Preexisting Poliovirus-Specific IgA in the Circulation Correlates with Protection against Virus Excretion in the Elderly

Anne-Marie Buisman,1 Frithjofna Abbink,2 Rutger M. Schepp,1 Jan A. J. Sonsma,1 Tineke Herremans,1 and Tjeerd G. Kimman3

1Laboratory for Infectious Diseases and Perinatal Screening, 2Department for Infectious Diseases Epidemiology, and 3Laboratory for Toxicology, Pathology, and Genetics, Center for Infectious Diseases Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Background. Epidemiological studies have indicated that at least 10% of the Dutch elderly do not have poliovirus serotype-specific neutralizing antibody titers and might be at risk for poliovirus infection. Previously we established that memory immunity does not protect the elderly against poliovirus replication. In this study, we investigated whether preexisting immunoglobulin (Ig) A protects against poliovirus infection.

Methods. Elderly individuals (n = 383), divided into seronegative and seropositive groups, were challenged with monovalent oral poliovirus vaccine (mOPV), either serotype 1 or serotype 3. After challenge, poliovirus serotype-specific circulating and salivary IgA responses were measured by enzyme-linked immunosorbent assays, and poliovirus excretion in stool was measured.

Results. The majority of elderly persons without preexisting IgA excreted poliovirus in the stool. In contrast, most elderly persons seropositive for IgA did not excrete poliovirus. Significant inverse correlations were found between preexisting titers of poliovirus serotype-specific circulating IgA and virus excretion. Challenge with mOPV (re)induced IgA responses; low salivary IgA responses correlated with that in the circulation but not with virus excretion.

Conclusions. These results indicate that preexisting IgA values in the circulation correlate with protection against poliovirus infection in the elderly. This further implies that persons without preexisting IgA might contribute to the circulation of poliovirus and therefore may threaten its eradication.

The live attenuated oral poliovirus vaccine (OPV) and the parenterally administered inactivated polio vaccine (IPV) have successfully eliminated poliomyelitis in most parts of the world [1–3]. However, both vaccines differ in the induction of mucosal immunity, which is important for protection against poliovirus infection (or reinfection) and is essential for reducing virus circulation. [4–6]. At the mucosal surfaces, IgA antibodies are the first line of defense against invasion of ingested pathogens, such as poliovirus.

OPV is more efficient than IPV in inducing IgA [5, 6], but it introduces live virus into the environment. This is a problem for poliovirus eradication, because OPV can evolve into circulating vaccine-derived polioviruses, which have recently caused outbreaks in Hispaniola and Egypt [7, 8]. Even so, >95% of the children in the world, mostly living in the developing countries, are immunized with OPV. One of the current World Health Organization (WHO) strategies to eradicate poliovirus from the last regions of endemicity is the use of monovalent OPV (mOPV), because circulation of poliovirus must cease completely [9, 10].

In The Netherlands, since 1957, vaccination with IPV has been offered to persons born in 1945 and thereafter, which has resulted in a dramatic drop in the incidence of poliomyelitis [11]. However, mucosal immunity provided by poliovirus-specific IgA has been demonstrated only in persons who have been in contact with live po-
Poliovirus, either wild type or OPV [12, 13]. At least 10% of Dutch persons born before 1950, when poliovirus was endemic, do not have detectable neutralizing antibodies against one of the poliovirus serotypes [14]. Moreover, although a proportion of these elderly persons have evidence of memory immunity, we recently demonstrated that they were not protected against virus excretion after challenge with mOPV [15]. Because IgA may be important to protect persons from infection or reinfection and to reduce poliovirus circulation, we measured IgA responses in these elderly persons. Salivary IgA production reflects the common mucosal immune response, whereas circulating IgA forms a second line of defense, mediating elimination of pathogens that have breached the mucosal surface [16]. A correlation between serum and gut IgA described for rotavirus infection indicates that circulating pathogen-specific IgA responses might reflect those at the mucosal surfaces [17, 18].

In the present study, we investigated whether IgA, either in the mucosa or in the circulation, correlates with protection against poliovirus replication in seronegative and seropositive elderly persons. As an infection model, we challenged 383 elderly persons with mOPV serotype 1 (mOPV-1) or serotype 3 (mOPV-3) and subsequently measured both plasma and salivary IgA responses as well as poliovirus shedding.

**MATERIALS AND METHODS**

**Study design.** Participants for the study, which was conducted in 1999, were selected from a pool of 1847 elderly persons born between 1925 and 1950, as described elsewhere [15]. In short, 383 participants were assigned to 4 groups on the basis of their preexisting poliovirus serotype–specific neutralizing antibody titer and vaccination history. Group 1 comprised persons seronegative for serotype 1 (SN-1; n = 98), with no recorded vaccination history; group 2, persons seronegative for serotype 3 (SN-3; n = 103), with no vaccination history; group 3, persons seropositive for all 3 serotypes, with no vaccination history (i.e., naturally immune [NI]; n = 94); and group 4, persons with a documented history of all IPV vaccinations (IPVV; n = 88). Some in the SN-1 and SN-3 groups (25% and 35%, respectively) were also seronegative for serotype 2 poliovirus. Persons with medical problems and/or an OPV vaccination history were not eligible for the study.

The SN-1 and SN-3 participants were challenged with mOPV-1 (1 × 10⁶ TCID₅₀) or mOPV-3 (1 × 10⁴ TCID₅₀), respectively (both Chiron Behring). Participants in the NI and IPVV groups were randomly assigned to either mOPV-1 (NI-1; n = 50) (IPVV-1; n = 43) or mOPV-3 (NI-3; n = 44) (IPVV-3; n = 45). In summary, 6 groups were studied.

Prechallenge samples were collected from blood and saliva; subsequently, saliva and stool samples were obtained on days 3, 7, 14, 21, 28, 35, 42, 49, and 56 after challenge, and blood samples were obtained on days 7, 28, and 56. Participants signed an informed-consent form. The study was approved by the Medical Ethical Committee of the Organisation for Applied Scientific Research, Leiden, The Netherlands, in 1998 (METC98/50).

**Poliovirus serotype–specific IgA ELISA.** Saliva samples were centrifuged for 10 min at 2000 g. Plasma and saliva supernatants were inactivated for 30 min at 56°C, a cocktail of protease inhibitors (Boehringer) was added to the saliva samples according to the manufacturer’s instructions, and all samples were stored at −20°C. To prevent possible interimmunoglobulin isotype competition, plasma samples were depleted of IgG by adding GullSORB (9:1 vol/vol; Gull Laboratories). The IgA ELISA was performed on plasma and saliva samples as described elsewhere [19]. Briefly, micotiter plates were coated with serotype-specific monoclonal antibodies to poliovirus in combination with serotype-specific inactivated poliovirus (D antigen). Plasma (1:50) and saliva (1:4) samples were serially diluted 2-fold. Goat anti–human IgA (α-chain specific) labeled with alkaline phosphatase (1:8000) was used, followed by p-nitrophenyl phosphate (1 mg/mL).

IgA responses were expressed as optical density values. Positive and negative control samples were included in all assays. Samples were considered positive when the values were above the cutoff level, defined as the average optical density value plus 3 SDs for the results obtained with negative control serum. To analyze further the influence of preexisting plasma IgA on virus excretion, values were divided into negative (OD <100), low (OD of 100–299), medium (OD of 300–600), and high (OD >600).

Salivary IgA values were expressed as a positive/negative ratio to correct for possible high sample-dependent background values, which were measured as optical density values found in control wells without serotype-specific D antigen. Salivary IgA ratios >1.5 were considered positive.

**Virus titration.** The amounts of excreted poliovirus were measured in stool samples at different time points. Virus isolation was done on L20B cells according to the WHO protocol [20].

**Statistical methods.** Mann-Whitney U nonparametric tests were performed to determine the significance of differences between groups. Relationships between data sets were determined by linear regression and Spearman correlation analysis. Differences with P < .05 were considered significant.

**RESULTS**

**Poliovirus serotype–specific salivary IgA responses.** Before challenge, almost all seronegative (SN-1) and vaccinated (IPVV-1) persons had no serotype 1–specific salivary IgA, and only a few seronegative persons had serotype 3–specific IgA (SN-3). Approximately 10% of the NI persons (NI-1 and NI-3) showed IgA responses, and 18% of vaccinated persons had serotype 3–specific salivary IgA (figure 1).
After challenge, the prevalence of salivary serotype 1–specific IgA in the SN-1 group increased to 50% on day 14 and stayed elevated (30%–40%) until day 56. The prevalence in the NI-1 group also increased on day 14 and was comparable to that in the SN-1 group, whereas that in the IPVV-1 group increased from day 21 (figure 1A). The prevalence of salivary IgA in the SN-3 group was slightly enhanced on day 14, reached a peak of 40% on day 28, and rapidly decreased to 20% on day 35. The prevalence in the IPVV-3 group and that in the NI-3 group were somewhat higher at 56 days after challenge (figure 1B).

Among the IgA-positive saliva samples of the seronegative groups, the mean IgA ratio (positive vs. negative) was determined. The IgA ratio was increased 2-fold on day 14 in the SN-1 group and on day 21 in the SN-3 group. Remarkably, salivary IgA ratios specific for the other 2 poliovirus serotypes also increased after challenge with mOPV-1 or mOPV-3 (data not shown).

Figure 1. Prevalence of poliovirus serotype–specific IgA in the saliva after challenge with monovalent oral poliovirus vaccine serotype 1 (mOPV-1) (A) or serotype 3 (mOPV-3) (B). IPVV-1 and IPVV-3, persons vaccinated with inactivated polio vaccine and challenged with mOPV-1 or mOPV-3, respectively; NI-1 and NI-3, naturally immune persons challenged with mOPV-1 or mOPV-3, respectively; SN-1 and SN-3, seronegative for serotype 1 and serotype 3, respectively.

Poliovirus-specific IgA in plasma and its correlation with salivary IgA. In all groups, the mean plasma IgA values were determined for all 3 polioviruses. Before challenge, very low serotype 1– and serotype 3–specific IgA values were detected in the SN-1 and SN-3 groups, respectively. Low IgA values for the other 2 poliovirus serotypes were also detected in both seronegative groups (figure 2A and 2B). No change was observed on day 7, but on days 28 and 56 after challenge of SN-1 and SN-3 persons, the IgA values were significantly increased for the corresponding poliovirus serotypes. Remarkably, the serotype 2–specific IgA values were also significantly increased on days 28 and 56 after challenge in both the SN-1 and SN-3 groups (figure 2A and 2B).

Persons in the IPVV-1 and IPVV-3 groups already had plasma IgA values specific for all 3 poliovirus serotypes, which did not increase after challenge with either mOPV1 or mOPV3. In all of these participants, IgA values specific for poliovirus serotype 2...
and serotype 3 were higher than those specific for serotype 1 (figure 2C and 2D).

Persons in the NI-1 group showed significantly lower prechallenge serotype 1–specific IgA values than those in the IPVV-1 group; however, these were significantly increased on day 28. In this group, serotype 2– and serotype 3–specific IgA values also increased during the next 56 days (figure 2E). Persons in the NI-3 group already showed elevated IgA values for all 3 poliovirus serotypes before challenge, which did not increase (figure 2F).

To compare circulating poliovirus serotype–specific IgA with salivary IgA, we applied linear regression. Despite the low prevalences of salivary IgA, we found significant linear regression and low but significant correlations ($P < .0001$) when comparing plasma IgA optical density values with ratios of salivary IgA in all groups at 28 days after challenge with mOPV-1 ($n = 188; r = 0.34$) (figure 3A) or mOPV-3 ($n = 192; r = 0.28$) (figure 3B).

**Inverse correlation between preexisting IgA in the circulation and virus excretion.** At 28 days after the challenge, 69% of individuals in the SN-1 group (figure 4A) and 60% of those in the SN-3 group (figure 4B) produced plasma IgA. These percentages were somewhat decreased at 56 days (figure 4). Before the challenge, most participants in the IPVV and NI groups already showed serotype 1– and serotype 3–specific IgA responses, respectively, although the seroprevalence of IgA was higher in the IPVV-3 (97%) and NI-3 (85%) groups (figure 4B) than in the IPVV-1 (69%) or NI-1 (58%) groups (figure 4A). At day 28, the prevalence of plasma IgA was increased in both the IPVV-1 and NI-1 groups (figure 4A) but not in the groups challenged with mOPV-3 (figure 4B).

The majority (80%) of participants in the SN-1 group excreted mOPV-1 virus at 3 and 7 days after challenge. These high numbers of virus shedders declined rapidly (figure 4A). Approximately half (55%) of those in the SN-3 group excreted mOPV-3 virus at day 3, with peaks of 80% at day 7 and 62% at day 14; the number of excretors declined after that. Far more seronegative persons excreted mOPV-3 from day 14 to day 49 (figure 4B), compared with persons challenged with mOPV-1 (figure 4A). In the seronegative groups, the mean duration of virus excretion was significantly longer ($P < .01$) after mOPV-3 challenge than after mOPV-1 challenge (mean ± SD, 20 ± 16 and 13 ± 12 days, respectively). This finding was reported recently, along with all individual virus titers [21]. The sum of the mean virus

![Figure 2](image-url)
The titer from day 3 to day 56 was also significantly higher ($P < .01$) after challenge with mOPV-3 than after challenge with mOPV-1 (25.4 and 23.0 log$_{10}$ TCID$_{50}$/g of feces, respectively).

In contrast, seropositive persons with medium or high serotype 1–specific IgA values or high serotype 3–specific IgA values did not excrete mOPV-1 or mOPV-3, respectively (figure 5), and only a few with medium serotype 3–specific IgA values excreted mOPV-3 (figure 5B).

For all participants, inverse linear regression was performed on the IgA values before challenge (day 0) and the peak in virus titers at day 7 after challenge with mOPV-1 (figure 6A) or mOPV-3 (figure 6B). There was a significant inverse correlation ($P < .0001$) between preexisting poliovirus serotype–specific IgA in the circulation and virus titers after challenge with either mOPV-1 or mOPV-3. Significant correlations ($P < .01$) were also found between prechallenge IgA values and duration of excretion for both serotypes ($R = -0.5$ for both mOPV-1 and mOPV-3), but inverse linear regression was significant only for mOPV-1.

### DISCUSSION

The purpose of the present study was to investigate whether poliovirus-specific circulating IgA and/or mucosal salivary IgA antibodies afforded protection against poliovirus replication in elderly persons after challenge with mOPV. The findings showed that preexisting circulating IgA correlates with protection against poliovirus replication in elderly persons. Most seronegative persons did not have detectable poliovirus-specific IgA in their circulation before challenge, and 80% of them excreted poliovirus serotype 1 or serotype 3 after challenge. The majority of either IPV-vaccinated or NI elderly persons had both polio-neutralizing antibodies and detectable poliovirus-specific plasma IgA and did not excrete virus after challenge. Importantly, the rest of the neutralizing antibody-positive group (20%) did not have serotype–specific IgA or had only low serotype 3–specific IgA values before challenge and did excrete poliovirus after challenge. Obviously, the detected neutralizing antibody or IgG titers in these persons were not enough to prevent virus infection and excretion. For all participants there were significant inverse correlations between serotype-specific preexisting plasma IgA values and virus excretion after poliovirus challenge. Although others have reported the importance of serum IgA induction after poliovirus infection or vaccination [4–6, 12] and the relationship between poliovirus excretion and intestinal IgA responses [22–24], ours are the first findings clearly indicating the role played by circulating IgA responses in protection against poliovirus replication. The results of our study are in agreement with those showing the importance of serum IgA for protection against other infectious diseases in humans, such as...
disease caused by rotavirus and HIV. For rotavirus, a correlation between gut and serum IgA has also been described [17, 18, 25]. Secretory IgA is the main antibody in secretions and exists as a dimeric complex containing a joining chain and a secretory component, whereas serum IgA is mainly monomeric. Serum IgA mediates the protection against pathogens that have breached the mucosal surface [26–28]. Recently, it has been shown that serum monomeric IgA, rather than secretory IgA, enables phagocytosis by Kupffer cells mediated by the receptor for IgA (FcaRI, or CD89), thereby providing a second line of defense in mucosal immunity for preventing disease [29]. Because FcaRI (CD89) is expressed on neutrophils, eosinophils, and especially monocytes in the circulation [30, 31], we speculate that circulating phagocytes play a role in the defense against poliovirus mediated by serum IgA.

To study the correlation of preexisting IgA and virus excretion, we combined results from persons originally defined as IPV vaccinated or NI, based on their high poliovirus neutralizing antibody titers at the start of the challenge, and considered them as a single group of seropositive persons. Antibody responses of both groups were comparable, which can be explained by the fact that IPV vaccinees lived during a period when poliovirus was still endemic. Indeed, most persons with high neutralizing antibody titers were already positive for plasma IgA, indicating earlier contact with live poliovirus [12]. Neutralization of the OPV virus by these IgA antibodies, either in the mucosa or in the serum, could explain the high number of seropositive persons who did not respond to the OPV challenge at all, that is, did not show either seroconversion or virus excretion. The presence of higher preexisting IgA values and prevalences in IPV-vaccinated persons than in those who are naturally immune can be explained by the boosting effect of IPV on IgA responses in already immune individuals [12].

We also studied the role played by mucosal IgA in protection against poliovirus infection. The presence of a common mucosal system implies that primed B cells in mucosal lymphoid tissues...
can enter the circulation and migrate to distant mucosal tissues [32, 33]. Previously, we reported that salivary IgA responses are easier to detect than fecal IgA responses [34]. Our results showed a correlation between plasma and salivary IgA responses, but the prevalence of salivary IgA was much lower than that found for plasma IgA and showed differences in kinetics between mOPV-1 and mOPV-3. Induction of salivary IgA is variable and of limited duration, which indicates that salivary IgA is less sensitive in reflecting poliovirus immune responses than circulating IgA. Conditions, such as the absorbent material for collection at home and temperature during transport by mail, may have contributed to the low association between circulating IgA and IgA in the saliva [35, 36].

Interestingly, we observed cross-reactivity in plasma IgA responses between poliovirus serotypes in both seronegative and seropositive participants after OPV challenge. This finding has not been described before, but it was also found for polio-specific neutralizing antibody titers after mOPV challenge [15]. A potential explanation might be that elderly persons have been in contact with other serotypes before, because poliovirus was endemic earlier during their lives. This would imply that the cross-reactive antibody responses to mOPV challenge were secondary responses in persons in whom waning immunity was observed initially. This phenomenon is confirmed by observations in patients infected with serotype 3 poliovirus during 1992–1993. Young patients showed only poliovirus serotype 3–specific antibodies, whereas older patients also had increased serotype 1–specific antibodies (T. Herremans et al., unpublished data).

To reduce the transmission of polioviruses, the WHO advocates the use of mOPV, because its immune response is stronger than that of trivalent OPV and no intertypic recombination can occur [8, 9, 37]. It was estimated that a single vaccination with mOPV-1 induces better seroconversion than 2 doses of trivalent OPV [38]. The induction of a more robust immune response by the mOPV strains is probably the result of a more widespread and longer replication in the human intestine. Our data showed that mOPV can induce or enhance IgA responses, although the response is higher after contact with mOPV-1 than for mOPV-3. This finding is consistent with those of studies showing lower rates of seroconversion for poliovirus serotype 3 in children vac-

**Figure 5.** Percentages of all seropositive persons (vaccinated with inactivated polio vaccine or naturally immune) excreting virus after challenge with monovalent oral poliovirus vaccine serotype 1 (mOPV-1) \((n = 93)\) (A) or serotype 3 (mOPV-3) \((n = 89)\) (B), divided into groups according to prechallenge serotype-specific plasma IgA values: negative (neg), low, medium (med), and high.

**Figure 6.** Inverse linear regression of prechallenge plasma IgA values specific for serotype 1 (A) and serotype 3 (B) poliovirus with poliovirus titers for the same serotypes (expressed as \(\log_{10}/\text{g of feces}\), at day 7 after challenge with monovalent oral poliovirus vaccine serotype 1 (mOPV-1) \((n = 191)\) (A) or serotype 3 (mOPV-3) \((n = 192)\) (B). Significant inverse correlation was found \((P < .0001; \text{Pearson’s correlation, } r = -0.57 \text{ for mOPV-1 and } r = -0.61 \text{ for mOPV-3})\).
minated with trivalent OPV, which contains an even higher dose of serotype 3 poliovirus than mOPV-3 [38].

Although we showed that preexisting plasma IgA is associated with protection against poliovirus infection, it is important to realize that the use of IPV alone is not sufficient to induce IgA antibodies [14]. All IPV recipients who not have been in contact with live poliovirus may be susceptible to poliovirus infection after (re)introduction of the virus and might contribute to the (silent) circulation of poliovirus [12, 13].

In addition, our findings indicate that poliovirus might circulate in seronegative elderly persons. These persons could contribute to the circulation of poliovirus after (re)introduction of poliovirus. Extrapolating our findings to the general Dutch population, at least 6% and 15% of the elderly population in The Netherlands are at risk for infection with type 1 and type 3 poliovirus, respectively [15]. Moreover, virus excretion will be considerably higher after exposure to wild poliovirus rather than OPV.

Acknowledgments

We are greatly indebted to all staff of the Rivierenland Municipal Health Services, the nurses recruited from Utrecht Doctor’s Laboratory Foundation, and all volunteers from Tiel who participated in this study. We thank Marina Conyn and Marion Koopmans for their ideas at the start of the study and Lia de Rond, Marion Barends, Anita Boelen, and Joan Kwakkel for technical laboratory assistance.

References


