Further Development of a New Transgenic Mouse Test for the Evaluation of the Immunogenicity and Protective Properties of Inactivated Poliovirus Vaccine

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Recently, we developed and optimized a new method for the evaluation of the protective properties of serotype 2 inactivated poliovirus vaccines (IPV). The method is based on the immunization and subsequent challenge of transgenic (Tg) mice susceptible to poliovirus. We describe a similar method for the assessment of the protectiveiveness of serotype 1 IPV and demonstrate that experimental IPV produced from attenuated Sabin strain (sIPV) of serotype 1 poliovirus induced serum neutralizing antibodies, immunoglobulin (Ig) G, IgM, and salivary IgA at titers comparable to those induced by conventional IPV (cIPV) produced from the wild-type Mahoney strain. In contrast to our previous results with serotype 2 sIPV, serotype 1 sIPV provided even better protection of Tg mice than cIPV against challenge with wild-type Mahoney strain.

The development and worldwide use of 2 highly efficient vaccines against poliomyelitis were among the most important advancements in public health in the past 50 years. Inactivated poliovirus vaccine (IPV) provided complete protection of recipients but failed to control the spread of the virus in human populations. By contrast, live oral poliovirus vaccine (OPV) induced protective immunity in both vaccine recipients and contacts and prevented transmission of the virus. However, OPV was shown to cause rare but serious complications, and it is capable of reverting to virulence and occasionally causing polio outbreaks. The worldwide campaign to eradicate poliomyelitis was based exclusively on OPV and resulted in the eradication of poliomyelitis in most of the world [1, 2]. This led to substantial changes in vaccination policies [3], with more countries switching from OPV to IPV. Increased demand for IPV can only be met by introducing additional manufacturing capacity, which will require the regulatory evaluation of new products. According to the World Health Organization (WHO) Global Action Plan, virulent polioviruses must be handled at biosafety level 3 with additional containment measures. Because conventional IPV (cIPV) is produced from wild-type (wt) strains, it has been proposed that IPV be produced from attenuated Sabin strains (sIPV), to mitigate the risk of the accidental release of wt virus into the environment [4]. The replacement of vaccine strains will require a scope of regulatory evaluation similar to that of new vaccines. In addition, combination vaccines with multiple antigens have been proposed, to reduce the number of injections. They also need thorough evaluation, but the virtual absence of poliovirus morbidity makes any direct clinical assessment of the efficacy of IPV impossible. Therefore, licensure of the new products must rely on surrogate end points and results obtained in animal experiments.

Recently, we developed and optimized a new assay for the evaluation of the protective properties of serotype 2 IPV in transgenic mice (Tg) susceptible to poliovirus [5, 6]. The Tg mouse assay was used for the comparison of the immunogenic and protective properties of sIPV produced from an attenuated strain recently developed by Doi et al. [7] at the Japanese Poliomyelitis Research Institute (JPRI) and those of cIPV produced from wt poliovirus. The results of that study [6] demonstrated that the protective properties of sIPV were inferior to those of cIPV and that the new sIPV may require further improvements. In the present article, we report our results on the development of a Tg mouse test for the comparative analysis of immunogenicity and protective properties of serotype 1 cIPV and sIPV.

Materials and methods. The pilot sIPV batches developed from Sabin 1 poliovirus stock were prepared at JPRI. Serotype 1 cIPV lots and purified concentrated Mahoney strains were obtained from a vaccine manufacturer.

TgPVR21 mice were obtained from the Central Institute for
Table 1. Protective properties of serotype 1 conventional inactivated poliovirus vaccine (cIPV) or inactivated poliovirus vaccine from the attenuated Sabin strain (sIPV) in TgPVR21 mice immunized twice and challenged with 25–50% paralytic doses of the Mahoney strain.

<table>
<thead>
<tr>
<th>Experiment, vaccine, dose</th>
<th>Not paralyzed, %&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IgM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IgG&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NT&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Salivary IgA, % positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cIPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 DU</td>
<td>100</td>
<td>15.0 ± 2.9</td>
<td>7.0 ± 0.7</td>
<td>1038 ± 550</td>
<td>100</td>
</tr>
<tr>
<td>20 DU</td>
<td>100</td>
<td>9.6 ± 5.7</td>
<td>6.9 ± 0.8</td>
<td>503 ± 375</td>
<td>75</td>
</tr>
<tr>
<td>10 DU</td>
<td>100</td>
<td>13.3 ± 2.8</td>
<td>6.7 ± 0.8</td>
<td>525 ± 479</td>
<td>12</td>
</tr>
<tr>
<td>5 DU</td>
<td>100</td>
<td>14.2 ± 2.0</td>
<td>6.7 ± 0.7</td>
<td>442 ± 493</td>
<td>0</td>
</tr>
<tr>
<td>sIPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 DU</td>
<td>100</td>
<td>7.0 ± 2.7</td>
<td>6.5 ± 0.7</td>
<td>235 ± 216</td>
<td>25</td>
</tr>
<tr>
<td>20 DU</td>
<td>100</td>
<td>7.4 ± 2.0</td>
<td>6.4 ± 0.8</td>
<td>160 ± 223</td>
<td>28</td>
</tr>
<tr>
<td>10 DU</td>
<td>100</td>
<td>6.9 ± 2.8</td>
<td>6.1 ± 0.9</td>
<td>223 ± 108</td>
<td>50</td>
</tr>
<tr>
<td>5 DU</td>
<td>100</td>
<td>9.7 ± 4.1</td>
<td>5.9 ± 0.8</td>
<td>426 ± 304</td>
<td>12</td>
</tr>
<tr>
<td>Control (medium)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>&lt;8</td>
<td>...</td>
</tr>
<tr>
<td>2 cIPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 DU</td>
<td>75</td>
<td>7.9 ± 3.5</td>
<td>13.5 ± 2.1</td>
<td>158 ± 235</td>
<td>42</td>
</tr>
<tr>
<td>1 DU</td>
<td>12</td>
<td>6.2 ± 2.9</td>
<td>8.2 ± 4.0</td>
<td>27 ± 41</td>
<td>33</td>
</tr>
<tr>
<td>sIPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 DU</td>
<td>100</td>
<td>8.0 ± 4.8</td>
<td>14.2 ± 4.0</td>
<td>320 ± 302</td>
<td>25</td>
</tr>
<tr>
<td>1 DU</td>
<td>100</td>
<td>5.6 ± 3.2</td>
<td>13.6 ± 2.7</td>
<td>341 ± 356</td>
<td>62</td>
</tr>
<tr>
<td>Control (medium)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>&lt;8</td>
<td>...</td>
</tr>
<tr>
<td>3 cIPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 DU</td>
<td>Before challenge</td>
<td>7.0 ± 3.5</td>
<td>6.6 ± 2.5</td>
<td>47 ± 103</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>After challenge</td>
<td>9.3 ± 3.2</td>
<td>5.9 ± 2.2</td>
<td>443 ± 579</td>
<td>ND</td>
</tr>
<tr>
<td>sIPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 DU</td>
<td>Before challenge</td>
<td>4.9 ± 2.7</td>
<td>5.7 ± 1.8</td>
<td>100 ± 92</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>After challenge</td>
<td>7.0 ± 2.0</td>
<td>9.4 ± 0.5</td>
<td>951 ± 1137</td>
<td>ND</td>
</tr>
<tr>
<td>Control (medium)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>&lt;8</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD, unless otherwise indicated. Serological data are shown for before challenge (experiments 1 and 2) and before and after challenge (experiment 3). DU, poliovirus D-antigen unit.

<sup>a</sup> For experiments 1 and 2, 8 mice/group; for experiment 3, 10 mice/group.

<sup>b</sup> Sample:control optical density ratio (positive result, >2.1).

<sup>c</sup> Microneutralization test, reciprocal titer.

Experimental Animals (Tokyo, Japan). Mice were tested for the absence of 22 common pathogens and monitored for the generational stability of genetic background and the introduced gene. Maintenance, containment, and transportation of mice were performed in accordance with recommendations of the WHO memorandum on transgenic mice susceptible to human viruses [8]. All mouse experiments were performed in accordance with the US *Guide for the Care and Use of Laboratory Animals* [9].

Equal numbers of male and female TgPVR21 mice were immunized intraperitoneally. The highest dose was equivalent to 1 human dose (40 poliovirus D-antigen units [DU]). Four to five weeks after the second immunization, mice were challenged intramuscularly. Mice were observed daily for 2 weeks for signs of paresis or paralysis. Then, 30–50 μL of blood was collected from tail vein and used for ELISA and the microneutralization test (NT). At the end of the observation period, saliva was collected as described elsewhere [6].

Immune serum and reagents were prepared as described elsewhere [6]. Poliovirus antigen–capture ELISA, α-capture ELISA for the determination of poliovirus-specific IgA in mouse saliva, ELISA for the detection of poliovirus-specific IgG and IgM in...
Simple regression analysis was used to compute the correlation coefficient \( r \) between results obtained by the NT and IgM and IgG ELISA.

**Results.** The Tg mouse immunization-challenge model was developed in our laboratory [5] and was recently adapted for comparative studies of protective properties of serotype 2 Sabin IPV [6]. For the evaluation of serotype 1 IPV, we further modified the technique. Mice were immunized twice at an interval of 15–16 days and challenged 30 days later with 25 50% paralytic doses of wild-type Mahoney strain.

In the first experiment, Tg mice were immunized with doses of 40, 20, 10, or 5 DU of serotype 1 cIPV or sIPV. None of the mice developed clinical signs after challenge with the Mahoney strain, whereas all control mice were paralyzed (table 1). All mice produced serum antibodies at approximately the same level, and neither IgM nor IgG immune responses depended on the immunization dose or the type of vaccine. Mice immunized with cIPV (especially at 40 DU/mouse) produced higher titers of neutralizing antibodies than mice immunized with sIPV, with apparent dose dependence. The same trend was observed for the production of IgA in saliva; titers ranged from 0% at a dose of 5 DU to 100% at a dose of 40 DU. In mice immunized with sIPV, there was no dose dependence in the production of serum neutralizing antibodies, IgM, or IgG or of salivary IgA. By contrast, a high correlation was observed for cIPV between neutralizing antibody titers and IgG (\( r = 0.72; P < .01 \)), and a low correlation was observed between neutralizing antibodies and IgM (\( r = 0.27 \)). For all doses of sIPV, a significant correlation (\( P < .01 \)) was found between neutralizing antibodies and IgG (\( r = 0.55 \)) and between neutralizing antibodies and IgM (\( r = 0.57 \)).

Because the 5-DU sIPV dose was more effective in producing neutralizing antibodies than higher doses lower doses of vaccines were used in the next experiment. Tg mice were immunized with 1 and 3 DU of cIPV or sIPV (table 1). sIPV was fully protective at both doses, whereas cIPV did not protect all mice. Lower protection at the low cIPV dose was also found to be the most discriminatory dose for the comparison of cIPV and sIPV, and this dose was also found to be the most discriminatory dose for the comparison of cIPV and sIPV. A good correlation (\( P < .01 \)) for both doses of cIPV was observed between neutralizing antibodies and IgG (\( r = 0.77 \)), between neutralizing antibodies and IgM (\( r = 0.61 \)), and for both doses of sIPV (neutralizing antibodies/IgG; \( r = 0.52 \) ) and (neutralizing antibodies/IgM; \( r = 0.72 \)).

The third experiment confirmed the results of the second experiment (table 1). Only 20% of mice immunized with 1 DU of cIPV resisted challenge, whereas 90% of mice immunized with sIPV did not develop paralysis. Although titers of IgM and IgG in both groups did not differ significantly, neutralizing antibody titers were higher in mice immunized with sIPV before and after challenge.

Our analysis of cross-reactivity (table 2) did not reveal any notable differences between the Sabin 1 and Mahoney strains. In antigen-capture ELISA tests that used heterologous capture and detection of antibodies, the Sabin-1 and Mahoney strains were almost fully cross-reactive. This cross-reactivity was confirmed in the NT, in which anti-Mahoney and anti–Sabin-1 antisera neutralized both Mahoney and Sabin-1 strains at similar titers. These results may reflect that the Mahoney and Sabin-1 strains are closely related and may explain the similar behavior of cIPV and sIPV in the TgPVR21 mouse protection test.

**Discussion.** The evaluation of vaccine efficacy in the absence of natural morbidity presents a substantial challenge that, according to the US Food and Drug Administration’s animal-experimentation rules, can be overcome by using data from animal models. New poliovirus vaccines should be tested in comparison with existing vaccines, to ensure that their efficacy can be reasonably expected to be the same. Antigenic differences between cIPV and sIPV cause us to question whether the correlation of serum antibody titers with protection will remain the same for new vaccines. Therefore, it is desirable to have an animal protection test that would provide at least some assurance that the old and new products not only elicit the same level of antibodies but that these antibodies are equally protective.

There is no convenient natural animal model of poliomyelitis.

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**Table 2. Cross reactivity between wild-type Mahoney and attenuated Sabin-1 poliovirus strains.**

<table>
<thead>
<tr>
<th>System, strain</th>
<th>Mahoney</th>
<th>Sabin-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen titer⁹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen-capture ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mahoney</td>
<td>1:185</td>
<td>1:81</td>
</tr>
<tr>
<td>Sabin-1</td>
<td>1:285</td>
<td>1:230</td>
</tr>
<tr>
<td>Neutralizing titer⁹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutralization test (rabbit antiserum)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mahoney</td>
<td>1:89,125</td>
<td>1:37,584</td>
</tr>
<tr>
<td>Sabin-1</td>
<td>1:11,220</td>
<td>1:15,849</td>
</tr>
</tbody>
</table>

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⁹ Titers were calculated using regression analysis (Microsoft Excel).

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litis. Recently, mice expressing human poliovirus receptors (PVR or CD155) were used in a Tg mouse OPV neurovirulence test [12]. Previous work from our laboratory also demonstrated that these mice could be used for immunization in a challenge test of vaccine efficacy [5]. Recently, we used this model to compare serotype 2 cIPV (produced from MEF-1 wt poliovirus) with the new experimental vaccine produced from the Sabin-2 strain [6]. The results revealed differences in the immunogenicity and protective properties of these products and suggested that further optimization of serotype 2 sIPV is needed.

In the present study, we compared the immunogenic and protective properties of IPV produced from serotype 1 Mahoney and Sabin poliovirus strains. Doi et al. [7], who developed the sIPV, reported that serotype 1 sIPV was immunogenic in humans—both adults and infants. They also showed, in a rat potency test, that sIPV and cIPV were equally immunogenic: titers of neutralizing antibodies were similar after immunization with either cIPV or sIPV. Our experiments in Tg mice confirmed this result. The production of neutralizing antibodies in Tg mice was on approximately the same level as that in rats. Thus, the sensitivities of the rat and Tg mouse models were comparable. In addition, we found that sIPV completely protected immunized mice against lethal challenge with wt poliovirus. This means that there was no discrepancy between the level of antibodies and their protective effect and that the level of neutralizing antibodies in humans could be used as the surrogate measurement of clinical efficacy of sIPV.

Therefore, unlike serotype 2 sIPV [6], serotype 1 sIPV had excellent immunogenicity and protective properties in Tg mice, which suggests that each strain may possess strictly individual properties and that evaluations of immunogenicity and protective properties must be done on a case-by-case basis. Unlike the serotype 2 strains MEF-1 and Sabin-2, which differ at >17% of their nucleotides [6], the Mahoney and Sabin-1 strains are closely related. Previously, we found that formaldehyde inactivation of the Sabin-1 strain (but not the wt Mahoney strain) impairs one of the neutralizing epitopes of the virus [13]. The full protective properties of sIPV in Tg mice suggests that epitope 1 may not play a significant role in antipolio immunity [14].

We also evaluated the utility of 4 serological markers: neutralizing antibody titers and titers of IgM and IgG in serum and of IgA in saliva. Despite the good correlation between neutralizing antibody titers and IgM or IgG levels, the neutralizing antibody titer reflecting functional affinity (avidity) is the most reliable marker of efficacy. Thus, in the present study, the neutralizing antibody titers were the focal point of the serological assessment of the vaccines. As a marker of local (mucosal) immunity, salivary IgA was found to be dose-dependent for cIPV but inadequate for sIPV (table 1). At the same time, sIPV was 100% protective against challenge with the Mahoney strain at all doses (1–40 DU). All the IgA-positive mice were found only at the highest dose of cIPV, 40 DU, which suggests that salivary IgA is not a good marker for the assessment of IPVs. The Tg mouse immunization-challenge model, supported with the appropriate serological tests, could be used for the comprehensive assessment of new IPVs produced from alternative strains and to predict their efficacy when clinical trials in humans are impossible.

Acknowledgment

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References