Excretion of Wild-Type and Vaccine-Derived Poliovirus in the Feces of Poliovirus Receptor-Transgenic Mice

Hein J. Boot,* Daniella T. J. Kasteel,1† Anne-Marie Buisman, and Tjeerd G. Kimman

Laboratory for Vaccine-Preventable Diseases, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands

Received 20 November 2002/Accepted 14 March 2003

The emergence of circulating vaccine-derived poliovirus (cVDPV) strains in suboptimally vaccinated populations is a serious threat to the global polio eradication. The genetic determinants for the transmissibility phenotype of polioviruses, and in particularly of cVDPV strains, are currently unknown. Here we describe the fecal excretion of wild-type poliovirus, oral polio vaccine, and cVDPV (Hispaniola) strains after intraperitoneal injection in poliovirus receptor-transgenic mice. Both the pattern and the level of fecal excretion of the cVDPV strains resemble those of wild-type poliovirus type 1. In contrast, very little poliovirus was present in the feces after oral polio vaccine administration. This mouse model will be helpful in elucidating the genetic determinants for the high fecal-oral transmission phenotype of cVDPV strains.

The global incidence of poliomyelitis has dropped spectacularly from an estimated 350,000 cases in 1988 to just 473 laboratory-confirmed cases in 2001 (6). This large decrease is due to the World Health Organization’s coordinated vaccination programs using the oral polio vaccine (OPV). A rare side effect of the usage of OPV is the so-called vaccine-associated paralytic poliomyelitis (VAPP). VAPP is a rare event (0.5 cases per 1 million first-time OPV doses), which occurs among first-time OPV recipients and contacts of first-time OPV recipients in roughly equal frequencies (16). Molecular analysis of the composition of the poliovirus strains causing VAPP revealed that the attenuating mutations are lost by the accumulation of specific point mutations, either alone or in combination with intertypic recombination between the three strains from the trivalent OPV vaccine (9, 12). Molecular analysis of a large panel of VAPP strains indicated that recombination of the OPV strains was not restricted to poliovirus strains but could also involve nonpoliovirus enteroviruses (NPEV) (13). Direct proof for the natural occurrence of such recombination events came from the molecular analysis of the strains causing a poliomyelitis epidemic on the island Hispaniola. Sequence alignments of the poliovirus serotype 1 isolates of this outbreak revealed that they contained the nonstructural protein-encoding part of the genome from a nonpoliovirus enterovirus and that multiple NPEV strains had acted as donors for these sequences (14).

The reversion of specific nucleotide and amino acids mutations present in the Sabin 1 derivatives of the wild-type polioviruses results in an increase in virulence (for a review, see reference 15). The danger of vaccine-derived poliovirus (VDPV) for the polio eradication campaign is, however, not primarily determined by its high-neurovirulence phenotype. It is the transmissibility phenotype of reverted poliovirus vaccine strains that poses the greatest risk to global poliovirus eradication. OPV strains have a low basic reproduction number (R0), although it is estimated that R0 is >1, meaning that these vaccine strains will circulate in a fully susceptible population (11). Vaccine-derived strains with an increased transmissibility will persist in a suboptimally vaccinated population when the effective reproduction rate (R) is >1. Such vaccine-derived strains have indeed been recovered from sewage (19) and from a patient with acute flaccid paralysis (7). During long-term circulation of vaccine-derived poliovirus strains, quasispecies will arise with an increased transmissibility and neurovirulence. In recent years several such strains, which have a substantial genetic drift (mutations) and shift (recombination with poliovirus or nonpoliovirus enteroviruses), have emerged. These genetic differences reflect the long-term transmission of VDPV strains in the suboptimally vaccinated populations of Egypt (4), Hispaniola (2), the Philippines (3), and Madagascar (5). Such OPV-derived strains, which possess both a high transmissibility and a high neurovirulence, are referred to as circulating vaccine-derived poliovirus (cVDPV) strains. Currently it is unclear what the correlation between the transmissibility and the neurovirulence phenotype is, i.e., whether all highly neurovirulent strains are also highly transmissible and whether all highly transmissible strains possess a high neurovirulence.

The lack of knowledge concerning genetic determinants of person-to-person transmissibility hampers the risk assessment of the isolated vaccine-derived poliovirus strains for the global poliovirus eradication. It is therefore important to develop ways to assess the transmissibility of naturally occurring and (artificially generated) vaccine-derived poliovirus strains. Recently it has been shown that mice who possess the human receptor for poliovirus (ICR-PRVTg21 mice [10]) excreted poliovirus in the feces after intraperitoneal injection (1). As fecal excretion is one of the main determinants for high transmissibility of picornaviruses, we have used these mice to analyze the fecal excretion pattern of the Sabin OPV strains.

* Corresponding author. Mailing address: Laboratory for Vaccine-Preventable Diseases, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Phone: 31 30 274 4596. Fax: 31 30 274 4449. E-mail: Hein.Boot@RIVM.NL.
pressed as TCID50 per 100 mg of feces (Fig. 1). Subsequently, digestion was determined on Hep-2C cells, concentrated by Amicon (Millipore) filtration (cutoff, 100,000 kDa), and purified by CsCl gradient centrifugation and gel filtration (PD-10; Pharmacia). Threefold serial dilutions in phosphate-buffered saline (PBS) were made, and the 50% tissue culture infectious dose (TCID50) of each dilution was determined on Hep-2C cells (17). Groups (n = 6) of ICR-PVRTg21 mice, with ages between 7 and 12 weeks, were housed in separate isolators. Each mouse was kept in its own cage. Groups had an average equal distribution of male and female animals. After intraperitoneal injection of poliovirus in 0.2 mL of PBS, the mice were monitored daily, and fecal samples of individual mice were collected daily up to 10 days postinoculation (p.i.). Feces were resuspended by vortexing in 10 volumes of PBS and 1 volume of chloroform. After centrifugation (1,800 g for 10 min), the poliovirus titer in the supernatant of each fecal sample was determined and expressed as TCID50 per 100 mg of feces (Fig. 1). Subsequently, we determined the number of poliovirus-excreting mice, the mean duration of excretion, and the mean poliovirus level in the positive fecal samples (Table 1). Furthermore, we determined the relative level of duration of excretion by calculation of the poliovirus excretion days coefficient (PEDC), which we defined as the number of virus-positive fecal samples (from days 2 to 6 p.i.) divided by the total number of fecal samples (from days 2 to 6 p.i.) per group of mice (Table 1). The reason for focusing on days 2 to 6 p.i. is that peak excretion occurred in this period (Fig. 1), although occasionally a mouse excreted poliovirus for a longer period (up to and most likely beyond day 10 p.i.). Analysis of the excretion data of male (n = 17) versus female (n = 16) mice receiving 10^3 TCID50 (Mahoney) showed no gender-related differences in the duration or level of poliovirus excretion (data not shown).

The duration of excretion of poliovirus showed a strong dose-response relation, as shown by poliovirus titers in the feces of mice of different concentrations of wild-type serotype 1 (Mahoney). Intraperitoneal injection of 10^3 TCID50 of Mahoney did not result in detectable amounts (TCID50 > 10^3.8) of poliovirus in the feces, while a 10-fold increase (TCID50 = 10^4) of the inoculation titer resulted in excretion of poliovirus between days 2 and 10 p.i. in two of the six injected mice (Fig. 1a and Table 1). The duration of poliovirus excretion was considerably longer when 10^5 TCID50 of Mahoney was administered, although still one of the six animals did not excrete poliovirus at all (Fig. 1a and Table 1). When 10^6 TCID50 of Mahoney was administered, all animals excreted virus (up to a TCID50 of 10^5.8 per 100 mg of feces) and occasionally a mouse died at 5 to 9 days p.i. The mean level of poliovirus in the positive samples, in contrast, did not show a dose-response relation, as mean TCID50 levels were around 10^3 TCID50 per 100 mg of feces, irrespective of the inoculation dose (Table 1). Although the representative wild-type strains for the three different serotypes of poliovirus all showed the same excretion pattern, it is clear that both the duration and the level of MEF (serotype 2) excretion are reduced in comparison with those of Mahoney and Saukett (serotype 3) (Fig. 1b and Table 1).

Next, we compared the excretion level and pattern of DOR00-016, a representative cVDPV strain of the largest genogroup of the Hispaniola outbreak (14), with the excretion of OPV-1 and the wild-type serotype 1 strain (Mahoney). Both the duration and the level of fecal excretion of the DOR00-016 strain mimicked those of the Mahoney strain after inoculation of 10^5 TCID50 (Fig. 1e). The differences in the PEDCs of DOR00-016 (PEDC = 0.8) and Mahoney (PEDC = 1.0) were observed after injection of a TCID50 of 10^5 result from the fact that one of the six mice receiving DOR00-016 did not respond (no virus excretion during the entire monitoring period and no serotype 1–specific immunoglobulin G [IgG] antibodies). When representative strains of the two other genogroups of the Hispaniola outbreak (HAI0-003 and HAI01-007) were inoculated at a TCID50 of 10^6, all mice excreted poliovirus for comparable durations.

Blood samples of all mice were taken just before euthanasia at 10 days p.i., and serotype-specific poliovirus IgG levels were determined after a 1:50 dilution of the serum, as described below (1). The relative level of poliovirus type 1–specific antibodies was expressed as the ratio of the optical density at 405 nm (OD405) of the sample (S) to that of the positive control (P), where we considered an S/P of <0.1 to be positive (Table 1). We found an almost perfect correlation between the level of virus excretion during the 10-day observation period (74% positive) and the presence of an IgG titer at 10 days p.i. (68% positive) after the inoculation of wild-type (or wild-type-like) poliovirus strains. However, when OPV strains were inoculated at high titers, we observed hardly any fecal excretion, whereas a high IgG titer was found (Table 1 and data not shown). Mice that did not develop an IgG titer excreted virus for shorter periods (i.e., PEDC of 0.4 with a standard deviation [SD] of 0.3 versus a PEDC of 0.9 with an SD of 0.2). In general, the level of IgG-specific antibodies was higher after inoculation with a higher dose, although relatively large differences were found among mice receiving the same poliovirus strain and dose (Table 1). This high difference in response to the same inoculum and the fact that OPV-1 induces intermediate to high IgG titers in mice that do not excrete virus excludes the use of this parameter to predict the fecal excretion level and the duration of excretion of different (vaccine-derived) polioviruses.

The global eradication of the highly contagious wild-type poliovirus is a big challenge for mankind. The recent identification of circulating OPV-derived strains, which combine a high neurovirulence with a high transmissibility, in suboptimally vaccinated populations (2–5), is a major threat to this eradication. The lack of knowledge concerning the genetic determinants for the high-transmissibility phenotype hampers a proper risk assessment of the use of trivalent OPV in comparison to bivalent or monovalent OPV or inactivated polio vaccine. Also, the range of potential NPEV strains, which can act as donor sequences for cVDPV strains, will remain unknown as long as the genetic determinants of transmissibility have not been characterized in more detail.

Unfortunately, the resistance to oral infection of poliovirus receptor (PVR) transgenic mice prohibits a direct analysis of the difference in transmissibility (R0 or Rn) of poliovirus strains in a mouse population. Although several different promoters for the expression of the PVR in transgenic mice have
FIG. 1. The group average TCID₅₀ of poliovirus in the feces of individual mice (six ICR-PVRTg21 mice per group) was determined at days 1 to 10 following intraperitoneal injection of different strains and concentrations of poliovirus. Data of representative experiments are shown. (a) Wild-type serotype 1 (Mahoney) was given at a TCID₅₀ of 10⁴, 10⁵, and 10⁶. (b) Intermediate doses (TCID₅₀ = 10⁵) of the three different serotypes of wild-type poliovirus strains. (c) Intermediate doses (TCID₅₀ = 10⁴) of three serotype 1 strains: a vaccine (OPV-1) strain, a wild-type (Mahoney) strain, and a cVDPV (DOR00-016) strain. The detection level of poliovirus in the individual fecal samples is at a TCID₅₀ of 10¹.8, and the error bars represent the standard errors of the means.
been used (i.e., the promoter of PVR itself [18], the rat intestine fatty acid binding protein [20], and the human β-actin protein [8]), none of these transgenic mice can be infected with poliovirus by the oral route. For this reason we have analyzed the fecal excretion of different poliovirus strains in transgenic mice after intraperitoneal injection, as the total excretion amount of virus is one of the major determinants of transmissibility of picornaviruses.

We observed large differences between the excretion of the OPV vaccine strains on one hand and that of the cVDPV and wild-type strains on the other hand. The combination of mutations and recombinations of the Hispaniola strains is apparently sufficient to turn the low fecal excretion phenotype of OPV into one which is indistinguishable from that of the wild-type poliovirus. We expect to achieve further insight in the poliovirus transmissibility determinants by analyzing in vitro-generated site-directed mutagenized and recombinant polioviruses in our PVR mouse fecal excretion model.

The technical assistance of Femke van Nuten, Rutger Scheppe, and Femke van den Berg in preparing the poliovirus stocks and performance of the titrations is greatly appreciated. We thank Harrie van der Avoort for helpful discussions and for critically reading the manuscript and Ole Kew (CDC) for providing the Hispaniola strains.

This work was supported by a grant from the Prinses Beatrix Fonds (The Netherlands).

REFERENCES


<table>
<thead>
<tr>
<th>TCD50*</th>
<th>Virus (serotype)</th>
<th>Phenotype</th>
<th>No. of poliovirus-excreting mice/total no. injected</th>
<th>Mean (SD) excretion duration (days)</th>
<th>Mean (SD) poliovirus excretion (log10 TCD50/100 mg of feces)</th>
<th>Mean PEDC (SD)</th>
<th>No. of positive mice/no. inoculated (S/P; SD) at day 10 p.i.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^3</td>
<td>Mahoney (1)</td>
<td>Wild type</td>
<td>0/6</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0/6</td>
</tr>
<tr>
<td>10^4</td>
<td>Mahoney</td>
<td>Wild type</td>
<td>2/6</td>
<td>2.2 (3.4)</td>
<td>3.1 (1.1)</td>
<td>0.3 (0.4)</td>
<td>2/6 (0.8; 0.4)</td>
</tr>
<tr>
<td>10^5</td>
<td>OPV-1 (1)</td>
<td>Vaccine</td>
<td>3/12</td>
<td>0.4 (0.8)</td>
<td>2.0 (0.2)</td>
<td>0.0 (0.1)</td>
<td>2/12 (0.4; 0.2)</td>
</tr>
<tr>
<td></td>
<td>Mahoney</td>
<td>Wild type</td>
<td>27/30</td>
<td>4.3 (2.1)</td>
<td>3.0 (0.8)</td>
<td>0.7 (0.3)</td>
<td>19/27 (0.7; 0.3)</td>
</tr>
<tr>
<td></td>
<td>DOR00-016 (1)</td>
<td>cVDPV</td>
<td>12/12</td>
<td>5.1 (1.3)</td>
<td>2.8 (0.7)</td>
<td>0.9 (0.2)</td>
<td>7/11 (0.7; 0.3)</td>
</tr>
<tr>
<td></td>
<td>MEF (2)</td>
<td>Wild type</td>
<td>5/6</td>
<td>2.0 (1.7)</td>
<td>2.4 (0.3)</td>
<td>0.4 (0.3)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>OPV-3 (3)</td>
<td>Vaccine</td>
<td>0/16</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Saukett (3)</td>
<td>Wild type</td>
<td>6/6</td>
<td>3.8 (0.4)</td>
<td>2.7 (0.6)</td>
<td>0.8 (0.1)</td>
<td>ND</td>
</tr>
<tr>
<td>10^6</td>
<td>Mahoney</td>
<td>Wild type</td>
<td>14/14</td>
<td>5.1 (1.0)</td>
<td>3.2 (0.8)</td>
<td>0.9 (0.1)</td>
<td>9/9 (0.9; 0.1)</td>
</tr>
<tr>
<td></td>
<td>DOR00-016</td>
<td>cVDPV</td>
<td>5/6</td>
<td>5.3 (2.8)</td>
<td>3.5 (0.8)</td>
<td>0.8 (0.4)</td>
<td>4/5 (0.9; 0.1)</td>
</tr>
<tr>
<td></td>
<td>HAI00-003 (1)</td>
<td>cVDPV</td>
<td>6/6</td>
<td>4.8 (1.6)</td>
<td>3.3 (0.9)</td>
<td>0.8 (0.4)</td>
<td>4/5 (0.6; 0.4)</td>
</tr>
<tr>
<td></td>
<td>HAI01-007 (1)</td>
<td>cVDPV</td>
<td>6/6</td>
<td>5.5 (0.5)</td>
<td>3.3 (0.9)</td>
<td>1.0 (0.1)</td>
<td>5/5 (0.5; 0.2)</td>
</tr>
<tr>
<td>10^7</td>
<td>OPV-1</td>
<td>Vaccine</td>
<td>1/9</td>
<td>0.1 (0.3)</td>
<td>1.9</td>
<td>0.0 (0.1)</td>
<td>8/8 (0.3; 0.1)</td>
</tr>
<tr>
<td>10^8</td>
<td>OPV-2 (2)</td>
<td>Vaccine</td>
<td>1/10</td>
<td>0.1 (0.4)</td>
<td>2.8</td>
<td>0.0 (0.0)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>OPV-3</td>
<td>Vaccine</td>
<td>3/10</td>
<td>1.0 (1.4)</td>
<td>2.0 (0.1)</td>
<td>0.1 (0.2)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Injected intraperitoneally with 200 μL of PBS.

a Mice that had at least one poliovirus-positive fecal sample between days 2 and 10 p.i. were considered poliovirus excreting. Up to four groups of mice (n = 6 to 10) were used per experiment. The data in this table are based upon multiple experiments in cases where n was >10.

b The results of day 1 have been omitted from this analysis, as we frequently observed shedding of the inoculum after intraperitoneal injection of high doses (e.g., TCD50 = 10^9) of OPV (data not shown).

c PEDC of mice receiving the same poliovirus (at least six mice per group).

d A mouse was considered positive when S/P was > 0.1.

d ND, not determined.


