Evaluation of Immunogenicity and Protective Properties of Inactivated Poliovirus Vaccines: A New Surrogate Method for Predicting Vaccine Efficacy

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An assay for the evaluation of protective properties of inactivated poliovirus vaccines (IPVs) in transgenic (Tg) mice susceptible to poliovirus has been developed and optimized for type 2 IPV. This method was used to compare the immunogenicity and protective properties of experimental IPV produced from the attenuated Sabin strain (sIPV) with those of conventional IPV (cIPV) produced from the wild-type (wt) poliovirus MEF-1 strain. Modified enzyme-linked immunosorbent assays (ELISAs) were used to measure immune response in serum and saliva samples from test mice. Tg mice were vaccinated and were challenged either with wt poliovirus or virulent poliovirus derived from the vaccine strain. Compared with cIPV, sIPV induced lower levels of antibodies and did not completely protect mice against challenge with wt virus but did protect mice against challenge with the virulent vaccine-derived strain. This may be due to an 18% nucleotide difference between the MEF-1 and Sabin 2 strains, resulting in 72 amino acid substitutions and leading to antigenic dissimilarity. Immunological properties of both strains, revealed by cross-neutralization tests and ELISAs, confirmed that MEF-1 possesses broader immunogenicity than does Sabin 2. This animal model may be used for the assessment of new IPVs and of combination vaccines containing an IPV component.

The worldwide campaign to eradicate poliomyelitis initiated at the World Health Assembly in 1988 led to a dramatic decrease in poliovirus morbidity across the world and may result in complete eradication of the disease in the coming years [1, 2]. This profound achievement will cause changes in the polio immunization strategy [3] and will require stricter containment measures for wild-type (wt) poliovirus stocks [4]. However, even strict security measures cannot completely eliminate the risk of accidental release of wt poliovirus into the environment. Some vaccine manufacturers started development of IPVs from attenuated Sabin strains (sIPVs), and, recently, promising results have been reported [5]. To characterize a new sIPV, it is important to compare its immunogenic and protective properties to the conventional IPVs (cIPVs) prepared from wt polioviruses. Doi and coworkers at...
the Japanese Poliomyelitis Research Institute (JPRI) reported that, immunogenically, the sIPV of type 1 was more effective than, the sIPV of type 3 was comparable to, and the sIPV of type 2 was less effective than their respective cIPVs, as tested in rats [5]. The ultimate measure of vaccine efficacy can be obtained only in clinical trials in human populations. However, rapidly declining poliomyelitis morbidity makes it impossible to evaluate new poliovirus vaccines in clinical trials, creating the need for the development of surrogate tests. Here we report our comparative evaluation of IPVs prepared from wt and attenuated strains of poliovirus and propose a new laboratory method for assessment of the protective properties of IPV-containing vaccine products.

MATERIALS AND METHODS

Vaccines and Viruses

The pilot sIPV batch, developed from Sabin 2 poliovirus stocks, was prepared at JPRI. Type 2 IPV lots and purified concentrated MEF-1 stock were obtained from a vaccine manufacturer. Strain 10660 of vaccine-derived type 2 poliovirus was isolated from a child with vaccine-associated paralytic poliomyelitis (VAPP) and was a gift from O. Ivanova (M. P. Chumakov Institute of Poliomyelitis, Moscow, Russia). The 10660 isolate and MEF-1 strain were grown in HEp-2 cells at 34°C and were cleared from debris by centrifugation, and titers were determined by the WHO procedure [6].

Mice

TgPVR21 mice were obtained from the Central Institute for Experimental Animals (CIEA; Tokyo, Japan). The mice were tested for the absence of 22 pathogens and were monitored for generational stability of genetic background and the introduced gene. Maintenance, containment, and transportation of the mice were performed in accordance with the recommendations of the WHO memorandum on transgenic (Tg) mice susceptible to human viruses [7]. All experiments using mice were performed in accordance with the Guide for the Care and Use of Laboratory Animals [8].

Immunization and Challenge

On arrival from Japan, the mice were randomized and were given 1 week to acclimatize. In protection tests, equal numbers of male and female mice were intraperitoneally (ip) immunized with varying doses of either cIPV or sIPV and were intramuscularly (im) challenged with 0.5 mL of either MEF-1 or strain 10660 dilutions. The mice were observed daily for 2 weeks, for signs of paesiasis and/or paralysis. Approximately 50 μL of blood was collected from the tail vein after the first and/or second immunization; saliva was collected at the end of the observation period (day 14 after challenge). To collect saliva, mice were first anesthetized by an ip injection of a 0.3–0.4 mL mixture of ketamine (final concentration, 10 mg/mL) and xylazine (final concentration, 0.4 mg/mL) in physiologic saline and then were injected ip with 100 μL of 0.05 mol/L pilocarpine, to induce salivation.

Serological Studies

Immune sera and reagents. Adult female rabbits (New Zealand white, strain NsdOkd) were immunized im (0.5 mL in each thigh) with either purified MEF-1 (90 μg/mL) or Sabin 2 (174 μg/mL) once or twice within an interval of 2 weeks. Two weeks after each immunization, blood samples were collected and were tested for poliovirus-specific IgG by ELISA (see below). The rabbits with poliovirus-specific IgG titers of 1:16,000–1:128,000 were exsanguinated under anesthesia, and IgG was affinity purified by use of the Protein A Antibody...
Purification Kit (Sigma). The IgG was used for both coating of immunoplates during the ELISAs and biotin labeling by use of the Micro-Biotinylation Kit (Sigma). To avoid possible idio-type–anti-idiotype reactions, the IgG used for coating and for biotinylation was collected from different rabbits. The IgG and IgG-biotin conjugates were kept in 50% glycerol at −20°C.

ELISAs

Poliovirus antigen–capture ELISA. Immunoplates (Costar, no. 3369) were coated overnight at 4°C with 2 μg/mL (100 μL/well) of anti-poliovirus IgG in coating buffer (Sigma). Twofold virus dilutions and control (mocking) antigen in ELISA buffer (PBS with 1% bovine serum albumin and 0.05% Tween-20) were added (100 μL/well), and the plates were incubated for 2 h at 37°C. After a wash step, 100 μL/well of IgG-biotin conjugate diluted in ELISA buffer was added. After 1 h of incubation at 37°C and a wash step, 100 μL/well of ExtrAvidin-peroxidase conjugate (Sigma) was added. After incubation for 30 min at room temperature, the wells were washed, and 150 μL/well of 3,3’,5,5’-tetrathylbenzidine substrate (Sigma) was added. After incubation for 30 min at room temperature, the reaction was stopped by the addition of 75 μL/well of 0.5 mol/L sulfuric acid. Optical density was registered at 450 nm by use of the ELISA reader Benchmark Plus (Bio-Rad). In all ELISAs described here (except for the block ELISA), a test sample was considered to be positive if the ratio of the optical density in the test well and that in the control well was $\geq 2.1$.

Block ELISA for anti-poliovirus summary antibodies. The block ELISA for anti-poliovirus summary antibodies was based on the antigen-capture ELISA. Immunoplates were sensitized as described above. The dose of standard poliovirus antigen was determined by cross-titration, and 100 μL/well of the antigen was incubated for 2 h at 37°C. After a wash step, serum samples diluted 1:20 in ELISA buffer (100 μL/well) were added and were incubated for 1 h at 37°C. Serum samples from non-immunized animals were used as controls in the same dilution. The remaining steps (starting from incubation with IgG-biotin conjugate) were the same as those described above for the antigen-capture ELISA. A serum sample was considered to be positive if it caused a $\geq 50\%$ reduction of the optical-density reading for standard antigen, compared with that in serum from nonimmunized animals.

α-Capture ELISA for anti-poliovirus IgA in saliva. Immunoplates were coated with 20 μg/mL (100 μL/well) of goat anti-mouse IgA (α-chain specific; Sigma). After a wash and a block step, 100 μL/well of saliva sample diluted 1:4 in ELISA buffer was added to duplicate wells and was incubated for 2 h at 37°C. Saliva samples from nonimmunized Tg mice were used as controls in the same dilutions. The wells were washed, and 100 μL/well of poliovirus D antigen units (100 μL/well) in ELISA buffer was added. After incubation for 2 h at 37°C and a wash step, 100 μL/well of the appropriate IgG-biotin conjugate diluted in ELISA buffer was added and was incubated for 1 h at 37°C. The wells were washed, and the remaining steps (starting from the addition of ExtrAvidin-peroxidase conjugate) were the same as those described above for the antigen-capture ELISA.

ELISA for the detection of poliovirus-specific IgG in rabbit serum samples and poliovirus-specific IgM and IgG in mouse serum samples. These assays were performed as described elsewhere [9]. The dose of standard poliovirus antigen was 40 DU/100 μL. Mouse serum samples were tested at a dilution of...
Figure 2. Response to challenge in TgPVR21 mice. The mice were immunized twice with either 8 DU of inactivated poliovirus vaccine (IPV) produced from the attenuated Sabin strain (A) or conventional IPV (B). Serum antibodies were determined before challenge and 2 weeks after challenge with 25 50% paralytic doses of MEF-1.

1:200. Serum samples from nonimmunized rabbits and mice were used as controls.

**Microneutralization assay.** Titers of poliovirus-neutralizing antibodies in mouse serum samples were determined by a microneutralization assay with 100 TCID₅₀ of challenge virus, performed according to the recommendations of the WHO [6].

**Determination of nucleotide sequence.** RNA from the MEF-1 strain was extracted by use of phenol-SDS, and cDNA was prepared by use of SuperScript II reverse transcriptase (Life Sciences) and random dN₆ primers. Eight overlapping DNA segments were amplified by polymerase chain reaction (PCR), as described elsewhere [10]. Nucleotide sequences were determined by use of a Prism 310 Genetic Analyzer and Big Dye chemistry (Applied Biosystems). Sequencing primers were located on both complementary DNA strands ~400 nt apart from each other, so that the entire sequence was determined with at least 2-fold redundancy. The GenBank accession number of the MEF-1 sequence is AY238473.

**Statistical methods.** Simple regression analysis was used to compute the correlation coefficients (r) between the results obtained by the block ELISA and by the IgM and IgG ELISAs.

**RESULTS**

**Genomic sequence of the MEF-1 strain.** The lower immunogenicity of sIPV in rats (compared to cIPV) that was observed by Doi et al. [5] may be a result of antigenic differences between the Sabin 2 and MEF-1 strains. There is no published data on the sequence of the MEF-1 strain, which prompted us to determine the complete nucleotide sequence of this strain. We found that the MEF-1 strain obtained from a major IPV manufacturer was very close to the published sequence of another wt type 2 poliovirus strain, Lansing [11], with only 16 nt differences between them and only 2 aa substitutions. Unlike the very high similarity between the MEF-1 and Lansing strains, there were 1310 nt differences (17.6%) between the MEF-1 and Sabin 2 strains (72 [3.3%] aa substitutions, including several in antigenic sites 1 and 3). This finding suggests that MEF-1 and Sabin 2 may have different antigenic properties, further justifying the need to compare the antigenicity of conventional and Sabin IPVs.

**Preliminary animal experiments.** The immunization-challenge model using Tg mice susceptible to poliomyelitis, previously developed in our laboratory [12], was adapted to study the relationship between immunogenicity and protective properties of type 2 IPVs and to compare cIPV and sIPV. Because the initial protocol was not optimized for type 2 IPV, we conducted preliminary experiments in Tg mice, to select the most appropriate virus strain(s), virus dose(s), and route for challenge as well as the clinical-signs observation period and the time at which to sample blood for the evaluation of immune response. The 50% paralytic dose (PD₅₀) for the MEF-1 strain was 6.6 log₈₀ TCID₅₀ for the im route but was 8.1 log₈₀ TCID₅₀ for the ip route. This result showed that im injection was more effective than ip injection, and it was therefore selected for further experiments. Because the Sabin 2 and MEF-1 strains may differ in their immunological profile, as suggested by our sequence data, it was necessary to evaluate an alternative challenge strain that would be antigenically closer to the Sabin strain. Use of an alternative strain had an additional benefit, because work with wt polioviruses requiring BSL-3/polio containment may not be possible in the posteradication period; therefore, attenuated strains might be used as a substitute in future experiments. The reference Sabin 2 strain and the vaccine-derived poliovirus (VDPV) strain 10660, isolated from a patient with VAPP, were tested in Tg mice, to determine PD₅₀s. Inoculation of mice (im) with varying doses of Sabin 2 revealed complete resistance of females and low sensitivity of males to the highest dose, 10¹⁰¹ TCID₅₀. The PD₅₀ for
strain 10660 was 7.0 log_{10} TCID_{50} for im inoculation, comparable to that for MEF-1.

Affected mice developed partial or complete paralysis of 1 or 2 rear legs on days 4–6 after inoculation. Very few mice developed paralysis during the second week after inoculation, and no mice developed paralysis during the third week after inoculation. Therefore, for protection tests, the observation period after challenge was limited to 2 weeks.

**Immunization and challenge.** In the first experiment, mice were immunized with either cIPV or sIPV at a dose of 8 or 16 DU once or twice (12 days after the first immunization). Challenge with 25 PD_{50} of MEF-1 was performed on day 19 after the final immunization. A strong correlation between clinical status and the level of systemic antibodies was observed in the mice immunized with cIPV: all of the mice that survived challenge with MEF-1 showed increased levels of antibodies, whereas no immune response was detected in 3 mice that developed paralysis (figure 1A). Interestingly, the mice immunized with the higher dose of cIPV (16 DU) produced lower levels of antibodies. A positive correlation between protection and immune response was observed in the mice immunized with sIPV: all of the mice that survived challenge with MEF-1 showed increased levels of antibodies, whereas no immune response was detected in 3 mice that developed paralysis during the second week after inoculation, or 2 rear legs on days 4–6 after inoculation. Very few mice developed paralysis during the third week after inoculation, and no mice developed paralysis after challenge with 25 PD_{50} of MEF-1 was performed on day 19 after inoculation. Therefore, for protection tests, the observation period after challenge was limited to 2 weeks.

### Table 2. Experiment 2: Protective properties of conventional inactivated poliovirus vaccine (cIPV) in TgPVR21 mice immunized twice and challenged with 25 50% paralytic doses of MEF-1.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Survived, %</th>
<th>IgM^{a}</th>
<th>IgG^{a}</th>
<th>NT^{b}</th>
<th>Saliva, IgA^{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 DU</td>
<td>100</td>
<td>2.8 ± 1</td>
<td>5 ± 1.5</td>
<td>621 ± 554</td>
<td>50</td>
</tr>
<tr>
<td>4 DU</td>
<td>100</td>
<td>3.6 ± 1.7</td>
<td>5.2 ± 0.9</td>
<td>716.8 ± 337</td>
<td>60</td>
</tr>
<tr>
<td>1 DU</td>
<td>100</td>
<td>3.9 ± 1.6</td>
<td>5 ± 1.4</td>
<td>600 ± 659</td>
<td>25</td>
</tr>
<tr>
<td>Medium (control)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** DU, poliovirus D antigen unit.

^{a} Mean sample:control optical-density ratio (positive result, >2.1).

^{b} Microneutralization test, reciprocal titer.

^{c} Percentage of positive mice.

Analysis of antibody production in mice was performed 2 weeks after challenge by measuring the total levels of IgM and IgG and by using a block ELISA, which determines, in a serum sample, the level of antibodies' blocking effect on the binding of biotinylated polyclonal IgG specific to different epitopes. Therefore, the percentage of the block reflects the level of respective antibodies in the serum sample. We found strong correlations between the results obtained by the IgM ELISA and the block ELISA (r = 0.75; P < .0001) and between the results obtained by the IgG ELISA and the block ELISA (r = 0.7; P < .0001). In mice immunized with sIPV, these methods revealed an obvious booster effect from MEF-1 challenge. After challenge, the level of anti-poliovirus antibodies distinctly increased in the mice immunized with sIPV, whereas it remained about the same in the mice immunized with cIPV (figure 2A and 2B and table 1).

Because the 8-DU dose produced a stronger immune response than did the 16-DU dose, another experiment was performed 2 weeks after challenge by measuring the total levels of IgM and IgG and by using a block ELISA, which determines, in a serum sample, the level of antibodies' blocking effect on the binding of biotinylated polyclonal IgG specific to different epitopes. Therefore, the percentage of the block reflects the level of respective antibodies in the serum sample. We found strong correlations between the results obtained by the IgM ELISA and the block ELISA (r = 0.75; P < .0001) and between the results obtained by the IgG ELISA and the block ELISA (r = 0.7; P < .0001). In mice immunized with sIPV, these methods revealed an obvious booster effect from MEF-1 challenge. After challenge, the level of anti-poliovirus antibodies distinctly increased in the mice immunized with sIPV, whereas it remained about the same in the mice immunized with cIPV (figure 2A and 2B and table 1).

### Table 3. Immunogenicity of conventional inactivated poliovirus vaccine (cIPV) or inactivated poliovirus vaccine produced from the attenuated Sabin strain (sIPV) in TgPVR21 mice immunized twice and challenged with 100 50% paralytic doses of the vaccine-derived strain 10660.

<table>
<thead>
<tr>
<th>Vaccine, dose</th>
<th>Serum</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>cIPV</td>
<td>IgG^{a}</td>
<td>Positive mice, %</td>
</tr>
<tr>
<td>1 DU</td>
<td>4.5 ± 0.5</td>
<td>100</td>
</tr>
<tr>
<td>4 DU</td>
<td>4.2 ± 1.4</td>
<td>100</td>
</tr>
<tr>
<td>sIPV</td>
<td>4.3 ± 0.6</td>
<td>100</td>
</tr>
<tr>
<td>8 DU</td>
<td>3.9 ± 0.4</td>
<td>100</td>
</tr>
<tr>
<td>Control, medium</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** DU, poliovirus D antigen unit.

^{a} Mean sample:control optical-density ratio (positive result, >2.1).
formed, to determine whether lower doses of IPV might be even more immunogenic. In the second experiment, mice were immunized twice with varying doses of cIPV, the interval between the first and second immunizations was increased to 16 days, and the interval between the last immunization and challenge was increased to 26 days. All of the mice that received doses of 1, 4, and 8 DU survived challenge with MEF-1, but the mice that received the 1- or 4-DU doses had higher titers of IgM and IgG than did the mice that received the 8-DU dose. The highest percentage of mice that developed mucosal IgA, as well as the highest neutralizing antibody production, were observed after immunization with 4 DU (table 2).

**Protection against homologous VDPV strain.** In a third experiment (table 3 and figure 3), the immunogenicity and protective properties of cIPV and sIPV were compared using the same immunization regimen as in the second experiment, but VDPV strain 10660 was used for challenge instead of wt MEF-1. PCR restriction fragment–length polymorphism analysis of regions VP1, 2C, and 3D, as well as partial sequencing of the genomic region coding for VP1, confirmed the close relatedness of the 10660 isolate and the Sabin 2 strain (E. Cherkasova, personal communication). All of the control mice, challenged with strain 10660 at a dose of 100 PD50, died. The mice immunized with either 1 DU or 4 DU of cIPV survived the challenge and produced high levels of IgG. Immunization with 8 DU of sIPV induced protection of all of the mice and generated a level of IgG in serum comparable to that observed for immunization with 1 DU of cIPV. sIPV was less immunogenic and less protective at a 4-DU dose than at an 8-DU dose. Mucosal immune response in Tg mice was evaluated in saliva by use of an α-capture ELISA developed for the present study. The highest level of salivary IgA was observed in the mice immunized with 4 DU of cIPV and was slightly less in the mice immunized with 1 DU of cIPV. In mice immunized with sIPV, levels of salivary IgA were lower, but the percentage of positive mice was higher than after immunization with cIPV (table 3).

Incomplete protection of mice by sIPV against challenge with wt MEF-1 and better protection against challenge with the homologous VAPP-derived isolate 10660 may be due to incomplete antigenic homology of the MEF-1 and Sabin 2 strains.

**Table 4. Cross-reactivity between the MEF-1 and Sabin 2 strains.**

<table>
<thead>
<tr>
<th>System</th>
<th>Antigen-capture ELISA</th>
<th>Neutralization titer with virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antigen titer</td>
<td></td>
</tr>
<tr>
<td>MEF-1</td>
<td>1:640</td>
<td>1:74.989</td>
</tr>
<tr>
<td>Sabin 2</td>
<td>1:80</td>
<td>1:2371</td>
</tr>
<tr>
<td></td>
<td></td>
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</table>

Figure 3. Serum IgG response in TgPVR21 mice immunized either with conventional inactivated poliovirus vaccine (cIPV) (circles) or inactivated poliovirus vaccine produced from the attenuated Sabin strain (sIPV) (squares). The mice were immunized twice with either 1 or 4 DU of cIPV or 4 or 8 DU of sIPV. Antibody levels were determined 4 weeks after the second immunization.
Our analysis of antigenic cross-reactivity between these 2 strains is presented in table 4. In ELISAs, anti–MEF-1 antibodies reacted with MEF-1 only slightly better than with Sabin 2, whereas anti–Sabin 2 antibodies exhibited an 8-fold difference in reactivity with Sabin 2, compared with that with MEF-1 (table 4A). This asymmetric reactivity was confirmed in a neutralization test, in which anti–MEF-1 antiserum neutralized both strains at an approximately equal level, whereas anti–Sabin 2 antiserum showed 5-fold lower neutralization activity against MEF-1 than against Sabin 2 (table 4B). This finding suggests that MEF-1 has a broader reactivity than does Sabin 2, partially explaining the results of the protection tests in Tg mice.

DISCUSSION

Progress in the global eradication of poliomyelitis and the need for stricter containment of wt poliovirus stocks stimulated some manufacturers to explore the possibility of making IPV from attenuated (Sabin) strains. Of 3 poliovirus serotypes, the sIPV of types 1 and 3 were found to be very similar to the respective cIPVs when tested for immunogenicity in rats, whereas the sIPV of type 2 appeared to be less immunogenic, compared with the cIPV [5]. By the time this experimental sIPV became available for the present study, we had determined the complete nucleotide sequence of the MEF-1 strain, which is the substrate for production of cIPV. The 1310-nt difference (∼18%) between the MEF-1 and Sabin 2 strains (including amino acid substitutions in antigenic sites) is consistent with the above observation of poor protection by type 2 sIPV, presumably because of the incomplete antigenic match between these strains. This was later confirmed in our study of cross-reactivity using ELISAs and neutralization tests with serum from rabbits immunized with the MEF-1 or Sabin 2 polioviruses.

The close similarity between the MEF-1 nucleotide sequence and the published sequence of another unrelated wt strain, Lansing, is unexpected. The Lansing strain was isolated in 1937 from human brain tissue in Lansing, Michigan [16]. Four years later, Van Rooyen isolated the MEF-1 virus from a patient with polio in the Mediterranean Expeditionary Force in Egypt [17]. It was transmissible to rodents and in early studies was found to be serologically identical to the Lansing strain [18]. The close match of nucleotide sequences between the 2 strains isolated 4 years apart on 2 continents suggests that the stocks may have been mixed up or mislabeled at some point in their passage history.

The only way to study the relationship between immunogenicity and protective properties is to conduct in vivo experiments. Tg mice expressing human poliovirus receptor [19]—and therefore susceptible to poliomyelitis—are an excellent model for such studies [12, 20]. The TgPVR21 mouse line created by Koike et al. [21] and established for mass production at the CIEA [22] was widely used in our laboratory [23, 24] and in the WHO collaborative study [25–27] for evaluation of neurovirulence of oral poliovirus vaccine. In the present study, we present the results of further development of the Tg mouse protection test with the specific goal of comparing type 2 cIPV and sIPV. Our results confirm a previous observation made in rats [5], that these 2 vaccines are antigenically different. Although cIPV elicited an immune response that protected mice against challenge with wt poliovirus, sIPV administered in similar doses induced a lower level of antibodies and did not completely protect against wt virus challenge. It is also notable that, when challenged with a homologous vaccine-derived strain of type 2 poliovirus, sIPV-immunized Tg mice exhibited a higher level of protection, suggesting that inadequate protection in experiments with MEF-1 challenge can be explained, at least in part, by the incomplete antigenic match of these 2 strains.

Although sIPV did not completely protect against challenge with MEF-1, cIPV protected against MEF-1 and type 2 vaccine–derived strain 10660, isolated from a patient with VAPP. This asymmetric response suggests that type 2 IPV prepared from wt MEF-1 possesses broader immunogenicity than does IPV prepared from attenuated Sabin poliovirus, which is consistent with previous observations for type 1 [20]. The antigen-capture ELISA and neutralization tests, in which MEF-1 or Sabin 2 antiserum were tested against MEF-1 or Sabin 2 antigens, also revealed these differences.

The Tg mouse protection test may also be used to evaluate immunogenic properties of cIPV or sIPV in more detail. When the same doses of cIPV and sIPV were used, sIPV was less immunogenic than was cIPV, with regard to production of both classes of serum antibodies, IgM and IgG. However, whereas sIPV was not totally protective in the Tg mouse model, a booster effect was observed in those mice that survived challenge with MEF-1. The titers of antibodies in challenge survivors reached the same level that was determined in mice immunized with cIPV before the challenge. Similar effects were reported by Doi et al. in a rat potency test after the second immunization [5]. In addition, it was demonstrated that 2 immunizations were more effective than a 1-time immunization, regardless of the administered dose. The period between immunizations also had an effect on the production of antibodies, because immunization repeated 4 weeks after the initial dose was administered was more effective than with a shorter period of time between immunizations. In the present study of local immunity in mice (IgA in saliva), we used a newly developed α-capture ELISA. We observed higher levels of local immune response to cIPV, compared with that to sIPV. A similar correlation between polioviruses and immune response was observed previously with type 1: TgPVR21 mice produced higher levels of secreted IgA in saliva after immunization with live wt type 1 Mahoney strain than after immunization with Sabin 1
In the present study, assessment of local immunity in mice contributed to the comparative analysis of cIPV and sIPV. In the mouse model, we could not find a regimen of immunization in which sIPV would be as effective as cIPV. However, double immunization with 4 DU of sIPV provided an immune response and protection comparable with that of 1 DU of cIPV. This observation suggests that an increase in the type 2 component in the final vaccine formulation (e.g., from 8 DU in cIPV to 30 DU in sIPV) may compensate for the lower immunogenicity of type 2 sIPV. The very important question is the relationship between the immunogenicity and protective properties determined in Tg mice and the field performance of these 2 vaccines in humans. One possibility is that the lower immunogenicity of sIPV, compared with cIPV, is a peculiarity of this rodent model and may not necessarily hold true for immune response in humans. However, the asymmetric immune response that we have observed in this system can also play a role in humans. For instance, some studies with human serum revealed a similar asymmetry in immune response to Sabin and wt polioviruses [28],

Another important question is what this incomplete antigenic match between the strains means in terms of protection of human populations against poliomyelitis. There has been almost no wt type 2 poliovirus circulation in the world since 1999, but there was a number of individual cases and at least 2 outbreaks of poliomyelitis caused by vaccine-derived strains of type 2 [29, 30]. Therefore, protection against vaccine-derived strains that have a close antigenic relationship to Sabin 2 may be a more important objective in the posteradication period, offsetting the lower potency of sIPV against challenge with heterologous wt strains.

The TgPVR21 mouse protection model may be also useful for the evaluation of new IPVs from different sources and for the assessment of immunogenicity of poliovirus component in new combination vaccines. Initial experiments not reported here found that a combination vaccine with IPV component was as immunogenic and protective in the mouse test as was cIPV.

In conclusion, the method reported in the present study can be used for the evaluation of different vaccine products containing IPV and for comparative studies of different IPVs made from alternative strains. It can also be used for the assessment of potential benefits of modified immunization regimens and use of adjuvants for improving both overall immunogenicity and its mucosal component. Similar studies of the modification of the Tg mouse test for the characterization of IPVs of types 1 and 3 are in progress.

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References

24. Dragunsky E, Gardner D, Taffs R, Levenbook I. Transgenic PVR Tg-


