

Poliovirus-Specific Immunoglobulin A in Persons Vaccinated with Inactivated Poliovirus Vaccine in The Netherlands

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In The Netherlands the inactivated poliovirus vaccine (IPV) is used for protection against poliomyelitis. It is not clear if parenteral vaccination with IPV can lead to priming of the mucosal immune system. We developed and evaluated enzyme-linked immunosorbent assays for the detection of poliovirus serotype-specific immunoglobulin A (IgA) and secretory IgA antibodies. Using these assays we examined the kinetics of the IgA response in sequential serum samples from 15 poliomyelitis patients after natural infection with serotype 3 poliovirus. In 36% of the patients IgA remained present for up to 5 months postinfection. Furthermore, we examined, in an IPV-vaccinated population, the presence of IgA antibodies in sera from young children (4 to 12 years of age; $n = 177$), sera from older children (between 13 and 15 years of age; $n = 123$), sera from healthy blood donors ($n = 66$), and sera from naturally immune elderly persons ($n = 54$). The seroprevalence of IgA to all three serotypes was low in young vaccinated children (5 to 7%), and the seroprevalence of IgA types 2 and 3 was low in older vaccinated children (2 to 3%). The seroprevalence of antibodies to type 1 was significantly higher (18%) in older children than in younger children. This higher seroprevalence is most likely explained by the persistence of IgA following infection with the serotype 1 wild-type poliovirus strain during the 1978 epidemic. In healthy adults, the seroprevalence of type 1- and type 2-specific IgA was significantly higher than that in young children. These results suggest that at least part of the IgA found in the older population is induced by infections unrelated to the IPV vaccination schedule. Finally, we found that parenteral vaccination with IPV was able to boost secretory IgA responses in 74 to 87% of a naturally exposed elderly population ($n = 54$). While the presence of secretory IgA in IPV-vaccinated persons has been documented previously, our findings suggest that mucosal priming with live virus is necessary to obtain an IgA response after IPV booster vaccination.

Systemic antibody responses to poliovirus infection and vaccination (with live or inactivated virus) have been studied extensively. The presence of circulating neutralizing antibodies is sufficient for protection from paralytic disease (13). In contrast, less is known about the induction of mucosal immunity, which is important in limiting virus circulation in the community along with protection from infection (7, 13). An important component of mucosal immunity is secretory immunoglobulin A (sIgA). The presence of sIgA on mucosal surfaces reduces viral excretion after oral poliovirus vaccine (OPV) challenge (12). In theory, intramuscular vaccination with inactivated poliovirus vaccine (IPV) is expected to induce little or no sIgA. However, in several studies some degree of local immunity was measured in IPV vaccinees, but it was less effective than that in people vaccinated orally with OPV or infected with wild-type virus (3, 4, 7, 8, 14). A difficulty in comparing results from different studies is that vaccination schedules and dosages have not been standardized between countries. As a result, it remains unclear if the superiority of OPV vaccination over vaccination with enhanced-potency IPV with respect to mucosal immunity applies to the situation in The Netherlands, where a total of six IPV vaccinations are given at 3, 4, 5, and 12 months and 4 and 9 years of age. In addition, studies of mucosal

immunity have mostly been done in recently vaccinated individuals, which may not reflect the situation in older age groups. The question is whether IPV vaccination confers sufficient mucosal immunity to prohibit virus circulation following introduction in the community. This is particularly important in The Netherlands, where pockets of religious communities with low vaccination coverage exist. In these groups, epidemics of poliomyelitis occurred in 1978 and 1992 (5, 16, 17). The purpose of this work was to develop poliovirus-specific IgA assays and to study the kinetics of IgA responses after infection and in an IPV-vaccinated population.

MATERIALS AND METHODS

Clinical samples. The specificity of the poliovirus-specific IgA enzyme-linked immunosorbent assays (ELISAs) was tested with serum samples from persons who were negative for neutralizing antibodies to poliovirus ($n = 114$). These samples were obtained from nonvaccinated children from a part of the population which refuses vaccination for religious reasons. Samples that had titers of neutralizing antibodies of $<1:2$ for all three serotypes of poliovirus and that were negative for antibodies to other components of the vaccine cocktail (diphtheria and tetanus toxoids) that is used in the routine immunization of children in The Netherlands were considered true negatives. The sensitivities of the assays were determined with a panel of sera collected from patients with a proven poliovirus infection within 2.5 months after the onset of paralysis. Patients included persons infected with poliovirus during the serotype 3 outbreak which occurred from 1992 to 1993 in The Netherlands ($n = 54$) and patients from Pakistan (all three serotypes; 1991 to 1995; $n = 98$). Infection was confirmed in all patients by isolation of wild-type poliovirus from stool samples and by detection of poliovirus-specific IgM in serum by ELISA (11). Isolated wild-type polioviruses were discriminated from vaccine-derived viruses by an ELISA with type-specific cross-absorbed antisera described previously (20).

To determine the kinetics of the IgA response after natural infection, sequential serum samples from 15 patients (age range, 1 to 36 years) from the 1992 and

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1993 serotype 3 epidemic in The Netherlands were tested for the presence of IgM and IgA antibodies. Poliovirus-specific IgM levels in serum samples were determined to confirm primary infection.

To determine the seroprevalence of poliovirus-specific IgA in an IPV-vaccinated population, sera from the following groups were investigated. (i) Sera were collected from schoolchildren between 4 and 12 years of age (collected shortly after IPV vaccination; $n = 177$) or between 13 and 15 years of age ($n = 123$). These serum samples had been collected during the 1992 and 1993 outbreak in The Netherlands to check the seroprevalence of neutralizing antibodies to poliovirus and to test for evidence of infection with poliovirus in this group. All children had been vaccinated with IPV, had protective levels of neutralizing antibodies to all three serotypes, and were positive for antibodies to diphtheria and tetanus toxoids (also included in the vaccine). The young children had received four to six doses of IPV and the older schoolchildren (ages, 13 to 15 years) had completed the IPV vaccination schedule several years ago.

(ii) Sera from fully IPV-vaccinated healthy blood donors were tested to determine the long-term persistence of IgA after vaccination ($n = 66$). This latter group was compared with age-matched blood donors from Belgium ($n = 66$), where OPV is used in the national vaccination program (in Belgium OPV is given at 3, 4, 5, and 12 months and 6 and 12 years of age). The age range of both groups of blood donors was between 18 and 65 years (average age, 39 years).

(iii) Sera from a group of unvaccinated older persons from The Netherlands (ages, 52 to 85 years; $n = 54$) who had been given a single dose of IPV were examined for the presence of neutralizing antibodies and poliovirus-specific IgA. This group was expected to be naturally exposed at a young age when poliovirus was endemic in The Netherlands. Sera were collected at the time of IPV vaccination and 1 and 4 weeks thereafter.

IgA ELISA. Wells of microtiter plates (Maxisorb; Nunc) were coated overnight at 4°C with serotype-specific monoclonal antibody to poliovirus at a concentration of 0.6 to 1.2 µg/ml in 0.04 M carbonate-bicarbonate buffer (pH 9.6). The monoclonal antibodies used were 5-18D8 for poliovirus type 1, 1-10C9E6 for type 2, and 2-13D9 for type 3 (15). Negative control wells were coated with a monoclonal antibody to influenza A virus [6-21/19-6, A/Singapore/6/86 (H1N1) strain specific]. After blocking for 1 h at 37°C with 5% BLOTTO (Pierce, Oud Beijerland, The Netherlands) in phosphate-buffered saline (PBS) containing 0.05% Tween 20, 40 to 70 D-antigen units (form found in the infectious virus) of formaldehyde-inactivated poliovirus was added to each well. We used the inactivated Mahoney strain for the type 1 assay, strain MEF for the type 2 assay, and strain Saukett for the type 3 assay; all strains were from the vaccine production facility of the National Institute of Public Health and the Environment. Poliovirus strains were originally derived from the American Type Culture Collection. The plates were incubated for 2 h at 37°C. PBS containing 0.5% Tween 20 and 2% BLOTTO was used as a dilution buffer. Volumes of 100 µl were used, and the plates were washed four times in PBS with 0.05% Tween 20 between each incubation step. In parallel wells, dilution buffer without virus was added to control for nonspecific binding of sera. Prior to testing, sera were depleted of IgG with Quik-Sep (Isolab, Mechelen, Belgium) according to the manufacturer's instructions to prevent possible isotype competition. Sera at dilutions of 1/50 were added to the plates, and the plates were incubated overnight at 4°C. After washing, an optimal dilution (1/8,000) of goat anti-human IgA labelled with alkaline phosphatase (alpha-chain specific; Sigma, Zwijndrecht, The Netherlands) was added, and the plates were incubated for 1.5 h at 37°C. The plates were washed, and 100 µl of *p*-nitrophenylphosphate at a concentration of 1 mg/ml in 0.1 M glycine buffer (pH 10.4) was added to each well. After incubation at room temperature for 30 min the plates were read at 405 nm by use of an Organon Teknika microwell system 510 spectrophotometer. A serum sample was considered positive if the optical density (OD) was above the cutoff level, defined as the average OD + 3 standard deviations of the results obtained with negative control sera from nonvaccinated persons ($n = 114$). At least one IgA-positive serum sample (derived from an OPV-vaccinated subject) and one IgA-negative control serum sample were included on each plate. Optimal dilutions of monoclonal antibodies, viral antigen, sera, and detector antibodies were established by checkerboard titrations. The dilutions of reagents that were chosen gave the highest OD differences between signal and background levels. The specificities of positive signals were confirmed by blocking experiments in which serum samples were preincubated with homologous poliovirus (± 120 D-antigen units) for 2 h at 37°C and centrifuged at $2,000 \times g$ for 3 min to remove immune complexes prior to testing by ELISA. A >50% reduction of the signal was considered confirmatory.

IgA capture ELISA. To determine if IgA detected after IPV vaccination was also present in its secretory form, a capture ELISA was used to investigate the sera from the population of older individuals. The assay was a modification of the IgM ELISA that has been described previously (11). Briefly, microtiter plates were coated with monoclonal antibody against the secretory component (Sigma, Zwijndrecht, The Netherlands) overnight at 4°C in carbonate buffer. The plates were blocked with 5% normal goat serum, serum dilutions (1:50) were added, and the plates were incubated for 1.5 h at 37°C. Formaldehyde-inactivated poliovirus type 1, 2, or 3 was added as described earlier, and bound antigen was detected with horseradish-peroxidase-labelled serotype-specific monoclonal antibody (1 h, 37°C). Tetramethylbenzidine (TMB) (0.1 mg/ml) in 0.11 M sodium acetate buffer was used as a substrate, and the reaction was stopped after 30 min with 2 M H₂SO₄. Serum samples were considered positive if the responses were

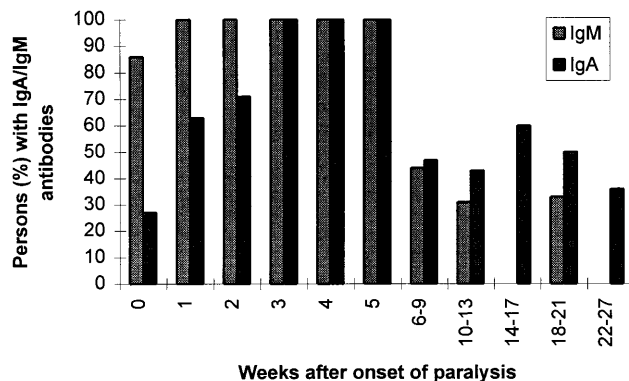


FIG. 1. Positivity rates of IgM and IgA antibodies to poliovirus type 3 in poliomyelitis patients. Results are from consecutive serum samples from 15 patients with poliomyelitis collected during the 1992 and 1993 outbreak in The Netherlands.

above the cutoff level, defined as the average OD + 3 standard deviations of the results obtained with negative control sera from nonvaccinated persons ($n = 114$). At least one sIgA-positive control serum sample and one sIgA-negative control serum sample were included on each plate.

Poliovirus type-specific IgM antibody capture ELISA. The IgM ELISA was performed as described previously (11). In brief, wells of microtiter plates were coated overnight at 4°C with 100 µl of μ -chain-specific monoclonal antibody to human IgM (Sanbio BV, Uden, The Netherlands) at a dilution of 1:100 in PBS supplemented with 0.5% Tween 20 and 5% fetal calf serum. Serum dilutions (1:50) were added, and the plates were incubated overnight at 4°C. An IPV suspension containing between 40 and 70 D-antigen units was added, and the plates were incubated for 2 h at 37°C. Bound antigen was detected with horseradish peroxidase-labelled serotype-specific monoclonal antibodies (1 h, 37°C). TMB was used as a substrate, and color development was stopped after 12 min by the addition of 2 M H₂SO₄. A positive control serum sample, a negative control serum sample, and a positive-negative cutoff control serum sample were examined in each assay. The positive-negative cutoff serum sample was prepared on the basis of comparison with the distribution of the values of the OD at 450 nm (OD₄₅₀) obtained with sera from both patients and healthy controls. A ratio of >1 between the OD₄₅₀ of the sample and the OD₄₅₀ of cutoff serum sample was considered to indicate the presence of poliovirus-specific IgM in the sample.

Neutralization assay. Titers of poliovirus-neutralizing antibody in sera were determined in the standard microneutralization test as recommended by the World Health Organization (21) by using virus strains Mahoney (serotype 1), MEF (serotype 2), and Saukett (serotype 3) as challenge viruses.

Statistical methods. To determine the significance of the difference in seroprevalence between two groups, a chi-square analysis was performed. *P* values of <0.05 were considered significant.

RESULTS

Specificity and sensitivity of the poliovirus-specific IgA ELISAs. The specificities of the poliovirus-specific IgA ELISAs were 100, 99, and 99% for poliovirus serotypes 1, 2, and 3, respectively. Poliovirus-specific IgA was detected in 89, 81, and 90% of the samples from serotype 1-, 2-, and 3-infected patients, respectively. Positive signals in the ELISA could only be blocked by preincubation with the homologous poliovirus and not with heterologous virus (data not shown).

Kinetics of IgA production in patients infected with wild-type poliovirus. At least one of the serum samples from all type 3-infected poliomyelitis patients ($n = 15$) had poliovirus serotype 3-specific IgA and IgM antibodies. Four patients had IgA antibodies to all three serotypes, and one patient had IgA against types 1 and 3. Poliovirus serotype 3-specific IgA reached a peak at 3 to 4 weeks after the onset of paralysis (Fig. 1) and decreased to low levels within 3 months. Thirty-six percent of the patients remained positive for IgA for up to 5 months postinfection. In individual patients the maximum levels of IgA were found later than the maximum levels of IgM (data not shown).

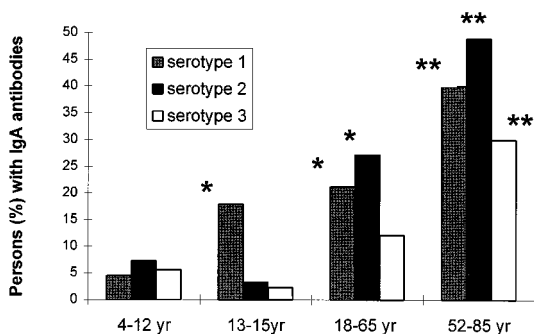


FIG. 2. Seroprevalence of IgA antibodies to poliovirus types 1, 2, and 3 in IPV-vaccinated persons of different ages in The Netherlands (groups I to III) and in persons born before the vaccination program (group IV). The populations were as follows: group I, 4- to 12-year-old schoolchildren ($n = 177$); group II, 13- to 15-year-old schoolchildren ($n = 123$); group III, 18- to 65-year-old IPV-vaccinated blood donors ($n = 66$); and group IV, 52- to 85-year-old persons without previous vaccination ($n = 54$). *, significant ($P < 0.05$) compared to 4- to 12-year-old schoolchildren; **, significant ($P < 0.05$) compared to schoolchildren and blood donors.

IgA in IPV-vaccinated children. Poliovirus-specific IgA to serotypes 1, 2, and 3 was found in 4.5, 7.3 and 5.6% of young children (ages, 4 to 12 years), respectively (Fig. 2). No correlation was found between the total number of IPV doses received and the number of children with detectable IgA levels (data not shown). Of the older schoolchildren (ages, 13 to 15 years), 18% had IgA antibodies to serotype 1, whereas the seroprevalences of IgA to serotypes 2 and 3 were low (3.3 and 2.3%, respectively) (Fig. 2).

IgA levels in IPV-vaccinated healthy adults. IgA antibodies to serotypes 1, 2, and 3 were found in 21.2, 27.3, and 12.1% of healthy adult blood donors, respectively (Fig. 2). The seroprevalence of poliovirus serotype 1- and 2-specific IgA was significantly higher ($P < 0.05$; chi-square test) in adults than in the young schoolchildren (ages, 4 to 12 years). Antibodies to serotypes 2 and 3 were significantly ($P < 0.05$; chi-square test)

more prevalent in the blood donors than in the older children (ages, 13 to 15 years).

IgA levels in OPV-vaccinated healthy adults. IgA was found more frequently in the OPV-vaccinated Belgian blood donors (33.8, 32.3, and 32.3% for serotypes 1, 2, and 3, respectively) than in age-matched IPV-vaccinated donors from The Netherlands (21.2, 27.3, and 12.1% for serotypes 1, 2, and 3, respectively), as mentioned earlier; the difference was only significant for serotype 3 ($P < 0.05$; chi-square test).

IgA antibodies in elderly individuals before and after IPV vaccination. Before IPV vaccination of a group of older persons (ages, 52 to 85 years; $n = 53$), 87, 83, and 79% had neutralization titers ($\geq 1:8$) to serotypes 1, 2, and 3, respectively. Four weeks after vaccination, 96% of these individuals had protective levels of neutralizing antibodies for all three serotypes. Only two persons did not develop neutralizing antibodies to serotype 3 after receiving the IPV booster. Before IPV vaccination, 40, 49, and 30% of the persons had detectable IgA antibodies to serotypes 1, 2, and 3, respectively (Fig. 2); in most cases the ELISA absorbance values were low, and median values were below the cutoff levels for all three serotypes (Fig. 3). After receiving the IPV booster, a strong increase in IgA levels occurred within 1 week in 93% of persons for serotype 1, 94% for serotype 2, and 83% for serotype 3. In 77% of persons a booster response to all three serotypes was found (Fig. 3). After 4 weeks following IPV vaccination, three persons (6%) had no detectable IgA antibody to poliovirus serotype 1, one (2%) had no IgA antibody to serotype 2, and 7 (14%) had no IgA antibody to serotype 3. All IgA-positive serum samples were also positive in the neutralization assay.

sIgA levels in elderly individuals before and after IPV vaccination. Only a small fraction of the older persons (ages, 52 to 85 years) had detectable sIgA to poliovirus serotype 1 (8.3%), serotype 2 (14.6%), and serotype 3 (10.4%) before IPV vaccination. In all cases except one (serotype 2), OD values were low (less than two times the cutoff value). One week after administration of one dose of IPV, 75, 87, and 74% of persons reacted with a rapid increase in sIgA antibody levels in serum

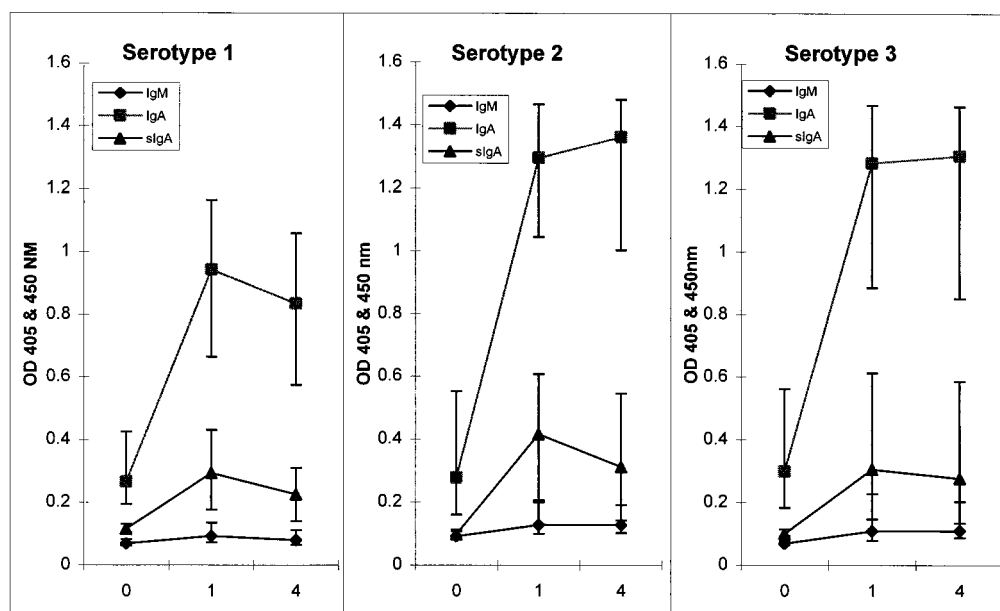


FIG. 3. Levels of IgM, IgA, and sIgA in an older population before and after vaccination with IPV. Dots indicate median values in each group. Error bars indicate upper (75%) and lower (25%) quartiles.

for serotypes 1, 2, and 3, respectively (Fig. 3). The levels of sIgA remained high until at least 4 weeks after the administration of IPV. All serum samples tested in the sIgA assay were IgM negative.

DISCUSSION

We studied serum IgA responses after poliovirus infection or vaccination in persons in different age groups vaccinated with IPV in The Netherlands. We developed ELISAs to measure poliovirus-specific sIgA circulating antibody levels. The specificities of both IgA ELISAs were high for all three serotypes (99 to 100%). A positive reaction could only be blocked with homologous virus, indicating that cross-reactivity with other poliovirus serotypes and probably also other enteroviruses did not occur. In the group of poliomyelitis patients from the 1992 and 1993 serotype 3 epidemic in The Netherlands, IgA antibodies to the other two serotypes were also detected in 4 of the 15 persons, suggesting cross-reactivity. A more likely explanation, however, is that this was caused by contact with the virus in OPV offered during the epidemic. The sensitivity was determined by testing a group of poliomyelitis patients and was rather low (81 to 90%). This may be explained by a short duration of the IgA response that may have been missed in the infrequent sampling schedule. The sensitivities of the ELISAs appear sufficient for the study of poliovirus immunity in well-defined populations.

In the group of poliomyelitis patients, IgA on average peaked at 4 weeks after the onset of paralysis and was back to low levels in the majority of persons after 3 months. It was striking that the poliovirus serotype 3-specific IgA antibodies persisted in approximately one-third of the patients beyond 5 months postinfection, confirming data from literature (12). Persisting IgA levels suggest a continuous or repeated antigenic stimulus of the immune system; however, there is little or no evidence of persistent poliovirus infection, in contrast to the persistence of other enterovirus infections. An alternative explanation might be the retention of viral antigen in dendritic cells in the bone marrow (1, 18). Studies are under way to test this hypothesis.

We examined sera from different age groups for IgA antibodies. The seroprevalence of poliovirus-specific IgA was low (5 to 7%) in young schoolchildren (ages, 4 to 12 years), and we found no increase after the administration of more doses of IPV. A drawback of this study is that the sera that were used for these experiments were not collected immediately after vaccination, and IgA induced by IPV could have disappeared from the circulation at the time of testing. The low number of fully IPV-vaccinated children found to be positive for IgA antibodies indicates that IPV may induce IgA antibodies, but only in a small proportion of individuals (5 to 7%), and that IgA may not be present in serum for an extended period of time. Similar to our findings, it has been reported that IPV vaccination can induce sIgA in saliva after the administration of at least three doses of IPV in children, but only in a minority of vaccinated children (9%) (3, 18, 19). This is not a result of the incapacity of children to produce sIgA, since children at 6 months of age in Pakistan produced levels of salivary IgA similar to those in adults (3).

Serotype 2- and 3-specific IgA was less prevalent in the older children than in the group of younger children, which may reflect waning immunity. The higher seroprevalence rates of IgA to serotype 1 poliovirus in the older schoolchildren most likely is explained by persistence of IgA following infection with the serotype 1 wild-type poliovirus strain during the 1978 epidemic (17). Since 1978, wild-type poliovirus type 1 has not

been found in this community, and any vaccine would have included all three serotypes. The older schoolchildren may have been infected in their first few years of life. In addition, healthy adults had a significantly higher seroprevalence of IgA for serotypes 1 and 2 than IPV-vaccinated children. This finding is not merely explained by a cohort effect, because the difference was also seen when blood donors born before the start of the national vaccination program were excluded from the data analysis. This strongly suggested that additional stimulation of the immune response to poliovirus with wild-type poliovirus or live vaccine strains has occurred. Similarly, an unvaccinated population of 52- to 85-year-old persons had the highest seroprevalence of IgA to all three serotypes. All IgA levels found in this group must have been induced by infection with live virus (vaccine or wild type). This may have occurred during childhood, when poliovirus was still endemic in The Netherlands, during holidays in countries where OPV is used, or through importation of wild-type or vaccine-derived polioviruses into The Netherlands. However, there is no evidence of an endemic circulation of wild-type or vaccine virus in The Netherlands in the period from 1979 to 1991 (16).

An interesting finding was that parenterally administered IPV in previously immunized persons could induce strong memory IgA and sIgA responses. Induction of memory sIgA responses by a parenterally administered inactivated vaccine has also been described for influenza virus (2, 10), *Pseudomonas aeruginosa*, and meningococci (6, 9). The investigators postulated that this most likely results from previous mucosal priming by infection with wild-type virus or bacteria (e.g., with influenza virus), as may have been the case for live poliovirus in the population that we studied. The rapid immune response in our own experiments and the lack of induction of poliovirus-specific IgM also suggests a secondary response and indicates that parenteral poliovirus vaccines can induce an sIgA response in persons previously exposed to live poliovirus. It remains to be determined how sIgA responses in serum correlate to sIgA responses at mucosal sites and to protection and whether IPV can also boost sIgA in persons without previous mucosal infection. Experiments are under way to examine these relations.

In summary, we have shown that in fully IPV-vaccinated children, IgA antibodies to poliovirus are present, but only in a small proportion of individuals, whereas in adult IPV vaccinees the seroprevalence is significantly higher. This suggests that most of the IgA present later in life in an IPV-vaccinated population was induced by continuous or additional exposure to live virus strains (wild-type or vaccine strains) and due to the persistence of IgA. In addition, we have shown that parenterally administered IPV is able to boost sIgA responses in a naturally exposed elderly population, suggesting a link between the systemic and mucosal immune systems.

In the near future, poliomyelitis due to wild-type infection will be eradicated and the circulation of live polioviruses will decrease. An important question is whether an IPV-vaccinated population will be able to mount a mucosal (booster) immune response under those circumstances. Our future work will focus on the capacity of parenteral IPV to prime for mucosal memory responses and to give protection against virus excretion.

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