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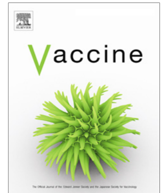

Intranasal and sublingual delivery of inactivated polio vaccine

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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. 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.

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1. 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

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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.

2. Materials and methods

2.1. 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).

2.2. 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 −80 °C 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* (4 °C). Supernatants, mentioned further as intestinal wash, were collected and stored at −80 °C 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 −80 °C 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.

2.3. 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 13,000 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 2 h at 37 °C. Subsequently, plates were washed and threefold sample dilutions in assay buffer were added and incubated for another 2 h 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 1 h 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 min., 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.

2.4. Virus neutralization (VN) assay

Neutralizing antibodies against all three poliovirus types were measured separately by inoculating Vero cells with 100 $TCID_{50}$ 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).

2.5. 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-St

reptomycin-L-Glutamine, 100× (Gibco, Invitrogen)). Erythrocytes were removed by ACK lysis buffer (Gibco, Invitrogen). After washing, cells were counted and 5×10^5 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.

2.6. 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).

3. Results

3.1. 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 (Fig. 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 $p < 0.001$) (Fig. 1A and B). 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) (Fig. 1A) and day 35 (3 immunizations) (Fig. 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) (Fig. 1A). For the induction of detectable systemic IgG after sublingual sIPV delivery, an adjuvant (Fig. 1A) and/or at least 3 vaccinations were needed (Fig. 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 (Fig. 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 (Fig. 2). Besides, significantly higher virus-neutralizing (VN) titers were

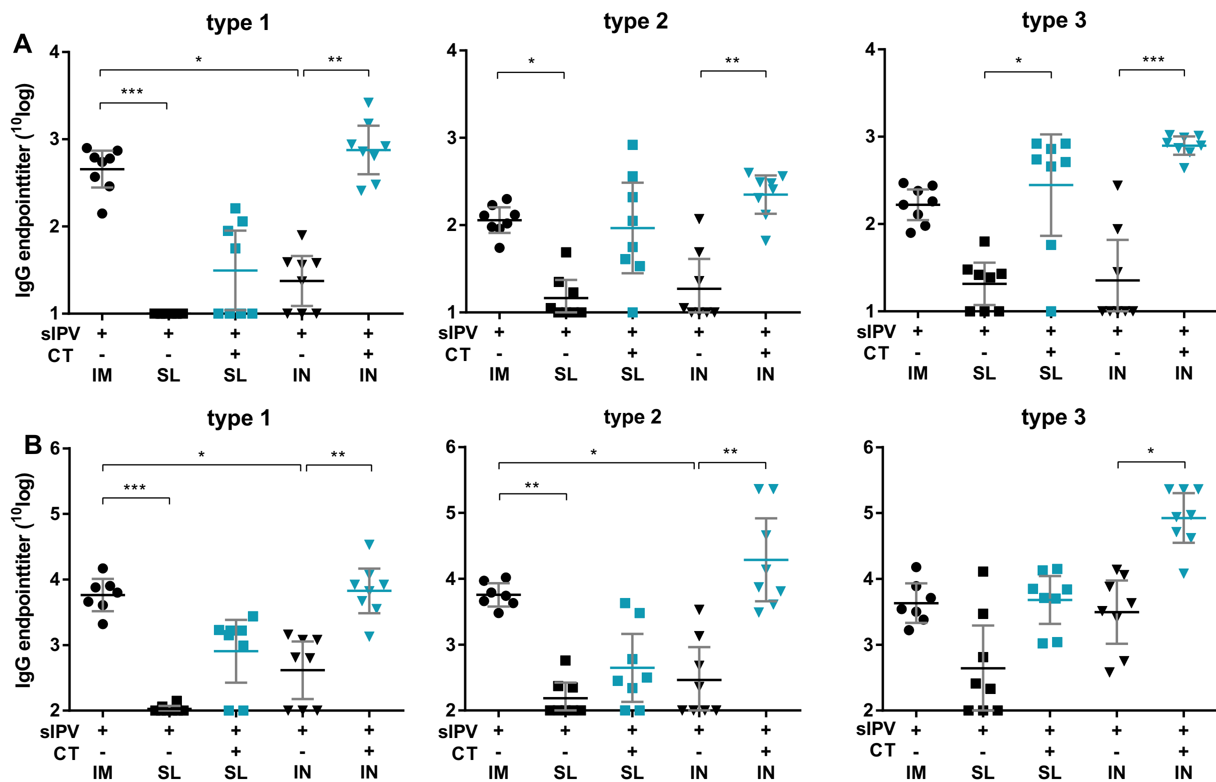

Fig. 1. Systemic immunity induced after vaccination with sIPV via parenteral or mucosal routes. Polio-specific IgG antibody endpoint titers in serum from mice (n = 8) immunized with sIPV via conventional intramuscular (IM, circles) injection or via sublingual (SL, squares) or intranasal (IN, triangles) route. For both mucosal routes, vaccinations were given in the absence (black symbols) or presence (blue 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). Mean values were depicted as horizontal line and error bars depicted 95% confidence interval values. Asterisks indicate significant differences between groups ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

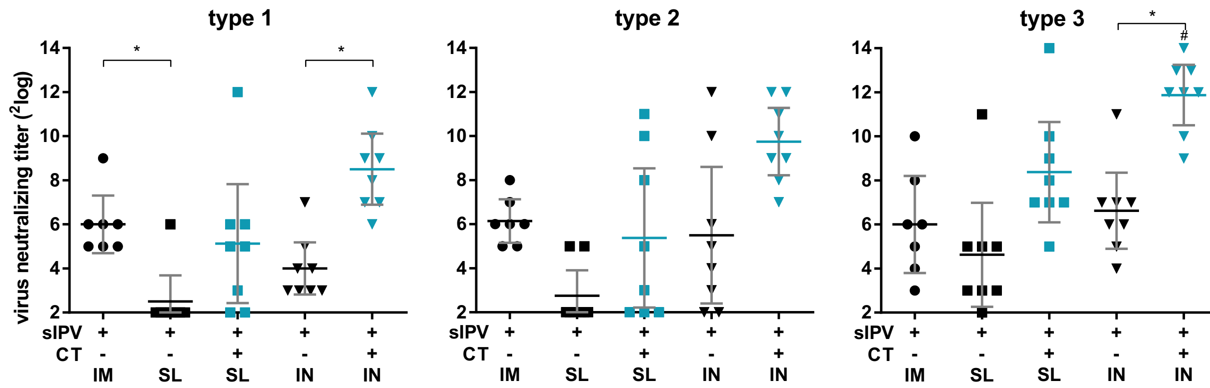

Fig. 2. Virus-neutralizing capacity of serum from mice immunized with sIPV via parenteral or mucosal routes. Virus-neutralizing (VN) serum antibody titers were measured from mice (n = 8) immunized three times with sIPV via conventional intramuscular (IM) injection or via sublingual (SL) or intranasal (IN) route. For both mucosal routes, vaccinations were given in the absence (black symbols) or presence (blue symbols) of the mucosal adjuvant cholera toxin (CT). Sera were collected one week after the third immunization (day 35). Mean VN titers were depicted as horizontal line and error bars showed 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 ($^{\#}p < 0.05$).

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 ($p < 0.05$). 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 (Fig. 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 ($p < 0.05$) (Fig. 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 (Fig. 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 (Fig. 1). Significantly improved IgG antibody titers against CT were observed after intranasal vaccination compared with those obtained after sublingual immunization ($p < 0.01$) (Fig. 3).

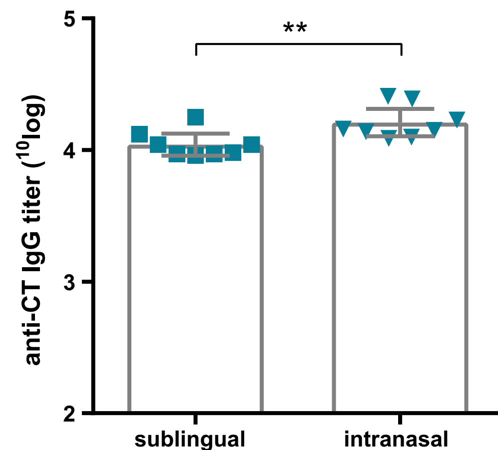

Fig. 3. Systemic antibody titers induced against cholera toxin (CT) delivered via sublingual or intranasal routes. CT-specific IgG antibody titers were measured in serum from mice (n = 8) immunized with sIPV plus CT as adjuvant via sublingual or intranasal route. Sera were collected one week after the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

3.2. 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 (Fig. 4A) and feces (Fig. 4B), whereas the intramuscular route was unable to do so. Non-adjuvated sIPV delivery via the sublingual route induced no detectable salivary IgA against polio type 1 and 2, and no polio-specific fecal IgA (Fig. 4A and B). 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 (Fig. 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 ($p < 0.001$) (Fig. 4A and B).

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 (Fig. 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 ($p < 0.001$) (Fig. 4C).

3.3. 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

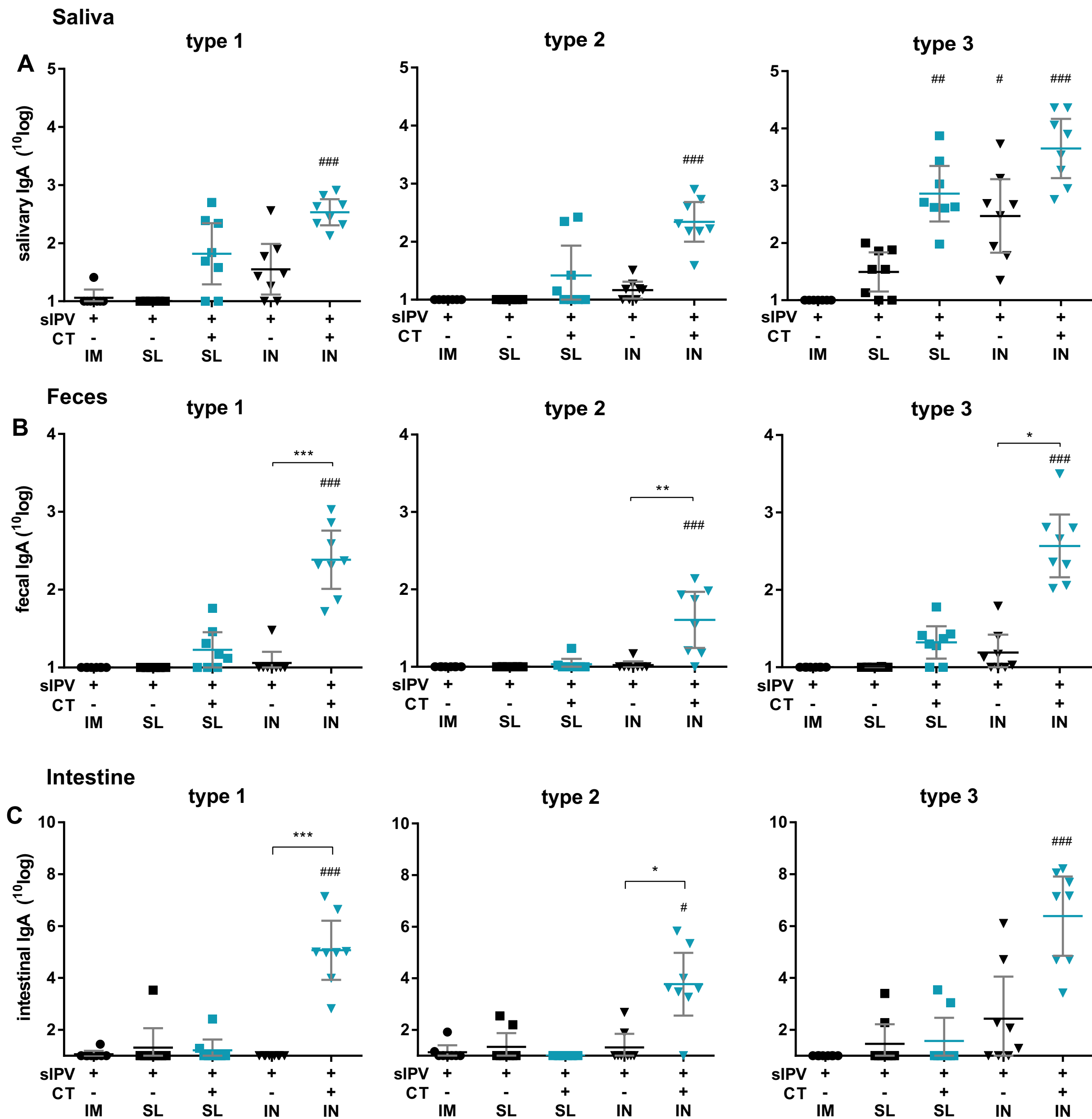

Fig. 4. Mucosal immunity induced after sIPV delivery via mucosal routes. Polio-specific IgA endpoint titers in saliva, fecal extracts and intestinal tissue samples from mice (n = 8) immunized with sIPV in the absence (black symbols) or presence (blue symbols) of the mucosal adjuvant cholera toxin (CT) 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. Mean values were depicted as horizontal line and error bars showed 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 ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$).

($p < 0.001$); type 2 ($p < 0.001$); type 3 ($p < 0.05$)) (Fig. 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 ($p < 0.01$) (Fig. 5).

4. 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

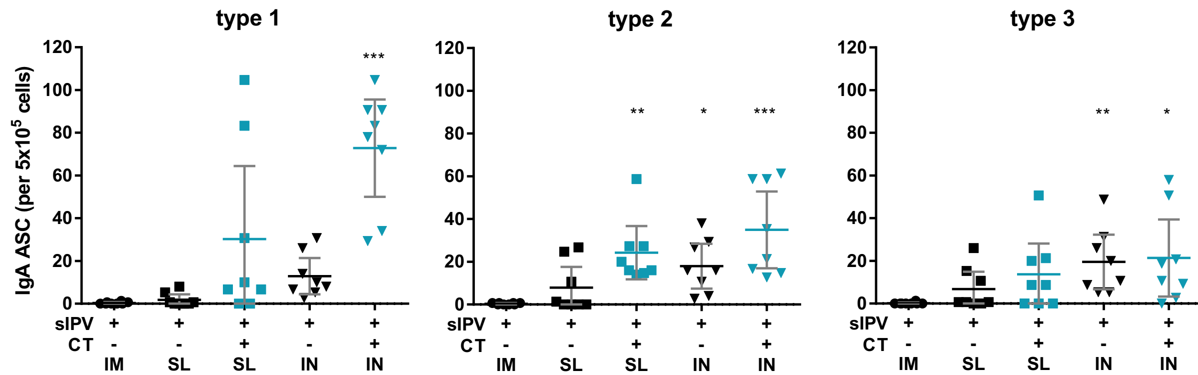

Fig. 5. IgA-secreting B cell responses elicited after mucosal sIPV immunization. An ELISpot assay was performed to detect polio-specific IgA-secreting B cells from spleens from mice (n = 8) immunized with sIPV in the absence (black symbols) or presence (blue symbols) of the mucosal adjuvant cholera toxin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

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.

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