Poliovirus-Specific Memory Immunity in Seronegative Elderly People Does Not Protect against Virus Excretion

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Background. Dutch people born between 1925 and 1945 were ineligible for vaccination with the inactivated poliovirus vaccine (IPV) introduced in 1957 and may have escaped natural infection because of reduced poliovirus circulation. We examined whether people with low or undetectable antibody levels are susceptible to infection and whether memory immunity provides protection against virus excretion.

Methods. A total of 429 elderly participants were challenged with monovalent oral poliovirus vaccine (type 1 or 3) and followed for 8 weeks. Immune responses and virus excretion were compared for 4 groups, defined on the basis of seronegativity for poliovirus type 1 or 3, natural immunity, and IPV-induced immunity.

Results. On the basis of the rapidity of the antibody response and the absence of immunoglobulin M, we saw clear evidence of memory immune responses in 33% of the participants without detectable antibodies against poliovirus type 1 and in 5% of the participants without detectable antibodies against poliovirus type 3. Fecal virus-excretion patterns were not significantly different for seronegative participants, regardless of whether they showed evidence of memory immunity.

Conclusions. Rapid antibody responses after challenge with oral polio vaccine provide evidence for poliovirus-specific memory immunity in seronegative elderly people. However, in contrast to preexisting immunity, memory immunity does not protect against virus excretion. These results have important implications for the poliomyelitis-eradication initiative, in particular for future immunization policies after eradication has been achieved.

Presented in part: 13th European Congress of Clinical Microbiology and Infectious Diseases, Glasgow, United Kingdom, 10–13 May 2003 (abstract P683).

Financial support: Health Research and Development Council (ZonMw), The Netherlands.

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Until the eradication of poliomyelitis is established throughout the world, countries that have been certified to be free of endemic poliovirus remain at risk for importation of wild-type (wt) polioviruses from regions of the world in which they still circulate. Reintroduction of the virus might also arise from laboratory stocks, environmental samples, or deliberate release [1, 2]. Moreover, recent unexpected findings in Hispaniola and Egypt have shown that outbreaks can be caused by revertant Sabin-vaccine strains [3, 4].

In The Netherlands, poliovirus vaccination with injectable inactivated poliovirus vaccine (IPV) was introduced in 1957 and was offered to everyone born in and after 1945. In general, the Dutch population is well protected against poliomyelitis [5]. However, despite the large number of people who are seropositive for antibodies against poliovirus and a national vaccination coverage rate of 97%, the threat of a poliomyelitis outbreak in The Netherlands is...
still real, especially because of a sociogeographically clustered group who choose to remain unvaccinated for religious reasons. Furthermore, the population born between 1925 and 1945 has comparatively low poliovirus seroprevalence levels [5]. The latter group was ineligible for the routine vaccination program and might have escaped natural infection because of reduced poliovirus circulation; in addition, natural or vaccine-induced immunity may have waned. It is unclear whether everyone with a low or undetectable level of antibodies is susceptible to infection and is therefore at risk during an outbreak. Some people, particularly the elderly, may be protected by memory immunity (an accelerated immune response after challenge), because the immune system was previously primed. Previous studies have suggested that protection against poliovirus infection—whether due to previous experience with wt polioviruses or with vaccine strains—may be present in the absence of detectable serum antibodies [6–10].

The main purpose of the present study was, therefore, to find evidence of memory immunity in seronegative elderly people after challenge with oral polio vaccine (OPV) and to examine the protective efficacy of memory immunity. Immune responses and virus excretion were compared for 4 groups of participants, defined on the basis of seronegativity for poliovirus type 1 or 3, natural immunity, and IPV-induced immunity.

**PARTICIPANTS, MATERIALS, AND METHODS**

**Study design.** Elderly people born in the birth cohort presumed to be at risk (1925–1945: hereafter, "the risk birth cohort") and IPV recipients born in the adjacent birth cohort (1945–1950) were challenged with a standardized dose of monovalent OPV type 1 (MOPV-1) or type 3 (MOPV-3) and were followed for 8 weeks. An outline of the study design is given in figure 1. All inhabitants of Tiel from the risk birth cohort (n = 6175) and a random sample of inhabitants from the adjacent birth cohort (n = 850) were invited to participate in the screening. Tiel is a medium-sized town in the center of the country and has 40,000 inhabitants and average rates of vaccination coverage. The purpose of the screening was to assess levels of antibodies against poliovirus types 1–3. During screening, participants signed an informed-consent form, provided a serum sample (10 mL), and completed a questionnaire. Within 8 weeks, the participants received written information about their antibody levels. Those who were not selected for the second part of the study and had antibody levels that were inadequate to provide protection were advised to obtain a booster inoculation of diphtheria, tetanus, and inactivated poliovirus vaccine (DT-IPV).

For the second part of the study, the OPV challenge, participants were selected on the basis of their prechallenge serum neutralizing antibody titers, IPV vaccination histories, and exclusion criteria. Those with major medical problems (particularly immunodeficiency disorders) and with a history of OPV vaccination were excluded from the study. The selected participants were assigned to 1 of the 4 following groups: those seronegative for poliovirus type 1 with no history of vaccination (SN-1), those seronegative for poliovirus type 3 with no history of vaccination (SN-3), those seropositive for all 3 poliovirus types with no history of vaccination (i.e., those who were naturally immune [NI]), and those seropositive for all 3 poliovirus types with a documented history of a full series of IPV vaccinations and no history of OPV vaccination (IPVV). The NI and IPVV participants were randomly assigned to be challenged with either MOPV-1 (hereafter, "NI-1" and "IPVV-1") or MOPV-3 (hereafter, "NI-3" and "IPVV-3"), so that 6 groups were used in the analyses. At the first appointment, prechallenge (day 0) serum samples were obtained, after which OPV was administered. Stool samples were obtained on days 3, 7, 14, 21, 28, 35, 42, 49, and 56 after challenge. Serum samples were obtained on days 7, 28, and 56 after challenge. On completion of their participation, the participants who belonged to the SN-1 and SN-3 groups were offered a full series of inoculations with DT-IPV. The Medical Ethics Committee of the Dutch Organization for Applied Scientific Research (Leiden, The Netherlands) approved the study proposal.
Study population, participation rates (pr), and design Tiel, The Netherlands, 1999. IPV, inactivated poliovirus vaccine; IPVV-1 and -3, participants seropositive for all 3 poliovirus types with a documented history of a full series of IPV vaccinations and no history of OPV vaccination who were challenged with MOPV-1 and -3, respectively; MHS, municipal health services; MOPV-1 and -3, monovalent OPV type 1 and 3, respectively; NI-1 and -3, participants considered to be naturally immune who were challenged with MOPV-1 and -3, respectively; OPV, oral polio vaccine; SN-1 and -3, participants seronegative for poliovirus type 1 and 3, respectively, with no history of vaccination.

**Vaccines.** To keep the study feasibly small, only 2 vaccines, for poliovirus types 1 and 3, were used in the challenge experiments. Poliovirus types 1 and 3 are the most important types, having caused epidemics during recent decades. MOPV-1 and MOPV-3 (Oral-Virelon...
T1 Type 1 and Oral-Virelon T1 Type 3; Chiron Behring) contained $5.8 \times 10^5$ TCID$_{50}$ of poliovirus type 1 and $1.0 \times 10^4$ TCID$_{50}$ of poliovirus type 3, respectively, according to the manufacturer. Vaccine was received from the manufacturer 1 week before the start of the challenge study (April 1999), was stored at 4°C until use, was administered orally in 1-mL volumes, and was used within the shelf-life expiration date assigned by the manufacturer (October 1999).

**Collection of samples.** At each of the 5 visits, venous blood (10 mL) was obtained for antibody determinations (neutralizing antibody titers and poliovirus type specific IgA and IgM). Serum was separated and stored at -20°C until testing. Stool samples, collected to determine viral excretion, were collected at home and kept in the refrigerator until delivery or were sent immediately by regular mail to the National Institute of Public Health and the Environment. Virus isolation was performed by use of L20B cells, as described elsewhere [11].

**Serological assays.** All serum samples were tested for poliovirus antibodies by use of an ELISA developed by Herremans et al. (PoBi assay; [12]). Titers obtained in the PoBi assay and the standard neutralizing antibody test (NT) are closely correlated [12]. Seronegativity for poliovirus types 1 and 3 was confirmed by a standard NT, as recommended by the World Health Organization (WHO) [11]. The Sabin strains of poliovirus types 1 and 3 were used for this purpose. Furthermore, a standard NT was used to confirm all PoBi assay results in the SN-3 group. In brief, serial 2-fold dilutions of serum samples and 100 50% cell-culture infective doses of virus were incubated in 96-microwell plates (Greiner) for 3 h at 37°C. After incubation, $1.75 \times 10^4$ HEp-2C cells were added to each well. After 6 days of incubation at 37°C, the plates were read. The results are given as log$_2$ reciprocal titers, expressed as the reciprocal of the greatest dilution showing complete neutralization of the cytopathic effect of 100% cell-culture infectious doses. A titer of 1 : 8 (i.e., a log$_2$ titer of 3) or more is a generally accepted and frequently used indication of protective immunity. A rapid (i.e., memory) response was defined as a $\geq 4$-fold increase in neutralizing antibody titer occurring during the first 7 days after OPV challenge [13].

A total of 42 participants (10%) were excluded from the analysis because of misclassification. Misclassifications were caused by small differences between the antibody titers determined during screening and the prechallenge antibody titers. All serum samples were tested for poliovirus-specific IgA and for poliovirus-specific IgM by use of ELISAs, as described elsewhere [14, 15].

**RESULTS**

**Participation.** In total, 7025 people were invited to the screening, and 1847 people (26%) participated. The level of participation in the challenge study (for the entire period of 8 weeks) was much greater, with an average participation rate of 48%. Figure 1 shows the number of participants in each group and the corresponding participation rates.

**Serum neutralizing antibody response after OPV challenge.** The percentage of participants experiencing a $\geq 4$-fold increase in neutralizing antibody titers for poliovirus types 1–3 (hereafter, the “responders”) after challenge with either MOPV-1 or MOPV-3 are given in table 1 for SN-1, SN-3, NI-1, NI-3, IPVV-1, and IPVV-3 participants, stratified by prechallenge antibody titer. The postchallenge antibody responses were classified as rapid ($\geq 4$-fold increase occurring within 7 days), slow ($\geq 4$-fold increase occurring between days 8 and 56) and none (<4-fold increase during the study period).
Table 1. Percentages of participants experiencing a 4-fold increase in serum neutralizing antibody titer for poliovirus types 1, 2, and 3, stratified by prechallenge (day 0) antibody titers (PCT) and rapidity of response, and prechallenge sample sizes, in seronegative (SN), inactivated poliovirus vaccine vaccinated (IPVV), and naturally immune (NI) participants challenged with monovalent oral polio vaccine type 1 (MOPV-1) or 3 (MOPV-3) Tiel, The Netherlands, 1999.

<table>
<thead>
<tr>
<th>Vaccine challenge, participant category</th>
<th>Poliovirus type 1</th>
<th>Poliovirus type 2</th>
<th>Poliovirus type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of participants</td>
<td>Rapidity of response</td>
<td>No. of participants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(day 7)</td>
<td>(day 8–56)</td>
</tr>
<tr>
<td>MOPV-1 challenge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN-1 total</td>
<td>98</td>
<td>33</td>
<td>60</td>
</tr>
</tbody>
</table>
| of 0                                    | 55               | 33               | 56               | 11               | 0                | ...             | ...              | ...              | 0                | ...             | ...              | ...
| PCT of 1-2                              | 43               | 33               | 65               | 2                | 25               | 0               | 56               | 44               | 48               | 6               | 31               | 63               |
| IPVV-1 total                            | 43               | 12               | 21               | 67               | 34               | 3               | 9                | 88               | 38               | 5               | 21               | 74               |
| of 3-6                                  | 29               | 17               | 31               | 52               | 23               | 4               | 13               | 83               | 31               | 6               | 26               | 68               |
| PCT of 7                                | 14               | ...              | ...              | ...              | 11               | ...             | ...              | ...              | 7                | ...             | ...              | ...
| NI-1 total                              | 50               | 10               | 36               | 54               | 49               | 2               | 22               | 76               | 43               | 0               | 21               | 79               |
| of 3-6                                  | 46               | 11               | 39               | 50               | 42               | 2               | 26               | 72               | 42               | 0               | 21               | 79               |
| PCT of MOPV-3 challenge                 |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
| SN-3 total                              | 10               | 30               | 20               | 50               | 43               | 9               | 49               | 42               | 103              | 5               | 68               | 27               |
| of 0                                    | ...              | ...              | ...              | ...              | ...              | ...             | ...              | ...              | ...              | ...             | ...              | ...
| PCT of 1-2                              | 10               | 30               | 20               | 50               | 43               | 9               | 49               | 42               | 46               | 7               | 59               | 34               |
| IPVV-3 total                            | 47               | 6                | 94               | 46               | 46               | 2               | 22               | 78               | 45               | 0               | 7                | 93               |
| of 3-6                                  | 37               | 8                | 0                | 92               | 38               | 0               | 26               | 74               | 39               | 0               | 8                | 92               |
| PCT of 7                                | 10               | ...              | ...              | ...              | ...              | ...             | ...              | ...              | ...              | ...             | ...              | ...
| NI-3 total                              | 55               | 0                | 7                | 93               | 53               | 0               | 15               | 85               | 44               | 0               | 25               | 75               |
| of 3-6                                  | 45               | 0                | 9                | 91               | 45               | 0               | 18               | 82               | 44               | 0               | 25               | 75               |
| PCT of 7                                | 10               | ...              | ...              | ...              | ...              | ...             | ...              | ...              | ...              | ...             | ...              | ...

**NOTE.** Data are percentages of participants, unless otherwise noted. Boldface values represent serological responses to the same type of virus as that used in the challenge. IPVV-1 and -3, participants seropositive for all 3 poliovirus types with a documented history of a full series of IPV vaccinations and no history of OPV vaccination who were challenged with MOPV-1 and -3, respectively; NI-1 and -3, participants considered to be NI who were challenged with MOPV-1 and -3, respectively; SN-1 and -3, participants seronegative for poliovirus type 1 and 3, respectively, with no history of vaccination.

* Excluded from the analysis because booster responses could not be evaluated for those with a PCT of 7 (the greatest dilution tested was 1 : 256 [log₂ titer of 8]).
Most of the seronegative participants challenged with OPV experienced a slow (i.e., primary) antibody response—60% of the SN-1 participants after MOPV-1 challenge and 68% of the SN-3 participants after MOPV-3 challenge. In contrast, 33% of the SN-1 participants and only 5% of the SN-3 participants experienced the expected rapid (i.e., secondary) antibody response after challenge. The remainder did not experience an antibody response to the challenge.

Overall, 93% of the SN-1 participants experienced a 4-fold increase in neutralizing antibody titers for poliovirus type 1 after challenge with MOPV-1, which was significantly more than the 33% of the IPVV-1 participants and the 46% of the NI-1 participants. The groups challenged with MOPV-3 showed a similar pattern but with a lower rate of 4-fold increases in neutralizing antibody titer. In the 2 seronegative groups, lower prechallenge antibody titers were not associated with a higher rate of 4-fold increases in neutralizing antibody titer.

The serological data showed clear evidence of cross-reactivity. A substantial percentage of the participants challenged with MOPV-1 experienced a 4-fold increase in neutralizing antibody titers for poliovirus types 2 and 3 as well. Similar results were found for the participants challenged with MOPV-3. The highest percentages of participants experiencing a cross-reactive 4-fold increase in neutralizing antibody titer were observed for poliovirus type 2 in the 2 seronegative groups 56% of the SN-1 participants and 49% of the SN-3 participants. In addition, booster responses for the virus types other than the one used in the challenge were also found for the IPVV and NI participants (up to 26%).

**IgM response.** Poliovirus-specific IgM was undetectable in all serum samples from the rapid responders and was almost absent in the serum samples from the slow responders experiencing a 4-fold increase in neutralizing antibody titer on day 56 (table 2). Poliovirus-specific IgM was, however, detectable in serum samples from slow responders experiencing a 4-fold increase in neutralizing antibody titer on day 28. The greatest prevalences were found in the 2 seronegative groups.

**Table 2. Percentages of participants positive for IgM, stratified by rapidity of response (day 7, 28, or 56), and prechallenge (day 0) sample sizes, in seronegative (SN), inactivated poliovirus vaccine vaccinated (IPVV), and naturally immune (NI) participants who were challenged with monovalent oral polio vaccine type 1 (MOPV-1) or 3 (MOPV-3) Tiel, The Netherlands, 1999.**

<table>
<thead>
<tr>
<th>Participant category</th>
<th>Rapid responder (day 7)</th>
<th>Slow responder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of participants</td>
<td>IgM positive, %</td>
</tr>
<tr>
<td>SN-1 (n = 103)</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>SN-3 (n = 115)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>IPVV-1 (n = 29)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>IPVV-3 (n = 39)</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>NI-1 (n = 46)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>NI-3 (n = 44)</td>
<td>0</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** IPVV-1 and -3, participants seropositive for all 3 poliovirus types with a documented history of a full series of IPV vaccinations and no history of OPV vaccination who were challenged with MOPV-1 and -3, respectively; NI-1 and -3, participants considered to be NI who were challenged with MOPV-1 and -3, respectively; SN-1 and -3, participants seronegative for poliovirus type 1 and 3, respectively, with no history of vaccination.
IgA response. As expected, poliovirus-specific IgA was absent in the majority of serum samples from seronegative participants before challenge and increased significantly after challenge, with a peak on day 28 (table 3). Of the IPVV and NI participants, the majority were already seropositive for IgA before challenge.

Table 3. Percentages of participants positive for IgA, in seronegative (SN), inactivated poliovirus vaccine vaccinated (IPVV), and naturally immune (NI) participants who were challenged with monovalent oral polio vaccine type 1 (MOPV-1) or 3 (MOPV-3) Tiel, The Netherlands, 1999.

<table>
<thead>
<tr>
<th>Participant category</th>
<th>IgA positive, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>SN-1 (n = 103)</td>
<td>3</td>
</tr>
<tr>
<td>SN-3 (n = 115)</td>
<td>5</td>
</tr>
<tr>
<td>IPVV-1 (n = 29)</td>
<td>69</td>
</tr>
<tr>
<td>IPVV-3 (n = 39)</td>
<td>97</td>
</tr>
<tr>
<td>NI-1 (n = 46)</td>
<td>59</td>
</tr>
<tr>
<td>NI-3 (n = 44)</td>
<td>86</td>
</tr>
</tbody>
</table>

NOTE. IPVV-1 and -3, participants seropositive for all 3 poliovirus types with a documented history of a full series of IPV vaccinations and no history of OPV vaccination who were challenged with MOPV-1 and -3, respectively; NI-1 and -3, participants considered to be NI who were challenged with MOPV-1 and -3, respectively; SN-1 and -3, participants seronegative for poliovirus type 1 and 3, respectively, with no history of vaccination.

Poliovirus excretion. Quantitative data on virus excretion are shown in figures 2 and 3. Most seronegative participants challenged with OPV excreted virus for a considerable period of time (figures 2A and 3A). In the SN-1 group, the highest percentage (81%) excreted poliovirus type 1 three days after challenge, whereas, in the SN-3 group, the highest percentage (75%) excreted poliovirus type 3 seven days after challenge. Although the maximum excretion rates for poliovirus type 3 were observed somewhat later, compared with those for poliovirus type 1, overall, the percentage of seronegative participants who excreted poliovirus type 3 was higher during the entire study period.
Figure 2  Percentage excreting poliovirus (bars), duration of poliovirus excretion, and quantity of poliovirus type 1 per gram of stool (expressed as log10 mean titer [e.g., 4 = 10^4]) (solid lines), for participants seronegative for poliovirus type 1 (SN-1) (A), participants seropositive for all 3 poliovirus types with a documented history of a full series of inactivated poliovirus vaccine (IPV) vaccinations and no history of oral polio vaccine (OPV) vaccination (IPVV-1) (B), and naturally immune (NI-1) participants (C) challenged with 5.8 × 10^5 TCID50 of monovalent OPV type 1, stratified by rapidity of serologic response Tiel, The Netherlands, 1999.
Figure 3  Percentage excreting poliovirus (bars), duration of poliovirus excretion, and quantity of poliovirus type 3 per gram of stool (expressed as log10 mean titers [e.g., 4 = 10^4]) (solid lines), for participants seronegative for poliovirus type 3 (SN-3) (A), participants seropositive for all 3 poliovirus types with a documented history of a full series of inactivated poliovirus vaccine (IPV) vaccinations and no history of oral polio vaccine (OPV) vaccination (IPVV-3) (B), and naturally immune (NI-3) participants (C) challenged with 1.0 × 10^4 TCID50 of monovalent OPV type 3, stratified by rapidity of serologic response. Tiel, The Netherlands, 1999.

Comparison of the excretion rates for poliovirus type 1 showed that rapid and slow responders excreted similar amounts of virus. For poliovirus type 3, the excretion rates of the rapid and slow responders showed a consistent pattern: the slower the increase in neutralizing antibody titer, the higher the percentage of individuals excreting poliovirus type 3. Furthermore, in both the SN-1 and SN-3 groups, there was no relationship between prechallenge titers and (1) duration of excreting or (2) the percentage of participants excreting virus (data not shown).

Figure 2B and 2C and figure 3B and 3C clearly show the effect of preexisting antibodies on virus excretion. As expected, only 17% of the IPVV-1 participants and 18% of the IPVV-3 participants excreted challenge virus, and the time of excretion was short (maximum, 28 days), compared with that in the 2 seronegative groups. Similar results were found for the NI participants. After challenge, only 22% of the NI-1 participants and 30% of the NI-3 participants excreted challenge virus, and the period of excretion was relatively short.

Finally, the mean titers of virus isolated from stool were not significantly different between poliovirus type 1 excreters and poliovirus type 3 excreters when SN-1, SN-3, IPVV, or NI participants were compared. There was no relationship between the titers of virus shed in stool and day after challenge.
DISCUSSION

In the present study, we found clear evidence of memory immunity in 33% of the elderly population without detectable antibodies against poliovirus type 1 (i.e., the SN-1 group) and in 5% of the elderly population without detectable antibodies against poliovirus type 3 (i.e., the SN-3 group). The complete absence of IgM responses among rapid responders is supplementary evidence for the existence of memory immunity, because IgM antibodies are characteristic of primary antibody responses. In contrast to preexisting antibodies, memory immunity, as measured in persons seronegative for the type of virus they are challenged with, does not protect against virus excretion. These results have important implications for the poliomyelitis-eradication initiative, in particular for future immunization policies after eradication has been achieved.

Our study was aimed at the elderly because lower seroprevalences were found in that age group [5]. To our knowledge, there are no published studies that have evaluated the antibody response among the elderly after artificial OPV challenge. All OPV challenge studies conducted to date have focused on children. However, it is important to mention that some of these studies have suggested that people with low or undetectable serum antibody levels are probably not in danger of developing clinical poliomyelitis, because they possess memory immunity [16, 17]. The concept of immunologic hyperreactivity, or immunologic memory, was invoked almost 50 years ago [18]. As this concept developed [19, 20], it was suggested that, when the level of neutralizing antibodies fell below detectability, immunologic memory would persist irreversibly, so that restimulation by vaccine or infection would result in a rapid and strong increase in antibody levels. This secondary response to infection was postulated to be rapid enough to protect against virus replication and paralytic disease. In other words, the individual who has immunologic memory, even if he or she does not have (detectable) circulating antibodies at the time of exposure, may respond sufficiently rapidly to block invasion of the central nervous system and prevent viral excretion in feces. In the present study, a rapid (i.e., memory) response was defined as a 4-fold increase in neutralizing antibody titer occurring within 7 days after OPV challenge, which is the definition most commonly used [13].

Our study found few rapid responders—33% of the SN-1 participants and 5% of the SN-3 participants. However, it is likely that this is an underestimation of the true effect, because antibody responses considered to be indicative of a rapid response were evaluated on day 7 only. Our choice of day 7 was based on the definition of memory response and on the results of studies by Herremans et al. [21] and Rumke et al. [10], in which IPV was used as the challenge virus.

Noteworthy is the small number of rapid responders observed after challenge with MOPV-3. Although one might argue that this finding was caused by the fact that the potency of the type 3 vaccine used in the present study was less than that recommended by the WHO for trivalent OPV, our results on virus-excretion patterns clearly show that this was not the case. After all, no differences in the virus-excretion patterns of the 2 seronegative groups were observed. Furthermore, the OPV type 3 in trivalent OPV is more highly dosed, compared with monovalent OPV type 3, to balance the dominance of OPV type 2 [22]. Finally, our results are consistent with those of an earlier study in children who received trivalent OPV; this study showed that rates of seroconversion are lowest for poliovirus type 3 [23].

The small number of antibody responses (4-fold increase) among the IPVV and NI participants in the present study seems to indicate an excellent level of protection—there was no increase in neutralizing antibody titer because poliovirus replication was absent. In these participants, titers of antibody (both IgG and IgA) against poliovirus were already present before OPV challenge. As described elsewhere [24], secretory IgA provides a local barrier to poliovirus infection, although the level of antibody that provides protection is not known. The presence of IgA in the serum of the IPVV participants can be explained by the fact that poliovirus was still endemic during the initial period of their lives, and so infection with poliovirus might have occurred. Because IPV vaccination alone is not able to induce IgA, previous mucosal priming with live virus is needed. Subsequently, IPV is able to boost IgA
responses, which explains the higher prevalence of IgA found in the IPVV participants, compared with that in the NI participants [21].

The prevalence of antibody responses was somewhat greater for the NI participants, which can be explained by the lower prechallenge antibody levels. Previous studies [8, 9] have suggested that higher prechallenge antibody levels prevent enteric re-infection by a vaccine virus after a challenge dose is administered, whereas lower prechallenge antibody levels permit boosting infection to take place.

In most previous challenge studies that used monovalent vaccine, serological responses were described only for the type of virus used for the challenge. In the present study, the existence of cross-reactivity has been clearly shown. In the seronegative groups, as many as 66% of the participants were found to experience a primary response for 1 of the 2 poliovirus types other than the challenge type. Although most participants experienced a primary response, we even found a noticeable number of participants who experienced a rapid response for a poliovirus type other than the challenge type. The high level of cross-reactivity observed in the present study is not a PoBi assay artifact, because all results in the SN-3 group and a sample of the results in the SN-1 group (25%) were confirmed by a standard NT. Moreover, this surprising level of cross-reactivity has not previously been observed with the PoBi technique [12]. A potential explanation is that elderly people have previously come into contact with the other types, given that poliovirus was endemic during their lives. These cross-reactive responses we measured could partly be secondary responses.

A unique feature of the present study was the challenge with OPV instead of the more commonly used IPV; challenge with OPV simulates a more natural route of infection and provided us with the opportunity to monitor virus excretion in feces as a parameter of protection. Failure to detect viral multiplication in the intestine (by excretion of virus in stool) after challenge with poliovirus indirectly demonstrates the presence of intestinal immunity. Conversely, fecal shedding of virus comparable with that seen in a nonimmune individual suggests absence of intestinal immunity and neutralization of the virus. One of the most striking results of the present study is that similar fecal virus-excretion patterns were found for the rapid and slow responders in the 2 seronegative groups. Furthermore, ~80% of the seronegative participants excreted virus after challenge with OPV, and, for 85% 100% of those who showed a memory response, virus excretion continued for up to 8 weeks after challenge. The effect that preexisting antibodies have on virus excretion was clearly shown: only ~20% of the IPVV participants and ~25% of the NI participants excreted challenge virus, and the period of excretion was relatively short.

Extrapolation of our findings to the general population results in estimates that at least 6% (for poliovirus type 1) and 15% (for poliovirus type 3) of the elderly population of The Netherlands is at risk for infection [5]. In addition to the individual risk of infection, the risk of the circulation of poliovirus among the population should be emphasized, because the vast majority of those protected by memory immunity will excrete virus for a substantial period of time after exposure. Moreover, virus excretion will be considerably higher after exposure to wt poliovirus, compared with that after exposure to OPV.

It can be debated whether protection against virus excretion (as measured in the present study) is correlated with protection against disease. Although the implications of our results will manifest themselves only in the event of the reintroduction of the virus—and the risk of reintroduction is small—in the event of an epidemic, we would consider vaccination for those born between 1925 and 1945 who live in the Bible Belt of The Netherlands.

In summary, on the basis of the rapidity of the antibody response and the absence of IgM, we conclude that there is clear evidence for the existence of memory immunity in 33% of the elderly population without detectable antibodies against poliovirus type 1 and in 5% of the elderly population without detectable antibodies against poliovirus type 3. However, the majority of seronegative individuals, whether showing evidence of memory immunity or not, are a threat to the poliomyelitis-eradication initiative, because they are not protected against virus replication after challenge with OPV.
Acknowledgments

We are indebted to all staff of the Rivierenland Municipal Health Services (Tiel, The Netherlands); the nurses recruited from the Utrecht Doctors' Laboratory Foundation; all volunteers from Tiel who participated in this study; Marion Koopmans, for helpful comments regarding the ideas for this study; Rutger Schepp and Tineke Herremans, for expert laboratory technical assistance; Bennie Bloemberg, for assisting with preparations for the fieldwork; and Hester de Melker, for help with the project and preparation of the manuscript.
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