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Novel formulations and delivery strategies for inactivated polio vaccines : new routes with benefits

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Citation

Kraan, H. B. (2018, October 18). *Novel formulations and delivery strategies for inactivated polio vaccines : new routes with benefits*. Retrieved from https://hdl.handle.net/1887/66318

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Author: Kraan, H.B.
Title: Novel formulations and delivery strategies for inactivated polio vaccines : new routes with benefits
Issue Date: 2018-10-18

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Vaccine, 2017; 35;2647-53

Intranasal and sublingual delivery of inactivated polio vaccine

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ABSTRACT

Polio is on the brink of eradication. Improved inactivated polio vaccines (IPV) are needed towards complete eradication and for the use in the period thereafter. Vaccination via mucosal surfaces has important potential advantages over intramuscular injection using conventional needle and syringe, the currently used delivery method for IPV. One of them is the ability to induce both serum and mucosal immune responses: the latter may provide protection at the port of virus entry.

The current study evaluated the possibilities of polio vaccination via mucosal surfaces using IPV based on attenuated Sabin strains (sIPV). Mice received three immunizations with trivalent sIPV via intramuscular injection, or via the intranasal or sublingual route. The need of an adjuvant for the mucosal routes was investigated as well, by testing sIPV in combination with the mucosal adjuvant cholera toxin.

Both intranasal and sublingual sIPV immunization induced systemic polio-specific serum IgG in mice that were functional as measured by poliovirus neutralization. Intranasal administration of sIPV plus adjuvant induced significant higher systemic poliovirus type 3 neutralizing antibody titers than sIPV delivered via the intramuscular route. Moreover, mucosal sIPV delivery elicited polio-specific IgA titers at different mucosal sites (IgA in saliva, fecal extracts and intestinal tissue) and IgA-producing B-cells in the spleen, where conventional intramuscular vaccination was unable to do so. However, it is likely that a mucosal adjuvant is required for sublingual vaccination. Further research on polio vaccination via sublingual mucosal route should include the search for safe and effective adjuvants, and the development of novel oral dosage forms that improve antigen uptake by oral mucosa, thereby increasing vaccine immunogenicity. This study indicates that both the intranasal and sublingual routes might be valuable approaches for use in routine vaccination or outbreak control in the period after complete OPV cessation and post-polio eradication.

INTRODUCTION

During the past decades, the global incidence of paralytic poliomyelitis has decreased by more than 99% since 1988. Type 2 wild poliovirus was eradicated in 1999 and the last reported case of type 3 wild poliovirus was from 2012. Since 2015, cases of type 1 wild poliovirus were only detected in the remaining endemic countries (i.e., Pakistan, Afghanistan and Nigeria) [1]. It is expected that wild poliovirus will be eradicated within a few years. However, to accomplish a polio-free world, eradication efforts should focus on both wild polioviruses as well as vaccine-derived viruses. Therefore, the endgame strategy of the Global Polio Eradication Initiative (GPEI) includes a phased withdrawal of the live-attenuated oral polio vaccine (OPV), the source of vaccine-derived viruses, and the worldwide inclusion of the inactivated polio vaccine (IPV) into all routine immunization programs [2]. The GPEI is pursuing several priority approaches for the development of a new generation of IPV [3]. To this extent, Intravacc has developed a new polio vaccine based on Sabin polio viruses, Sabin IPV (sIPV), that is being transferred to local vaccine manufacturers to support post-eradication goals in terms of biosafety and IPV availability [4-7].

A new generation of sIPV should not only be affordable and safe to produce, but preferably should also induce mucosal immunity, remain stable, and be easy to administer. This is important with regard to stockpiling and outbreak management in the period after cessation of OPV and after eradication. Several alternative polio vaccine delivery strategies are in development, with a focus on dermal delivery of polio vaccines [8]. Vaccination via mucosal sites has the benefits of needle free vaccine delivery . Moreover, mucosal immunization is able to elicit strong mucosal immunity, even at distant effector sites. As we know from OPV, polio-specific mucosal immunity in the gut is a powerful mechanism for protection and interruption of polio transmission [9].

The current study evaluated the potential of different mucosal routes, i.e., intranasal and sublingual, in mice. It was investigated whether intranasal or sublingual vaccination with sIPV is able to elicit functional systemic immunity (serum) as well as local immune responses at different mucosal sites.

MATERIALS AND METHODS

Vaccine

Monovalent Sabin IPV bulk material used in this study was produced as described previously [10]. For the preparation of trivalent sIPV, monovalent type 1, type 2 and type 3 were mixed and diluted in M199 medium (Bilthoven Biologicals, The Netherlands) to a nominal concentration of 1000-1600-3200 D-antigen units (DU) per mL for type 1, type 2 and type 3, respectively. Cholera toxin from Vibrio Cholerae was purchased from Sigma-Aldrich (St. Louis, MO).

Immunization study

The animal experiment was performed according to the guidelines provided by the Dutch Animal Protection Act, and was approved by the Committee of Animal Experimentation (DEC) of the National Institute of Public Health and Environment (RIVM). Balb/cOlaHsd mice (8-10 weeks old from Envigo, The Netherlands) were anesthetized with ketamine-xylazine, and received a single human dose (based on previous clinical studies [6, 11]) trivalent sIPV (10-16-32 DU/dose) via the intramuscular (IM, injection of 50 μL in hind limb), intranasal (IN, pipetting 10 μL in the nose) or sublingual (SL, pipetting 10 μL under the tongue) route at day 0, 7 and 28. Adjuvanted groups received 5 μg/dose cholera toxin. Upon SL immunization, mice were maintained in upright position to minimize the risk of swallowing. Blood samples were taken at day 0 (prior to immunization) and day 14 (after second immunization). At day 35, anesthetized animals received an intraperitoneal injection of 0.1 mL of 0.05 M pilocarpine (Sigma-Aldrich, St. Louis, MO) in PBS to induce saliva production. Saliva was collected and, subsequently, animals were sacrificed by bleeding. Post-mortem, fecal samples were isolated from the large intestine, weighted and stored at -80ºC until analysis. Spleens were placed in RPMI-1640 medium (Gibco, Invitrogen) supplemented with 5% fetal bovine serum and placed on ice for the B-cell ELISPOT. Small intestines were harvested and placed in 3 mL PBS containing 50 mM EDTA (Gibco, Invitrogen) and protease inhibitors (Complete, Mini, EDTA free, Roche Applied Sciences). Small intestines were extensively vortexed and centrifuged for 15 min at 300 g (4ºC). Supernatants, mentioned further as intestinal wash, were collected and stored at -80ºC until analysis (IgA ELISA). Subsequently, small intestines were cut into small pieces, transferred to cryotubes, and 2 μL PBS containing 2% saponin (Sigma Aldrich, St. Louis, MO) and protease inhibitors was added per mg intestinal sample. After a fast freezing step, samples were centrifuged for 20 min at 4600 rpm and supernatants

were filtered through 0.22 µm filters (Merck Millipore, Darmstadt, Germany). Intestinal tissue samples were stored at -80ºC until further analysis. The presence of both excreted (intestinal washes) and intracellular (intestinal tissue samples) polio-specific IgA in small intestine was assessed by ELISA.

IgG and IgA ELISA

Enzyme linked immunosorbent assays (ELISA) were performed to determine polio-specific antibody titers in sera, saliva, feces, intestinal washes and intestinal tissue samples. Fecal extracts were prepared by adding fecal extract buffer, PBS containing 10% normal goat serum (Sigma Aldrich, St. Louis, MO) and protease inhibitors, to the fecal pellets (0.2 g/mL). Fecal extracts were extensively vortexed and, subsequently, centrifuged for 15 min at 13000 g. Supernatants were filtered through 0.22 µm filters and immediately tested. For the ELISA, polystyrene 96 wells microtiter plates (Greiner Bio-One, Alphen a/d Rijn, The Netherlands) were coated overnight at 4°C with bovine anti-poliovirus serum (Bilthoven Biologicals, Bilthoven, The Netherlands) in PBS (Gibco from Invitrogen, Paisley, UK). After washing coated plates with 0.05% (v/v) Tween 80 (Merck, Darmstadt, Germany) in tap water, trivalent inactivated polio vaccine diluted in assay buffer, PBS containing 0.5% (w/v) Protifar (Nutricia, Zoetermeer, The Netherlands) and 0.05% (v/v) Tween 80 (Merck, Darmstadt, Germany), was added and incubated for 2h at 37°C. Subsequently, plates were washed and threefold sample dilutions in assay buffer were added and incubated for another 2h at 37°C. After washing, plates were incubated with horse-radish peroxidase (HRP)-conjugated goat-anti-mouse IgG or HRP-conjugated goat-anti-mouse IgA (Southern Biotech, Birmingham, AL). After 1h incubation at 37°C, plates were washed and TMB substrate solution, containing 1.1 M sodium acetate (Bilthoven Biologicals, Bilthoven, The Netherlands), 100 mg/mL 3,3’,5,5’-tetramethylbenzidine (Sigma-Aldrich, St. Louis, MO), and 0.006% (v/v) hydrogen peroxide (Merck, Darmstadt, Germany), was added to each well. After 10-15 minutes, the reaction was stopped with 2 M sulfuric acid (Bilthoven Biologicals, Bilthoven, The Netherlands) and absorbance was measured at 450 nm by using a Biotek L808 plate reader. For the CT-specific ELISA, plates were coated with 1 µg/mL CT and blocked with 1% Protifar in PBS. ELISA was further performed as described above. Endpoint titers were determined by 4-parameter analysis using the Gen5™ 2.0 Data Analysis software (BioTek Instruments, Inc., Winooski, VT) and defined as the reciprocal of the serum dilution producing a signal identical to that of negative control samples at the same dilution plus three times the standard deviation.

Virus neutralization (VN) assay

Neutralizing antibodies against all three poliovirus types were measured separately by inoculating Vero cells with 100 TCID50 of the wild-type strains (Mahoney, MEF-1 and Saukett) as described previously [12, 13]. Twofold serial serum dilutions were made and serum/ virus mixtures were incubated for three hours at 36°C and 5% CO_2 followed by overnight incubation at 5°C. Subsequently, Vero cells were added and after 7 days of incubation at 36°C and 5% CO_2, the plates were stained and fixed with crystal violet and results were read macroscopically. Virus neutralizing (VN) titers were expressed as the last serum dilution that has an intact monolayer (no signs of cytopathogenic effect).

B-cell ELISPOT

MultiScreen-HTS IP 96 wells filter plates (Merck Millipore, Darmstadt, Germany) were wet by adding 35% ethanol, immediately washed twice with PBS and, subsequently, coated overnight with 5 µg/mL monovalent IPV type 1, 2 or 3. As a positive control, wells were coated with a mixture of 7 µg/mL purified goat-anti-mouse kappa and 7 µg/mL purified goat-anti-mouse lambda (Southern Biotech). As a negative control, wells were left uncoated (PBS). After washing with PBS, plates were blocked with RPMI-1640 medium (Gibco, Invitrogen) with 2% Protifar (Nutricia, Zoetermeer, The Netherlands) for 1 hour at room temperature. Spleens were homogenized using a 70-µm cell strainer (BD Falcon, BD Biosciences) and cells were collected in RPMI-1640 supplemented with 10% fetal bovine serum and antibiotics (Penicillin-Streptomycin-L-Glutamine, 100x (Gibco, Invitrogen)). Erythrocytes were removed by ACK lysis buffer (Gibco, Invitrogen). After washing, cells were counted and 5x105 cells/ well were added to coated plates. After overnight incubation at 37°C and 5% CO_2 plates were washed extensively and wells were developed by stepwise incubations with AP-conjugated goat-anti-mouse IgA (Southern Biotech) and washing with PBS followed by the addition of BCIP-NBT liquid substrate (Sigma Aldrich, St. Louis, MO). Plates were kept in dark during spot development and thereafter, the reaction was stopped by discarding the substrate and extensively washing of both sides of the filter with tap water. Plates were dried overnight at 37°C and spots were counted using EliSpot reader (AID iSpot FluoroSpot Reader System, Autoimmun Diagnostika GmbH, Strassberg, Germany) and AID EliSpot software.

Statistical analysis

Data was statistically analyzed by comparing all groups by a Kruskal-Wallis test followed by a Dunn's multiple comparisons test. Probability (p) values of $p < 0.05$ were considered

statistically significant. Statistics were performed using GraphPad Prism version 6.07 (GraphPad Software Inc., La Jolla, CA).

RESULTS

Systemic immunity elicited after mucosal sIPV administration

To assess whether systemic immunity was induced after vaccination of mice with sIPV via conventional intramuscular (IM) injection, via the nose (intranasal, IN), or under the tongue (sublingual, SL), polio-specific IgG antibodies were measured in serum. For both mucosal routes (SL and IN), the vaccine was also given in the presence of cholera toxin (CT), which is known as a strong mucosal adjuvant [14-16]. Prior to immunization, no polio-specific immune responses were detected (data not shown). After two immunizations (day 14), evident polio-specific IgG antibody titers were already induced in the group of mice vaccinated with sIPV via the conventional intramuscular route using needle and syringe (Figure 1A). Intranasal and sublingual administered sIPV without adjuvant induced significantly lower anti-polio type 1 IgG titers than the intramuscular control group (respectively, $p<0.05$ and $p<0.001$) (Figure 1A and 1B). However, the inclusion of CT as adjuvant significantly improved systemic IgG responses after intranasal sIPV vaccination. Animals that received sIPV plus CT via the intranasal route induced polio-specific IgG antibody titers similar to those obtained after intramuscular vaccination, both at day 14 (2 immunizations) (Figure 1A) and day 35 (3 immunizations) (Figure 1B). For the sublingual route, higher numbers of responders were observed after immunization with sIPV plus CT with a significant enhanced IgG antibody titers against polio type 3 induced after 2 immunizations (day 14) (Figure 1A). For the induction of detectable systemic IgG after sublingual sIPV delivery, an adjuvant (Figure 1A) and/or at least 3 vaccinations were needed (Figure 1B).

To investigate the functionality, the virus-neutralizing capacity of the sera was determined after three immunizations. All mice from the intramuscular control group showed distinct neutralizing antibody titers against all three poliovirus types (Figure 2). The presence of CT adjuvant was beneficial for the induction of virus-neutralizing antibodies after sIPV vaccination via the sublingual or intranasal route. For all serotypes higher numbers of animals responded after mucosal sIPV immunization plus CT (Figure 2). Besides, significantly higher virus-neutralizing (VN) titers were induced after intranasal vaccination of sIPV plus CT compared to intranasal vaccination in the absence of an adjuvant, both for type 1 ($p<0.05$) and type 3

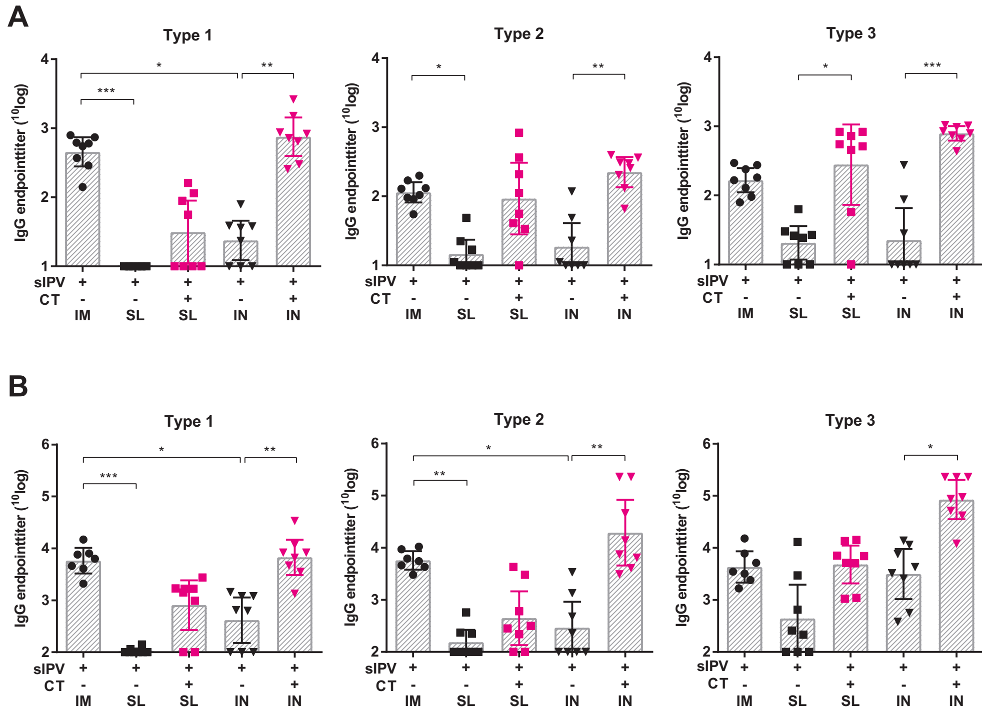


Figure 1 **Systemic immunity induced after vaccination with sIPV via parenteral or mucosal routes.** Polio-specific IgG antibody endpoint titers in serum from mice (n = 8) immunized with sIPV via conventional intramuscular (IM, circles) injection or via sublingual (SL, squares) or intranasal (IN, triangles) route. For both mucosal routes, vaccinations were given in the absence (black symbols) or presence (pink symbols) of the mucosal adjuvant cholera toxin (CT). Sera were collected one week after the second (day 14, panel A) and third immunization (day 35, panel B). Bars represent mean values and error bars depict 95% confidence interval values. Asterisks indicate significant differences between groups (* p<0.05, ** p<0.01, *** p<0.001).

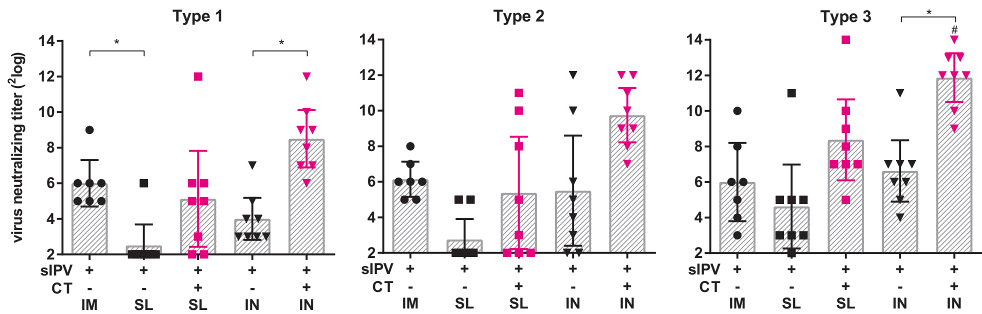


Figure 2 **Virus-neutralizing capacity of serum from mice immunized with sIPV via parenteral or mucosal routes.** Virus-neutralizing (VN) serum antibody titers were measured from mice (n = 8) immunized three times with sIPV via conventional intramuscular (IM) injection or via sublingual (SL) or intranasal (IN) route. For both mucosal routes, vaccinations were given in the absence (black symbols) or presence (pink symbols) of the mucosal adjuvant cholera toxin (CT). Sera were collected one week after the third immunization (day 35). Bars represent mean VN titers and error bars depict 95% confidence interval values. Asterisks indicate significant differences between groups (* p<0.05, ** p<0.01). Hashtags indicate a significant difference with the conventional IM group (# p<0.05).

($p<0.05$). Similar VN titers were obtained after sublingual vaccination in the presence of an adjuvant compared to conventional intramuscular immunization (without adjuvant), although some non-responders were observed after sublingual vaccination (Figure 2). Interestingly, superior type 3-specific VN titers were observed after sIPV (plus adjuvant) delivery via the nose, even when compared to intramuscular injected sIPV ($p<0.05$) (Figure 2).

To evaluate whether differences in systemic immune responses could be assigned to a difference in antigen penetration through and uptake by mucosae, antibody titers against the adjuvant, the highly immunogenic cholera toxin (CT), were measured in sera from mice that received sIPV plus CT. After three immunizations, all animals induced evident CT-specific IgG antibody titers (Figure 3). Even after vaccination via the sublingual route, no non-responders were observed, whereas in some animals no detectable polio-specific IgG titers were found (Figure 1). Significantly improved IgG antibody titers against CT were observed after intranasal vaccination compared with those obtained after sublingual immunization ($p<0.01$) (Figure 3).

Mucosal immune responses induced after SL and IN immunization with sIPV

Polio-specific IgA antibody endpoint titers were determined in different mucosal samples to evaluate mucosal immunity after sIPV immunization of previously mentioned administration routes. Both sublingual and intranasal administration of sIPV induced polio-specific IgA antibody responses in saliva (Figure 4A) and feces (Figure 4B), whereas the intramuscular route was unable to do so. Non-adjuvated sIPV delivery via the sublingual

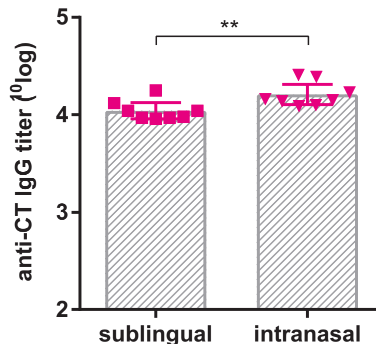


Figure 3 **Systemic antibody titers induced against cholera toxin (CT) delivered via sublingual or intranasal routes**. CT-specific IgG antibody titers were measured in serum from mice (n = 8) immunized with sIPV plus CT as adjuvant via sublingual or intranasal route. Sera were collected one week after the third immunization (day 35). Individual (pink symbols) and mean (bars) are depicted. Error bars represent 95% confidence interval values. Mean values differ significantly ($p=0.0093$) as analyzed by Mann-Whitney test.

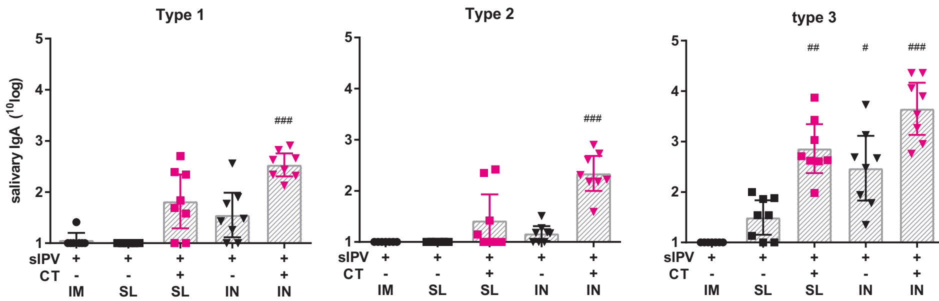
route induced no detectable salivary IgA against polio type 1 and 2, and no polio-specific fecal IgA (Figure 4A and 4B). Sublingual vaccination of sIPV in combination with CT resulted in higher numbers of mice having detectable IgA titers in mucosal samples. For type 3, significant salivary IgA titers were elicited after sublingual administration of sIPV plus CT (Figure 4A). Also significantly enhanced polio-specific IgA titers (all serotypes) were observed in mice immunized intranasally with sIPV plus CT, both in saliva ($p<0.001$) and feces ($p<0.001$) (Figure 4A and 4B).

To evaluate the intestinal immune responses further, local IgA antibody production was determined by ELISA on detergent extractions of small intestinal samples. No intestinal immunity was induced after intramuscular vaccination with sIPV. Only for type 3, 25% of the animals induced polio-specific IgA in the intestine after sublingual delivery of sIPV, with or without adjuvant (Figure 4C). Again, animals immunized via the intranasal route with sIPV plus CT showed significantly improved IgA antibodies against polio type 1 ($p<0.001$), type 2 ($p<0.05$) and type 3 ($p<0.001$) (Figure 4C).

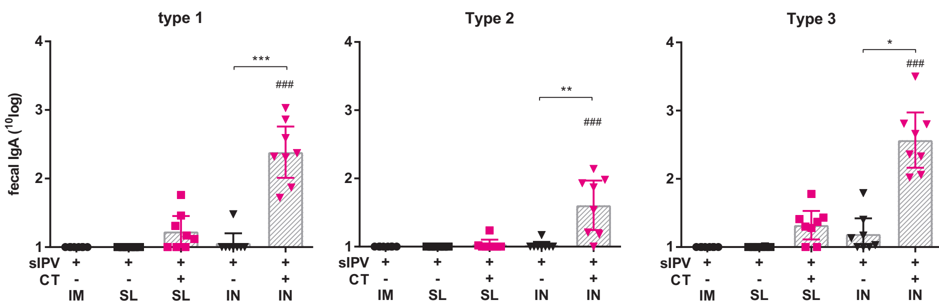
Polio-specific B cell responses elicited after SL and IN immunization with sIPV

The effect of the different immunization routes on the numbers of polio-specific plasma cells was evaluated in single cell suspensions from spleens. Whereas no IgA-secreting plasma cell responses were found in splenocytes from mice immunized via intramuscular injection, significantly enhanced numbers of IgA-producing B-cells were found in spleens of mice immunized via the intranasal route with either unadjuvanted sIPV (type 2 ($p<0.05$); type 3 ($p<0.01$)) or CT-adjuvanted sIPV (type 1 ($p<0.001$); type 2 ($p<0.001$); type 3 ($p<0.05$)) (Figure 5). The presence of CT enhanced the B cell responses after sublingual delivery of sIPV, since a higher number of animals showed IgA-producing B cell responses compared to the unadjuvanted sublingual group. Moreover, mice vaccinated sublingually with sIPV plus CT showed significantly enhanced numbers of polio type 2-specific IgA-secreting B cells ($p<0.01$) (Figure 5).

A **Saliva**



B **Feces**



C **Intestine**

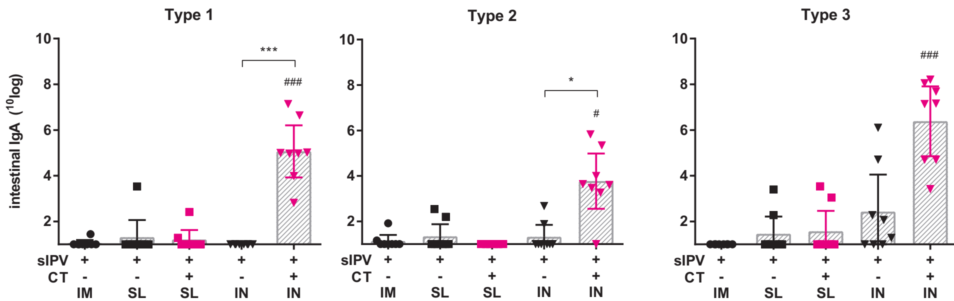


Figure 4 **Mucosal immunity induced after sIPV delivery via mucosal routes.** Polio-specific IgA endpoint titers in saliva, fecal extracts and intestinal tissue samples from mice (n = 8) immunized with sIPV in the absence (black symbols) or presence (pink symbols) of the mucosal adjuvant cholera toxin (CT) were measured. Immunizations were given via conventional intramuscular (IM) injection or via sublingual (SL) or intranasal (IN) routes. One week after the third immunization polio-specific IgA antibody titers were measured in saliva (panel A), fecal extracts (panel B) and intestinal tissue samples (panel C) were measured. Bars represent mean values and error bars depict 95% confidence interval values. Asterisks indicate significant differences between groups (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) and hashtags indicate significant differences with the conventional IM control group (# $p<0.05$, ## $p<0.01$, ### $p<0.001$).

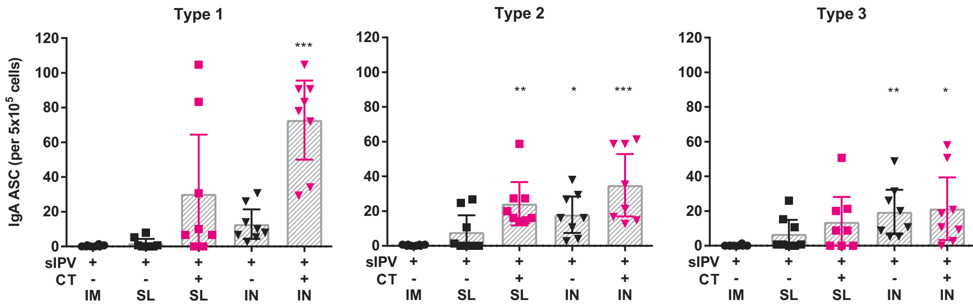


Figure 5 **IgA-secreting B cell responses elicited after mucosal sIPV immunization.** An ELISpot assay was performed to detect polio-specific IgA-secreting B cells from spleens from mice (n = 8) immunized with sIPV in the absence (black symbols) or presence (pink symbols) of the mucosal adjuvant cholera toxin (CT). Immunizations were given via conventional intramuscular (IM) injection or via sublingual (SL) or intranasal (IN) route. Bars represent mean antibody-secreting cell (ASC) numbers and error bars depict 95% confidence interval values. Asterisks indicate significant differences with the conventional IM control group (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

DISCUSSION

The goal of the current study was to evaluate whether mucosal administration of sIPV could elicit both systemic immunity and polio-specific mucosal IgA at distinct mucosal sites. sIPV delivered via the sublingual or intranasal route was able to induce systemic polio-specific IgG responses with poliovirus-neutralizing capacity. Besides, mucosal vaccination of sIPV elicited polio-specific IgA antibody titers at distinct mucosal sites including strong intestinal responses after IN immunization and IgA-producing B cell responses in the spleen both after IN and SL immunization.

Mucosal compartmentalization restricts the induction of intestinal immunity upon intranasal immunization [17, 18]. The existing paradigm that only gut-resident dendritic cells (DCs) can recruit T cells to the gastrointestinal tract is difficult to reconcile with our findings and other reports of gastrointestinal T cell responses and protective gut immunity after intranasal immunization of influenza [19, 20] or Salmonella antigen [21]. Ruane *et al.* showed that lung DCs, which were targeted by intranasal immunization, stimulated gut-homing integrin α4β7 expression on CD4+ T cells in the lungs, and induced cell migration to the gastrointestinal tract in mice [22]. Therefore, it might be that intranasal vaccine delivery is

able to recruit antigen-specific immune cells to the gut and thereby inducing local intestinal immunity, which is in agreement with our findings.

Mucosal vaccine delivery has several practical advantages over vaccination via parenteral routes using needle and syringes, as previously mentioned. However, only relatively few mucosal vaccines for human use are licensed [23]. With the exception of some cholera vaccines, which have a very strong intrinsic immune potentiating capacity [24], all these mucosal vaccines are live attenuated vaccines, like OPV or intranasal influenza vaccines (Flumist/Fluenz) [8, 25]. In contrast to OPV, mucosal polio vaccination based on IPV is expected to require the inclusion of an adjuvant to evoke appropriate immunity against polio [13], which was confirmed in the current preclinical study. Both intranasal and sublingual vaccination of sIPV plus cholera toxin (CT) as adjuvant were able to significantly enhance functional systemic immunity and polio-specific IgA titers in mucosal samples compared to immune responses obtained after mucosal sIPV vaccination without adjuvant. However, CT and the Escherichia coli-derived heat-labile toxin (LT) are well known as potent mucosal adjuvants, but are also associated with adverse effects in humans. Concerns has been raised after an undesired association between facial nerve paralysis (Bell's palsy) and the intranasally delivered inactivated influenza vaccine (Nasalflu) containing an enzymatically active LT adjuvant [26]. Probably the neuronal-binding capacity of the LT-derived adjuvant was the cause of this adverse effect suggesting that nasal administration of LT or CT molecules is inadvisable [27]. Migration to or accumulation in the central nerve system might be avoided by vaccine administration under the tongue [28-30]. Moreover, in a recent Phase 1 study (NCT00820144) conducted in France, the sublingual administration of recombinant CT B subunit in healthy subjects was found to be safe. Therefore, a new safe mucosal adjuvant with strong immune potentiating capacity should be included in the further development of a mucosal (Sabin) IPV although adverse effects after sublingual immunization are not documented as far as we know. Since the mucosal route is minimally addressed for IPV yet, current experience is limited to the use of a double mutant of LT (dmLT) in combination with the sublingual route as described by White *et al*. [31]. Adjuvants (e.g., LPS derivative PagL, oil-in-water emulsions, CpG ODN) that have shown their potential for (Sabin) IPV via the parenteral route could also be evaluated for mucosal vaccination [12, 32].

In the last decade, sublingual vaccine delivery has gained significant attention as shown by the numerous published preclinical studies that provide a strong base for further testing of this non-invasive route [33]. Our findings and earlier research indicates that the inclusion of an adjuvant might be needed to avoid tolerance or low-to-undetectable immune responses after

sublingual delivery of IPV [31]. Besides the inclusion of an adjuvant as immune potentiator, novel oral dosage forms to improve contact time or to facilitate transport through the mucosal barrier, may be required to achieve successful vaccination. White *et al*. studied the sublingual route for IPV (based on Salk strains) using a thermoresponsive gel (TRG) delivery system [31]. Whereas sublingual administration of IPV as a liquid or as TRG in the absence of an adjuvant was not able to induce any immune response in mice, sublingual administered IPV as TRG in combination with the mucosal adjuvant dmLT led to systemic poliovirus-neutralizing antibody titers, and salivary and fecal IgA production [31]. A comparison between the liquid and TRG formulation (both with or without adjuvant) is missing, but it is expected that mucoadhesive dosage forms that prolong the residence time of the vaccine at the oral mucosa and thereby facilitate antigen uptake by local antigen-presenting cells, are needed [13, 33]. Research on sublingual polio vaccination may, besides the use of novel adjuvants, comprise development of extended release formulations, including solid dosage forms that at the same time improve the thermostability of the vaccine as well. Earlier studies revealed that dried IPV can be more resistant to higher temperatures compared to liquid IPV [34-36].

The phased withdrawal of OPV and inclusion of IPV into all global routine immunization programs will create a market for non-invasive delivery of polio vaccines, even a considerable time after eradication either for routine immunization or stock piling. Improved polio vaccine delivery strategies should be suitable for the final target population: infants. The current study demonstrated the potential of both the intranasal and sublingual routes for polio vaccination with IPV based on Sabin strains. Intranasal vaccination showed to be more efficient in eliciting both systemic and mucosal immune responses compared with the sublingual route. However, besides possible redirection to olfactory bulbs (Bell's palsy), the risk of wheezing in young children exists [8]. The sublingual route could be an easy and safe polio immunization approach. Nevertheless, for the induction of evident immunity upon sIPV vaccination under the tongue, strong mucosal adjuvants might be required. Therefore, further research on polio vaccination via the sublingual route should include the search for a safe and effective adjuvant and the development of novel oral dosage forms that improve antigen uptake by the oral mucosa.

ACKNOWLEDGMENTS

The authors would like to thank the colleagues from the animal research center of Intravacc for their assistance with the animal studies, and Geert-Jan Willems for performing the anti-CT IgG ELISA.

REFERENCES

1. Global Polio Eradication Initiative. History of Polio. Available from: http://polioeradication.org/; [accessed on 3 January 2017].
2. Global Polio Eradication Initiative. Polio Eradication & Endgame Strategic Plan 2013-2018. Available from: http://polioeradication.org/wp-content/uploads/2016/07/PEESP_EN_A4.pdf; 2013; [accessed on 3 January 2017].
3. Okayasu H, Sutter RW, Jafari HS, Takane M, Aylward RB. Affordable inactivated poliovirus vaccine: strategies and progress. J Infect Dis. 2014;210 Suppl 1:S459-64.
4. Thomassen YE, van 't Oever AG, van Oijen MG, Wijffels RH, van der Pol LA, Bakker WA. Next generation inactivated polio vaccine manufacturing to support post polio-eradication biosafety goals. PLoS One. 2013;8:e83374.
5. Verdijk P, Rots NY, van Oijen MG, Oberste MS, Boog CJ, Okayasu H, *et al*. Safety and immunogenicity of inactivated poliovirus vaccine based on Sabin strains with and without aluminum hydroxide: a phase I trial in healthy adults. Vaccine. 2013;31:5531-6.
6. Verdijk P, Rots NY, van Oijen MG, Weldon WC, Oberste MS, Okayasu H, *et al*. Safety and immunogenicity of a primary series of Sabin-IPV with and without aluminum hydroxide in infants. Vaccine. 2014;32:4938-44.
7. Resik S, Tejeda A, Fonseca M, Alemani N, Diaz M, Martinez Y, *et al*. Reactogenicity and immunogenicity of inactivated poliovirus vaccine produced from Sabin strains: a phase I Trial in healthy adults in Cuba. Vaccine. 2014;32:5399-404.
8. Kraan H, van der Stel W, Kersten G, Amorij JP. Alternative administration routes and delivery technologies for polio vaccines. Expert Rev Vaccines. 2016;15:1029-40.
9. Hird TR, Grassly NC. Systematic review of mucosal immunity induced by oral and inactivated poliovirus vaccines against virus shedding following oral poliovirus challenge. PLoS Pathog. 2012;8:e1002599.
10. Thomassen YE, Rubingh O, Wijffels RH, van der Pol LA, Bakker WA. Improved poliovirus D-antigen yields by application of different Vero cell cultivation methods. Vaccine. 2014;32:2782-8.
11. Soonawala D, Verdijk P, Wijmenga-Monsuur AJ, Boog CJ, Koedam P, Visser LG, *et al*. Intradermal fractional booster dose of inactivated poliomyelitis vaccine with a jet injector in healthy adults. Vaccine. 2013;31:3688-94.
12. Westdijk J, Koedam P, Barro M, Steil BP, Collin N, Vedvick TS, *et al*. Antigen sparing with adjuvanted inactivated polio vaccine based on Sabin strains. Vaccine. 2013;31:1298-304.
13. Kraan H, van der Stel W, Kersten G, Amorij JP. Alternative administration routes and delivery technologies for polio vaccines. Expert Rev Vaccines. 2016:1-12.
14. Amorij JP, Westra TA, Hinrichs WL, Huckriede A, Frijlink HW. Towards an oral influenza vaccine: comparison between intragastric and intracolonic delivery of influenza subunit vaccine in a murine model. Vaccine. 2007;26:67-76.
15. Cuburu N, Kweon MN, Hervouet C, Cha HR, Pang YY, Holmgren J, *et al*. Sublingual immunization with nonreplicating antigens induces antibody-forming cells and cytotoxic T cells in the female genital tract mucosa and protects against genital papillomavirus infection. J Immunol. 2009;183:7851-9.
16. Sjokvist Ottsjo L, Jeverstam F, Yrlid L, Wenzel AU, Walduck AK, Raghavan S. Induction of mucosal immune responses against Helicobacter pylori infection after sublingual and intragastric route of immunization. Immunology. 2017;150:172-83.
17. Quiding-Jarbrink M, Granstrom G, Nordstrom I, Holmgren J, Czerkinsky C. Induction of compartmentalized B-cell responses in human tonsils. Infect Immun. 1995;63:853-7.
18. Quiding-Jarbrink M, Nordstrom I, Granstrom G, Kilander A, Jertborn M, Butcher EC, *et al*. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses. J Clin Invest. 1997;99:1281-6.
19. Masopust D, Choo D, Vezys V, Wherry EJ, Duraiswamy J, Akondy R, *et al*. Dynamic T cell migration program provides resident memory within intestinal epithelium. J Exp Med. 2010;207:553-64.
20. Esplugues E, Huber S, Gagliani N, Hauser AE, Town T, Wan YY, *et al*. Control of TH17 cells occurs in the small intestine. Nature. 2011;475:514-8.
21. Pigny F, Lassus A, Terrettaz J, Tranquart F, Corthesy B, Bioley G. Intranasal Vaccination With Salmonella-Derived Serodominant Secreted Effector Protein B Associated With Gas-Filled Microbubbles Partially Protects Against Gut Infection in Mice. J Infect Dis. 2016;214:438-46.
22. Ruane D, Brane L, Reis BS, Cheong C, Poles J, Do Y, *et al*. Lung dendritic cells induce migration of protective T cells to the gastrointestinal tract. J Exp Med. 2013;210:1871-88.
23. Holmgren J, Svennerholm AM. Vaccines against mucosal infections. Curr Opin Immunol. 2012;24:343-53.
24. Cong Y, Bowdon HR, Elson CO. Identification of an immunodominant T cell epitope on cholera toxin. Eur J Immunol. 1996;26:2587-94.
25. Amorij JP, Hinrichs W, Frijlink HW, Wilschut JC, Huckriede A. Needle-free influenza vaccination. The Lancet Infectious diseases. 2010;10:699-711.
26. Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, *et al*. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. The New England journal of medicine. 2004;350:896-903.
27. Lewis DJ, Huo Z, Barnett S, Kromann I, Giemza R, Galiza E, *et al*. Transient facial nerve paralysis (Bell's palsy) following intranasal delivery of a genetically detoxified mutant of Escherichia coli heat labile toxin. PloS one. 2009;4:e6999.
28. Cuburu N, Kweon MN, Song JH, Hervouet C, Luci C, Sun JB, *et al*. Sublingual immunization induces broad-based systemic and mucosal immune responses in mice. Vaccine. 2007;25:8598-610.

29. Song JH, Nguyen HH, Cuburu N, Horimoto T, Ko SY, Park SH, *et al*. Sublingual vaccination with influenza virus protects mice against lethal viral infection. Proc Natl Acad Sci U S A. 2008;105:1644-9.
30. Shim BS, Stadler K, Nguyen HH, Yun CH, Kim DW, Chang J, *et al*. Sublingual immunization with recombinant adenovirus encoding SARS-CoV spike protein induces systemic and mucosal immunity without redirection of the virus to the brain. Virol J. 2012;9:215.
31. White JA, Blum JS, Hosken NA, Marshak JO, Duncan L, Zhu C, *et al*. Serum and mucosal antibody responses to inactivated polio vaccine after sublingual immunization using a thermoresponsive gel delivery system. Human vaccines & immunotherapeutics. 2014;10:3611-21.
32. Yang C, Shi H, Zhou J, Liang Y, Xu H. CpG oligodeoxynucleotides are a potent adjuvant for an inactivated polio vaccine produced from Sabin strains of poliovirus. Vaccine. 2009;27:6558-63.
33. van der Maaden K, Trietsch SJ, Kraan H, Varypataki EM, Romeijn S, Zwier R, *et al*. Novel hollow microneedle technology for depth-controlled microinjection-mediated dermal vaccination: a study with polio vaccine in rats. Pharmaceutical research. 2014;31:1846-54.
34. Kraan H, van Herpen P, Kersten G, Amorij JP. Development of thermostable lyophilized inactivated polio vaccine. Pharmaceutical research. 2014;31:2618-29.
35. Kraan H, Ploemen I, van de Wijdeven G, Que I, Lowik C, Kersten G, *et al*. Alternative delivery of a thermostable inactivated polio vaccine. Vaccine. 2015;33:2030-7.
36. Kraan H, Ten Have R, van der Maas L, Kersten G, Amorij JP. Incompatibility of lyophilized inactivated polio vaccine with liquid pentavalent whole-cell-pertussis-containing vaccine. Vaccine. 2016;34:4572-8.