(with a lesion score of <3), it was difficult to make a correct inference for the severe paralysis from the overall lesion scores only. We estimated that the proportion of critical motor neurons was, at most, 1·4 % of susceptible neurons in the lumbar cord (Table 1). Limbs affected by poliomyelitis in humans showed a mean of 40·8 % remaining motor units (McComas *et al.*, 1997). Therefore, a small population of the motor neurons in the lumbar cord seemed to be critical for severe paralysis in TgPVR21 mice.

In summary, we have developed a *trans*-encapsidation system for a PV replicon in 293T cells and analysed the poliomyelitis-like paralysis of TgPVR21 mice induced by the PV replicon. This model would be useful for the analysis of *in vivo* cell death induced by PV infection and for the development of effective therapies for poliomyelitis (Dodd *et al.*, 2005).

ACKNOWLEDGEMENTS

We are grateful to Ms Ayako Harashima for her excellent assistance. We are grateful to Dr Akio Nomoto for his continuous support. We appreciate Drs Koji Sakai and Shigeru Morikawa for kindly providing the pKS435 expression vector and Dr Naokazu Takeda for his generous gift of anti-2C antibody. This study was supported in part by Grants in Aid for the Promotion of Polio Eradication and for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labour and Welfare, Japan.

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JOURNAL OF VIROLOGY, May 2006, p. 4313–4325 Vol. 80, No. 9
0022-538X/06/$08.00+0 doi:10.1128/JVI.80.9.4313–4325.2006

Role of the Alpha/Beta Interferon Response in the Acquisition of Susceptibility to Poliovirus by Kidney Cells in Culture

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Received 27 September 2005/Accepted 3 February 2006

Replication of poliovirus (PV) is restricted to a few sites, including the brain and spinal cord. However, this neurotropism is not conserved in cultured cells. Monkey kidney cells become susceptible to PV infection after cultivation in vitro, and cell lines of monolayer cultures from almost any tissue of primates are susceptible to PV infection. These observations suggest that cellular changes during cultivation are required for acquisition of susceptibility. The molecular basis for the cellular changes during this process is not known. We investigated the relationship between PV susceptibility and interferon (IFN) response in primary cultured kidney and liver cells derived from transgenic mice expressing human PV receptor and in several primate cell lines. Both kidneys and liver in vivo showed rapid IFN response within 6 h postinfection. However, monkey and mouse kidney cells in culture and primate cell lines, which were susceptible to PV, did not show such rapid response or showed no response at all. On the other hand, primary cultured liver cells, which were partially resistant to infection, showed rapid IFN induction. The loss of IFN inducibility in kidney cells was associated with a decrease in expression of IFN-stimulated genes involved in IFN response. Mouse kidney cells pretreated with a small dose of IFN, in turn, restored IFN inducibility and resistance to PV. These results strongly suggest that the cells in culture acquire PV susceptibility during the process of cultivation by losing rapid IFN response that has been normally maintained in extraneural tissues in vivo.

Poliovirus (PV), belonging to the *Picornaviridae*, is the causative agent of poliomyelitis (44). The replication of PV is limited to a few sites, including the brain and spinal cord. Severe pathological lesions are not observed in most extraneural tissues, despite the presence of virus in many tissues during the viremic phase of infection (2, 52). PV was initially isolated by Landsteiner and Popper (32) in 1908 as a transmittable pathogen. In those days, PV could be transferred by inoculating a suspension of the spinal cord of a paralyzed monkey into another, because of the neurotropic nature of PV. In 1949, Enders et al. (8) for the first time succeeded in propagating PV in primary cultured cells from various human embryonic tissues. Later, Dulbecco and Vogt (7) found that monkey kidney developed permissivity to infection after cultivation in vitro and that PV titers could be quantified by plaque assay. PV, therefore, is able to replicate in monolayer cells in primary culture and in cell lines derived from almost any tissue of primates, although PV cannot replicate well in the extraneural tissues in vivo. This new technology for propagating PV in cell cultures in vitro had a great impact on virology and allowed revolutionary progress in PV studies. Attenuated Sabin strains were developed by a number of passages of virulent PV strains in cultured cells (53). Large-scale production of PV vaccines has been done using monkey kidney cells. The molecular mechanisms of PV replication have been studied using cultured cells with strict control of the experimental conditions (49). Although we have benefited greatly from this, the molecular basis for this paradoxical change in susceptibility is still unknown.

The factors that control PV susceptibility have been studied. The research focused on identifying the determinants of tissue and cell tropism. Holland and colleagues thought that susceptibility was determined at the level of virus entry into the cell. They proposed that the PV receptor (PVR) is a major determinant of tissue and cell tropism based on observations that a single round of replication occurred in nonpermissive mouse cells after transfection of PV genomic RNA (20) and that PV was adsorbed by a homogenate of neural tissues (19). Molecular cloning of the human *PVR* gene revealed that PVR is a membrane protein that belongs to the immunoglobulin superfamily (29, 40). Transgenic (tg) mice that carry the human *PVR* gene were produced. PV selectively replicated in the central nervous system (CNS), and the mice showed paralytic disease that resembled human poliomyelitis upon PV infection (31, 51). If Holland's hypothesis were correct, one could expect that PVR would not be expressed in the extraneural tissues at high levels in vivo but would be expressed in the cells after cultivation in vitro. However, PVR mRNA was detected in various tissues of human and PVR-tg mice (43). Furthermore, when Ren and Racaniello (50) investigated the distribution of PVR

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transcripts in the kidneys of tg mice by in situ hybridization, they showed that epithelial cells in the Bowman's capsule, podocytes in the glomerulus, and some of the tubular epithelial cells in the medulla expressed human PVR mRNA at high levels, although these cells were not susceptible to PV in vivo. They also observed that the freshly dispersed kidney cells expressed PVR at the cell surface, as judged by a PV binding assay, but susceptibility developed after cultivation in vitro for 24 h. They concluded that expression of PVR is not sufficient for PV susceptibility and predicted that other factors that might change during the cultivation process are also needed.

Another possibility was that susceptibility was determined at the level of translation initiation of the viral protein. Translation initiation of picornaviruses is controlled by an internal ribosome entry site (IRES) located in the 5′ noncoding region of the genome (45). The PV IRES interacts with canonical translation initiation factors and noncanonical translation initiation factors, IRES *trans*-activating factors (ITAFs), to achieve efficient translation initiation (17). To date, polypyrimidine tract binding protein (PTB) (18, 46), neural cell-specific PTB (27, 35, 37, 48), La autoantigen (38, 39), poly(rC) binding protein 2 (PCBP-2) (1), and upstream of N-ras (Unr) (3) have been identified as ITAFs for PV. There is some evidence for tissue-specific or cell-type-specific translation initiation mediated by the IRES in picornaviruses. Chimeric PVs containing replacements of IRES sequences with corresponding sequences from human rhinovirus type 2 or hepatitis C virus did not propagate in the CNS of PVR-tg mice (14, 63). The foot-and-mouse disease virus IRES was not active in neurons because it required $ITAF_{45}$, which was expressed only in proliferating cells (47). The PV Sabin 3 strain did not grow efficiently in the CNS, in which PTB was not expressed at high levels, and a translation deficit of the Sabin 3 IRES was rescued by increased expression of PTB in the CNS (15). These results may be good examples demonstrating that a lack or shortage of ITAFs resulted in tissue- or cell-specific failure of replication of viruses. If the expression of ITAFs determines susceptibility, we would expect that IRES activity would not be observed in the kidneys in vivo. Kauder and Racaniello (26) reported contradictory results. They constructed a recombinant adenovirus that expressed biscistronic reporter luciferase genes under the control of PV IRESs. They infected mice intravenously with the recombinant adenovirus vector and determined reporter gene expression by luciferase assay. They showed that the PV IRES was active in neural, as well as in extraneural, tissues, including the kidneys. This implies that kidney cells in vivo express sufficient levels of all ITAFs for PV. Furthermore, they did not observe the neural-tissue-specific reduction of translational activity of the attenuated Sabin IRES. Sabin 3 virus and hepatitis C virus/PV recombinant virus propagated efficiently in the CNS of neonatal PVR-tg mice and caused paralysis. They then concluded that PV tropism and attenuation are determined after internal ribosomal entry. Since direct measurement of expression profiles of ITAFs in adult and developing tissues has not been reported, the contribution of the ITAFs to tissue- or cell-specific infection of PV is still controversial (56; see reference 55 for a review).

Finally, we have recently demonstrated that PV tissue tropism is strongly influenced by antiviral activity mediated by alpha/beta interferons (IFN-α/β) (21). PVR-tg mice deficient in the alpha/beta IFN receptor 1 (*Ifnar-1*) gene were produced by crossing PVR-tg mice and *Ifnar* knockout mice (41). Although PV replication sites were restricted to the CNS in the wild-type PVR-tg mice, extensive PV replication was observed in a wider range of tissues in the PVR-tg/*Ifnar* knockout mice. This result suggests that extraneural tissues, such as the liver, spleen, and pancreas, are potentially susceptible to PV infection and that they are normally protected by the IFN response. In the wild-type PVR-tg mice, neural tissues expressed very low levels of IFN-stimulated genes (ISGs) and did not show rapid IFN response upon PV infection. However, extraneural tissues expressed slightly higher levels of ISGs, even in the uninfected mice. They showed sufficient IFN response and were protected from PV infection. From these results, we consider that the difference in IFN responses among the tissues influences differential PV susceptibility.

We hypothesized that the acquisition of PV susceptibility by kidney cells after cultivation may be a consequence of changes in either the IFN response, PVR expression, or ITAF expression. Using primary cultured kidney cells and liver cells from PVR-tg mice, we investigated the relationship between PV susceptibility and expression of the factors mentioned above. Here, we present evidence that the loss of rapid IFN inducibility associated with the decrease in expression of ISGs involved in IFN response during the cultivation process plays an important role in the change in susceptibility of kidney cells.

MATERIALS AND METHODS

Mice. Six-week-old C57BL/6 mice, B6.PVR-Tg21 mice, and B6.PVR-Tg21/*Ifnar* knockout mice, described by Ida-Hosonuma et al. (21), were used in this study. All experiments using mice were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Tokyo Metropolitan Institute for Neuroscience.

Preparation of mouse kidney cells. Mice were deeply anesthetized with diethyl ether and perfused with 10 ml of phosphate-buffered saline and 10 ml of 0.25% trypsin in Eagle's minimum essential medium (MEM). The kidneys were then removed, sliced into small pieces, and incubated in 0.25% trypsin in MEM at 37°C for 20 min. The cells were dispersed in MEM with 10% fetal calf serum (FCS) by pipetting, washed twice, and seeded on a plate at a density of 3×10^4 cells/cm^2. After 1 week, cells grown as a monolayer culture were used for the experiments.

Preparation of mouse liver cells. Mice were deeply anesthetized with diethyl ether and perfused with 20 ml of Hanks' balanced salt solution (HBSS; Sigma) containing 5 mM EGTA and then with 200 ml of HBSS containing 0.5 mg/ml collagenase (Wako Pure Chemical Ltd.) and 0.05 mg/ml trypsin inhibitor. The liver was removed and sliced in Gey's balanced salt solution (Sigma). The dispersed cells were passed through a 100-μm cell strainer (Falcon). The cells were then washed with 20 ml of Gey's balanced salt solution three times. The cells were suspended in William's medium E (Sigma) containing 10% FCS and seeded on a collagen-coated plate at a density of 1.25×10^5 cells/cm^2. The cells were used the following day.

Preparation of African green monkey kidney (AGMK) cells. The kidneys of an African green monkey were removed, sliced into small pieces, and digested with 0.3% trypsin in HBSS at 37°C or at 4°C with continuous stirring for an appropriate period. The dispersed cells were collected and washed twice with HBSS. The cells were suspended in Dulbecco's modified MEM supplemented with 10% FCS, seeded on a plate, and incubated at 37°C. The cells grown as a monolayer culture were passaged, and the cells at passages 3 to 5 were used in the experiments.

Cell lines. An African green monkey kidney cell line, JVK-03 (30), and human embryonic kidney 293 (HEK293) (12), COS-7 (11), HepG2 (28), and HeLa (55) cells were maintained in MEM containing 5% FCS.

Virus. PV type I Mahoney strain derived from infectious cDNA pOM (58) were used. The virus titer was determined by a plaque assay on JVK-03 cells.

Quantitative real-time PCR. Isolation of total RNA, DNase I treatment, and cDNA synthesis were performed as described previously (21). Total human

kidney and liver RNAs were purchased from BD Biosciences. Real-time PCR was performed using an ABI PRISM 7500. The quantification of mouse 18S rRNA, IFN-α mRNA, and human IFN-α mRNA was performed by the SYBR green method using 18S-rRNA-F (5′-GTA ACC CGT TGA ACC CCA TT-3′), 18S-rRNA-R (5′-CCA TCC AAT CGG TAG TAG CG-3′), mIFNα-F (5′-TCC TGA ACC TCT TCA CAT CAA A-3′), mIFNα-R (5′-ACA GGC TTG CAG GTC ATT GAG-3′), hIFN-α-F (5′-GTA CTG CAG AAT CTC TCC TTT CTC CTG-3′), and hIFN-α-R (5′-GTG TCT AGA TCT GAC AAC CTC CCA GGG CAC A-3′) as primers. The mIFNα-F and mIFN-α-R primers amplified all mouse IFN-α mRNA species, and hIFN-α-F and hIFN-α-R primers amplified all human IFN-α mRNA species. The quantification of mouse IFN-β, 2′-5′ oligoadenylate synthetase (OAS), RIG-I, MDA-5, IRF-3, IRF-7, IRF-9, STAT-1, STAT-2, TBK-1, IKKε, IFNAR-1, La, PTB, PCBP-2, UNR, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), PVR mRNAs, and 18S rRNA was performed using Assay-on-Demand PCR probes (Applied Biosystems). The amounts of mRNAs were determined by comparison with the standard templates of cloned cDNAs of known copy number. The expression levels were then normalized to the levels of 18S rRNA. The data are represented as the copy number of mRNA per 10^7 copies of rRNA.

Immunohistochemistry. Detection of PV antigens in infected mice was performed as described previously (31).

Effect of IFN treatment of kidney cells. Mouse kidney cells were treated with recombinant mouse IFN-β (Toray) at concentrations of 0.1 to 100 IU/ml for 6 h. The cells were washed with MEM three times. The IFN-treated cells were infected with PV (at a multiplicity of infection[MOI] of 0.01 to 10) in the presence or absence of anti-mouse IFN-α (17.5 μg/ml) and anti-mouse IFN-β (1.8 μg/ml) monoclonal antibodies (Yamasa Shoyu Co. Ltd, Choshi, Japan). The antibodies at the above concentrations can block 10 and 1,000 IU of IFN-α and IFN-β, respectively. The expression levels of the IFN-α, IFN-β, and Oas1a mRNAs at the indicated times were determined by reverse transcription (RT)-PCR. The PV titer was determined by plaque assay. Mouse IFN activity was measured by the cytopathic effect (CPE) dye uptake method using L929 cells (22, 67). The NIH research reference reagent for mouse IFN-β (National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md.) was used as the standard for unit definition.

RESULTS

PV susceptibility and IFN response in the kidneys and liver in vivo. We previously reported that most of the extraneural tissues in the PVR-tg mice are potentially susceptible to PV infection but that they are normally protected by the IFN response. We investigated PV replication and IFN response in the kidneys and liver in vivo in detail. Mice were intravenously inoculated with 10^7 PFU of PV and were sacrificed daily. Virus titers in the tissues were determined. The PV titers in the kidneys (Fig. 1A) and livers (Fig. 1B) of PVR-tg mice decreased similarly to those of non-tg mice, which suggests that PV cannot replicate well in these tissues of PVR-tg mice. However, the virus titers in the kidneys of the PVR-tg/*Ifnar* knockout mice increased over time (Fig. 1A). The PV titer in the livers of the PVR-tg/*Ifnar* knockout mice remained at approximately 10^7 PFU/g tissue until 3 days postinfection (p.i.), with only a slight decrease observed (Fig. 1B).

We inoculated 10^8 PFU of PV intravenously into PVR-tg and PVR-tg/*Ifnar* knockout mice and detected PV antigens by immunohistochemistry. PV antigens were detected in the glomeruli of the kidneys obtained from the PVR-tg/*Ifnar* knockout mice (Fig. 1D), but not from those of PVR-tg mice (Fig. 1C). This indicates that at least cells in the glomeruli of kidneys in vivo are also potentially susceptible to PV infection. These cells may be exposed most easily to PV circulating in the blood. Consistent with the profile of the PV titer, a few PV antigen-positive cells were detected in the livers of the infected PVR-tg mice (Fig. 1E), but a large number of PV antigen-positive cells were detected in those of the PVR-tg/*Ifnar* knockout mice (Fig. 1F). These results indicate that PV can replicate in these sites if IFN signaling is disrupted.

In order to determine the IFN response in the kidneys and livers of the PVR-tg mice, we examined the expression of IFN-α, IFN-β, and OAS1a mRNAs after intravenous inoculation of PV (10^8 PFU) (Fig. 1G to J). We detected very low levels of IFN-α and IFN-β mRNAs in the uninfected kidneys and in the kidneys at 4 h p.i. However, at 6 h p.i., we observed expression of IFN-α and IFN-β mRNAs and induction of OAS1a mRNA by 14.4-fold compared to the value for the uninfected mice, suggesting that an antiviral state had been established by that time (Fig. 1G and H). In the liver, expression of IFN-α and IFN-β mRNAs and induction of OAS1a mRNA were clearly detected at 6 h p.i. (Fig. 1I and J). It should be noted that both kidneys and liver establish the antiviral state within 6 h p.i. Considering the number of cells in the living animal and the amount of PV inoculated, infection in the tissues must occur at a very low MOI. Therefore, only a small number of cells are infected at the beginning. IFN detected before 6 h p.i. might have been produced by these cells. Although the amounts of IFN mRNAs detected by RT-PCR are small, the IFN mRNA per infected cell must be significant. Since a single round of PV replication in the cells takes approximately 6 h, it is likely that the multiple rounds of PV replication are strongly inhibited in the kidneys and liver due to the IFN response.

PV susceptibility and IFN response in kidney cells in vitro. We prepared primary cultured cells by dispersing the mouse kidneys with trypsin. Approximately 6×10^7 viable cells per mouse, as judged by trypan blue staining, were obtained after trypsinization. The cells were seeded in plates at a density of 3×10^4 cells/cm^2. Approximately 1% of the cells began to grow and formed a monolayer 1 week later. As shown in Fig. 2B, C, and D, we observed at least three kinds of cells with different morphologies. When PV was inoculated into cultured cells at an MOI of 0.001, the PV titer increased rapidly (Fig. 2A), and all types of cells showed CPE by 24 h p.i. (Fig. 2E and F). When cells were infected with PV at an MOI of 10, rounding and detachment of the cells were observed at 6 h p.i. The results indicate that the kidney cells derived from the PVR-tg mice were susceptible to PV, although the kidneys in vivo are not the sites of PV replication. This result further shows that the kidneys of the PVR-tg mice acquire PV susceptibility after cultivation in vitro, which is the same as monkey kidneys. We then prepared kidney cells in culture from PVR-tg/*Ifnar* knockout mice. The increase in the PV titer and the appearance of CPE in the kidney cells derived from PVR-tg/*Ifnar* knockout mice were indistinguishable from those in the kidney cells derived from wild-type PVR-tg mice (Fig. 2A, E, and F). This, in turn, suggests that replication of PV in kidney cells in culture derived from wild-type PVR-tg mice is not affected by the IFN response.

In order to confirm the conclusions mentioned above, we infected kidney cells with PV at an MOI of 10 and measured the IFN response (Fig. 2G and H). We detected very low levels of IFN-α and IFN-β mRNAs in uninfected kidneys and in the kidneys at 4 h p.i. At 6 h p.i., expression of IFN-β mRNA increased slightly. However, we did not detect induction of OAS1a mRNA or IFN activity in the supernatants of the infected cells by a standard IFN assay (data not shown). It is

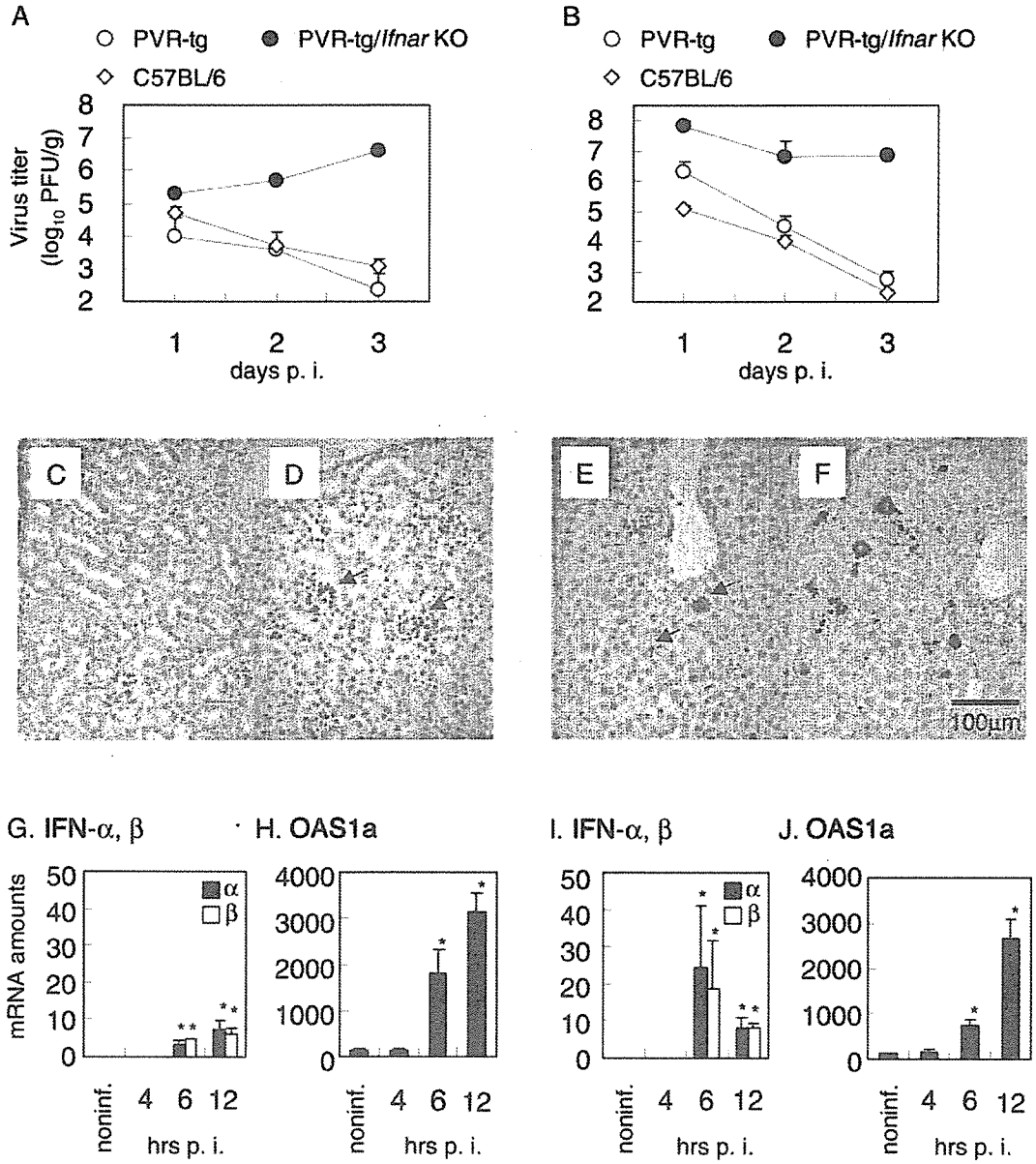


FIG. 1. PV replication and IFN response in vivo. (A and B) PV replication in mouse kidneys (A) and liver (B). C57BL/6 mice, PVR-tg mice, and PVR-tg/*Ifnar* knockout (KO) mice were inoculated intravenously with 10^7 PFU of the Mahoney strain. Mice were sacrificed daily. The mice were perfused with 20 ml of phosphate-buffered saline, and the virus titer in the kidneys was determined by plaque assay. The error bars indicate standard errors of the means (SEM). (C to F) Immunohistochemical detection of PV antigens. PVR-tg mice (C and E) and PVR-tg/*Ifnar* knockout mice (D and F) were intravenously inoculated with 10^8 PFU of PV, and the kidneys and livers were examined 1 day p.i. PV antigens were detected in a few cells on two glomeruli of the kidneys (arrows) of the PVR-tg/*Ifnar* knockout mice (D), but not in the wild-type PVR-tg mice (C). Only a few PV antigen-positive cells were detected in the livers of the PVR-tg mice (E), but a large number of PV antigen-positive cells were detected in the livers of PVR-tg/*Ifnar* knockout mice (F). (G to J) IFN response in vivo. PVR-tg mice were inoculated with 10^8 PFU of PV. The total RNAs of the kidneys or livers of the uninfected (noninf.) and infected mice were isolated. IFN-α/β (G) and OAS1a (H) mRNA levels in the kidneys and IFN-α/β (I) and OAS1a (J) mRNA levels in the liver were determined by quantitative real-time PCR analysis. The copy numbers of the mRNA per 10^7 copies of rRNA are shown. The values represent the averages (± SEM) of three mice. The asterisks indicate a significant difference ($P < 0.05$; Student's t test) in comparison with the uninfected samples.

possible that the amounts of IFN-α and IFN-β mRNAs detected at 6 h p.i. were not sufficient to bring about ISG induction. Alternatively, it is also likely that the IFN mRNA detected at 6 h p.i. and later was not translated or secreted as IFN protein, since the shutoff of host protein synthesis begins to inhibit the translation of IFN mRNAs approximately 2 h after PV infection (9) and the PV 3A protein disrupts normal ER-Golgi trafficking, preventing the secretion of cytokines (4, 6).

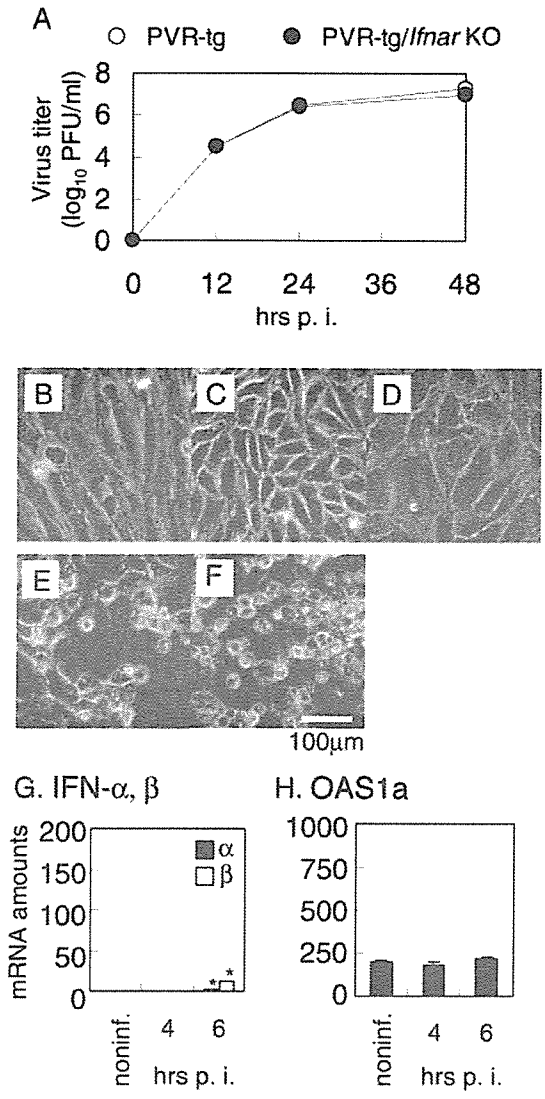


FIG. 2. PV replication and IFN response in cultured kidney cells. (A) Time course of PV titers in cultured kidney cells. Primary cultured kidney cells from PVR-tg mice and PVR-tg/*Ifnar* knockout (KO) mice were infected with PV at an MOI of 0.001. At each time point, the cells were disrupted by three cycles of freezing and thawing, and virus titers were determined by plaque assay. The PV propagation profiles in the two types of cell were indistinguishable. (B to D) Morphology of uninfected primary cultured kidney cells. At least three different kinds of cells were present in the uninfected culture. (E and F) Kidney cells from PVR-tg mice (E) and from PVR-tg/*Ifnar* knockout mice (F) were infected with PV at an MOI of 1.0. CPE was observed at 24 h p.i. (G and H) IFN response in cultured kidney cells from PVR-tg mice. The cells were infected with PV at an MOI of 10. Total RNA of the cells was isolated at the indicated times p.i., and the IFN-α/β (G) and OAS1a (H) mRNA levels were determined by quantitative real-time PCR analysis. The asterisks indicate a significant difference ($P < 0.05$; Student's t test) in comparison with the uninfected (noninf.) samples. The error bars indicate SEM. Note that the vertical scales in panels G and H are different from those in Fig. 1G to J.

Thus, they did not contribute to establishing the antiviral state. We therefore postulate that the acquisition of PV susceptibility in the kidney cells after cultivation is due to the loss of IFN response during the process of cultivation.

PV susceptibility in liver cells in vitro. We then prepared liver cells in culture by collagenase treatment. We obtained 1×10^7 viable liver cells per mouse and plated them onto plastic plates at a density of 1.25×10^5 cells/cm^2. The live cells readily attached to the plates and could be maintained for several days, after which the cells were deformed and died. Based on the morphology, most of the cells were hepatocytes, and other cells were not included in the culture (Fig. 3B and D). We then compared the PV susceptibilities of liver cells derived from PVR-tg mice and of those derived from PVR-tg/*Ifnar* knockout mice. The cells from wild-type PVR-tg mice also acquired susceptibility to PV but, unexpectedly, were not fully susceptible. After PV inoculation at an MOI of 0.001, the titer in the liver cells derived from wild-type PVR-tg mice increased much more slowly than in cultured kidney cells and other cell lines (compare Fig. 2A, 3A, and 4A). Cells with CPE were rarely observed at 24 h p.i. (Fig. 3C). The PV titer continued to increase until 72 h p.i. We stopped observation at that time, since the cells were starting to deteriorate, and therefore, it was difficult to distinguish cellular damage from CPE caused by PV infection. On the other hand, PV replication in the cells from PVR-tg/*Ifnar* knockout mice was as efficient as in the cultured kidney cells and other cell lines (Fig. 2A, 3A, and 4A). The infected cells showed CPE with shrinkage and loss of the nuclear membrane at 24 h p.i. (Fig. 3E). When the cells of PVR-tg mice were infected with PV at an MOI of 50, the titer reached a plateau at 6 h p.i., but it was approximately 10 times lower than that in liver cells of PVR-tg/*Ifnar* knockout mice (data not shown). These results indicate that the liver cells derived from wild-type PVR-tg mice are partially resistant to PV.

We then examined the IFN response in primary cultured liver cells of PVR-tg mice infected with PV at an MOI of 10. Significant levels of IFN-α and IFN-β mRNAs were observed at 4 h p.i., and induction of OAS1a mRNA was observed at 6 h p.i. (Fig. 3F and 3G). It is worth noting that IFN response occurred in liver cells as early as 6 h p.i. These results suggest that the primary cultured liver cells derived from PVR-tg mice still retain the capability for a rapid IFN response, as they did in vivo. Although the amounts of IFN mRNAs detected in the liver cells in culture are larger than those detected in the liver in vivo (Fig. 1I), this does not necessarily mean that IFN inducibility in the cultured cells is higher than that in the liver in vivo. Since the cells were infected with PV at an MOI of 10 in the in vitro experiment, the IFN mRNAs produced per infected cell may be lower than those in vivo. It is possible that this early response contributes to the partial resistance of the liver cells to PV infection.

IFN induction in primate cells after PV infection. The above-mentioned results suggested that primary cultured mouse cells were susceptible to PV infection when they could not establish the antiviral state mediated by rapid IFN response. We therefore investigated whether this is true for the cultured AGMK cells and several other cell lines. AGMK cells at the third to fifth passages from the primary culture were used in the experiments. JVK-03 is a cell line spontaneously immortalized from a primary culture of AGMK cells (30).

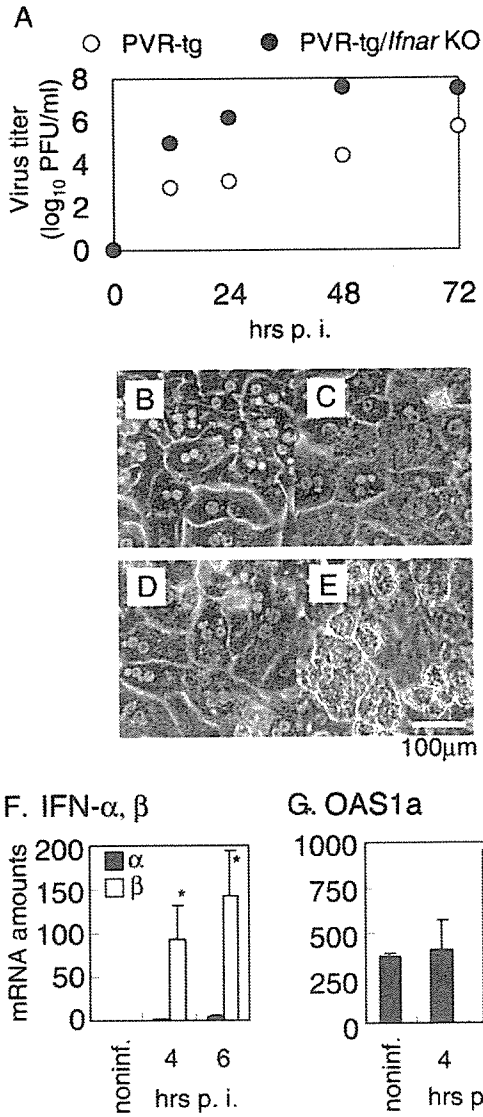


FIG. 3. PV replication and IFN response in cultured liver cells. (A) Time course of PV titers in cultured liver cells. Primary cultured liver cells from PVR-tg mice and PVR-tg/*Ifnar* knockout (KO) mice were infected with PV at an MOI of 0.001. At each time point, PV titers were determined by plaque assay. Note that the titer in the cells from PVR-tg mice increased much more slowly than that in cells from PVR-tg/*Ifnar* knockout mice. (B to E) Morphology of primary cultured liver cells. Liver cells from PVR-tg mice (B and C) and from PVR-tg/*Ifnar* knockout mice (D and E). Uninfected liver cells are monotonic (B and D). The cells were infected with PV at an MOI of 1.0 and observed at 24 h p.i. (C and E). The CPE was rarely observed in the liver cells from PVR-tg mice (C) but was clearly observed in liver cells from PVR-tg/*Ifnar* knockout mice (E). (F and G) IFN response in liver cells from PVR-tg mice. The cells were infected with PV at an MOI of 10. Total RNA of the cells was isolated, and the IFN-α/β (F) and OAS1a (G) mRNA levels were determined by quantitative real-time PCR analysis. The asterisks indicate a significant difference ($P < 0.05$; Student's *t* test) in comparison with the uninfected (noninf.) samples. The SEM are indicated by vertical bars. Note that the vertical scales in panels F and G are different from those in Fig. 1G to 1J.

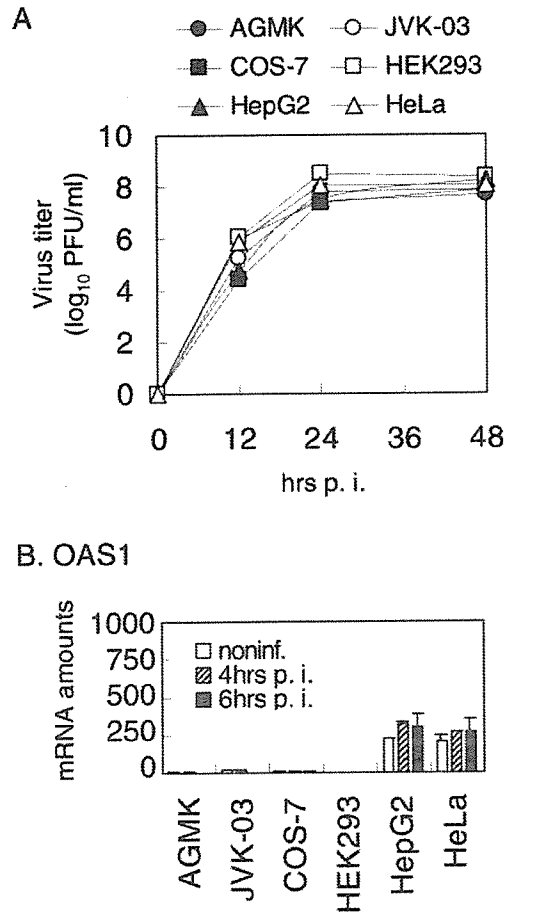


FIG. 4. PV replication and IFN response in cultured cells. (A) Time course of PV titers in AGMK cells and primate cell lines. AGMK cells, JVK-03 cells, COS-7 cells, HEK293 cells, HepG2 cells, and HeLa cells were inoculated with PV at an MOI of 0.001. The cells were disrupted at the indicated time points, and PV titers were determined. (B) Expression of OAS1 mRNA in infected cells. Cells were infected with PV at an MOI of 10. At 4 and 6 h p.i., total RNA was isolated from the cells, and mRNA levels were determined. Note that OAS1 mRNA levels did not change during the course of infection in all cells. The SEM are indicated by vertical bars. noninf., uninfected.

AGMK cells, JVK-03 cells, HEK293 cells, COS-7 cells, HepG2 cells, and HeLa cells were tested for PV propagation and IFN response. All the cells were highly susceptible, and PV infection spread easily when the cells were infected at an MOI of 0.001 (Fig. 4A). Almost no IFN-α or IFN-β mRNA expression was detected within 6 h after PV infection at MOIs of 0.001, 0.1, and 1 (data not shown). An increase in OAS1 mRNA levels was not observed upon PV infection at an MOI of 10 (Fig. 4B) or at the different MOIs (data not shown). The results suggest that these cells do not respond to PV rapidly or do not respond at all.

Reduced expression levels of ISGs in kidney cells. We hypothesized that kidney cells have lost the rapid inducibility of IFNs because of the decrease in the expression levels of genes involved in IFN production. We compared the expression levels of various genes in the kidneys in vivo and kidney cells in

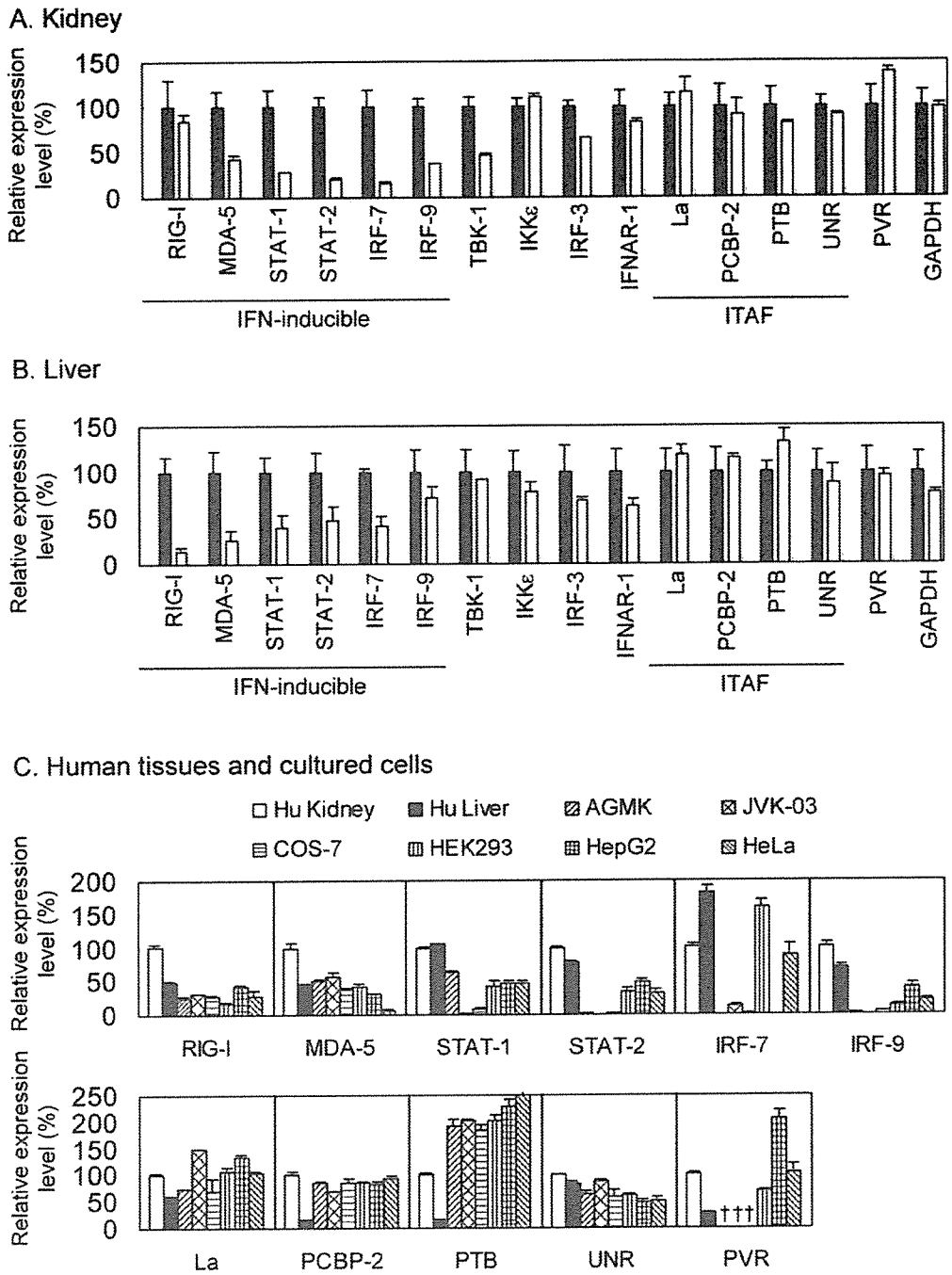
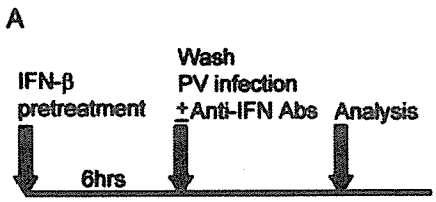


FIG. 5. Comparison of expression levels of ISGs, ITAFs, and PVR mRNAs. (A) Levels of mRNAs in kidney cells in vivo (closed bars) and in vitro (open bars). RNA levels were determined by quantitative real-time PCR. The level of expression of each gene in the kidneys in vivo is represented as 100%. (B) Levels of mRNAs in liver cells in vivo (closed bars) and in vitro (open bars). The level of expression of each gene in the liver in vivo is represented as 100%. (C) mRNA levels involved in the IFN response (top) and mRNA levels of ITAFs and PVR (bottom) in human (Hu) and monkey cells. The level of expression of each gene in the human kidney in vivo is represented as 100%. †, PVR mRNAs in the monkey cells could not be measured by the TaqMan probe for human PVR because of the sequence difference in the probed region. The SEM are indicated by vertical bars.

vitro by RT-PCR. RIG-I, MDA-5, STAT-1, STAT-2, IRF-7, and IRF-9 are involved in the IFN response and are induced by IFNs (25, 33, 36, 54, 65). As shown in Fig. 5A, the levels of MDA-5, STAT-1, STAT-2, IRF-7, and IRF-9 mRNAs were reduced to less than 50% of their original levels after cultivation. TBK-1, IKKε, IRF-3, and IFNAR-1 are also involved in the IFN response (41, 57, 66) but are not IFN inducible. Their expression levels, and that of a housekeeping gene, the

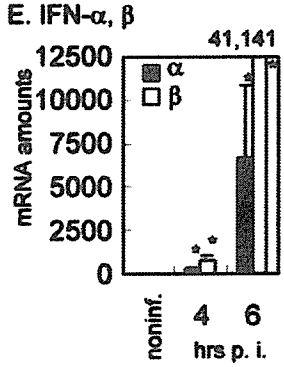


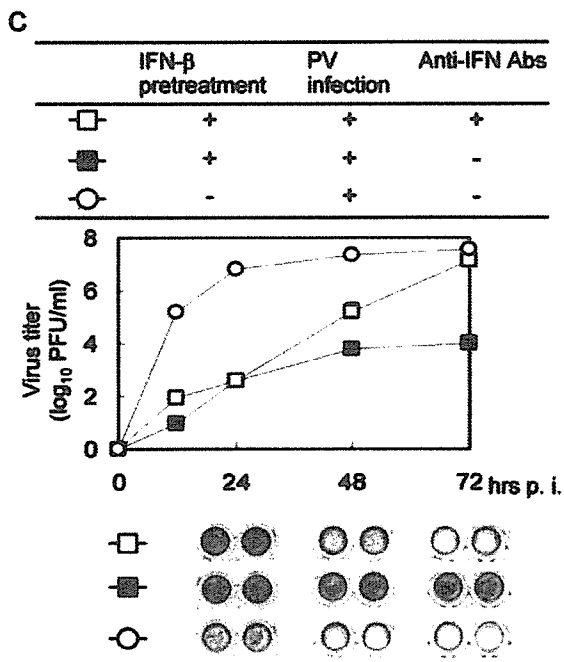
D

	Induction level ± SEM
RIG-I	15.8 ± 2.5
MDA-5	23.9 ± 4.3
STAT-1	16.6 ± 2.7
STAT-2	18.5 ± 1.3
IRF-7	129.1 ± 12.1
IRF-9	15.0 ± 0.7

B

IFN-β pretreatment (IU/ml)		IFN activities in the culture medium ± SEM (IU/ml)
10.0		31.3 ± 20.2
3.2		16.5 ± 6.6
1.0		20.1 ± 0.5
0.3		8.8 ± 0.1
0.1		5.6 ± 0.4
0		<3
noninf.		<3





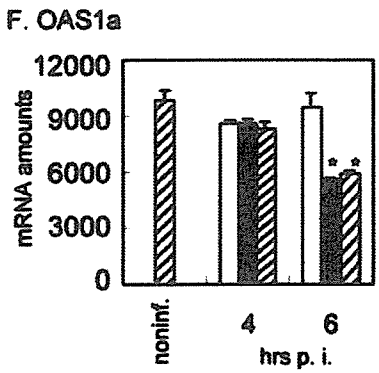


FIG. 6. Effect of priming on the susceptibility of cultured mouse kidney cells. (A) Experimental procedure. Kidney cells were pretreated with IFN-β at the indicated doses for 6 h. The cells were washed with MEM three times and challenged with PV with or without anti-IFN-α and -β antibodies (Abs) to neutralize the effects of newly synthesized IFNs. The cells were tested for survival, PV titers, and IFN response at the indicated time points. (B) Resistance of mouse kidney cells to PV infection after pretreatment with IFN-β. The cells in duplicate were treated with IFN-β at the indicated doses (left column). The cells were then infected with PV at an MOI of 0.1 for 3 days, and surviving cells were visualized by staining them with Amido black 10B. The IFN activities in the culture medium at 72 h after challenge were measured by the CPE dye uptake method (right). Mean values (± SEM) of two wells are indicated. Note that IFNs were induced only in the pretreated cells. noninf., uninfected. (C) Time course of PV replication in pretreated kidney cells. The cells pretreated with 1 IU/ml of IFN-β were infected with PV at an MOI of 0.001 in the presence (+) or absence (−) of anti-IFN antibodies, and the PV titer was determined at each time point. Open circles indicate the PV titer of untreated kidney cells infected at the same MOI. Note that the PV titer did not increase after 48 h p.i. if the anti-IFN antibodies

GAPDH gene, did not show considerable change. Notably, a decrease in the level of IRF-7 after cultivation was most evident in the kidneys. Similarly, in the liver, the expression levels of these IFN-inducible mRNAs also decreased (Fig. 5B). RIG-I, MDA-5, STAT-1, STAT-2, and IRF-7 mRNA levels decreased to less than 50% after cultivation. The decrease in the expression levels of these genes may be responsible for the acquisition of susceptibility.

With regard to the ITAFs and PVR, we directly measured the mRNA levels of PTB, La, PCBP-2, UNR, and PVR and compared the expression levels in the kidneys and livers of PVR-tg mice in vivo and in vitro (Fig. 5A and B). The expression levels of these mRNAs in vivo and in vitro did not differ significantly. In situ hybridization and immunofluorescence studies revealed that PVR mRNA is not expressed in all cells in the kidneys of PVR-tg mice in vivo (23, 50). However, all the primary cultured cells are susceptible to PV infection. We therefore could not exclude the possibility that additional expression of PVR in the kidney cells that had no PVR expression in the kidneys in vivo also occurred during the cultivation process.

In addition, we determined the levels of ISGs involved in the IFN response in primate cells. The human TaqMan probes worked sufficiently in measuring mRNA for these genes of the African green monkey. Since it was not possible to compare the expression levels of ISGs before and after cultivation directly, we used human kidney and liver RNAs that were commercially available as controls. The mRNA levels for each ISG in AGMK cells, JVK-03 cells, COS-7 cells, and HepG2 cells were different from cell to cell. However, in general, they were lower than those of the human kidney and liver in vivo (Fig. 5C). In the HEK293 cells and HeLa cells, IRF-7 mRNA levels were much higher than or similar to those in human kidneys. In these cells, however, the levels of other ISG mRNAs tested were lower than those in the human kidneys. The fact that HEK293 cells and HeLa cells failed to induce rapid IFN response upon PV infection suggested that high-level expression of IRF-7 alone was insufficient for rapid IFN response. Rapid and robust IFN response was not achieved unless a positive-feedback loop of IFN response was formed (60). This idea is supported by the experimental results showing that depletion of the gene products involved in the IFN response using RNA interference or dominant-negative techniques resulted in a decrease in IFN response (5, 64). Our results also suggest that the shortage of one of the ISGs may be rate limiting for the whole IFN response.

Expression levels of ITAF mRNAs in cultured human and monkey cells were also determined. The expression levels of ITAF mRNAs were not greatly different from cell to cell (Fig. 5C). The expression levels of La autoantigen, PCBP-2, and UNR mRNAs were almost the same as those in human kidneys in vivo. The levels of PTB mRNA were approximately twofold higher than those in human kidneys in vivo. However, the absolute values for PTB mRNA levels were the same as those for PTB mRNA levels in mouse kidneys and livers in vivo (4,000 to 10,000 copies/10^7 copies of 18S rRNA). Thus, the PTB levels observed in the cultured cells were not exceptionally high. We therefore consider that the changes in mRNA levels for La, PCBP-2, PTB, and UNR do not contribute to the acquisition of susceptibility to PV infection in these cells in culture. We could not measure the PVR mRNA levels for monkey cells by the TaqMan probe designed for the human PVR gene. In the HEK293, HepG2, and HeLa cells, the PVR mRNA levels were not significantly higher than those in the human kidney or liver. Thus, the acquisition of PV susceptibility is associated with a decrease in ISG expression levels. These results strongly suggest that the kidney cells in culture, as well as many cell lines of primate origin, have lost rapid IFN response due to the loss of factors that regulate this response.

Effect of priming of mouse kidney cells with IFN. We tested if pretreatment of mouse kidney cells with a small amount of IFN reconverted the cells to PV resistance by acquisition of rapid IFN inducibility. The experimental procedures are shown in Fig. 6A. Kidney cells were treated with various concentrations of recombinant mouse IFN-β for 6 h. They were then infected with PV at an MOI of 0.1. The culture medium was removed for IFN assay, and the cells were fixed with 4% paraformaldehyde at 3 days p.i. and stained with Amido black 10B. The cells pretreated with more than 0.3 IU of IFN-β were protected from PV infection (Fig. 6B). Consistent with the protection phenomenon, IFN activity was observed in the culture medium of the protected cells (Fig. 6B). Similarly, cells were protected when they were infected with PV at an MOI of 1 (data not shown). The infected cells had been alive for more than a week on the day uninfected control cells died because of overgrowth. However, the cells were killed by PV when infected at an MOI of 10 (data not shown). The cells pretreated with IFNs became resistant to PV infection, except when they were infected at a very high MOI.

We compared the virus growth in the pretreated (1 IU/ml for 6 h) and untreated kidney cells after PV infection at an MOI of 0.001 (Fig. 6C), and the viabilities of the cells were monitored by Amido black 10B staining (Fig. 6C, bottom). PV replication was greatly inhibited in the pretreated cells compared to the untreated cells. Notably, the increase in PV titer was not observed after 48 to 72 h p.i. (Fig. 6C, middle), despite the presence of many uninfected cells (Fig. 6C, bottom). This indicated that the infection did not spread after 48 h p.i.,

were not added, despite the presence of viable cells in the wells. At the bottom, survival of the cells at indicated time points was displayed by Amido black 10B staining. (D) ISG levels in pretreated cells. Kidney cells were pretreated with 1 IU/ml of IFN-β for 6 h. mRNA levels of each gene relative to the untreated cells are indicated. (E) IFN-α and -β mRNA induction in pretreated cells. The cells were pretreated with 1 IU/ml of IFN-β as described above and infected with PV at an MOI of 10. The total RNA was isolated at the indicated time points, and the IFN-α/β mRNA levels were determined. The asterisks indicate a significant difference ($P < 0.05$; Student's t test) in comparison with the uninfected samples. The error bars indicate SEM. Note that the vertical scale is different from those in Fig. 1G and I, 2G, and 3F and that very large amounts of IFN mRNAs are expressed in the primed cells. (F) OAS1a levels in pretreated cells. The pretreated cells (1 IU/ml of IFN-β) were infected with PV at an MOI of 10 in the absence (open bars) or in the presence (solid bars) of anti-IFN antibodies or were kept uninfected (hatched bars). The asterisks indicate a significant difference ($P < 0.05$; Student's t test) in comparison with the infected cells (open bars) at each time point.

possibly because newly synthesized IFNs accumulated in the culture medium. Pretreated cells were also infected in the presence of anti-IFN antibodies. In the presence of anti-IFN antibodies, the PV titer increased over time, indicating that the PV infection could spread. In parallel with the increase in PV titer, protection was not observed after 48 h p.i. The results suggest that the pretreatment of IFN-β confers rapid IFN inducibility on the cells and that the antiviral state is maintained by the newly produced IFNs. We then determined the mRNA levels of the IFNs and ISGs after pretreatment with 1 IU of IFN-β and subsequent PV infection. As expected, all the ISG mRNA levels increased due to the pretreatment and became higher than the mRNA levels of ISGs in the kidney in vivo (Fig. 6D). Furthermore, induction of IFN-α and IFN-β mRNAs at high levels was detected as early as 4 h p.i. (Fig. 6E). Consistent with this result, IFN activity was detected in the supernatant of PV-infected cells at 6 h p.i. (data not shown). OAS1a mRNA was also induced at high levels by the pretreatment, and the levels were maintained during the infection period (Fig. 6F). OAS1a mRNA levels in the pretreated cells were not maintained unless IFN was continuously supplied. In fact, the levels decreased over time after removal of IFN-β without PV infection or after PV infection in the presence of anti-IFN antibodies (Fig. 6F). This suggests that the expression levels are maintained by newly synthesized IFNs during this period. All these results indicate that loss of rapid IFN response after cultivation in kidney cells is reversible and that primed kidney cells restore rapid IFN inducibility and resistance to PV infection.

DISCUSSION

In this study, we demonstrated that the change in susceptibility of cells during the process of cultivation in vitro is controlled most strongly by the level of IFN response. Kauder and Racaniello and Ren and Racaniello predicted that a factor(s) other than PVR is needed for the acquisition of PV susceptibility after cultivation. They also showed that the PV IRES is functional in the mouse kidney in vivo, suggesting that a factor(s) other than ITAFs is important for the change in susceptibility (26, 50). In the cases of kidney cells and primate cell lines, rapid IFN response is lost in association with a decrease in at least some ISG expression. Although it is obvious that the expression of PVR and ITAFs influences the pathogenicity of PV in vivo in some specific situations, it did not change significantly during the cultivation process, and thus, it may not have a strong influence on this phenomenon.

PV is a highly cytopathogenic virus, and the infected cells are destroyed by lytic replication of the virus. PV has several mechanisms to inhibit IFN action. The 2A protease cleaves eIF-4G, leading to the suppression of cap-dependent protein synthesis (9). Host proteins, including IFNs, cannot be translated after shutoff. This shutoff phenomenon occurs as early as approximately 2 h p.i. in HeLa cells. The 3C protease cleaves the p65-RelA subunit of the NF-κB complex (42). Since the IFN-β gene is also positively regulated by NF-κB through positive regulatory domain II on the IFN-β gene promoter (10, 34, 62), cleavage of p65-RelA suppresses the NF-κB-mediated expression of IFN-β. Cleavage of p65-RelA is observed 3 h p.i. in HeLa cells. The 3A gene product inhibits the trafficking of secreted proteins, including IFNs (4, 6). The PV-infected cells in monolayer cultures either do not produce a large amount of IFNs or do not produce them at all (59) (Fig. 2G), probably due to inhibition by these viral proteins. Because of these anti-IFN effects of PV, the effects of IFNs on susceptibility to PV have not been taken into consideration.

As the inhibition by PV proteins operates within a few hours p.i., host cell defense must operate rapidly. It is known that treatment with a small dose of IFNs can enhance the amount of IFN produced and bring about IFN production at an earlier time in many cell lines. This phenomenon is known as priming (59). Using embryonic fibroblasts, Hata et al., Sato et al., and Taniguchi and Takaoka have shown that cells expressing very low levels of IRF-7 cannot induce a rapid and strong IFN response upon virus infection, while the cells expressing certain levels of IRF-7, possibly together with other ISGs, by a spontaneous weak IFN stimulation are able to form a positive-feedback loop of IFN action and induce a rapid and strong IFN response (Fig. 7A and B) (16, 54, 60). The former and the latter states may correspond to the "unprimed state" and "primed state," respectively. If PV infection occurs in the unprimed cells, the viral proteins can accumulate to the threshold levels quickly, IFN action is strongly inhibited by the viral proteins, and the cells will not be able to establish the antiviral state (Fig. 7A). Conversely, if PV infection occurs in the primed cells, the ISGs have already accumulated above the threshold level before infection, and a rapid and strong IFN response will occur, strengthening the cellular antiviral state (Fig. 7B). Since both expression of viral proteins after PV infection and expression of ISG induction by IFNs occur rapidly, which of them gains the initiative first is very important in determining susceptibility. The cells must be primed to produce IFNs in response to PV infection (Fig. 6) (24). From this point of view, "primed or not primed" is a very important factor in determining the fate of the cells and tissues.

We previously showed that the cells in some extraneural tissues are normally protected by the IFN response (21). The cells in the extraneural tissues in vivo expressed ISGs to a certain degree before PV infection. Expression of OASs and PKR may inhibit viral replication. In addition, ISGs that are involved in IFN production, like RIG-I and IRF-7, support a rapid and strong IFN response. Therefore, it is possible to consider that cells in the extraneural tissues in vivo are normally in the "primed state." Indeed, constitutive expression of IFNs under physiological conditions is known in humans (13, 61). It is likely that the cells in the extraneural tissues may be exposed to the constitutive IFNs. This weak IFN signal may be important for maintaining the basal ISG levels (60). On the other hand, down-regulation of ISG expression in cultured cells occurred during the process of cultivation (Fig. 5). The acquisition of susceptibility was associated with this decrease in expression of ISGs. Priming of the kidney cells with a small amount of IFN increased the basal ISG levels (Fig. 6C). This, in turn, restored rapid IFN response and resistance to PV (Fig. 6). The results suggest that cultured cells in vitro do not receive constitutive IFNs or do not produce constitutive IFNs by themselves. Therefore, we consider that the acquisition of PV susceptibility by cells in vitro is due to the transition of the cells from the "primed state" to the "unprimed state."

PV exhibits a paradoxical tropism. The replication sites of

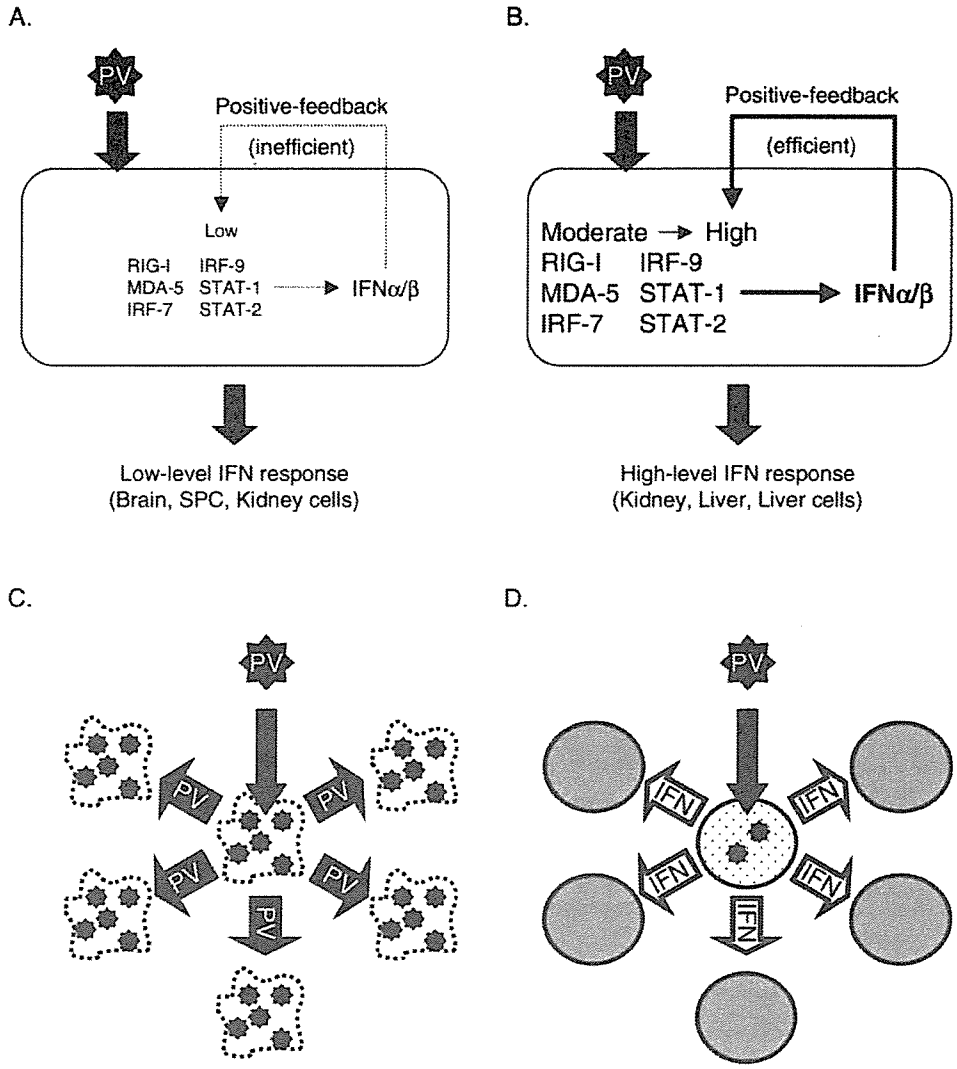


FIG. 7. Schematic diagram of PV susceptibility as controlled by IFN response. (A and B) Difference between IFN responses in unprimed and primed cells. In the unprimed cells (A), ISGs are expressed at very low levels. Upon PV infection, PV can replicate without antiviral action by the cell. The accumulated viral proteins inhibit IFN action, and ISGs cannot be expressed at sufficient levels to form a positive-feedback loop of IFN action. Thus, only a poor IFN response occurs. In the primed cells (B), ISGs are expressed before PV infection and they contribute to rapid induction of IFN by forming a positive-feedback loop. Induced ISGs further strengthen the antiviral state. (C and D) PV replication in the unprimed (susceptible) tissue or cells in culture and in the primed (nonsusceptible) tissue or cells in culture. In the susceptible tissues or cell cultures (C), PV can replicate efficiently in the initial infected cells (center) and spread easily to the surrounding cells. Pathological lesions are produced when this chain of events occurs. In the nonsusceptible tissue or cell cultures (D), a small number of infected cells (center), which have been primed, act as victims to produce IFN rapidly. The infected cells supply IFN to the surrounding cells before they release a large amount of progeny virus. The antiviral state in the surrounding cells (gray) is strengthened by the IFN. The chain of infection does not occur in the primed tissues and cells.

PV in vivo are restricted to a few tissues, including the CNS. However, PV is able to propagate in monolayer cultures derived from almost any tissue of primates. By taking the IFN response of the cells and tissues into consideration, it is now possible to explain this situation. The behavior of the cells and tissues after PV infection is largely dependent on the expression levels of ISGs, namely, the degree of priming. Infection in the tissues occurs at a very low MOI in natural infection in humans and experimental infection in tg mice in vivo. Therefore, initial infection in the tissues occurs in only a small number of cells (Fig. 1B). In the tissues where the cells are primed, these small numbers of infected cells become victims to produce IFNs rapidly (Fig. 1G and H). PV cannot spread easily, and severe pathological lesions are not produced, since the surrounding cells receive IFNs and strengthen the antiviral state before they are exposed to PV (Fig. 7D). Thus, the IFN response prevents the chain of events leading to massive infection. This was also true for the primed kidney cells in vitro (Fig. 6). On the other hand, in susceptible tissues, PV will spread from the initial infectious center to surrounding cells

because the IFN response is not sufficient (Fig. 7C). Similarly, PV can spread very easily in cultured cells because they are not in the primed state (Fig. 2). Therefore, the IFN response has a strong influence on the susceptibility of the cells both in vivo and in vitro. The paradox is now elucidated, since both tissue and cell specificities of PV infection are regulated, at least in part, by the same mechanism mediated by the IFN response.

ACKNOWLEDGMENTS

We thank Akio Nomoto, Atsushi Kato, Sou Hashizume, and Masayoshi Kohase for helpful discussions and Takaji Wakita for advice in preparing primary liver culture.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology and by a Grant-in-Aid for Research on Emerging and Reemerging Infectious Diseases from the Japanese Ministry of Health, Labor and Welfare.

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ウイルスは現在まで分離されていない。

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ポリオワクチン株ウイルスの家族内感染――静岡県

2005年5月末日，9カ月児が初めてポリオ予防接種を受け，その後下痢が続き，便の処理を行った40代父親（ポリオワクチン接種歴不明）が，2005年6月初めに，発熱（39℃），全身倦怠感の臨床症状を呈し，県内の医療機関を受診した。

2005年6月中旬に，ポリオ（急性灰白髄炎）の疑いで検体（9カ月児から糞便，父親からは糞便と咽頭ぬぐい液）が採取されたので，Vero, RD-18およびHEp-2細胞を用いてウイルス分離，ウイルス同定，およびPCR-RFLP法を用いたポリオウイルスの型内株鑑別を試みた。

糞便については3,000rpm 20分間遠心後，上清を採取し，さらに10,000rpm 20分間冷却遠心した上清，咽頭ぬぐい液は3,000rpm 20分間遠心した上清を試料とし，3種類の細胞に接種したところ，9カ月児の糞便，父親の咽頭ぬぐい液からウイルスが分離され，ウイルス同定検査の結果，ポリオウイルス1型であることが確認された。また，分離されたポリオウイルスがワクチン株か野生株かの鑑別を，VP3/VP1を標的としたPCR-RFLP法により実施したところ，*Dde*Iで360・120bp，*Hae*IIIで229・140・111bp，*Hpa*IIで278・202bpに切断され，いずれもワクチン株であるSabin 1型と判定された。

また，Sabin株特異的モノクローナル抗体を用いた鑑別試験でもワクチン株と同定された。

本事例は，麻痺等後遺症を残すことなく完全に回復したと報告されているが，ポリオワクチン接種児からの家族内感染が推定され，ポリオワクチン接種後は十分な注意が必要と思われる。

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＜外国情報＞

風疹と先天性風疹症候群の排除（elimination），1969～2004――米国

2004年10月，CDCは，米国における風疹と先天性風疹症候群（congenital rubella syndrome; CRS）の排除（elimination）に向けた進行状況を評価するために，公衆衛生，感染症，予防接種の各分野で国際的権威を有する専門家による，独自の委員会を招集した。1969年に風疹ワクチンが認可されてから，風疹，CRSの実質的な減少が認められた。米国内における風疹の常在性（endemic）の流行がなくなったことは，以下の最近のデータに裏付けられている。1）2001年以降，風疹の報告数は毎年25例以下，2）学童期の小児においては少なくとも95％の接種率，3）推定で人口の91％が免疫を保有，4）風疹の集団発生を探知できる適切なサーベイランスの実施，5）ウイルスの遺伝子型が，世界の他地域に由来するウイルスの遺伝子型と一致。これらのデータから，委員会のメンバーは満場一致で，風疹はもはや米国の常在性疾患ではないと結論付けた。

1962～1965年の世界的な風疹流行の際には，米国内における風疹患者は約1,250万例に上った。結果として，2,000例の脳炎，11,250例の死亡，2,100例の新生児死亡，そして，20,000例のCRSを記録した。その経済的打撃は，米国内だけで約15億ドルにも達すると推定された。この大規模な流行により，風疹ワクチンの開発が促進され，このワクチンを利用した予防接種政策の必要性が強く促された。

ワクチン導入前，風疹の罹患率は9歳以下の小児が最も高かった。1969年の風疹ワクチン導入時には，1歳～思春期までの小児に対する1回接種を実施した。学童期の小児における接種率を早急に上げるため，特に学校で，大規模なキャンペーンが企画実施された。風疹患者の報告数は，1969年の57,686例から1976年の12,491例まで，78％減少した。同時に，CRS報告数も1970年の68例から1976年の23例に減少した。1970年代後半までに，風疹患者の報告数は減少し続けたが，一方で，高校や大学，軍，医療現場などで，年長児や青壮年を中心に集団発生が起き，風疹の罹患率は青壮年で最も高くなった。CRSの報告数も増加し，1976年の23例から，1979年の57例に上った。当時の血清疫学調査によれば，成人の10～20％が風疹感受性者として蓄積していることが推測された。

このことを受け，1978年，Advisory Committee on Immunization Practice（ACIP）は，小児に加えて，感受性のある思春期後の女性と，軍関係者，大学生，職場で感染の可能性がある者も接種の対象として推奨した。小児におけるワクチン予防可能疾患のすべてにつき，小児期の接種率を90％以上に引き上げようとす

微生物学

経口生ポリオワクチンと薬剤の併用禁忌

Q 経口生ポリオワクチンと副腎皮質ステロイド薬、免疫抑制薬とは併用禁忌となっている。併用禁忌とすべき投与間隔が日本ポリオ研究所作成の文献には記載されておらず、不明である。どの程度を考えたらよいのか。ワクチンの初回、2回目投与別に。（徳島県　K）

A 経口生ポリオワクチン（oral polio vaccine：OPV）は、3種類の血清型の弱毒化ポリオウイルス（Sabin Ⅰ、Ⅱ、Ⅲ株）を混合したワクチンで、日本では1960年代初頭より、ポリオの予防接種に用いられている。OPVは、安全性および有効性に優れたワクチンとして日本におけるポリオ流行の制圧に寄与し、また世界ポリオ根絶計画の達成のために、現在も世界の多くの地域で用いられている。

OPV接種後、弱毒化ポリオウイルスは腸管で増殖し、腸管免疫および血中中和抗体を誘導することによりポリオウイルスに対する免疫を付与し、ポリオ発症を予防する。弱毒化ポリオウイルスは腸管で一定期間増殖し、その期間糞便中にポリオウイルスが排出されるが、多くの健常児ではOPV接種後2カ月程度で糞便中からポリオウイルスは検出されなくなる。RNAウイルスであるポリオウイルスは増殖過程で変異を蓄積しやすい性質（genetic instability）を有するため、腸管でのウイルス増殖の過程で、弱毒化ポリオウイルスと比較して病原性が増加した病原性復帰変異株の割合が増加する。健常児でも病原性復帰変異株が出現する場合があるが、腸管でのウイルス増殖が短期間で終息するため、ワクチン接種者および接触者に対する重篤な副作用（ワクチン由来麻痺）のリスクはきわめて小さい。

一方、免疫不全患者の場合、OPV接種後長期間、腸管でのウイルス増殖が維持される場合があり、ウイルスの病原性復帰およびポリオウイルスに対する免疫不全により、ワクチン由来麻痺を発症するリスクが健常児に比べて大きい。これまで報告されている免疫不全によるワクチン由来麻痺症例の多くは先天性の免疫不全患者であり[1]、過去に免疫不全の診断がなされている者、および近親者に先天性免疫不全患者がいる者は「接種要注意者」に区分されている[2]。

免疫低下をもたらす可能性のある治療（ステロイド大量療法、放射性治療、抗癌剤の使用等）を受けている場合も、ワクチン由来麻痺のリスクを増加させる可能性があるため、治療中止後6カ月以内はOPV接種を行わないことが推奨されているが[3]、少量および短期のステロイド使用はこの限りではない[4]。同様の理由から、OPV接種後、腸管でポリオウイルスが増殖している期間は、治療の必要性にもよるが、免疫低下をもたらす可能性のある治療を控えるべきであると考えられる。腸管でのポリオウイルス増殖期間は個体差が大きく一様ではないが、通常2～6週間程度と報告されている[5]。

OPV接種後3カ月以上経過した場合、多くのワクチン接種児では、糞便中のポリオウイルスは検出されずワクチン由来麻痺のリスクは小さい。OPV初回接種後は1型および2型ポリオウイルスの増殖効率がよく、2回目接種後は3型の増殖効率がよい傾向があり、2回目接種後は、3型ポリオウイルスが高頻度に検出される[6]。2回目接種後のウイルス排出期間についてのまとまった報告はないため、初回接種と同様、OPV接種後3カ月が免疫低下をもたらす可能性のある治療開始の目安となる。

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◆◆◆回　答◆◆◆

国立感染症研究所ウイルス第2部
第2室室長　清水博之

Vol. 22, No. 9, 2006 化学療法の領域

特集 ワクチンの今日的問題点

4. ポリオワクチン

清水 博之[*1] 武田 直和[*2]

世界ポリオ根絶計画は，徹底したOPV接種によりこれまでに大きな成果を挙げている。しかし，根絶の最終段階では有効性および安全性の観点からtrivalent OPVに替わる新たなワクチン戦略が必要とされている。現在，インド等，残された野生株ポリオ流行地では効果的な集団免疫の誘導のためmonovalent OPVの導入が進められており，一方ポリオフリーの地域では，ワクチン由来麻痺およびワクチン由来株によるポリオ流行のリスクを低下させるため，OPVからIPVへの変更が行われている。日本でもSabin株に由来するIPV抗原を含む混合ワクチンの開発が進められている。

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／弱毒化経口ポリオワクチン

Ⅰ はじめに

世界保健機関（World Health Organization：WHO）を中心として進められている世界ポリオ根絶計画は，現在大きな岐路にさしかかっている。経口生ポリオウイルスワクチン（oral poliovirus vaccine：OPV）接種の徹底により，地域固有の野生株ポリオウイルス流行地域は2006年現在4カ国（インド，パキスタン，アフガニスタン，ナイジェリア）にまで減少したが，世界全体のポリオ症例数の減少はここ数年顕著でない[1]。その大きな原因は，ポリオ流行国に由来する野生株ウイルスによるポリオ再流行で，2002～2006年にかけて，いったんポリオフリーを達成した多くの地域でポリオ流行が再燃した[2]。また，ワクチン由来ポリオウイルス（vaccine-derived poliovirus：VDPV）に由来するポリオ流行が，2000年以降世界各地で報告されており[3]，これまでと同様の戦略でポリオ根絶計画を進めることについて疑問視する意見もある[4]。ポリオワクチンの諸課題およびワクチン戦略は，ポリオ根絶計画の進展と密接に関連している。そのため，本稿ではまずポリオ根絶計画の現状を概説したのち，ポリオワクチンに関する現在および将来的な諸課題について解説する。

Ⅱ 世界ポリオ根絶計画の現状

1988年にWHOにより世界ポリオ根絶計画が提唱されて以来，ポリオ症例数および流行地域は着実に減少し，2006年時点におけるポリオ流行国は4カ国となった。一方，ポリオ症例数の推移から見ると，ポリオ根絶計画はここ数年必ずしも順調に進捗しているとは言いがたい（表1）。残されたポリオ流行国であるインド，パキスタン，アフガニスタン，ナイジェリアはそれぞれ固有の地域問題を有しており，近い将来ポリオフリーを達成できるか予断を許さない。特にナイジェリア北部のポリオ流行は深刻で[5]，2006年上半期は前年同期の3倍余のポリオ症例数が報告されており，1型だけでなく3型ポリオウイルス伝播も継続している（2006年5月末現在，http://www.polioeradication.org/）。

Polio vaccine

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表1　各国のポリオ確定症例数の推移（WHOによる集計）

国	年次別野生株ウイルスによるポリオ症例数							地域固有のポリオ流行国／ポリオ輸入国（由来）**	
	2000	2001	2002	2003	2004	2005	2006*		
ナイジェリア	28	56	202	355	782	802	436		流行国
インド	265	268	1,600	225	134	66	45		流行国
パキスタン	199	119	90	103	53	28	4		流行国
アフガニスタン	27	11	10	8	4	9	11		流行国
ニジェール	2	6	3	40	25	10	3		流行国（2004年まで）
ブルキナファソ	0	0	1	11	9	0	0	輸入	ナイジェリア
レバノン	0	0	0	1	0	0	0	輸入	インド
ガーナ	1	0	0	8	0	0	0	輸入	ブルキナファソ
トーゴ	0	0	0	1	0	0	0	輸入	ガーナ
チャド	4	0	0	25	24	2	0	輸入	ナイジェリア
カメルーン	0	0	0	2	13	1	0	輸入	ナイジェリア等
ベニン	1	0	0	2	6	0	0	輸入	ナイジェリア
中央アフリカ	3	0	0	1	30	0	0	輸入	チャド
コートジボアール	1	0	0	1	17	0	0	輸入	ブルキナファソ
ボツワナ	0	0	0	0	1	0	0	輸入	ナイジェリア
マリ	0	0	0	0	19	3	0	輸入	ナイジェリア等
ギニア	0	0	0	0	7	0	0	輸入	コートジボアール
スーダン	4	1	0	0	127	27	0	輸入	チャド
サウジアラビア	0	0	0	0	2	0	0	輸入	スーダン
エチオピア	3	1	0	0	1	22	3	輸入	スーダン
イエメン	0	0	0	0	0	478	1	輸入	スーダン
インドネシア	0	0	0	0	0	303	2	輸入	サウジアラビア
エリトリア	0	0	0	0	0	1	0	輸入	スーダン
アンゴラ	55	1	0	0	0	10	0	輸入	インド
ソマリア	46	7	3	0	0	185	25	輸入	イエメン
ネパール	4	0	0	0	0	4	1	輸入	インド
コンゴ民主共和国	28	0	0	0	0	0	2		輸入？

* 2006年の症例数は，6月13日時点での集計

** 塩基配列の相同性により推定した輸入ポリオウイルスの由来国（文献2より引用）

さらに，近年大きな問題となっているのは，2002～2006年にかけて世界各地で報告された輸入野生株ポリオウイルスによるポリオ再流行の発生である。主としてナイジェリアに由来する1型野生株ポリオウイルスの伝播により，アフリカおよびアジアの多くの国々でポリオ患者の発生が認められ，スーダン，ソマリア，イエメン，インドネシアでは大規模なポリオ再流行を引き起こした

(表1)[2]。これらの地域でのポリオ再流行は，いったんポリオフリーを達成した多くの地域においてワクチン接種率が低下していることを示しており，世界ポリオ根絶の成否に関わる大きな問題を提起している。大きな流行となってはいないが，2006年にはバングラディシュ，ネパール，ミャンマー，シンガポール等で野生株ポリオ症例が報告され，野生株によるポリオ流行のリスクはポリオフリーを達成した地域を含めて継続している。2005年にポリオ再流行が発生したインドネシアでは，1型野生株ポリオウイルスの広範囲の伝播と同時期に，限局した地域(Madura島)において1型VDPVによる大規模なポリオ流行が発生しており，野生株ポリオウイルスのみならずVDPVによるポリオ流行もまたポリオ根絶最終段階における大きなリスク要因であることが改めて示された(http://w3.whosea.org/EN/Section1226.asp)。

Ⅲ　OPVとIPV

OPVおよび不活化ポリオワクチン(inactivated poliovirus vaccine：IPV)は，50年余の使用経験により確固たる実績を有する安全性および有効性に優れたポリオワクチンである。安価で接種しやすいという利点から，途上国におけるポリオコントロールの標準的手法であるmass immunizationに適しているため，OPVは現在も世界ポリオ根絶計画の主要なツールとして用いられている。一方，世界のほとんどを占めるポリオフリーの地域では，OPVによるワクチン由来麻痺(vaccine-associated paralytic poliomyelitis：VAPP)のリスクを無視できないため，IPVの導入が進められている。欧米先進国の多くが現在IPVを使用しており，日本でもIPVの早期導入が強く望まれている[6]。ポリオ根絶後，OPV接種停止前後に想定されるOPVに由来するポリオ流行のリスクを最小限とするためにも，可能な国・地域ではIPV導入が必要とされており，日本もその例外ではない[7]。

Ⅳ　tOPVとmOPV

インドやパキスタン等，残されたポリオ流行国では，頻回のキャンペーンにより多くのOPV接種歴を有する子供がしばしばポリオに罹患することが知られている。衛生・栄養状態，常在する非ポリオエンテロウイルス等他の腸管ウイルス感染，OPVのコールドチェーン，虚偽報告や記録漏れ等々，様々な要因がOPV頻回投与後のポリオ発症に関与する可能性が指摘されている[8]。3種類の血清型の弱毒化ポリオウイルスを抗原として含むtrivalent OPV (tOPV)は有効性，安全性および価格の面から優れたワクチンであることは，世界のほとんどの地域においてtOPV接種の徹底によりポリオ根絶が達成されたという実績からも明らかである。しかし，特定の血清型のポリオウイルスに対する効率的な免疫誘導の観点から，単一の血清型のポリオウイルス抗原を含むmonovalent OPV (mOPV)の導入が進められている[9]。1997年のインドの症例を最後に2型野生株はすでに地球上から一掃されており，1型および3型それぞれの血清型のmOPVを野生株ポリオウイルス流行地に効果的に導入することにより，残された野生株の伝播を遮断することが期待されている。mOPVおよびtOPVの集団レベルの有効性を比較評価するのは困難であるが，1型mOPV初回接種後の抗体保有率は，熱帯地方においても80%以上に達するとの報告がある[10]。1型および3型mOPVの有効性がインド等残されたポリオ流行地で実証されるか，mOPV導入後のポリオ発生動向の推移について注目が集まっている。

1型mOPV導入により，ポリオフリー地域においてもVAPPおよびVDVP発生のリスクを低下させることができると考えられ，今後のポリオワクチン戦略において検討すべきオプションのひとつである。しかし，3型ポリオウイルス(野生株およびVDPV)伝播の可能性，ワクチンメーカーからのmOPVの安定供給，および各国におけるmOPVのライセンス取得など，導入にあたっての課題も少なくない。

Ⅴ　VDPVによるポリオ流行のリスク

2000年以降，野生株ポリオフリーを維持している多くの地域でcirculating VDPV(cVDPV)によるポリオ流行が報告されている。1997年のカンボジアの症例を最後に野生株ポリオウイルス伝播が終息した西太平洋地域においても，2001年のフィ