7–8·2), respectively. We isolated the last poliovirus from a stool sample collected 1 month after the OPV/IPV switch. We did not detect any polioviruses in 223 stool samples collected during the post-transitional period. The Cochram-Armitage test shows a significant trend ($p<0·0001$) in prevalence across the three periods. We recorded the demographic features of the 24 poliovirus-positive and 609 poliovirus-negative children (webappendix 1). All polioviruses were isolated from vaccinees who had OPV within 10 weeks of being admitted to hospital and who were younger than age 6 months. Poliovirus isolation rates were similar across all socioeconomic groups. 24 inpatients yielded 30 Sabin-like polioviruses (seven type 1, 12 type 2, 11 type 3).

See Lancet Online for webappendix 1

Since 1997 there has been continued monitoring of acute flaccid paralysis in children younger than age 15 years.[4] Between January, 2001, and September, 2003, we analysed stool samples from 22 of 33 reported cases of acute flaccid paralysis. Only one child, aged 2 months and with spinal muscular atrophy, had Sabin-like polioviruses type 1 and type 2 isolated from each of two stool samples collected on Feb 28 and March 1, 2001. She had received her first dose of OPV 11 days previously.

Enterovirus surveillance used the national laboratory network, which investigates mainly inpatients with febrile illnesses not associated with acute flaccid paralysis (about 1200 stool samples annually).[5] We analysed data for 33 months (13 months before, 2 months during, and 18 months after the switch) and noted that polioviruses disappeared rapidly after the OPV/IPV switch (webappendix 2). Before the switch, we identified 38 poliovirus-positive children. In the transition period, we isolated Sabin-like polioviruses type 1 and type 2 from a 2-month-old boy without acute flaccid paralysis, whose stool sample was collected 5 days after the OPV/IPV switch. 19 months later, we identified a Sabin-like poliovirus type 2 in a 10-month-old girl with conjunctivitis. Sequencing in the VP1 region showed 99·9% homology to the parental Sabin strain. Almost all poliovirus-positive cases from enterovirus surveillance were aged 6 weeks to 5 months. 40 children without acute flaccid paralysis yielded 48 Sabin-like polioviruses (19 type 1, 18 type 2, 11 type 3).

See Lancet Online for webappendix 2 and webappendix 3

For environmental surveillance, we collected weekly sewage samples over 18 months (3 months before, 2 months during, and 13 months after the switch) from three sewage treatment plants in Auckland, Hamilton, and Porirua (a satellite city of Wellington) where the surveillance hospitals were located. The catchment populations were 900 000, 100 000, and 65 000, respectively. Before the OPV/IPV switch, the poliovirus isolation rate was 94%. This proportion decreased after the switch, but not as rapidly as with other surveillance methods (figure 2). The decline was maintained in the post-transitional period (April, 2002, to April, 2003), such that after May, 2002, polioviruses were only detected once every 3 months.

We isolated 71 Sabin-like polioviruses as a result of environmental surveillance (nine type 1, 36 type 2, 26 type 3). Sequencing of environmental polioviruses during the post-transitional period confirmed these as Sabin-like with more than 99% homology with parental Sabin strains (webappendix 3). In particular, the five Sabin-like polioviruses identified by environmental surveillance 6, 9, and 12 months after the OPV/IPV switch had 99·7–100% homology with parental strains.

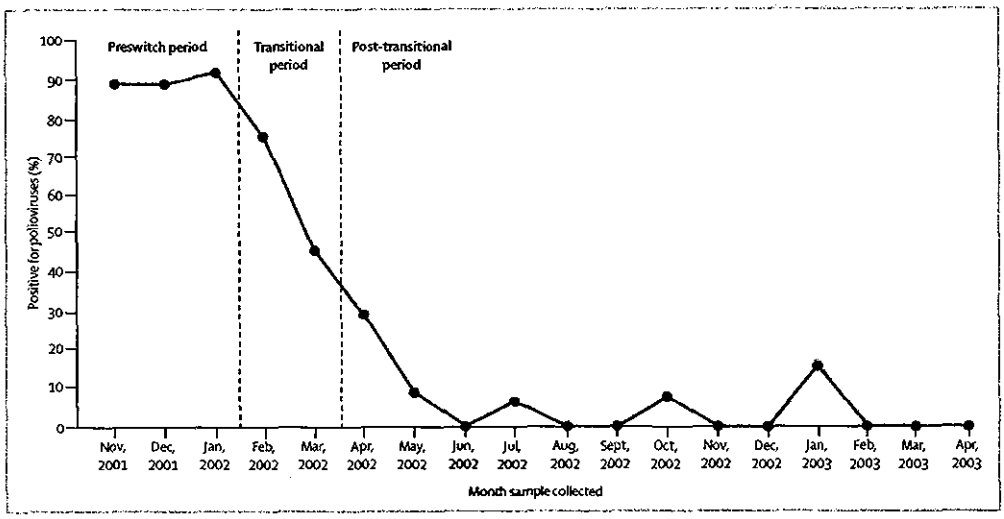


Figure 2: Poliovirus prevalence between November, 2001, and April, 2003, by environmental surveillance

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We noted limited circulation of OPV strains in New Zealand after the switch to IPV. First, with one exception, all polioviruses isolated from enterovirus surveillance during 2001–02 were from children aged 6 weeks to 5 months who should have received at least one dose of OPV. Second, OPV viruses detected by paediatric-inpatient surveillance were found only in vaccinees. Third, intratypic differentiation and sequence data for polioviruses obtained from paediatric-inpatient, acute flaccid paralysis, and enterovirus surveillance confirmed that all polioviruses were Sabin-like.

Since polioviruses evolve at a constant rate of 1% nucleotide substitutions per year,[6] environmental isolates 6–12-months post-switch with 99·7–100% sequence homology to parental Sabin strains infer that these viruses were derived from OPV administered 1–3-months previously. Rather than being from either the last cohorts of OPV immunised children or immune-deficient long-term excretors,[7] these viruses are more likely to have originated in recently vaccinated children or their close contacts from an OPV-using country. This finding shows that New Zealand remains vulnerable to vaccine or wildtype virus importation.

Every surveillance method revealed a different rate of OPV virus decline. The sensitivity of surveillance of acute flaccid paralysis for detection of sporadic vaccine-derived poliovirus is limited, examining about one in 100 000 children younger than age 15 years—ie, 0·1–0·5% of poliovirus-infected children manifesting paralytic poliomyelitis. Every year, enterovirus surveillance examines stool samples from roughly one in 3000 (1200 of 3 737 277) New Zealanders suspected of enteroviral infections. Paediatric-inpatient surveillance attempted to measure poliovirus excretion in a moderately representative population by sampling one in 648 (633 of 410 181) children living in three cities. Environmental surveillance obtained composite samples from sewage systems that serve 28% of the population. The higher and more prolonged poliovirus detection rates in sewage indicate the increased sensitivity of this method of surveillance over that of paediatric-inpatient surveillance in the same urban areas.

OPV strains do not persist for long after an OPV/IPV switch in a developed country with a temperate climate. Our study should be repeated in tropical, developing countries, however, where transmission of OPV viruses is likely to be more intense. The findings of such studies are vital to formulate polio immunisation policies in the postcertification era. Simultaneous global cessation of OPV after a mass immunisation campaign to maximise population immunity and minimise vaccine-derived poliovirus circulation could be adopted if there is minimum risk of sustained vaccine-derived poliovirus circulation.[2] Meanwhile, the continued risk of poliovirus importation means that New Zealand should maintain high IPV coverage. Finally, multiple surveillance methods, particularly environmental surveys, provide increased sensitivity for detection of poliovirus circulation, which will be essential in the posteradication era.

Contributors

Q S Huang, G Greening, M G Baker, and K Grimwood designed the study, supervised the virological, clinical, and environmental components, interpreted the data and their analysis, and wrote the report. *J Hewitt, D Hulston, L van Duin, and A Fitzsimons established* and did the laboratory tests. N Garrett did the study size calculations, contributed to the design, and undertook statistical analyses. K Grimwood, D Graham, and D Lennon established paediatric-*inpatient surveillance. H Shimizu and T Miyamura* undertook the sequence analysis and interpretation. M A Pallansch assisted in study design and interpretation.

Conflict of interest statement

We declare that we have no conflict of interest.

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特集

不活化ポリオワクチン

Inactivated poliovirus vaccine

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ワクチン

Key words ポリオウイルス ポリオ根絶計画 不活化ポリオワクチン 経口生ポリオワクチン

日本では，1960年代前半の大流行を最後として，弱毒化経口ポリオワクチン(oral poliovirus vaccine；OPV)の導入により，ポリオは完全に制圧されている．さらに，日本を含む西太平洋地域では，世界ポリオ根絶計画の進展により，2000年に野性株ポリオフリーが達成された．OPVは，日本および世界の多くの国々において，野性株ポリオウイルスの根絶とポリオフリーの維持に大きな役割を果たしてきた．OPVの集中的な投与により，インド，アフリカ等に残された世界で最後の野性株ポリオウイルスも，ここ数年以内には，その伝播が終息するものと期待されている．

有効性および安全性に優れたもう一つのポリオワクチンとして，不活化ポリオワクチン(inactivated poliovirus vaccine；IPV)が，欧米諸国を中心に実用化されている．IPVそのものは，けっして「新しいワクチン」ではなく，もっとも古くに実用化され今日まで一部の国で使われてきた伝統的なワクチンのひとつである[1]．近年，世界ポリオ根絶計画の進展および近い将来のポリオ根絶を見すえて，OPVに替わるIPVの新たな役割がクローズアップされている．本稿では，ポリオ根絶後のワクチン戦略におけるIPVの位置づけについて解説するとともに，現在日本で進められているSabin株に由来する新しいタイプのIPVの開発の現状について簡単に触れる．

Ⅰ．ポリオ根絶計画の現状

1988年に世界保健機関(World Health Organization；WHO)により提唱された世界ポリオ根絶計画は，現在最後の正念場を迎えている．WHOが当初目標としていた2000年までの世界レベルのポリオ根絶は達成できなかったが，1994年に南北アメリカ，2000年には日本を含む西太平洋地域，さらに2002年にはヨーロッパ地域において，地域固有の野生株ポリオウイルス伝播の終息，いわゆるポリオフリーが宣言された[2]．2002年に，ポリオ流行の再燃により1600例以上のポリオ症例が報

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0371-1900/04/¥50/頁/JCLS

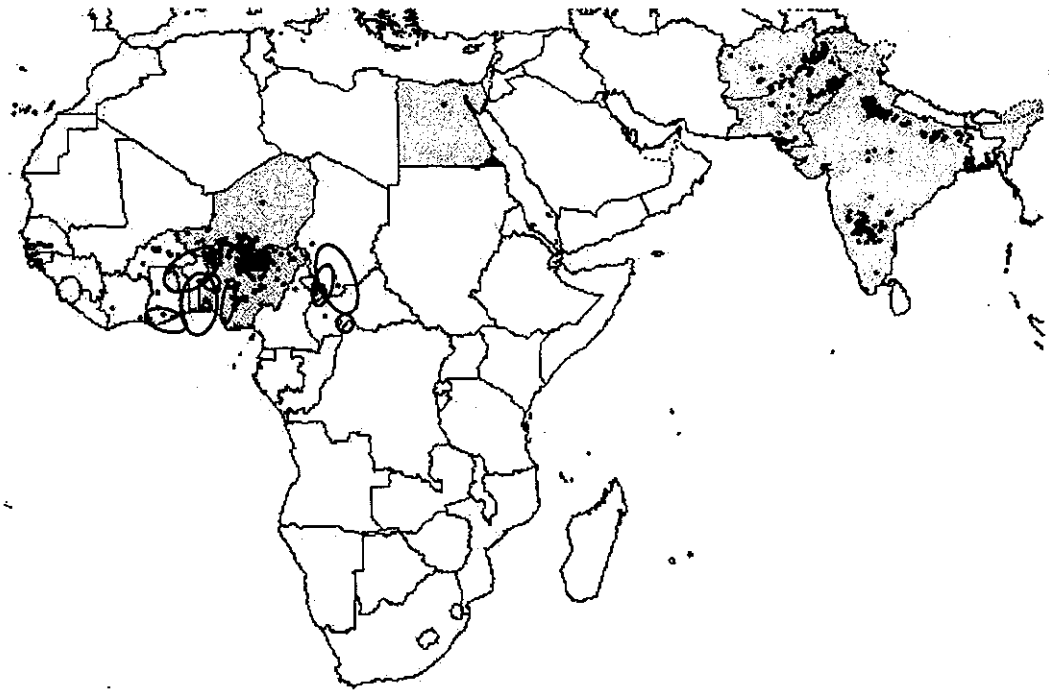

図1 野性株ポリオウイルスの分布(WHO本部提供資料を一部修正)
2003年4月7日～2004年4月6日までの1年間の集計．ひとつのドットが1例のポリオ確定症例を示す．薄い灰色は2003年時点でのポリオ流行国，楕円のかこいは，流行国からの移入によりポリオ再流行が起きている地域を示す．

告されたインドでは，2003年の症例数は225例まで減少し，インドにおけるポリオ根絶は最終局面をむかえている．2003年の時点でポリオ流行国は，インド，パキスタン，アフガニスタン，ナイジェリア，ニジェール，エジプトの6ヵ国であり，確定症例数は全世界で784例となっている(2004年4月現在，**図1**)．WHOは，残された流行地域での野性株ポリオウイルス伝播を2005年までに終息させ，2008年に世界ポリオ根絶宣言を行うことを目標として，集中的なワクチン強化接種キャンペーンを展開している[3]．

Ⅱ．OPV接種継続の問題点

OPVが安価で有効性に優れ接種が容易な，きわめて優れたワクチンであることに異論の余地はないが，世界中のほとんどの国でポリオフリーが達成されている現状では，OPV接種による潜在的なリスクが新たな社会問題を引き起こす可能性がある．ポリオ根絶後にOPV接種を継続する場合に生じるリスクについて，具体的な議論が始められている[4](**表1**)．OPVの継続による社会的なリスクのひとつは，ワクチン接種者および接触者における麻痺(vaccine-associated paralytic poliomyelitis；VAPP)の発生である．VAPPの発生頻度はきわめて低く，日本ではOPV接種者で約440万doseに1症例，2次感染が約580万doseに1症例とされている．国内では，おおむね1年に1例弱のVAPPが発生していることとなる．VAPP発生率は調査地域および調査方法でバラツキが認められるが，世界全体で推定した場合には，新生児コホート100万人に対して，2～4例のVAPPの発生が推定されている[5]．

また，近年世界各地で明らかにされたOPV由来株(vaccine-derived poliovirus；VDPV)の伝播によるポリオ流行は，OPV接種を継続する場合に無視できない社会的リスクをもたらす．VDPVは，ワクチン接種率が低い地域でウイルスが一定期間伝播する間に野性株と同等の病原性を獲得したcirculating VDPV(cVDPV)[6][7]とポリオウイ

表1 ポリオ根絶後にOPVを継続する場合およびIPVを導入する場合の潜在的リスク(文献4 Table 1を要約し和訳した)

ワクチン接種	想定される事例	現状	将来的なリスク
OPV	VAPP	250～500件/年	●OPVを使用する人口に依存する ●世界的なVAPPのリスクは新生児コホート100万人に対し2～4件/年と推定される
	cVDPVの流行	1件/年 (平均10症例/1つの流行)	●流行のリスクは集団免疫レベルと集団における他人との接触頻度に依存する ●低いワクチン接種率，不十分な公衆衛生環境および高い人口密度においてリスクが高まる
	cVDPVの長期間の流行	1例の報告がある (1988～1993年のエジプトにおける2型cVDPVの流行)	●リスクは，おそらく伝播期間に依存する(Sabin → VDPV → cVDPV) ●cVDPVの伝播や流行が検出されない場合あるいはcVDPV発生に適切に対応できない場合，世界の広い範囲へcVDPVが伝播する可能性がある ●cVDPVのリスクは，OPVからIPVへの切り替え，またすべてのワクチン接種停止に関連する
	持続感染者から接触者への感染によるポリオ	これまで報告は無い	●免疫不全を伴うVDPV排泄者(iVDPV)として，これまで19例がWHOへ報告されている ●数例が，野性株ポリオの長期排泄者として報告されている ●伝播のリスクは，持続感染者の発生率，接触者の免疫状態および環境に依存する
	不完全なOPV封じ込めによるOPV由来ウイルスの接触者あるいは集団への流出	これまで報告は無い (OPV接種を停止するまで，OPV接種に由来するウイルスと封じ込め施設からのウイルスを区別するのは不可能)	●すべてが先進国に存在するIPV製造施設と異なり，OPVは現在，ブラジル，中国，エジプト，インド，インドネシア，メキシコ等で製造あるいは出荷されている． ●現在の封じ込め計画は，Sabinウイルスを対象としていない
IPV	不完全なIPV封じ込めによる野性株ポリオウイルスの接触者あるいは集団への流出	封じ込めエリアからのウイルス流出が数例報告されているが，それらによるポリオ流行は報告されていない	●現在すべてのIPV製造施設は，より厳格なバイオセーフティー基準に対応した施設に改良されている ●封じ込め計画では従業員の免疫の維持を要求している ●IPV製造施設を有する工業国はワクチン接種を継続することが期待できるので，リスクは低減される

ルス持続感染者から長期間排泄されるimmunodeficient VDPV(iVDPV)[8]に分けられる．cVDPVによるポリオ流行は，ハイチ/ドミニカ(2000～2001年)，フィリピン(2001年)およびマダガスカル(2002年)での発生が報告されており，頻繁に起きる事例ではないとしても，OPV接種を継続するかぎり一定のリスクが存在する．特に，OPV接種を継続し，かつ，その地域のワクチン接種率が低下した場合にcVDPV流行のリスクが大きくなる．ポリオ根絶後一定期間の後，すべてのポリオワクチン接種を世界的に停止することを想定した場合も，ポリオ感受性人口が増加するため，VDPVによるポリオ流行のリスクが増大する．

Ⅲ．IPVの現状

ポリオ根絶の最終局面では，VAPP，cVDPVおよびiVDPVのリスクを低下させるため，少なくとも可能な国・地域ではできるだけ速やかにIPVを導入する必要がある．特にOPV接種の完全停止を前提とした場合は，VDPVによるポリオ流行の可能性を考慮して，緻密なサーベイランスの下で，ポリオウイルス伝播の完全な終息が確認されるまで，高い集団免疫を維持する必要がある．このためには，IPVの導入が唯一の方策である．

ポリオフリーが確立されている先進国の多くで

はすでに，VAPP のリスクを考慮して，OPV から IPV への変更が着々と進められている．アメリカでは，3年間の IPV/OPV の併用接種期間を経て，2000年からは IPV の4回接種となった．ヨーロッパでは，スウェーデン，フィンランド，フランスなど長年 IPV 接種が行われていた国を含め，多くの国々で IPV 接種が行われている．2003年時点で，IPV のみのポリオワクチン接種を行っている国は，欧米を中心に世界中で22ヵ国，IPV/OPV の併用接種を行っている国が9ヵ国と報告されでいる[4]．日本を含む西太平洋地域では，いまのところほとんどすべての国々で OPV によるワクチン接種が続けられているが，ニュージーランドでは，2002年2月に OPV から IPV への切り替えが行われた[9]．

IPV は OPV と異なり注射によるワクチン接種であり，IPV 単独接種を既存のワクチン接種スケジュールに組込むのは，社会的および金銭的なコストを，増大させることになる．そのため，IPV は他のさまざまな不活化ワクチンと組み合わせた混合ワクチンとして用いるのが合理的である．ジフテリア，破傷風，百日咳の3種混合ワクチン(DPT)＋IPV を基本として，b 型インフルエンザ菌ワクチンあるいは B 型肝炎ワクチン等を加えた混合ワクチンも開発されている．IPV 導入時は，他のワクチン接種スケジュールを考慮し，適切な混合ワクチンおよびワクチン接種スケジュールを選択する必要がある．

Ⅳ．Sabin-IPV

現在実用化されているすべての IPV は，強毒株ポリオウイルスを原料として製造されている．強毒株ポリオウイルスを大量に培養する必要がある IPV 製造施設では，ポリオ根絶後は厳重なウイルス封じ込めが要求される．最低限 Biosafety level-3/polio (BSL-3/polio) の基準を満たす製造施設が必要であり，管理面からも厳重な封じこめを徹底する必要がある[10]．そのため，強毒株の代わりに弱毒 Sabin 株を原料としたユニークな IPV (Sabin-IPV) の開発が，世界的に注目されている．Sabin-IPV 開発は実験室レベルでの報告はあるが[11)12)]，製造可能レベルでの開発は，いまのところ世界で唯一，わが国の日本ポリオ研究所により進められている[13)14)]．Sabin-IPV は，製造施設からのウイルス流出が起きた場合のリスクを大幅に減らすことができるとともに，IPV 製造過程で製造される Sabin ウイルスを，ポリオ根絶後の備蓄ワクチンとして利用できるという利点もある．

Sabin-IPV は，既存の IPV 同様，精製したウイルス粒子をホルマリン処理することにより不活化する．Micro-carrier 法により培養した Vero 細胞を用いて Sabin 1，2および3型ウイルス液をそれぞれ調整し，限外ろ過および DEAE-Sepharose カラムにより，ウイルス精製を行う．37℃，12日間のホルマリン処理によりウイルスを完全に不活化し，1，2および3型の不活化抗原を所定量混合することにより最終製品とする．IPV としての有効性を保証するためには，各血清型に対して適切な抗原性を有することが必要である．強毒株と Sabin 株では，各血清型に対する抗体誘導能が必ずしも同等ではなく，ホルマリン不活化による抗原性の変化も多少異なることから，Sabin-IPV 最終製品の抗原量はラットにおける免疫原性試験に基づいて独自に定められている[13]．ポリオウイルスに対する感染防御に主要な役割を果たしているのは中和抗体であり，Sabin-IPV 接種によって既存の IPV と同等の中和抗体価の上昇が認められれば，既存の IPV と同様の有効性が期待できる．日本ポリオ研究所により実施された臨床試験の結果によると，Sabin-IPV の2回接種により，1，2および3型の各血清型のポリオウイルスに対して，顕著な中和抗体価の上昇が認められている[13]．

Sabin-IPV は，新たに開発された生物製剤として安全性および有効性を中心とした製造承認を受ける必要があり，IPV 単独のワクチンとして，現在製造承認の審査が行われている．Sabin-

IPV の承認までには，今後臨床試験を含む追加データの再提出が予定されており，上市まで，いましばらく時間がかかることが予想される．また，DPT と Sabin-IPV の混合ワクチンについても，数社の国内メーカーにより積極的な開発が進められているが，導入時期は依然不透明である．

V．Polio Endgame とワクチン戦略

現在のレベルのワクチン接種とサーベイランスを，ポリオ根絶後も未来永劫続けていくことは，とくに途上国にとっては，きわめて困難である．OPV 接種を継続しワクチン接種率が低下した場合，VDPV によるポリオ流行のリスクは増加する．このジレンマから抜け出すためには，何らかの形で OPV 接種を停止するというシナリオが支持されており，世界的な OPV 接種停止を前提とした場合はとくに，IPV の導入の可否およびタイミングが重要となる．ポリオ根絶の最終段階での IPV の導入は，VAPP の発生をなくし，VDPV によるポリオ流行のリスクを低下させるための重要な選択枝のひとつである．世界的に見ると，IPV 導入の最大の問題点は，現在 OPV を使用している途上国において，IPV 導入による金銭的および社会的コストの大幅な増大を負担することが，きわめて困難であるという点である．現在でさえ定期接種によるワクチン接種率が低いアジア，アフリカ諸国では，IPV への切り替えにより，ポリオワクチン接種率が大幅に低下する可能性が危惧されている．

ポリオ根絶を間近にひかえ，これまで想定されていなかった，さまざまな困難が明らかにされている．野性株ポリオ根絶後のワクチン戦略についての世界的なコンセンサスは得られていないが，日本はもとより世界的にも，できる限り IPV を導入していくことが，スムーズな OPV 接種停止にとって重要である．そのため，Sabin-IPV 開発に関しても日本国内よりむしろ国際的な注目が大きい．Polio Endgame においては，Sabin-IPV を含めて使用可能なツールの選択肢を多く持つことが重要だと考えられる．日本では，かねてより OPV から IPV への切り替えの必要性が専門家から提言されてきた[15]．残念ながら，Sabin-IPV はいまだに実用化されておらず，現時点で認可された IPV は存在しないため，IPV 導入時期を含めたワクチン切り替えのスケジュールを明示できない状況である．世界的なワクチン戦略と歩調を合わせて，IPV への切り替えを含む中長期的ポリオワクチン戦略を，日本でも早急かつ具体的に策定する必要がある．

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特集

Ⅱ．ワクチン各論 —1．現行のワクチン

ポリオワクチン

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要旨

WHOは，2008年に世界ポリオ根絶宣言を行うことを目標として，残されたポリオ流行地域のワクチン接種キャンペーンを強化している．野生株ポリオ根絶後，世界的に一斉にOPV接種を停止することにより，真のポリオ根絶が達成されるが，ワクチン由来株によるポリオ流行など，さまざまな課題が浮かび上がっている．スムーズなOPV接種停止の実現のため，IPV導入の必要性が高まっている．

Key Words
ポリオウイルス
世界ポリオ根絶計画
不活化ポリオワクチン
経口生ポリオワクチン

はじめに

1988年に世界保健機関（World Health Organization，以下WHOと略す）により提唱された世界ポリオ根絶計画は，当初の予定であった2000年までの根絶宣言は達成できなかったものの着実に成果をあげつつある[1]．WHOは，世界ポリオ根絶宣言の新たな目標を2008年として，アジア，アフリカに残されたポリオ流行地域におけるワクチン接種キャンペーンの徹底，強化をはかっている．

ポリオ根絶計画の主要なツールとなっているのは，安価で安全性に優れ接種が容易である経口生ポリオワクチン（oral poliovirus vaccine，以下OPVと略す）であり，OPVの存在なしには，これまでのポリオ根絶計画の進展は考えられない．ポリオ根絶計画の最終段階においてもOPV接種キャンペーンによるワクチン接種率の向上が根絶の成否を決めるカギである．しかし，いまや世界のほとんどの地域を占めようになったポリオフリーの国々では，OPVによるワクチン由来麻痺（vaccine-associated paralytic poliomyelitis，以下VAPPと略す）のリスクを無視できないため，欧米諸国を中心として，もうひとつのポリオワクチンである不活化ポリオワクチン（inactivated poliovirus vaccine，以下IPVと略す）が導入されている．

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本稿では，ポリオ根絶最終段階になり明らかとなった，さまざまな課題に対して，OPVおよびIPV，2種類のポリオワクチンをどのように使い分けて対処していくべきかについて，世界および日本のポリオワクチンの現状をふまえて考察する．

世界ポリオ根絶計画の現状

2003年時点でのポリオ流行国は，6カ国にまで減少した．とくに2002年に，ウタプラディシュおよびビハール州を中心とした北部で1,600例ものポリオ症例が認められポリオ流行が再燃したインドでは，積極的なワクチン接種キャンペーンの展開により，2003年は225症例（2004年は，5月18日時点で8例）まで減少し，2004年後半における野生株ポリオ流行の終息の可能性が出てきた[2]（**表1**）．パキスタンおよびアフガニスタンでは，インドと異なる固有の1型および3型野生株の伝播が認められている．現時点での世界最大のポリオ流行地はナイジェリアであり，2003年のポリオ確定症例数は355例を数えた．

さらに問題なのは，いったんはポリオ流行が終息した西アフリカのナイジェリア周辺国（チャド，ニジェール，コートジボアールなど）において，ナイジェリアに由来する野生株ウイルスによるポリオ流行が再燃していることである[3]（**表1**）．貧しいイスラム教徒が多く暮らす北部ナイジェリアでは，貧困・治安の悪さ・根強いワクチン接種拒否等々さまざまな要因により，質の高いワクチン接種キャンペーンを展開

表1　野生株ポリオウイルス分離によるポリオ確定症例数

WHO本部による2004年5月18日現在の集計

国名	確定症例数			1/1～5/18の症例数の比較	
	2001	2002	2003	2003	2004
ナイジェリア	56	202	355	32	133
ニジェール	6	3	40	1	12
インド	268	1600	225	77	8
パキスタン	119	90	103	23	12
アフガニスタン	11	10	8	0	2
エジプト	5	7	1	0	1
チャド*	0	0	25	0	6
コートジボアール*	0	0	1	0	5
ブルキナファソ*	0	1	11	0	3
ベニン*	0	0	2	0	2
ボツワナ*	0	0	0	0	1
中央アフリカ*	0	0	1	0	0
カメルーン*	0	0	2	0	0
ガーナ*	0	0	8	1	0
トーゴ*	0	0	1	0	0
レバノン*	0	0	1	1	0
ソマリア	7	3	0	0	0
ザンビア*	3	2	0	0	0

*：輸入株ポリオウイルスによる症例．2003～2004年の西アフリカのポリオ症例のほとんどはナイジェリアに由来する野生株ポリオウイルスによる

灰色で示した6カ国は，いまだ地域固有の分離株の伝播が認められるポリオ流行国

することが困難な状況にある[4]．しかし，似たような問題点を抱えていたインド北部でのポリオ根絶の急速な進展を考えると，ナイジェリアでも，国内外行政機関のポリオ根絶に対するコミットメントを集中的に強化することにより，早急にポリオ根絶の目途をつけることが期待されている．WHOは，2005年中に最後の野生株ポリオ症例が検出され，3年余の確認期間を経て2008年中に世界ポリオ根絶宣言を達成することを目標として，残された流行地域のポリオ根絶を積極的に進めている[5]．

OPV接種継続の問題点

野生株ポリオ根絶達成が間近となり，ポリオ根絶後，いつ，どのようにOPV接種停止を行うかが現実問題となってきている．もちろん，野生株ポリオ根絶後もOPV接種を継続するという選択肢は残されているが，OPV接種を継続した場合，以下にあげるような問題点を考慮せねばならない．

①OPV単独接種を継続する場合，OPV接種者やその接触者にまれにおきるVAPPの発生は不可避である．日本では，VAPPの発生頻度はOPV接種者で440万接種に1例，接触者で580万接種に1例程度であるとされている．**表2**に示したように，野生株ポリオウイルスによるポリオ流行が終息した後日本で散発的に認められるポリオ症例は，ほとんどがVAPP症例であり，OPV単独接種を継続するかぎり，同様の頻度（平均して年間1例弱）でのVAPP発生が予想される[6]．低所得国も含めた世界全体として考えた場合，WHOは新生児コホート100万人に対して，年間2～4例のVAPPが発生すると推定しており，その結果，年間250～500人程度のVAPP患者が発生するとされている[7]．このVAPP発生数は，2003年の野性株ポリオウイルスによる患者数と大差ない．

表2　日本における年次別定型ポリオ患者数（1980～2002年）

平成14年度感染症流行予測調査報告書より一部抜粋

年	症例数		分離ウイルス血清型			
	計	ウイルス分離陽性数	1	2	3	2,3
1980	4	4	1*	1	-	2
1981	4	2	-	1	-	1
1982	0	0	-	-	-	-
1983	2	1	-	1	-	-
1984	0	0	-	-	-	-
1985	1	1	-	1	-	-
1986	1	1	-	-	1	-
1987	0	0	-	-	-	-
1988	0	0	-	-	-	-
1989	0	0	-	-	-	-
1990	0	0	-	-	-	-
1991	1	1	-	-	-	1
1992	2	2	-	-	2	-
1993	3	3	-	2	1	-
1994	1	1	1	-	-	-
1995	0	0	-	-	-	-
1996	0	0	-	-	-	-
1997	0	0	-	-	-	-
1998	2	2	1	-	1	-
1999	0	0	-	-	-	-
2000	1	1	-	-	1	-
2001	0	0	-	-	-	-
2002	0	0	-	-	-	-

*：非ワクチン型ポリオウイルス（他はワクチン型ポリオウイルス）

②OPV接種を継続しながら，一方でその地域のワクチン接種率が低下した場合，ワクチン由来ポリオウイルス（circulating vaccine-derived poliovirus：cVDPV）によるポリオ流行のリスクが高まることが，近年の研究で明らかにされた．cVDPVによるポリオ流行は，2000～2001年のヒスパニオーラ（1型），2001年のフィリピン（1型）および2002年にマダガスカル（2型）での発生が報告されている[8]（図）．さらに，過去の流行株の解析から，1983～1993年のエジプトでのポリオ流行もまた，2型cVDPVによるものであった[9]．ゲノム構造が解析されたcVDPVはすべて，神経毒力復帰に重要であるとされている野生株特有の塩基置換（reversion）を有し