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ALTERNATIVE DELIVERY OF POLIO VACCINES

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OUTLINE

ABSTRACT

Since global polio eradication is coming up, there is an urgent need for an affordable inactivated polio vaccine (IPV) for its use in the post-eradication era. However, to reach the final goal of a polio-free world, it is believed that the routine use all live-attenuated oral poliovirus vaccines (OPV) should be discontinued, where the use of (Sabin) IPV is preferable in the period thereafter. A new generation of IPV should overcome the major drawbacks of OPV and should therefore ideally be administered through alternative (needle-free) delivery routes, provide mucosal immunity, and be affordable for low-income countries. In this review, we will discuss different alternative delivery routes for polio vaccines. First, we will focus on the mucosal vaccine delivery route, in which lessons learned from OPV are highlighted and the potential of nasal, sublingual and buccal delivery are described. Secondly, dermal delivery of polio vaccines, like using jet injection and microneedle approaches, is addressed. Finally, future perspectives, including the potential of improved vaccine formulations, the use of adjuvants and promising delivery systems are given.

INTRODUCTION

Since the start of the WHO campaign in 1988 to eradicate polio globally, impressive progress has already been achieved in the fight against wild type poliovirus. After infection by one of the three serotypes (type 1, 2 and 3) via the fecal-oral route, the virus invades the nervous system and can cause total paralysis within a couple of hours. Poliomyelitis is a highly infectious disease that can strike at any age, but mainly affects children under five years old.

Given that there is no specific treatment, the only way to combat poliomyelitis is by prevention through vaccination. The majority of industrialized countries use trivalent inactivated polio vaccine (IPV) in their routine vaccination programs, in which IPV may be formulated as a combination vaccine with other antigens, such as diphtheria/tetanus/pertussis (DTP), Haemophilus influenzae type B (Hib) and hepatitis B surface antigen [1]. In developing countries, currently, the trivalent oral polio vaccine (OPV) is the vaccine of choice to strive for global eradication of poliomyelitis. Nevertheless, new approaches may greatly enhance the impact of polio eradication strategies; among them is the potential role of new generations of IPV, ideally delivered through alternative delivery routes like intradermal, sublingual or oral delivery.

NEED FOR AN IMPROVED POLIO VACCINE

The incidence of poliomyelitis has dramatically been decreased since the introduction of the inactivated polio vaccine (IPV) in 1955 and the live-attenuated oral polio vaccine (OPV) in 1961. IPV was first developed by Jonas Salk [2, 3] and most often based on the virulent wild polio strains Mahoney (type 1), MEF1 (type 2) and Saukett (type 3), whereas OPV was developed by Albert Sabin [4, 5]. Eradication of poliomyelitis by using these vaccines relies on herd immunity, whereby unimmunized children are less likely to become infected since neighboring children have been vaccinated. The success of the Global Polio Eradication Initiative (GPEI) to eliminate the wild type poliovirus can mainly be credited to the mass vaccination campaigns with OPV [5]. This vaccine was chosen because of its important advantages over inactivated vaccines: lower costs, ease of administration and its superior ability to induce local intestinal mucosal immunity [6]. Vaccination with OPV, a live attenuated virus, mimics a natural infection and thus leads to the induction of a local secretory antibody (sIgA) response in the gastrointestinal tract. This local IgA response is associated with a reduction in the shedding of poliovirus from the intestine [7,

8]. However, a major concern of the Sabin strains of OPV is the ability to revert to a neurovirulent form that can cause paralysis, the so-called vaccine-associated paralytic poliomyelitis (VAPP). An additional risk is the sustained circulation of vaccine-derived polioviruses (VDPV) and their ability to cause disease [9-11].

The rapid progress achieved by the GPEI raising the hope that a polio-free world would soon be realized after the eradication of wild polio virus type 2 in 1999 [12]. Nevertheless, since 2000, circulating VDPV outbreaks have occurred in 18 countries, with the majority (84%) of the reported cases related with circulating VDPV type 2 [13], which appears to have recovered important biological properties of wild type polioviruses to be transmissible and neurovirulent [14]. Hence, coordinated global cessation of OPV is required to reach complete eradication of polio. The worldwide use of IPV is believed to be the only way to avoid the risks of VAPP and emergence of VDPVs, and seemed to be the preferable vaccine because of its high efficacy and safety [15-18] (Table 1). However, the administration by injection brings some disadvantages, e.g., pain at the site of administration, logistical difficulties, trained medical staff, and safety and disposal concerns [19]. Further on, different studies indicate that IPV is inferior to OPV in inducing mucosal intestinal immunity, which is crucial to provoke a strong herd immunity effect and stop the spread of poliovirus in developing countries [20-22]. In contrast to that, the ability of IPV to prevent poliovirus outbreaks and provide herd protection has been demonstrated in different settings [23-25]. As a result, it is expected that IPV is less effective to stop transmission of poliovirus although it provides herd immunity.

Most of the higher-income and some middle-income countries that previously used OPV and have been free of wild type poliovirus transmission for several years have already switched to IPV in their routine immunization programs. A major issue for the introduction of IPV is the costs per vaccine dose, which is currently too high to be affordable for low-income countries [23, 26]. The high cost prize for IPV is mainly due to requirements for: (i) more virus per dose, (ii) additional down-stream processing (i.e. concentration, purification and inactivation), and the related QC-testing and (iii) containment [27]. Moreover, in the post-eradication era, the use of wild poliovirus strains for the production of IPV will be discouraged because of biological safety constrains and related risks [28].

Due to aforementioned reasons the GPEI has supported a comprehensive research program for the development of an improved IPV, which must be efficacious, inexpensive, safe to

manufacture, and easy to administer [4]. The latest achievement of GPEI is the development of IPV based on the non-neurovirulent Sabin strains, Sabin IPV (sIPV), in order to increase IPV production capacity in low and middle-income countries [29]. At the end of the last century, the potential of Sabin IPV was already shown by Kersten *et al*. [30]. In this case the Sabin strains are chosen since the cost-prize reduction is a primary objective and production of IPV using Sabin polio strains is less critical in terms of biosafety [31]. Currently, technology transfer of the sIPV production process to vaccine manufacturers in developing countries is performed by the Vaccinology unit of RIVM [27].

In addition to this sIPV, enhanced affordability of IPV might be achieved by the development of dose-sparing strategies, for example by the use of adjuvants and/or alternative vaccine delivery.

Table 1 *Oral polio vaccine (OPV) versus inactivated polio vaccine (IPV).*

	OPV	IPV
Pro's	Low costs (low dose) Ease of administration Local immunity (sIgA)	Safe Relatively stable High efficacy (1-2 dose)
Cons	Vaccine derived polioviruses (VDPV) Vaccine-associated paralytic poliomyelitis (VAPP) Highly unstable (frozen) Low efficacy (>3 doses)	High costs (high dose) No local immunity (sIgA) Administration by injection

NEED FOR ALTERNATIVE DELIVERY OF (S)IPV

Vaccine delivery is a crucial aspect in addressing the challenges in vaccine development as it encompasses both administration of the vaccine formulation to specific target sites and delivery of the antigen to and activation of relevant cells of the immune system [32]. Since alternative delivery methods and improved formulations have the potential to make vaccine delivery easier and safer, several alternatives for needle-based vaccination are currently being developed. Needle-free delivery approaches are preferred for multiple reasons; (i) it might reduce costs since it does not require trained health-care personnel, (ii) logistic problems associated with

supply and disposal of syringes and needles, and safety risks related to injection would be diminished [33], (iii) vaccine logistics could be further simplified by the use of dried vaccine formulations, which can be smaller and lighter than liquid formulations when packaged correctly, and (iv) the independence of a cold-chain for storage and distribution could further reduce the costs.

Besides the route of administration, other aspects need to be considered in the design of an affordable (needle-free) polio vaccine, such as the use of an adjuvant serving as delivery system and/or immune potentiator and an acceptable shelf-life, which will require formulation excipients. At the end, the proper designed delivery device, primary packaging, and the vaccine formulation together determine the storage conditions and shelf-life [32].

In this review, different alternative delivery routes for polio vaccines will be discussed, in order to develop an affordable and safe polio vaccine for use in the post-eradication era. First focus will be on the mucosal vaccine delivery route, in which lessons learned from OPV are highlighted and the potential of nasal, sublingual and buccal delivery are described. Secondly, dermal delivery of polio vaccines, like using jet injection and microneedle approaches, is addressed. Finally, future perspectives, including the potential of improved vaccine formulations, the use of adjuvants and promising delivery systems are given.

MUCOSAL IMMUNIZATION

Due to their large surface area and immunological competence, mucosal tissues are attractive target sites for vaccination. An important characteristic of mucosal vaccination is the ability to provoke local immune responses, which already can protect against infection at the point of pathogen entry. Because mucosal surfaces are generally exposed to loads of environmental antigens, over-reaction of the immune system is prevented by tolerance mechanisms. Therefore, deliberate vaccination by a mucosal route can effectively induce immune suppression. To overcome tolerance and obtain a protective immune response, strong mucosal adjuvants and/or special antigen delivery systems are required for mucosal vaccine formulations unless vaccination is done with live attenuated viruses, like OPV (see paragraph ‘Oral polio vaccine’)

The migration of immune cells from the inductive mucosa-associated lymphoid tissues (MALT) to effector tissues is the cellular basis for the common mucosal immune system. The MALT contains T-cell zones, B-cell enriched areas containing a high frequency of sIgA-positive B cells and a subepithelial area with antigen-presenting cells (APCs) for the induction of specific immune responses. Microfold epithelial cells (M cells) take up the antigen and transport them to the underlying APCs, including dendritic cells (DCs), which play a central role in bridging the innate with the adaptive immune system [34, 35]. Three major adaptive mechanisms participate in the immune defense of mucosal surfaces; secretory antibody formation (sIgA) and antigen-specific cell-mediated cytotoxicity are the primary mechanisms, whereas regulatory cells act mainly through the production of cytokines and chemokines. Although these cells may regulate IgA antibody formation and the cytotoxic T cell responses, they have also an important anti-inflammatory effect, and thus participate in the maintenance of mucosal tolerance against common pathogens [36].

TRADITIONAL MUCOSAL VACCINE DELIVERY – ORAL AND INTRANASAL

Traditional routes of mucosal immunization include the oral and nasal routes. The Peyer's patches, in the gut, and nasopharynx-associated lymphoid tissue (NALT) are two of the main components of the MALT and important inductive sites for the generation of mucosal immunity through delivery of antigen in the intestinal and respiratory tracts respectively.

ORAL AND NASAL IMMUNE SYSTEM

Oral and nasal vaccination have the potential to address all the prerequisites for a successful needle-free vaccine and may facilitate vaccine efficacy when formulated appropriately [32]. The gastro-intestinal and respiratory tracts are common sites of entrance for many pathogens, so the immune surveillance at these sites is high. On or just beneath the epithelial linings all the machinery is present to elicit optimal protective immunity in both mucosal and systemic immune compartments and to generate cross-protective immunity at more distant mucosal sites as well as systemic immunity. The mucosal immune system has a certain level of compartmentalization. As a result, depending on the inductive site and the immunogenicity of the antigen (local) immune responses can be induced at more distant effector sites (Figure 1). In the case of polio it is expected that immunity in the gut will give rise to protection against polio, as a result nasal vaccination, which induces mucosal immunity in the gut (that is more distant from the inductive site), is an interesting vaccination route for polio vaccination (Figure 1).

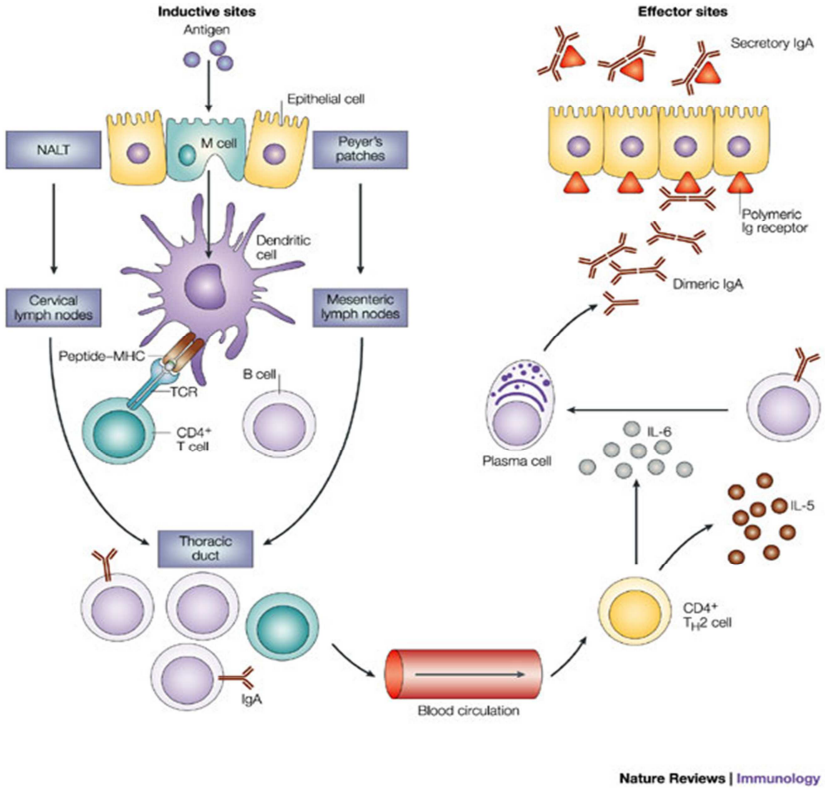

Figure 1 The mucosal immune response. *Pathogens are transported to the nasopharynx-associated lympoid tissue (NALT) and Peyer's patches through microfold (M) cells that are present in the epithelium covering NALT and Peyer's-patch follicles. Dendritic cells (DCs) process and present antigens to T cells and after stimulation, $CD4^+$ T cells induce IgA-committed B cell development. After IgA class switching, B cells migrate from NALT and Peyer's patches to the regional cervical and mesenteric lymph nodes respectively. Finally, antigen-specific $CD4^+$ T cells and IgA^+ B cells migrate to effector sites, such as the nasal passage and intestinal lamina propria, through the thoractic duct and blood circulation. IgA^+ B cells differentiate into IgA-producing plasma cells in the presence of cytokines (e.g. IL-5 and IL-6) that are produced by T helper 2 (Th2) cells, and they subsequently produce dimeric forms of IgA. These dimeric forms of IgA then become secretory IgA by binding to polymeric Ig receptors, which become the secretory component in the process of secretory IgA formation, that are exposed on the monolayer of epithelial cells lining the mucosa. Secretory IgA is then released into the nasal passage and intestinal tract. (from Kyono and Fukuyama, 2004, Nat Rev Immun) [37]*

EFFICACY OF ORAL AND NASAL VACCINATION

Despite the logistic advantages of mucosal (needle-free) vaccine delivery over injectable parental vaccines, only relatively few vaccines for human use are available: oral vaccine against cholera, typhoid, rotavirus, and polio, and a nasal vaccine against influenza [38]. With the exception of the cholera vaccines, which have a very strong intrinsic immuno-potentiating capacity [39], all these vaccines are live or live attenuated vaccines. However, they have been associated with effective development of both systemic (serum) and local mucosal immune responses, superior protection against re-infection, persistence of immunological memory,

better herd immunity (because of secondary spread and contact immunization) and the ease of administration [40]. To date marketed mucosal vaccines are administered via the mucosa where protection is required. This is in contrast to vaccination strategies that are under development in order to generate mucosal immunity at distant effector sites, like nasal vaccination against polio.

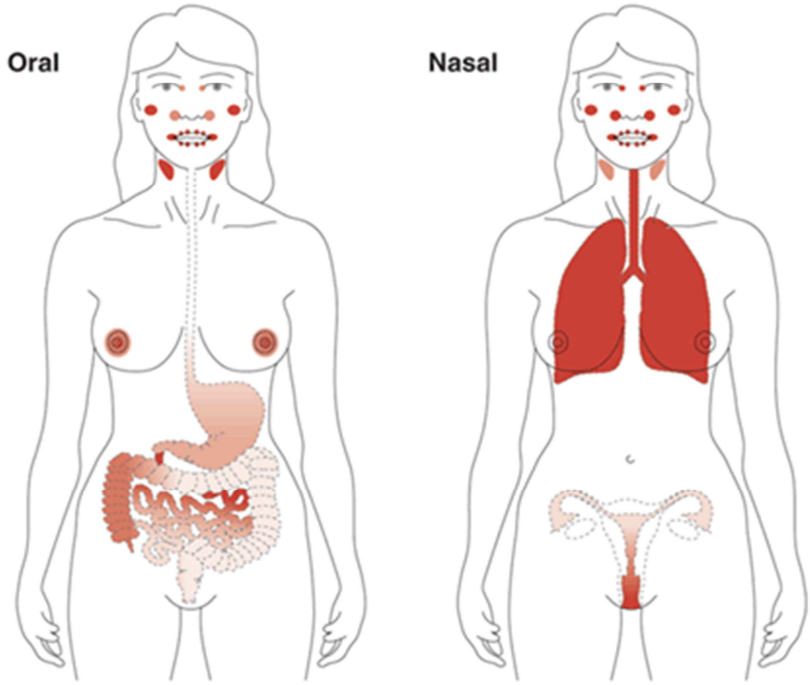

Figure 2 ***Expression of mucosal IgA immune responses after oral and nasal route of vaccination.*** *The 'common mucosal immune system' is more restricted than previously thought. In humans, immunization studies with cholera toxin B subunit by different mucosal routes have clearly shown that the strongest response takes place at the directly vaccine-exposed mucosa and the second-best responses at adjacent mucosa or at specifically interconnected inductive-expression mucosal systems such as the gut-mammary gland link in lactating women. A notable exception is the fact that nasal mucosal immunization not only stimulates an immune response in the respiratory tract, but also can give rise to a strong genital-vaginal mucosal immune response. However, different studies have been shown that intranasal vaccination induces antigen-specific antibody responses in the gastro-intestinal tract as well [41], which is not depicted in this figure. Shading indicates strength of response. (Adapted from Holmgren & Czerkinsky 2005, Nature Medicine) [42]*

ORAL POLIO VACCINE (OPV)

The only marketed needle free vaccination strategy against polio to date is oral vaccination by OPV. As mentioned before, OPV is the preferred vaccine in the developing world due to its important advantages over IPV (table 1). Currently, it is recommended by the WHO to give the trivalent OPV in the routine immunization program already at birth followed by three doses of

the same vaccine with intervals of at least four weeks. Nevertheless, the majority OPV is administered in additional mass vaccination campaigns in order to reach high proportion of children within the target population with the aim to provoke herd immunity and stop polio transmission.

The success of the live-attenuated OPV is attributed to the capability of the virus to replicate in the intestine, and thus generate an increasing antigen load that elicits strong antibody responses (serum IgG and local sIgA) [7, 43] and long-term persistence of neutralizing antibodies against poliovirus after three or four doses of trivalent OPV [44, 45]. The mucosal sIgA confers protection from poliovirus entry and multiplication in the intestine [46]. However, reduced immunogenicity of OPV in developing countries is identified as a major obstacle for global polio eradication [47]. Whereas rates of seroconversion following trivalent OPV administered at 2, 4 and 12 months of age, move toward 100% in industrialized countries, only 73% and 70% of children in developing countries have detectable neutralized antibodies against poliovirus type 1 and 3, respectively [48]. The explanation for these dissimilarities has not been found yet, but it was suggested that type 2 vaccine virus may interfere with responses to both type 1 and 3 vaccine viruses [48] or is possibly due to differences in the intestinal barrier caused by small bowel bacterial overgrowth and/or heavy intestinal helminth infestation [49, 50].

Monovalent [1, 51-53] and bivalent (type 1 and 3) [54] OPV formulations have been demonstrated to be more immunogenic per serotype than trivalent OPV. Therefore, the development of these new OPV formulations could overcome the interference of the immune response by type 2 Sabin virus. However, the risks of VAPP and VDPVs by reversion of the Sabin strains to a pathogenic strain still remain, and thus the global eradication of polio by using these vaccines impossible.

As a result, conversion to vaccination with inactivated polio vaccines is required. Substitution of OPV by an oral inactivated poliovirus vaccine seems ideal. However, such a proven strategy is not available yet for polio vaccination. In fact it is very challenging to develop an effective oral vaccine on the basis of inactivated pathogens or subunits/proteins. Several theories why live (attenuated) vaccines are superior in inducing protective immunity via mucosal sites are described; active receptor mediated virus entry, optimal particulate antigen presentation, cytosolic delivery of (replicating) RNA to induce Toll like receptors (e.g. TLR3, TLR7 and/or TLR9), co-delivery of viral proteins (as a result of RNA transcription), and increasing dose of virus on the

site of vaccination [32]. However, many of these hypotheses are limited proven by proper study design and are limited addressed in vaccine delivery design. In addition to the missing features described above, oral vaccination can hamper by digestion of the vaccine compounds in the gastro intestinal tract, making dose optimization very complicated and requirement of sophisticated delivery approaches.

NASAL VACCINATION

Intranasal vaccination can avoid degradation of vaccine antigen by digestive enzymes and low pH. As a result nasal vaccination may require smaller doses of antigen when compared to oral immunization [37]. However, for nasal vaccination also to date no vaccine is on the market on the basis of inactivated pathogens or subunits/proteins. A major drawback of intranasal immunization is the possible deposition of antigen in the central nervous system through the olfactory bulbs and olfactory nerves, which can cause temporary facial paralysis (Bell´s palsy) [55, 56]. This has been seen with a marketed virosomal influenza vaccine that was adjuvated by heat labile enterotoxin of E.Coli (LT) and has been withdrawn from the market due to this side effect. To date no efforts have been published that address nasal vaccination with polio vaccine formulations. Extensive reviews on nasal delivery of vaccines are available: [57-59].

NOVEL MUCOSAL VACCINE DELIVERY – SUBLINGUAL AND BUCCAL

Additional to the traditional mucosal routes, i.e., oral and nasal administration, other routes for inducing mucosal immunity against polio include the sublingual and buccal routes. These routes have been used for many years for the delivery of low-molecular weight drugs to the bloodstream. During the past few years, these routes become more and more popular in research on vaccine delivery. Important advantages of sublingual and buccal delivery over the oral route are the relatively low enzymatic activity in the mouth, and avoidance of the low gastric pH, which could affect the antigen.

Several studies demonstrated the efficacy and safety of sublingual vaccine delivery. Sublingual delivery of non-replicating antigens, inactivated or live viruses can induce protective immune responses by both systemic IgG and mucosal sIgA antibodies, and CTL responses [60-63]. Moreover, it has been shown that a high virus dose administered sublingually, does not redirect to the olfactory bulb [61], which gives the suggestion that this route offers a convenient and probably safer alternative to nasal delivery of vaccines. The buccal cavity is also known as

suitable and easily accessible site for the delivery of therapeutic agents. Buccal mucoadhesive vaccine formulations can be readily attached to the buccal cavity and retained for longer time.

To date no studies are published on the delivery of polio vaccines by these routes. However, the delivery of OPV may involve delivery of part of the dose in the sublingual and buccal sites. The 'side effect' via mouth mucosa of this oral delivered live polio vaccine is not investigated thus far. In order to achieve successful vaccination via the sublingual or buccal route, enhanced vaccine formulations are essential to target these mucosal inductive sites, but also to prevent the antigen from physical elimination and enzymatic degradation in the gastro-intestinal tract. In addition, it is expected that mucosal adjuvants are needed to induce proper immune responses in the case inactivated polio virus vaccines or subunit vaccines are used. Finally, sublingual and buccal vaccine delivery will depend on vaccine formulations that contain mucoadhesive agents to maintain prolonged contact with the oral mucosa [64].

DERMAL IMMUNIZATION

Driven by the fact that the dermis and epidermis of the human skin are rich in antigen-presenting cells, and the ease of access to the skin, there is renewed interest in dermal vaccine delivery. The skin's structural and cellular composition enables it to function as a physical and immunological barrier, suggesting that delivery of vaccines to the dermal layers, rather than parenteral vaccine delivery, should be more efficient and induce protective immune responses with smaller amounts of vaccine antigen [65].

As mentioned earlier, DCs are professional APCs that serve to efficiently amplify innate and adaptive immune responses. In the normal human skin two distinct populations of immature DCs are found, each within a specific layer, i.e., Langerhans cells (LCs) in the epidermis, and dermal DCs in the deeper skin layers [66]. However, the skin is equipped with an impressive barrier, the stratum corneum, which makes it almost impossible to induce an immune response through transcutaneous vaccination without disrupting this first defense line. Therefore, effective, safe, and convenient methods to achieve disruption of the stratum corneum are needed [32].

The different intradermal (ID) delivery methods that are currently available can be classified into three categories: administration by (1) needle and syringe; (2) jet injectors; and (3)

microneedles [67]. At present, the ID route of immunization is only used for the administration of two currently-licensed vaccines: Bacille Calmette-Guérin (BCG vaccination against tuberculosis) and rabies. Nevertheless, a PATH and WHO report reviewed the capability of ID delivery with vaccines against 11 diseases, including polio [68]. This section will discuss current research on ID polio vaccination and the potential benefits and challenges of dermal delivery devices for the use with IPV.

NEEDLE AND SYRINGE

ID injection methods using needles and syringes require considerable expertise and is therefore not ideal for routine vaccinations [69]. The Mantoux technique, originally used as a diagnostic for tuberculosis, involves the insertion of a needle parallel to the skin to produce a wheal.

Although millions of intramuscular (IM) doses of IPV have been administrated in the developing world, the initial experiments of Jonas Salk anticipated its use via the ID route. In 1953, Salk demonstrated the immunogenicity of IPV administrated both intramuscularly and intradermally [70]. Despite these and more promising results in the mid-1950s [70-73], the ID route was only in Denmark the most abundant route for IPV vaccination at that time [73, 74]. With the purpose of developing a more affordable IPV for the lower-income countries and increase its use in the post-eradication era, different studies investigated ID polio vaccination [75]. Trials of ID administration of the enhanced-potency IPV, which was, with its higher content of poliovirus antigen, responsible for highly improved seroconversion rates for all three serotypes [76], have been ongoing in India since the early 1990s. Satisfactory seroconversion rates were obtained with fractional (one-fifth) doses delivered ID in subjects who had been previously immunized [77], or had never been immunized against polio [78]. In 1998, a trial among 69 Indian infants demonstrated that two or three fractional doses ID were equivalent in terms of seroconversion to two full doses of IPV delivered IM or five doses of OPV (based on historical data). All infants who had no preexisting maternal antibodies seroconverted to all serotypes [79]. None of these studies using enhanced-potency IPV, however, included a comparator IM group. Therefore, recently a randomized controlled trial was conducted in the Philippines, to compare the primary and booster immunogenicity of IPV by ID injection to the IM route. These data demonstrated non-inferiority of fractional dosing by the ID route, and thus confirmed the medical validity of this IPV vaccination strategy [80].

JET INJECTOR

While different studies reinforce the observations of Salk about the potential of fractional dose strategy by ID injection, the mass usage of needle ID injections is not foreseeable. The need for skilled personnel, which is a major limitation for large-scale campaigns, and the safety and disposal concerns related to the use of needles remain. To overcome these problems and increase the affordability of IPV, needle-free devices for ID injection, which can be manually reset and used by volunteers, has been developed [19]. The WHO sponsored two studies in Cuba and Oman with two different IPV vaccines used with two different immunization schedules. The vaccines were administered either ID using a disposable syringe jet injector, the Bioject™ 2000 (Bioject Inc., Portland, OR, USA), or by IM route using conventional syringe and needle. Although the primary objective of these trials was to demonstrate non-inferiority in terms of seroconversion for the ID route compared to the IM route, this purpose was not fulfilled in the Cuban study; significantly lower seroconversion rates (ID: 52.9%, 85.0%, and 98.9% vs. IM: 89.3%, 95.5%, and 98.9% for serotypes 1, 2, and 3 respectively) and lower median antibody titers ($p<0.001$) were achieved in the ID arm [81]. In the Omani study, similar levels of seroconversion for serotypes 1 and 3 were after ID delivery of fractional doses as after IM vaccination of the full dose, whereas serotype 2 showed a statistically significant difference between the delivery routes (95.7% vs. 100%, $p=0.01$). However, for all serotypes, the median antibody titers were significantly lower in the fractional dose group [82], but it remains unclear whether the differences have practical implications since any detectable titer of neutralizing antibody against poliovirus would be expected to prevent against paralytic disease [83]. Given the well-characterized interference of maternally derived antibodies with IPV immunogenicity [84, 85], ID administration of fractional doses of IPV is unlikely to serve as an optimal antigen-sparing strategy when given at the routine ages of 6, 10, and 14 weeks, rather than a schedule that administers the first dose at 2 months of age [81, 82].

A randomized controlled trial of healthy infants aged 6-9 months in India showed that a single ID fractional dose IPV (one-fifth), using the spring-powered PharmaJet injector, was less effective than full-dose IPV in seroconverting seronegative children and in increasing antibody titers in seropositive children [86]. These findings are in contrast with the results found in the three-dose trial in Oman, which is described above. Nevertheless, all data together suggest that, when given at the correct interval, the fractional dose strategy with use of a needle-free device

may solve two existing problems of IPV by being safer to administer and decreasing costs by using less antigen. In addition, it has been hypothesized that ID delivery could improve protection against infection in the gut, since it may stimulate IgA mucosal immunity [87]. Further studies with different jet injectors should be done to establish whether the route of administration, the number of doses, or the device were responsible for the suboptimal immune response in the Indian study [86].

MICRONEEDLE APPROACHES

Another approach for ID vaccine delivery makes use of microneedle arrays that can penetrate the stratum corneum. These arrays are designed to disrupt the stratum corneum and target Langerhans cells in the epidermis, but are minimally invasive, since the nerves in the underlying tissue were not reached, and caused therefore no pain and only minimal irritation [88]. Currently, different microneedle strategies are being exploited in different research groups, i.e., the straightforward methods by pre-treating the skin with solid microneedles followed by application of a vaccine containing patch on the pretreated skin surface or using hollow microneedles to inject the vaccine into the epidermis, or the more recent strategies by the use of dissolvable microneedles or antigen-coated microneedle arrays. However, most microneedle technologies are still in the preclinical phase and the optimal microneedle strategy (material, shape) to deliver a vaccine into the skin has not yet been established [32].

Since ID administration of IPV has been shown to have great potential, a microneedle approach appears to be a useful delivery method for IPV vaccination. To guarantee the stability and immunogenicity of a transcutaneous polio vaccine by using coated or dissolvable microneedles, the development of a solid IPV formulation is required, which is a major hurdle to overcome. Furthermore, adjuvants can improve the potency of the vaccine and could steer the type of immune response that is elicited.

FUTURE PERSPECTIVES

Since global polio eradication is coming up, there is an urgent need for an affordable inactivated polio vaccine (IPV) for its use in the post-eradication era. However, to reach the final goal of a polio-free world, it is believed that the routine use all live-attenuated oral poliovirus vaccines (OPV) should be discontinued, where the use of (Sabin) IPV is preferable in the period

thereafter [17, 89]. A new generation of IPV should overcome the major drawbacks of this vaccine (Table 1), and should therefore ideally be administered through alternative (needle-free) delivery routes, provide mucosal immunity, and be affordable for low-income countries.

Selecting an appropriate vaccine delivery strategy for polio is based on the route of administration, the selection of a proper adjuvant, an improved vaccine formulation and the use of a (non-invasive) delivery system [32]. Mucosal vaccine delivery, like the sublingual and buccal routes, has the potential to elicit local immune responses at the point of virus entry, but induces in the absence of an adjuvant generally tolerance or low-to-undetectable immune responses [36]. Therefore, efforts on mucosal vaccine design should focus on *(i)* overcoming physiological barriers at mucosal routes, *(ii)* targeting local APCs for appropriate processing of the antigens that lead to specific T and B cell activation, and *(iii)* controlling the kinetics of antigen and adjuvant presentation to promote long-lived, protective adaptive immune memory responses [40].

Different adjuvants have already proven their potential for (s)IPV though via the parenteral route; CpGoligodeoxynucleotides (ODN) [90] aluminum hydroxyde[27], oil-in-water emulsions [91], and chitosan [92]. TLR ligands, including CpG ODN (TLR9) and monophosphoryl lipid A (MPL; TLR4), exhibit immuno potentiating activity when co-administered with antigens since they induce the maturation of immature dendritic cells (DCs), which is followed by T cell activation. However, up to the present time no data is available on preclinical evaluation of these adjuvants for mucosal vaccination. Especially, the by CDC developed E.Coli heat labile toxin with 2 mutant (dmLT) in order to decrease their toxicity may be very interesting for evaluation for vaccination via the oral mucosa.

Special attention should be given to restrictions related to the final target population for polio vaccination: infants. The delivery method chosen and the selected delivery device and formulation should be suitable for application in infants. For example sublingual tablets are not suitable for infants since they can give rise in risk of suffocation by a swollen tongue. New improved ways of delivery to the buccal and/or sublingual mucosa are under way. These include sticking formulations, like gels that become solid upon contact with the mucosa (temperature) or thin films that can be applied below the tongue. Advantage of these formulations is that they prolong the contact time with the mucosa and thereby may decrease the dose needed for induction of immunity.

Dermal delivery might be a more suitable alternative for vaccination of infants. An disadvantage of dermal delivery is that in general no mucosal immunity is elicited by this route. However, for certain vaccine adjuvant combinations there seem to be evidence that ID vaccination may also have the potential of inducing mucosal immunity [93-96]. Currently, no polio vaccines are combined with adjuvants for dermal vaccination. One of the adjuvants interesting for dermal delivery is dmLT from CDC that bears minimal side effects and as mucosal adjuvant may help to generate mucosal immunity. New approaches, such as biodegradable or coated microneedles, hold promise for dermal delivery since they may contribute to the stability of the vaccine, which is welcome for transport and vaccination in the developing countries where polio is still endemic.

To date only limited clinical studies with IPV via alternative delivery routes are performed. Future studies have to answer which delivery route is most suitable for polio eradication by new generation IPV vaccines. Questions that have to be addressed are the doses needed for induction of immunity.

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