Induction of Mucosal Immunity by Inactivated Poliovirus Vaccine Is Dependent on Previous Mucosal Contact with Live Virus

Tineke M. P. T. Herremans, Johan H. J. Reimerink, Annemarie M. Buisman, Tjeerd G. Kimman and Marion P. G. Koopmans

*J Immunol* 1999; 162:5011-5018; ;
http://www.jimmunol.org/content/162/8/5011

References
This article cites 29 articles, 6 of which you can access for free at:
http://www.jimmunol.org/content/162/8/5011.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Induction of Mucosal Immunity by Inactivated Poliovirus Vaccine Is Dependent on Previous Mucosal Contact with Live Virus

Tineke M. P. T. Herremans, Johan H. J. Reimerink, Annemarie M. Buismann, Tjeerd G. Kimman, and Marion P. G. Koopmans

The inactivated poliovirus vaccine (IPV) is used for protection against poliomyelitis in The Netherlands. It is not clear, however, whether IPV vaccination can lead to priming of the mucosal immune system and the induction of IgA. It has been demonstrated that IPV vaccination is able to induce strong memory IgA responses in the serum of persons who have been naturally exposed to wild-type poliovirus. This has led to the hypothesis that IPV vaccination is able to induce poliovirus-specific IgA at mucosal sites in persons who have been naturally primed with live poliovirus at mucosal sites. To test this hypothesis, the kinetics of the IgA response in serum and saliva after IPV vaccination were examined in persons previously vaccinated with oral poliovirus vaccine (OPV) or IPV. ELISA and enzyme-linked immunospot assays were used for the detection of poliovirus-specific IgA responses. In addition, B cell populations were separated on the basis of the expression of mucosal (α4β7 integrin) and peripheral homing receptors (L-selectin). Parenteral IPV vaccination was able to boost systemic and mucosal IgA responses in previously OPV-vaccinated persons only. None of the previously vaccinated IPV recipients responded with the production of IgA in saliva. In agreement with this finding, a large percentage of the poliovirus-specific IgA-producing lymphocytes detected in previous OPV recipients expressed the α4β7 integrin. It is concluded that IPV vaccination alone is insufficient to induce a mucosal IgA response against poliovirus. In mucosally (OPV-) primed individuals, however, booster vaccination with IPV leads to a strong mucosal IgA response.


Poliomyelitis has been effectively controlled through the use of two different vaccines: the inactivated poliovirus vaccine (IPV) and the attenuated oral poliovirus vaccine (OPV) (1). Mucosal immunity protects from (re)infection and is essential for the reduction of poliovirus circulation within the population (2–5). Therefore, induction of mucosal immunity is of particular importance for the poliomyelitis eradication program, because both poliovirus-induced paralysis and poliovirus circulation must come to a complete stop to reach the target of a polio-free world.

Whether wild-type poliovirus can remain circulating in vaccinated populations (silent circulation) is an important question for the eradication program. In theory, silent circulation is possible in IPV-vaccinated populations because i.m. vaccination with IPV probably induces little or no secretory IgA (S-IgA) at mucosal sites. Several studies, however, indicate that some degree of mucosal immunity can be measured in IPV vaccinees, albeit less than in people who have been vaccinated with the OPV or infected with wild-type virus (4–9). Most information comes from studies that were conducted at times when poliovirus was still endemic, or in regions where OPV was also used. Therefore, the results of these studies are likely to be confounded by additional priming of the mucosal immune system by infection with live poliovirus (vaccine or wild-type). Some of the more recent studies have also included IPV-vaccinated subjects recruited from endemic regions (7, 10). Therefore, it is still unclear whether the IPV vaccination alone is able to induce mucosal immunity and is responsible for the induction of S-IgA in saliva or stool samples.

We have previously shown that IPV vaccination can induce strong memory IgA responses in the serum of persons who have previously been naturally exposed to live (wild-type) poliovirus (11). An age-dependent increase in the presence of IgA in the circulation of the IPV-vaccinated population in The Netherlands, one that cannot be explained by IPV vaccination alone, has also been described (11). Based on these results, we have postulated that a memory IgA response after IPV vaccination is dependent on previous mucosal infection with live poliovirus (vaccine or wild-type).

To test this hypothesis, both IPV and OPV recipients were given a booster vaccination with one dose of IPV. The group of OPV recipients served as a model for previous mucosal priming with live poliovirus. Induction of poliovirus-specific IgA was measured in the plasma, saliva, and stool samples of the volunteers. Poliovirus-specific IgG and IgA Ab-producing cells isolated from the circulation were enumerated by enzyme-linked immunospot (ELISPOT) assays. The homing potentials of the poliovirus-specific IgG- and IgA-producing lymphocytes found in the circulation were also examined to determine their final destination.
Materials and Methods

Vaccine recipients and booster immunization

Fourteen IPV-vaccinated volunteers from The Netherlands (average age 25.8, range 20–41 yrs) and 11 OPV-vaccinated volunteers (average age 32.5, range 25–44 yrs) from different countries where OPV is used in national programs were enrolled in the study. Most OPV recipients were from countries where circulation of wild-type poliovirus has been absent or at low levels for some time, including Canada, Germany, Belgium, Italy, New Zealand, Austria, Spain, and Curacao. One OPV recipient was from Morocco, where wild-type poliovirus has been detected as recent as 1995. All volunteers were injected i.m. with a standard dose of the IPV (diphtheria, tetanus, poliomyelitis vaccines) (National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands) containing 40, 4, and 7.5 D-antigen units for serotypes 1, 2, and 3, respectively. This vaccine is also used in the regular immunization program in The Netherlands, where a total of six IPV vaccinations are given at 3, 4, 5, 12 mo, and at 4 and 9 yr of age. Blood specimens were collected before booster vaccination and at 3, 7, and 28 days postvaccination and were immediately processed. Saliva samples were collected in plastic vials containing a proteinase inhibitor mixture (Boehringer-Mannheim, Mannheim, Germany) at each of the first 10 days after vaccination and every week thereafter until week 8 postimmunization. Three stool specimens (wk 0, 1, and 2) were collected in special containers and examined for poliovirus-specific Abs. Stool and saliva samples were stored at –80°C until use.

The study was reviewed and approved by the Ethical Review Committee of the Netherlands Central Organization for Applied Scientific Research (Zest, The Netherlands). An informed consent form was signed by all volunteers at the start of the study.

Isolation of lymphocytes

Blood samples were collected in containers using EDTA as an anticoagulant. The blood samples were layered on an equal volume of ficoll (Histopaque; Sigma, Zwijndrecht, The Netherlands) to separate the lymphocytes into homing receptor-positive and -negative cell populations. The composition of the negatively selected cell populations was examined after cell separation by FACS analysis. Cells were incubated for 30 min at 4°C under 5% CO2. Plates were washed four times with PBS and 1% bovine serum albumin (BSA) in PBS. After washing, the cells were incubated overnight at 4°C. The wells were then saturated with 10% FCS in RPMI 1640 for 1 h at 37°C. Ag was added in a concentration of 40–120 DU/ml IPV and incubated for 2 h at 37°C. Plates were washed four times with PBS supplemented with 0.5% Tween 20. Serial dilutions (2-fold) of the PBMC in a volume of 100 μl starting at 106 cells/ml were incubated for 4 h, allowing the lymphocytes to secrete Abs. Plates were washed, and the Abs bound to the viral Ag on the plate were detected by alkaline phosphatase-conjugated IgG or Ig class-specific Igs (Sigma). Plates were incubated for 2 h at 37°C. After washing (3 × PBS; Sigma, Zwijndrecht, The Netherlands) containing 40, 4, and 7.5 D-antigen units for serotypes 1, 2, and 3, respectively. This vaccine is also used in the regular immunization program in The Netherlands, where a total of six IPV vaccinations are given at 3, 4, 5, 12 mo, and at 4 and 9 yr of age. Blood specimens were collected before booster vaccination and at 3, 7, and 28 days postvaccination and were immediately processed. Saliva samples were collected in plastic vials containing a proteinase inhibitor mixture (Boehringer-Mannheim, Mannheim, Germany) at each of the first 10 days after vaccination and every week thereafter until week 8 postimmunization. Three stool specimens (wk 0, 1, and 2) were collected in special containers and examined for poliovirus-specific Abs. Stool and saliva samples were stored at –80°C until use.

The study was reviewed and approved by the Ethical Review Committee of the Netherlands Central Organization for Applied Scientific Research (Zest, The Netherlands). An informed consent form was signed by all volunteers at the start of the study.

Separation of homing receptor-positive and -negative cell populations

The lymphocytes were isolated from the peripheral blood using magnetic beads coated with sheep anti-mouse IgG (Dynal M-450, Oslo, Norway). The beads with the attached cells were separated from the receptor-negative population through the application of a magnet. The beads were washed once and the separation was repeated.

The receptor-positive cells attached to the beads were suspended in medium. Both positively and negatively selected cell populations were used in assays was done. Cells were washed twice in RPMI 1640/10% FCS (10 min, 250 × g), counted, and adjusted to the required concentration.

Poliovirus-specific total IgG, IgA1, and IgA2 ELISA (plasma, saliva, and stool)

The IgA ELISA was performed as described (11). Presence of poliovirus serotype-specific IgA was determined in plasma, saliva, and stool samples. Plasma samples were inactivated (30 min at 56°C) before use in the IgA-ELISA and depleted of IgG Abs with Quik-Sep (Isolab, Mechelen, Belgium), according to the manufacturer’s instructions, to prevent possible interassay competition. Saliva samples were centrifuged (10 min, 3500 rpm) and inactivated for 30 min at 56°C. A 10% w/v suspension of the stool samples was added to the IgA ELISA at a 1:2 dilution. ELISA assays were performed with IgA1- and IgA2-specific conjugates (Southern Biotechnology Associates, Uithoorn, The Netherlands) to determine the subclasses of poliovirus-specific IgA. The results obtained with the saliva and stool IgA assay are expressed as a positive:negative ratio to correct for high background levels that were observed in some recipients. Optimal dilutions of reagents were determined by checkerboard titration. Positive and negative control serum samples were included in all IgA assays.

Poliovirus-specific secretory Ab capture ELISA

A capture ELISA was used as described to determine whether IgA detected in plasma samples after IPV vaccination was also present in its secretory form (11). Briefly, microwell plates were coated with a mAb against the secretory component (Sigma) by overnight incubation at 4°C in carbonate buffer. Plates were blocked with 5% Blotto (Pierce, Oud Beijerland, The Netherlands). Plasma dilutions (1:50) were added, and the plates were incubated for 1.5 h at 37°C. IPV was added, and bound Ag was detected with horseradish peroxidase-labeled serotype-specific mAb (1 h, 37°C). Tetramethylbenzidine was used as a substrate (0.1 mg/ml) in 0.11 M sodiumcarbonate buffer, and the reaction was stopped after 30 min with 2 M H2SO4.

Poliovirus-specific subclass and total IgG ELISA (saliva and plasma)

Saliva and serum samples were tested for the presence of poliovirus serotype-specific IgG Abs. Assays were performed as described for the IgA ELISA but with anti-human IgG-alkaline phosphatase-labeled conjugate or with biotin-labeled Abs to the different subclasses of IgG (IgG1, -2, -3, and -4; Sigma). Optimal dilutions of reagents were obtained by checkerboard titration. Avidin conjugated with alkaline phosphatase was added to the plates that were then incubated for 1 h at 37°C. The plates were washed, and 100 μl of p-nitrophenylphosphate was added to the plates at a concentration of 1 mg/ml in 0.1 M glycine buffer was added to each well after incubation at room temperature for 30 min, the plates were read at 405 nm.

Poliovirus-binding inhibition test (PoBI)

The PoBI was performed as described to determine the poliovirus serotype-specific Ab titer in the plasma samples (16). The reciprocal of the first serum dilution that was positive in the inhibition test was taken as the titer of the test sample.

Poliovirus-specific IgM capture ELISA

The IgM-ELISA was performed as described (17). A positive and a negative control serum samples were examined in each assay

Statistical methods

Student’s t tests were performed to determine the significance of the difference between IPV and OPV recipients. The p values of <0.01 were considered significant.
Results

Poliovirus-specific IgA- and IgG-producing cells in volunteers before and after IPV booster vaccination

The number of poliovirus-specific IgG- and IgA-producing cells in the circulation was determined for all three serotypes of poliovirus at days 0, 3, 7, and 28 (Fig. 1). Both IPV- and OPV-vaccinated subjects responded with IgG-producing cells that were detectable only at day 7 after booster vaccination with IPV (Fig. 1, A and B). High numbers of IgA-producing cells were detected in OPV-vaccinated persons 7 days after vaccination (Fig. 1 D). In contrast, none of the IPV recipients had IgA-producing cells to serotypes 1 and 2, and only one IPV-vaccinated subject responded with 230 serotype 3-specific IgA-producing cells/10^6 cells at day 7 (Fig. 1 C). No poliovirus-specific IgG- and IgA-producing cells were detected at 0, 3, and 28 days after booster vaccination in either group. On average, the levels of Ag-specific IgA-secreting cells were higher than for IgG secreting cells, but this was not observed in all OPV recipients.

The L-selectin and α4β7 positive and negative cell populations were tested in serotype 3-specific ELISPOT assays (Fig. 2). The majority (77.3%) of the poliovirus-specific IgA-producing cells detected at day 7 after booster vaccination in the OPV recipients expressed the α4β7 integrin on their surface (Fig. 2A). A median level of 2744 and 808 poliovirus-specific IgA-producing cells/10^6 cells was measured for α4β7 integrin-expressing and -nonexpressing cells, respectively. Poliovirus-specific IgA-producing cells were detected in both the L-selectin positive and negative populations in the OPV-vaccinated group (Fig. 2B). A total of 39% of the poliovirus-specific IgA-producing cells expressed L-selectin on their surface. There was no significant difference in the proportion of poliovirus-specific IgG-producing cells expressing the α4β7 integrin between the IPV and OPV recipients (72.3% vs 72.6%; data not shown). However, 80.9% of the poliovirus-specific IgG-producing cells expressed L-selectin in the IPV-vaccinated group, while only 46.5% were found positive with L-selectin in the OPV recipients (data not shown).

Poliovirus-specific IgA in saliva

A significant difference (p < 0.01) was seen in the poliovirus-specific salivary IgA response for the three serotypes between OPV and IPV recipients after the IPV booster vaccination (Fig. 3). Nine of eleven OPV recipients developed a salivary IgA response to all three serotypes of poliovirus after the IPV booster vaccination. The poliovirus-specific IgA appeared in the saliva within 5–6 days after the booster vaccination. None of the IPV-vaccinated volunteers (n = 14) responded with poliovirus-specific IgA in the saliva (Fig. 3).

Poliovirus-specific IgA in stool

Poliovirus-specific IgA to all three serotypes was detected in the stool samples of three out of nine of the OPV-vaccinated subjects. A mucosal IgA response was not detected in any of the IPV-vaccinated subjects’ stool samples. This difference was not significant (data not shown).

Poliovirus-specific IgA in plasma

Two subjects in the OPV group had detectable IgA to all three serotypes in their circulation before the IPV booster vaccination was given (Fig. 4B), and one subject in the IPV group had detectable poliovirus-specific IgA to serotypes 2 and 3 at day 0. There
was a clear increase in levels of circulating plasma IgA to all three serotypes in the OPV-vaccinated group at day 7 after the IPV booster vaccination, and the response remained elevated up to day 28 (Fig. 4). IgA responses to all three serotypes of poliovirus were also detected in the IPV-vaccinated group, but the levels were significantly lower than those observed in the OPV recipients (p < 0.01).

Poliovirus-specific IgA1 and IgA2 in plasma
Poliovirus-specific Abs were clearly present in both IgA1 and IgA2 subclasses in the OPV recipients (Fig. 4, D and F). IgA responses in the IPV recipients were seen at very low levels and appeared to be mainly of the IgA1 subclass. No poliovirus-specific IgA2 was detected in the majority of IPV recipients.

Poliovirus-specific secretory Abs in plasma
The IPV and OPV recipients also differed in the induction of poliovirus-specific Abs bound to the secretory component in their circulation (Fig. 5). Poliovirus-specific secretory Abs appeared in 7 of 10 OPV recipients for all three serotypes. Such responses were absent in the IPV recipients for serotype 1, and only 2 of 11 IPV recipients had detectable poliovirus-specific secretory Abs for serotype 2 and 3 (Fig. 5A). In all cases, the secretory Ab responses were at low levels and of short duration, with an apparent peak at day 7.

Poliovirus-specific (subclass) IgG in plasma and saliva
In general, the total IgG response in the plasma samples was not significantly different in both groups and consisted mainly of the

---

**FIGURE 2.** Expression of the homing receptors L-selectin and α4β7 integrin on the poliovirus serotype 3-specific IgA-producing cells in the circulation at 7 days after an IPV booster vaccination in previously IPV- and OPV-vaccinated recipients. Horizontal lines indicate the median values.

**FIGURE 3.** Poliovirus serotype 1-, 2-, and 3-specific IgA in the saliva after an IPV booster vaccination of previously IPV and OPV vaccinees. Results are expressed in median values, and the 25% and 75% percentiles of the ratio of positive reaction in ELISA readings in the presence of Ag divided by the ELISA readings in the absence of Ag (p/n ratio).
IgG1 subclass (Fig. 6). However, the IgG3 subclass response was more prominent in the IPV group compared with the OPV recipients, and the difference was significant ($p < 0.05$) for serotype 1 and 3 at day 7 and day 28 after vaccination (Fig. 6, E and F). A low level IgG2 response was induced in several individuals by the IPV booster vaccination in both groups (data not shown). No clear response was seen for IgG4 for all serotypes in both IPV and OPV recipients (data not shown). No poliovirus-specific IgG was seen in the saliva after IPV booster vaccination in most of the IPV and OPV recipient groups. However, two IPV recipients and one subject in the OPV group responded with an IgG response in the saliva to all three serotypes (data not shown).

**PoBI**

At the start of the study, the median levels of PoBI titers were generally higher in the IPV-vaccinated group than in the OPV recipients. This difference was significant for serotype 3 ($p < 0.01$). However, the OPV recipients responded with a similar increase in PoBI titer after IPV vaccination and reached the same levels at day 28 (data not shown).
The poliovirus-specific IgG levels detected in the saliva of IPV recipients were low or absent, suggesting that salivary IgG does not play a role in protection from mucosal infection with poliovirus. We cannot exclude isotype competition in our saliva assay between IgG- and IgA-poliovirus-specific Abs. However, in the IPV recipients with no detectable poliovirus-specific IgA in their saliva, this isotype competition is very unlikely. IgG is thought to enter the mucosal secretions nonspecifically through paracellular transport. It still remains to be investigated whether or not circulating IgG is able to exert an influence on protection to a mucosal poliovirus challenge. It has been postulated that a critical level of specific serum IgG may be sufficient to protect against infectious diseases by inactivating the inoculum of the pathogen (18).

Assuming that the absence of mucosal IgA reflects a lack of mucosal protection, our observation may have implications for the poliomyelitis eradication program in The Netherlands. In the absence of an efficient mucosal barrier, IPV recipients will remain sensitive to poliovirus infection. These infections will go unnoticed because fully vaccinated persons will not develop any symptoms of disease. Under these circumstances, IPV recipients will contribute to the (continuous) circulation of poliovirus. This poses a special risk to the religious communities with low vaccine coverage that presently exist in The Netherlands. Epidemics of poliomyelitis occurred within these groups in 1978 and 1992, despite the high national vaccine coverage (19–22). During the advancing stages of poliomyelitis eradication, accompanied by a decrease in the incidence of the mucosal infection of IPV recipients by live poliovirus, this effect is likely to be even more pronounced, resulting in the waning of presently existing mucosal immunity in the general population.

Parenteral IPV vaccination induced a strong and rapid IgA response in previously OPV-vaccinated persons both at mucosal sites and in the circulation. However, the presence or absence of Abs in stool extracts might not be truly representative due to prompt digestion of intestinal immunoglobulins by the enzymes present in the stool samples (23). Similar memory IgA responses in the circulation after IPV vaccination were detected in a group of nonvaccinated but naturally exposed persons (11). Induction of memory S-IgA responses by parenterally administered inactivated vaccines has also been described for influenza virus (24, 25), *Pseudomonas aeruginosa*, and meningococci (26, 27). In these cases, natural infection with the agents is very common, and the authors postulated that the memory IgA response most likely resulted from previous mucosal infection with the wild-type virus or bacteria, based on the dogma that mucosal Ag presentation is required for an effective mucosal immune response.

The mechanism by which inactivated parenteral vaccination can reestablish the mucosal IgA responses is unknown. The memory IgA-producing cells detected in our experiments most likely have originally been primed at mucosal sites. IPV is applied i.m., therefore, the peripheral lymph nodes are theoretically the first lymphoid location for Ag presentation to memory cells (28). Expression of L-selectin (indicating homing to peripheral lymph nodes) on poliovirus-specific IgG- and IgA-producing cells was demonstrated in this study. The memory lymphocytes will proliferate after restimulation and leave the peripheral lymph nodes. A large proportion (73%) of the circulating poliovirus-specific IgA-producing cells expressed the α4β7 mucosal homing receptor, indicating a preference for homing to the mucosal surfaces. This was underscored by the induction of poliovirus-specific IgA in saliva. At least a proportion of the poliovirus-specific IgA-producing cells in this experiment expressed both the α4β7 integrin and L-selectin on their surface. It is known that some homing receptors are expressed continuously, while others are induced by local activating

---

**FIGURE 5.** Induction of poliovirus serotype 1-, 2-, and 3-specific Abs in association with secretory component after an IPV booster vaccination in the plasma of previously IPV- and OPV-vaccinated recipients at 0, 3, 7, and 28 days after IPV booster vaccination. Horizontal lines indicate the median values.

**Polio-specific IgM in plasma**

Low positive IgM responses only were detected in recipients in both study groups at 7 and 28 days after booster vaccination. In the IPV-vaccinated group, 3, 2, and 4 of 11 had an IgM response to serotype 1, 2, and 3, respectively. In the OPV recipients, 3, 5, and 5 persons of 10 had an IgM response for serotype 1, 2, and 3, respectively. No significant differences were detected in IgM responses between the OPV recipients and IPV recipients (data not shown).

**Discussion**

Although low levels of mucosal immunity have been found after IPV vaccination in previous studies, these responses have been less effective in reducing viral shedding after a challenge with OPV than those observed after OPV vaccination (4–10). This study was conducted to determine mucosal immune responses following IPV vaccination in a country with almost no circulating poliovirus. Under these circumstances, it is clear that IPV vaccination alone is not sufficient to induce mucosal IgA. From this and previous work, we conclude that the previously reported mucosal responses after IPV vaccination are more likely to be the effect of previous mucosal priming with live viruses than of IPV vaccination alone. It remains to be determined whether or not IPV vaccinees, in the total absence of a mucosal IgA response, are partially protected in challenge experiments. Reduced virus shedding in IPV recipients after challenge with OPV has been previously reported, but again, it was unclear in these experiments whether poliovirus immunity was solely induced by IPV vaccination as opposed to a mixed immunization of IPV combined with mucosal infection (2, 8–9).
signals acting on both circulating and local cells (15). The final combination of homing receptors on the cells’ surface is likely to account for the regional preference of the activated cells. The presence of memory cells that express both the L-selectin and α4β7 homing receptors are an ideal immune surveillance mechanism to control infections both at the systemic and mucosal level.

The poliovirus-specific subclass response of IgA and IgG was determined to investigate whether there was a qualitative and quantitative difference in the humoral immune response after IPV booster vaccination in previously IPV- and OPV-vaccinated persons. Poliovirus-specific IgA2 in the circulation was clearly present in the OPV recipients, and might be derived from lymphocytes that were originally primed at mucosal sites. Presence of poliovirus-specific IgA2 in the plasma might serve as a systemic marker for mucosal memory rather than the presence of total poliovirus-specific IgA, since an IgA1 response, albeit small, was also seen in some of the IPV recipients.

We detected an IgG1 and IgG3 subclass response before and after IPV booster vaccination in both the OPV and IPV recipients (Fig. 6). Similar results were reported in poliomyelitis patients and for other enteroviral Ags (29, 30). However, in this study, the IgG3 response in the OPV recipients was significantly lower than in IPV recipients. These results might indicate a difference in preference for an IgG1 switch over an IgG3 switch after mucosal priming with OPV compared with systemic vaccination with IPV. No clear IgG4 response was observed, and only a few individuals responded with IgG2 poliovirus-specific Abs. Mechanisms for the observed differences remain to be investigated.

Not much is known about the induction and presence of circulating S-IgA. Immunoassays indicate that S-IgA can be detected at
relatively low levels (+/-10 µg/ml) in serum (31, 32). In the present study, we detected poliovirus-specific S-IgA after booster vaccination with IPV in previously OPV-vaccinated recipients. The response was at low levels and decreased rapidly. The biological relevance of this finding is speculative and remains to be investigated. We speculate that it represents an overload of the secretory system.

Very little is known about the effects of a combination schedule of IPV and OPV vaccination on systemic and mucosal immunity. A combination of both vaccines is able to overcome some of the disadvantages that can occur when each vaccine is used separately (such as vaccine-associated cases) and is able, at the same time, to achieve both the high serum Ab levels provided by enhanced IPV and the intestinal protection provided by enhanced OPV. Recent studies in the United States have employed a combination of eIPV and OPV that effectively induced high neutralization titers, as well as mucosal immunity (33).

Vaccination with OPV is a source of live virus introduction into the environment, and must, therefore, cease completely in the future. This work has demonstrated that after initial vaccination with OPV, IPV booster vaccinations are able to maintain the mucosal IgA response at high levels for years thereafter. These findings indicate that a combination schedule of OPV and IPV vaccination could serve as a powerful tool in the final stages of the eradication program.

Acknowledgments

We thank all of the volunteers that participated in this study; Dr. Lazarovitz and the LeukoSite Company (Cambridge, MA) for supplying the Act-1; Albert Ras for help with virus cultures and typing; Dick van Breuk and Dr. Osterhaus for assisting us in finding all of the needed volunteers; and Drs. Tajco Ossewaarde and Afke Brandenburg for their excellent medical assistance.

References