Poliovirus-specific IgA in persons vaccinated with inactivated poliovirus vaccine (IPV) in The Netherlands

Tineke Herremans, Anton van Loon, Johan Reimerink, Hans Rümke, Harrie van der Avoort, Tjeerd Kimman, and Marion Koopmans

Clinical and Diagnostic Laboratory Immunology 1997; 4: 499-503
Abstract

The inactivated poliovirus vaccine (IPV) is used for protection against poliomyelitis in The Netherlands. It is not clear if parenteral IPV vaccination can lead to priming of the mucosal immune system. We developed and evaluated ELISA assays for the detection of poliovirus-serotype-specific 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. IgA remained present in 36% of the patients for up to five months post infection. Furthermore, the presence of IgA antibodies was examined in an IPV-vaccinated population using sera from young children (4-12 years of age, n=177), older children (13-15 years of age, n=123), healthy blood donors (n=66) and naturally immune elderly persons (n=54). Seroprevalence of IgA was low in young vaccinated children for all three serotypes (5%-7%), and in older vaccinated children for types 2 and 3 (2%-3%). Seroprevalence for 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. The seroprevalence of type 1- and 2-specific IgA was significantly higher in healthy adults than 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 IPV vaccination was able to boost (secretory) IgA responses in 74%-87% of a naturally exposed elderly population (n=54). While the presence of (s)IgA in IPV-vaccinated persons has been previously documented, our findings suggest that mucosal priming with live virus is necessary to obtain an IgA response after IPV booster vaccination.

Introduction

Systemic antibody responses to poliovirus infection and vaccination (with live or inactivated virus) have been extensively studied. The presence of circulating neutralizing antibodies is sufficient for protection against paralytic disease [13]. In contrast, less is known about the induction of mucosal immunity, which is important for the limitation of virus circulation in the community as well as for protection from infection [7,13].

An important component of mucosal immunity is secretory IgA (sIgA). The presence of sIgA on mucosal surfaces reduces viral excretion after oral poliovirus vaccine (OPV) challenge [12]. In theory, intramuscular vaccination by inactivated poliovirus (IPV) is expected to induce little or no sIgA. However, several studies have measured some degree of local immunity in IPV vaccinees, albeit less effective immunity than in people vaccinated orally with OPV or infected with wild virus [3,4,7,8,14].

An inherent difficulty in the comparison of results from different studies lies in the fact that vaccination schedules and dosages have not been standardised between countries. It therefore remains unclear whether the superiority of OPV vaccination over enhanced potency IPV (eIPV) 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 subsequently at 4 and 9 years of age. In addition, most studies of mucosal immunity have been conducted with recently vaccinated individuals. This may not reflect the situation as it applies to older age groups.
The issue at hand 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. Epidemics of poliomyelitis occurred in these groups 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-ELISAs was tested using serum samples from persons negative for neutralising antibodies to poliovirus (n=114). These samples were obtained from non-vaccinated children from a segment of the population that refuses vaccination for religious reasons. Samples that had titers of neutralising 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 toxoid) used in the routine immunisation of children in The Netherlands were considered as true negatives. The sensitivity of the assays was determined using a panel of sera collected from patients with a proven poliovirus infection within 2.5 months after the onset of paralysis. Patients were from the 1992-1993 serotype 3 outbreak in The Netherlands (n=54) and from the outbreak in Pakistan during 1991-1995 involving all three serotypes (n=98). Infection was confirmed in all patients by the isolation of wild-type poliovirus from stool samples and by the detection of poliovirus-specific IgM in serum by ELISA [11]. Isolated wild-type polioviruses were discriminated from vaccine-derived viruses by an ELISA using type-specific cross-absorbed antisera as described previously [20].

Sequential serum samples from 15 patients (age range 1-36 years) from the 1992-1993 serotype 3 epidemic in The Netherlands were tested for the presence of IgM and IgA antibodies in order to determine the kinetics of the IgA response after natural infection. Poliovirus-specific IgM levels in serum samples were determined to confirm primary infection. Sera from the following groups were investigated to determine the seroprevalence of poliovirus-specific IgA in an IPV-vaccinated population:

1] Sera 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 1992-1993 outbreak in The Netherlands in order to check the seroprevalence of neutralising antibodies to poliovirus and to test for evidence of poliovirus infection within this group. All children had been vaccinated with IPV, had protective levels of neutralising antibodies to all three serotypes, and were positive for antibodies to diphtheria and tetanus toxoid (also included in the vaccine). The younger children (4-12 years) had received four to six doses of IPV and the older schoolchildren (13-15 years) had completed the IPV vaccination schedule several years previously.

2] 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 (OPV at 3, 4, 5 12 months, and at 6 and 12 years of age). The age range of both groups of blood donors was between 18 and 65 years (average 39 years).

3] Sera from a group of non-vaccinated older persons from The Netherlands (52-85 years, n=54) who had been given a single dose of IPV were examined for the presence of neutralising 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 at 1 and 4 weeks thereafter.

**IgA-ELISA**

Wells of microtiter plates (Nunc, Maxisorb) 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 hour at 37°C with 5 % Blotto (Pierce, Oud Beijerland, The Netherlands) in PBS containing 0.05 % Tween 20, 40 to 70 D-antigen units (the form found in the infectious virus) of formaldehyde-inactivated poliovirus were added to each well. We used the inactivated Mahoney strain for the type 1, MEF for the type 2, and Saukett for the type 3 assay, all from the vaccine production facility of the National Institute of Public Health and the Environment (RIVM). Poliovirus strains were originally derived from the American Type Culture Collection (ATCC). The plates were incubated for two hours at 37°C. Phosphate-buffered saline containing 0.5% Tween 20 and 2% Blotto was used as a dilution buffer. Volumes of 100µl were used, and 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 non-specific 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 inter-isotype competition. Serum dilutions of 1/50 were added to the plates and incubated overnight at + 4°C. After washing, an optimal dilution (1/8000) of goat-anti-human IgA labelled with alkaline phosphatase (alfa-chain specific, Sigma, Zwijndrecht, The Netherlands) was added and incubated for 1.5 hours at 37°C. The plates were washed and 100 µl per well of P-nitrophenylphosphate was added at a concentration of 1 mg/ml in 0.1 M glycine buffer (pH 10.4). After incubation at room temperature for 30 minutes the plates were read at 405 nm by use of an Organon Teknika microwell spectrophotometer. A serum sample was considered positive if the optical density (OD) was above the cut-off level, defined as the average OD + 3 standard deviations of results obtained with negative control sera from non-vaccinated persons (n=114). At least one IgA positive (derived from an OPV-vaccinated subject) and one IgA negative control serum was included on each plate. Optimal dilutions of monoclonal antibodies, viral antigen, sera, and detector antibodies were established by checkerboard titrations. The reagent dilutions chosen for this test provided the highest OD differences between signal and background levels. Specificity of positive signals was confirmed by blocking experiments in which serum samples were pre-incubated with homologous poliovirus (± 120 D-antigen units) for 2 hours at 37°C,
and centrifuged for 3 minutes at 10,000 rpm to remove immune complexes prior to testing in ELISA. A reduction of the signal of >50% was considered confirmative.

**Secretory IgA capture ELISA**

A capture ELISA was used to investigate the sera of the older population to determine if IgA detected after IPV vaccination was also present in its secretory form. The assay was a modification of the IgM ELISA that has been described previously [11]. Briefly, microtiter plates were coated with monoclonal antibody against secretory component (Sigma, Zwijndrecht, The Netherlands) overnight at 4°C in carbonate buffer. Plates were blocked with 5% normal goat serum, serum dilutions (1:50) were added, and the plates were incubated for 1.5 hours at 37°C. Formaldehyde-inactivated poliovirus type 1, 2 or 3 was added as described, and bound antigen was detected with horseradish-peroxidase-labelled serotype-specific monoclonal antibody (1 hour, 37°C). Tetramethylbenzidine (TMB) was used as a substrate (0.1 mg/ml) in 0.11 M sodiumacetate buffer, and the reaction was stopped after 30 minutes with 2M H2SO4. Serum samples were considered positive if responses were above the cut-off level, defined as the average optical density (OD) + 3 standard deviations of results obtained with negative control sera from non-vaccinated persons (n=114). At least one sIgA and one sIgA negative control serum was included on each plate.

**Poliovirus type-specific IgM-antibody capture ELISA**

The IgM-ELISA was performed as described previously [11]. In brief, wells of microtiterplates were coated overnight at 4°C with 100µl 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 were incubated overnight at 4°C. Inactivated poliovirus suspension containing between 40 and 70 D-antigen units was added and incubated for 2 hours at 37°C. Bound antigen was detected by horseradish-peroxidase-labelled serotype-specific monoclonal antibodies (1 hour, 37°C). TMB was used as substrate, and colour development was stopped after 12 minutes by the addition of 2M H2SO4. A positive and a negative control serum and a positive/negative cut-off control serum were examined in each assay. The positive/negative cut-off serum was prepared on the basis of comparison with the distribution of the OD450 values obtained with sera from both patients and healthy controls. A ratio of >1 between the sample OD450 and the cut-off serum OD450 was considered to indicate the presence of poliovirus-specific IgM in the sample.

**Neutralisation assay**

Poliovirus neutralising antibody titers (NT) of sera were determined in the standard microneutralisation test as recommended by the WHO [21], using Mahoney (serotype 1), MEF (serotype 2) and Saukett (serotype 3) virus strains as challenge viruses.

**Statistical Methods**

A Chi-square analysis was performed to determine the significance of the difference in seroprevalence between two groups. P values <0.05 were considered significant.
Results

Specificity and sensitivity of the poliovirus-specific IgA ELISAs

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

Kinetics of IgA production in patients infected with wild type poliovirus

All type 3-infected poliomyelitis patients (n=15) had poliovirus serotype 3-specific IgA and IgM antibodies in at least one of the serum samples. Four patients had IgA antibodies to all three serotypes, and one patient had IgA against type 1 and 3. Poliovirus serotype 3-specific IgA reached a peak at three to four weeks after onset of paralysis [Figure 1] and decreased to low levels within three months. Thirty-six percent of the patients remained positive for IgA for up to five months post infection. Maximum levels of IgA were found at a later date post-infection than the maximum levels of IgM in all patients (data not shown).

![Figure 1](image-url). 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 1992-1993 outbreak in The Netherlands.

IgA in IPV-vaccinated children

Poliovirus-specific-IgA was found in 4.5%, 7.3% and 5.6% of young children (4-12 years) for serotypes 1, 2 and 3 [Figure 2]. No correlation was found between the total number of IPV doses received and the number of children with detectable IgA levels (results not shown). Eighteen percent of the older schoolchildren (13-15 years) had IgA antibodies to serotype 1, whereas the seroprevalence of IgA to serotypes 2 and 3 was low (3.3% and 2.3 % respectively) [Figure 2].
Figure 2. Seroprevalence of IgA antibodies to poliovirus type 1, 2 and 3 in different age groups of IPV vaccinated persons in The Netherlands (group I-III) and in persons born before the vaccination programme (group IV). The populations were I) 4-12 year old school children (n=177), II) 13-15 year old school children (n=123), III) IPV vaccinated blood donors (n=66, labelled 18-65 years) and IV) 52-85 year old persons without previous vaccination (n=54). *= significant (p<0.05) compared to 4-12 year old schoolchildren, **= significant (p<0.01) compared to schoolchildren and blood donors.

IgA levels in IPV-vaccinated healthy adults

IgA antibodies were found in 21.2%, 27.3% and 12.1% of healthy adult blood donors for serotypes 1, 2 and 3 respectively [Figure 2]. The seroprevalence of poliovirus serotype 1- and serotype 2-specific IgA was significantly higher ($\chi^2$, p<0.05) in adults than in the young schoolchildren (4-12 years). Antibodies to serotypes 2 and 3 were significantly more prevalent ($\chi^2$, p<0.05) in the blood donors than in the older children (13-15 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 as mentioned (21.2%, 27.3% and 12.1% for serotypes 1, 2 and 3 respectively). The difference was only significant for serotype 3 ($\chi^2$, p<0.05).

IgA antibodies in elderly adults pre- and post-IPV vaccination

Before IPV vaccination, the group of older subjects (52-85 years old, n=53) had neutralisation titers ($\geq 1:8$) to serotypes 1, 2 and 3 (87%, 83% and 79% respectively). Four weeks after vaccination, 96% of these subjects had protective levels of neutralising antibodies for all three serotypes. Only two subjects did not develop neutralising antibodies to serotype 3 after the IPV booster. Before IPV vaccination,
40%, 49% and 30% of the subjects had detectable IgA against serotype 1, 2 and 3 respectively [Figure 2]. In most cases the ELISA absorbance values were low, and median values were below cut off levels for all three serotypes [Figure 3].

After the IPV booster, a strong increase in IgA levels occurred within one week for serotype 1 (93% of the subjects), serotype 2 (94% of the subjects) and serotype 3 (83% of the subjects). A booster response to all three serotypes was found in 77% of the subjects [Figure 3]. Four weeks following IPV vaccination, three subjects (6%) had no detectable IgA antibody to serotype 1 poliovirus, one subject (2%) had no detectable IgA antibody to serotype 2 and seven subjects (14%) had no detectable IgA antibody to serotype 3. All IgA positive serum samples were also positive in the neutralisation assay.

**Secretory IgA levels in elderly adults pre- and post-IPV vaccination**

Only a small fraction of the older subjects had detectable secretory-IgA against 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 (< 2 times the cut-off value). One week after the administration of one dose of IPV, 75%, 87%, and 74% of the subjects reacted with a rapid increase in sIgA in serum for serotype 1, 2 and 3 respectively [Figure 3]. Levels of sIgA remained high until at least four 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 different age groups of persons vaccinated with IPV in The Netherlands. We developed ELISA assays to measure poliovirus-specific (secretory) IgA circulating antibodies. The specificity of both IgA ELISAs was 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 other enteroviruses as well, did not occur.

IgA antibodies to the other two serotypes were also detected in four out of the 15 persons from the group of poliomyelitis patients from the 1992/1993 serotype 3 epidemic in The Netherlands, suggesting cross-reactivity. A more likely explanation, however, is that this was caused by contact with the OPV virus 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 during the infrequent sampling schedule. The sensitivity of the ELISAs appears sufficient for the study of poliovirus immunity in well-defined populations.

IgA in the group of poliomyelitis patients peaked, on average, at four weeks after the onset of paralysis, and was back at low levels in the majority of persons after three months. It was striking that the poliovirus serotype 3-specific IgA antibodies persisted in approximately one third of the patients beyond five months post infection, confirming data from the 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 other enterovirus infections. An alternative explanation might be the retention of viral antigen in dendritic cells in the bone marrow [1,18].

We examined sera from different age groups for IgA antibodies. The seroprevalence of poliovirus-specific IgA was low (5%-7%) in young schoolchildren (4-12 years), and we found no increase after more dosages of IPV. A drawback of this study is that the sera 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 that tested positive for IgA shows that IPV may induce IgA antibodies, but only in a small proportion (5%-7%), and that IgA may not be present in serum for an extended period of time. In keeping with our findings, it has been described that IPV vaccination in children can induce sIgA in saliva after at least three dosages, but only in a minority of vaccinated children (9%) [3,18,19]. This is not a result of the incapability of children to produce sIgA, since children at six months of age in Pakistan produced levels of salivary IgA similar to those in adults [3].

Serotype 2- and serotype 3-specific IgA was less prevalent in the older children than in the group of younger children, a finding that may reflect waning immunity. The higher seroprevalence rates of IgA to serotype 1 poliovirus in the older schoolchildren is most likely explained by the persistence of IgA following infection with the serotype 1 wild-type poliovirus strain during the 1978 epidemic [17]. After 1978, wild poliovirus type 1 was not 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 did IPV vaccinated children. This
finding is not merely explained by cohort effect, as the difference was also seen when blood donors born before the start of the national vaccination program were excluded from the data analysis.

This finding strongly suggests 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 compared to all other age groups. 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 OPV-countries, or through the importation of wild or vaccine-derived polioviruses into The Netherlands. However, there is no evidence for the endemic circulation of wild or vaccine virus in The Netherlands during the period 1979-1991 [16].

Interestingly, parenterally administered IPV in elderly, non-immunized persons was able to induce strong memory IgA and secretory IgA responses. Induction of memory secretory IgA responses by a parenterally administered inactivated vaccine has also been described for the influenza virus [2,10], pseudomonas aeruginosa and meningococci [6,9]. The authors postulated that the cause of these responses is most likely to be previous mucosal priming with wild type infection (e.g. with the 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 combined with the lack of induction of poliovirus-specific IgM also suggests a secondary response and shows that parenteral poliovirus vaccines can induce a secretory IgA response in persons previously exposed to live poliovirus. It remains to be determined how sIgA responses in serum correlate to sIgA at mucosal sites and to protection, and whether IPV can also boost sIgA in persons without previous mucosal infection. Experiments are underway to examine these relations.

In summary, we have shown that IgA antibodies to poliovirus are present in fully IPV-vaccinated children, but only in a small proportion, whereas the seroprevalence is significantly higher in adult IPV vaccinees. 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 or vaccine) and/or due to the persistence of IgA. In addition, we have shown that parenteral IPV was able to boost secretory IgA responses in a naturally exposed elderly population, suggesting a link between the systemic and mucosal immune system.

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

We gratefully acknowledge the help of Cecile Holweg and Albert Ras for the determination of neutralisation titers and virus titrations, and of Dr. Guy Berbers and Carin Knipping for the determination of antibodies to diphtheria and tetanus toxoid. We also thank the Utrecht blood bank for providing us with serum samples of IPV-vaccinated adults. This work was supported by a grant from The Foundation for the Advancement of Public Health and Environment (SVM), Bilthoven, The Netherlands.
References


