Review

Mucosal immunity and poliovirus vaccines: Impact on wild poliovirus infection and transmission

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A B S T R A C T
Since the resolution of the World Assembly in 1988 to eradicate polio globally, substantial progress toward this target has been achieved, but the final goal remains elusive. India and other tropical developing countries present a unique challenge because of the much lower oral poliovirus vaccine (OPV) immunogenicity compared to industrialized countries, both in terms of humoral and mucosal immunity. To overcome this challenge, further research is needed to elucidate the causes for the suboptimal OPV immunogenicity, better defining the optimal vaccine schedules and delivery strategies, developing and evaluating adjuvants to boost OPV immunogenicity, and improving the methods for directly measuring mucosal immunity.

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Contents

1. Introduction ......................................................................................................................... 8206
2. Section 1: poliomyelitis: the virus, the disease, and disease prevention ........................................... 8206
   2.1. Poliovirus ...................................................................................................................... 8206
   2.2. Poliomyelitis ............................................................................................................... 8206
   2.3. Disease prevention: available vaccines ........................................................................... 8206
3. OPV .................................................................................................................................. 8207
   3.1. Vaccine efficacy against paralytic poliomyelitis .............................................................. 8207
   3.2. Humoral immunity ....................................................................................................... 8207
   3.3. Mucosal immunity ....................................................................................................... 8207
   3.4. Other forms of OPV ...................................................................................................... 8207
   3.5. Adverse events: vaccine associated paralytic poliomyelitis (VAPP) .................................. 8207
   3.6. Adverse events: vaccine derived polioviruses (VDPVs) .................................................. 8207
4. IPV .................................................................................................................................. 8208
   4.1. Vaccine efficacy against paralytic poliomyelitis .............................................................. 8208
   4.2. Systemic immunity ....................................................................................................... 8208
   4.3. Mucosal immunity ....................................................................................................... 8208
5. OPV and IPV in combination .............................................................................................. 8209
   5.1. Vaccine efficacy against paralytic poliomyelitis .............................................................. 8209
   5.2. Systemic immunity ....................................................................................................... 8209
   5.3. Mucosal immunity ....................................................................................................... 8209
   5.4. Effect of IPV, OPV, or a sequential schedule on viral shedding ....................................... 8209
6. Section 2: immune functions in the neonate, the infant and in early childhood ......................... 8209
   6.1. Innate immunity .......................................................................................................... 8209
   6.2. Adaptive immunity ...................................................................................................... 8210

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1. Introduction

Since its inception in 1988, the Global Polio Eradication Initiative (GPEI) has significantly reduced the number of paralysis caused by poliovirus, from an estimated 350,000 cases per year in 125 countries [1] to 1349 cases in 20 countries in 2010 [2].

Despite the significant gain, four endemic countries (India, Nigeria, Pakistan, and Afghanistan) continue to be foci of infection, and populations in all countries are vulnerable to imported virus. Of special note is northern India, where oral poliovirus vaccine (OPV) per dose appears to be less effective at inducing systemic antibodies than in industrial countries.

This article provides a comprehensive analysis of the available information on: (1) poliomyelitis and the virus, the disease, and disease prevention; (2) immune functions in the neonate, the infant, and in early childhood; (3) environmental factors: impacts on induction of effective immunity after OPV; and (4) possible interventions to overcome focal OPV vaccine failure.

2. Section 1: poliomyelitis: the virus, the disease, and disease prevention

2.1. Poliovirus

Poliomyelitis is an enterovirus belonging to the family of Picornaviridae. A naked icosahedral structure approximately 27 nanometers in diameter, the virion is composed of about 60 copies of each of four capsid proteins (VP) subunits (VP1, VP2, VP3, and VP4), and contains a single stranded RNA genome about 7500 nucleotides in length.

The capsid proteins VP1, VP2, and VP3 are located on the surface and constitute important antigens for induction of neutralizing antibody. VP4 appears to be completely internal and probably plays no part in induction of neutralization. Three major neutralization antigenic sites, known as N-Ag I, II, and III, and possibly a fourth, N-Ag IV, have been identified on the capsid proteins [3].

Poliomavirus exists as three antigenic types or serotypes 1, 2, and 3. Most epitopes of N-Ag sites are clustered on VP1 for all virus serotypes. The most prominent antigenic site, N-Ag I, is located at the pentameric apex of the virus. In mice and presumably in humans, about 90% of type 2 and type 3 antibodies are directed against this site. Limited antigenic evolution does occur despite the weak selective pressure for substitution into the N-Ag sites, and unrelated wild-type poliovirus isolates commonly vary at one or more N-Ag sites [4].

N-Ag I contains a protease cleavage site, and if cleaved via the enzyme trypsin, all antigenic properties of the site in native virus are lost. Thus proteases potentially cleave virus antigenic sites and prevent development of mucosal neutralizing antibodies [5].

After entry via the oral cavity, the virus attaches to and enters epithelial cells that express the poliovirus receptor (PVR) CD155 on their surfaces. CD155 is a single-span, glycosylated peptide with three immunoglobulin-like ectodomains (NH2-V-C2-C2). CD155 mRNA has been detected in brain, spinal cord, leukocytes, lung, ileum, placenta, heart, skeletal muscle, kidney, and liver. However, the majority of detectable infections occur in the gastrointestinal (GI) tract and central nervous system (CNS).

The development of a CD155 transgenic mouse model has enabled numerous studies on the molecular aspects of virus-host cell interaction. However, specific studies on mucosal immunity and virus replication have been limited in scope because the mice have limited or no expression of CD155 in the oropharynx or the intestine [6].

After initial replication in the mucosal epithelium, poliovirus is shed in nasopharyngeal secretions and saliva for one to two weeks and in feces for three to six weeks. Immune compromised individuals have been shown to secrete poliovirus for prolonged periods of time, occasionally three years or more, and in some extreme situations for as long as 20 years after mucosal infection [7].

2.2. Poliomyelitis

Poliomavirus infection is asymptomatic in 90–95% of infected subjects. Approximately 4–5% may exhibit an abortive infection, similar to other enteric viral infections or aseptic viral meningitis, and roughly 0.5% may present paralytic disease, bulbar involvement, respiratory arrest, and occasionally death. Occasionally a patient may experience a secondary illness resembling motor neuron disease 15–40 years after a silent or clinically symptomatic poliovirus infection.

In 2010, 20 countries reported at least one case of clinical infection with wild-type poliovirus (WPV), according to the Global Polio Eradication Initiative (GPEI). To date, four endemic countries (India, Nigeria, Pakistan, and Afghanistan) are considered to have never eliminated WPV infection. These countries have continued to experience cases of WPV type 1 and type 3. In 16 of these countries, infection had been previously eliminated only to be reintroduced by travellers from other countries. In 2011, further progress has been recorded. In India (data as of 12 July 2011), only a single case of poliomyelitis due to poliovirus type 1 has been reported with onset
of paralysis on 13 January 2011 from West Bengal. In addition, no poliovirus type 3 has been detected in 2011 in India.

Despite the recent progress toward interrupting poliovirus transmission, India presents a unique challenge to eradication because of the low efficacy of OPV. Frequent and high-quality mass immunization rounds were required to achieve this progress (e.g., more than 10 SIA round per year with more than 90% coverage), in stopping transmission in all geographic areas, including the highest risk communities. In the states of Uttar Pradesh and Bihar [8,9], OPV has failed to protect vaccinated individuals in some situations even after repeated vaccination. Mucosal immunity induced by OPV is known to be short-lasting. In Uttar Pradesh and Bihar, about 15% of children who had been given ten or more doses of OPV lacked effective gut immunity, as demonstrated by the shedding of WPV type 1 [10].

2.3. Disease prevention: available vaccines

Two types of poliovirus vaccines are available for routine childhood immunization: trivalent inactivated poliovirus vaccine (IPV) and trivalent live attenuated oral poliovirus vaccine (OPV).

Both OPV and IPV vaccines are effective at inducing humoral immunity, measured by serum poliovirus-specific neutralizing antibodies.

3. OPV

OPV is the preferred vaccine in most of the world because of its ease of use, low cost, and potential to quickly halt viral transmission by more closely mimicking naturally acquired infection because of its oral route of administration. The World Health Organization (WHO) Expanded Program for Immunization (EPI) currently recommends trivalent OPV in the routine immunization program at birth plus three doses of the same vaccine spaced at least four weeks apart. However, the vast majority of OPV (either as trivalent, bivalent or monovalent vaccine) is administered in supplemental immunization campaigns with the aim to reach high proportion of targeted children in a very short period. This approach is required to achieve eradication, as it increases the population immunity rapidly and reduce complexity of logistics (e.g., cold chain, vaccinators).

OPV contains live attenuated poliovirus strains developed after repeated passage of wild-type virus strains in tissue culture. These attenuated Sabin vaccine strains are considerably less neurovirulent and differ from the “consensus” WPV at several antigenic sites, for example N-Ag I for Sabin type 1 and N-Ag IIIb for Sabin type 3. Other attenuating mutations include “non-consensus” capsid amino acid substitutions on the virion surface that potentially alter virus–receptor interactions. The N-Ag substitutions and other attenuating mutations are under negative selection during replication of the poliovirus vaccine strain in the intestines [11].

OPV also has the potential to indirectly vaccine the close contacts of vaccine recipients, who shed OPV virus in their nasopharyngeal secretions and feces. In a study in Yaoundé, Cameroon, an OPV campaign resulted in a decrease of 85% of paralytic poliomyelitis cases even though only 35% of children 12–13 months of age received three doses of OPV [12].

3.1. Vaccine efficacy against paralytic poliomyelitis

A vast body of empirical evidence supports the effectiveness of OPV. The vaccine has been successful in interrupting poliovirus circulation wherever it was used appropriately and wherever adequate coverage was achieved. Numerous studies also confirmed the effectiveness of OPV in preventing paralytic disease in industrialized and some developing country settings. A study in Oman estimated that OPV’s effectiveness in preventing paralytic disease was 90% [13]. However, a number of outbreaks of paralytic disease have occurred in developing countries where relatively high percentages (70–85%) of children have been immunized with OPV. The per dose efficacy of a single trivalent OPV vaccination is 50% in North America and Europe, but only 21% in India and only 9% in Uttar Pradesh [14].

3.2. Humoral immunity

The trivalent form of OPV is highly effective at stimulating systemic immune responses to all serotypes in industrialized countries. Studies in these countries have demonstrated that three doses of trivalent OPV administered at 2, 4, and 12 months of age result in antibody seroconversion in nearly 100% of vaccine recipients. However, in developing countries, three doses of trivalent OPV resulted in only 73% (range, 36–99%) and 70% (range, 40–99%) of vaccinated individuals developing serum antibodies to type 1 and type 3 poliovirus, respectively [15]. More recently, a study conducted in India found that two doses of trivalent OPV resulted in serum antibodies against type 1 and type 3 poliovirus in 63% and 52% of subjects, respectively [16].

3.3. Mucosal immunity

Mucosal immunity can be evaluated either directly by measuring secretory antibody or indirectly by determining viral shedding in feces or oropharynx secretions after challenge with OPV. When challenged with OPV, an individual with poliovirus-specific mucosal immune responses will exhibit less shedding of the challenge virus. Detection of intestinal poliovirus-specific secretory IgA has been correlated to a reduction in the amount of virus excreted [17,18].

A number of studies have demonstrated that OPV induces mucosal immunity. A study by Zhoaui et al. found that OPV vaccination resulted in 70% of recipients having nasopharyngeal neutralizing antibodies [19]. Other studies found that two or three doses of OPV induced a higher mucosal response, measured by nasopharyngeal slgA responses or OPV challenges, than the same number of doses of IPV [20,21]. A study by Faden et al. in an industrialized country setting found that IPV was less likely than OPV to induce a mucosal immune response. After three doses of either vaccine, 43–90% of IPV recipients developed either poliovirus-specific slgA or neutralizing antibodies versus 70–90% of OPV recipients. IPV recipients also exhibited significantly lower titers of nasopharyngeal neutralizing antibody and secretory IgA than the individuals given OPV [20].

3.4. Other forms of OPV

In recent years, monovalent (either type 1, 2 or 3) and bivalent (types 1 and 3) formulations of OPV have been developed. Monovalent and bivalent OPV have been demonstrated to be more immunogenic per serotype than trivalent OPV (tOPV) by removing interference by type 2 Sabin virus. For example, in a study in Egypt, monovalent OPV type 1 (mOPV1) was more effective at inducing antibodies against type 1 poliovirus than was tOPV [22]. In Uttar Pradesh, India, the estimated per-dose efficacy was 30% for mOPV1 versus 11% for tOPV [23]. In Nigeria, a case control study found that the per-dose efficacy was 67% for mOPV1 versus an estimated 16% for tOPV [24].

A recent trial of 900 babies in India found that bivalent type 1 and type 3 OPV (bOPV) was superior to tOPV and as good as mOPV1 and mOPV3 at inducing type-specific serum immunity after two vaccine doses [16].
3.6. Adverse events: vaccine derived poliovirus (VDPVs)

Vaccine-derived poliovirus (VDPV) strains are vaccine strains that have regained replicative ability and/or neurovirulence due to antigenic drift or spontaneous recombination of vaccine serotypes [26]. VDPVs have been subdivided into two groups; (1) immunodeficient VDPVs (iVDPVs) isolated from immunodeficient individuals; and (2) circulating VDPVs (cVDPVs) that arise after prolonged replication of the vaccine virus (Fig. 1). Although the prevalence of VDPV cases is low, these cases pose a risk to eradication, since patients with B-cell deficiencies, especially those with common variable immunodeficiency disorder (CVID) may excrete poliovirus chronically for a decade or more, and could constitute a reservoir from which poliovirus could emerge again. cVDPVs are another concern because of the epidemic potential of these strains. Preventing the emergence of cVDPVs requires maintaining high levels of vaccination coverage against poliovirus in all countries before polio is eradicated globally.

4. IPV

IPV was the first poliovirus vaccine to be licensed in 1955. It is derived from selected wild-type poliovirus strains that are inactivated with formalin. The potency of the original vaccine was enhanced in the 1970s and today roughly 60 mainly industrialized countries use either only IPV or a sequential OPV/IPV schedule. IPV may be formulated as a combination vaccine with other antigens, such as DTP, Hib, and Hepatitis B [23]. IPV preparations cost more per dose and require injection by trained healthcare workers using sterile needles.

4.1. Vaccine efficacy against paralytic poliomyelitis

IPV has been shown to be efficacious in both industrialized countries and developing countries. A study in Senegal demonstrated that a two-dose IPV schedule resulted in protection of over 89% of vaccine recipients during a type 1 poliovirus outbreak [28]. A study in the North Arcot region of India found that three IPV doses had an efficacy of 92% versus 66% for OPV [29]. IPV is also effective in preventing vaccine-associated paralytic poliomyelitis (VAPP). In the United States during the period of 1997–2000, an IPV–OPV sequential schedule (two doses of IPV followed by OPV) prevented VAPP in infants that received IPV before OPV [30].

4.2. Systemic immunity

IPV is highly effective at stimulating circulating antibody responses to poliovirus in both the industrialized country and developing country settings. For example, a study conducted in the United States found that a two-dose schedule resulted in a seroconversion rate of 99% [20]. A study conducted in Puerto Rico found that three doses of IPV resulted in seroconversion rates of 86–100% depending on the timing of vaccination [31]. To remove possibility of the interference by OPV or WPV, a study was conducted in Cuba, a country where WPV is no longer circulating and OPV use is limited. The study found that three doses of IPV given at 6, 10, and 14 weeks induced antibodies to type 1, type 2, and type 3 in 94%, 83%, and 100%, respectively of vaccine recipients [32].

4.3. Mucosal immunity

As mentioned above, IPV is less effective than OPV in stimulating mucosal immune responses [18]. While IPV seems less effective
in inducing immunity against mucosal infection than natural infection, protection against mucosal re-infection with WPV in such IPV recipients may be complemented by the possible diffusion of circulating IgG virus-specific antibody into mucosal sites [33].

5. OPV and IPV in combination

Vaccination schedules involving sequential or simultaneous use of OPV and IPV have been explored to prevent OPV-associated paralytic poliomyelitis and to close the immunity gap observed with the use of OPV or IPV alone.

5.1. Vaccine efficacy against paralytic poliomyelitis

Sequential administration of IPV and OPV has proven effective in both industrialized and developing country settings. Studies in Denmark and Hungary found that one or three doses of IPV followed by three doses of OPV resulted in protection against paralytic poliomyelitis and reduced cases of VAPP [34,35]. A study in the Gaza Strip found that switching from OPV-only to simultaneous administration of OPV and IPV resulted in reduction in the number of cases of paralytic poliomyelitis [36].

5.2. Systemic immunity

Sequential vaccinations using IPV and OPV have proven efficacious at stimulating systemic antibodies. A study of 123 children in Buffalo, New York, immunized using either IPV–OPV–OPV or IPV–IPV–OPV resulted in 100% of children with detectable serum neutralizing antibodies to all three serotypes.

In developing country settings, the simultaneous use of OPV and IPV has also induced uniformly high antibody response to all three poliovirus types. For example, a study conducted in Oman, The Gambia, and Thailand comparing a birth dose of OPV plus either OPV at weeks 6, 10, and 14 or simultaneous OPV and IPV at weeks 6, 10, and 14 found that in Oman and The Gambia seroconversion rates and viral geometric mean titers were highest in the infants that received the simultaneous vaccines. In Thailand, however, the immune response was similar in both the OPV-only and OPV–IPV simultaneous groups [37]. Studies in Pakistan also found enhanced immunological responses from simultaneous OPV–IPV immunizations [38]. A study in the Ivory Coast showed that a single, booster dose of IPV after three OPV doses was able to effectively close the remaining immunity gap.

5.3. Mucosal immunity

In both industrialized and developing country settings, the sequential administration of IPV and OPV resulted in mucosal responses. For types 1, 2, and 3 respectively, the percentage of children in the Buffalo study with nasopharyngeal sIgA was 100%, 100%, and 100% after three doses of OPV; 89%, 91%, and 89% after three doses of IPV; 94%, 100%, and 100% after IPV–OPV–OPV; and 75%, 81%, and 81% after IPV–IPV–OPV. The sIgA titers in nasopharyngeal secretions were highest in the three-dose OPV group. The three-dose IPV group and the IPV–IPV–OPV combination group had much lower sIgA titers [20]. In another study in the United States, two doses of IPV after OPV reduced mucosal shedding of poliovirus types 1 and 3 [39].

The above-mentioned study conducted in Oman, The Gambia, and Thailand, found that when vaccinated children were challenged with OPV, virus excretion was as low in the OPV/IPV treatment group as in the OPV group, indicating that both OPV/IPV and OPV alone induced mucosal immunity.

IPV appears capable of boosting circulating and mucosal sIgA levels in individuals previously inoculated with OPV or exposed to WPV. In OPV-primed individuals, a booster immunization with IPV has been shown to induce a strong mucosal sIgA response [40]. Also, in IPV-vaccinated individuals, a study by Hovi found that OPV challenge boosted the amount of poliovirus-specific sIgA detected in the intestines [41].

5.4. Effect of IPV, OPV, or a sequential schedule on viral shedding

Vaccine-induced mucosal antibodies can neutralize poliovirus in the gut, resulting in reduction of viral shedding in the feces. A variety of regimens have been evaluated for their impact on viral shedding, including IPV, OPV, or combinations. In seronegative children, a challenge with type 1 OPV virus resulted in greater than 80% excreting the vaccine virus in feces for a mean of 20 days, which is similar to the duration that non-immune children will excrete WPV. In children previously vaccinated with OPV or naturally immune due to WPV exposure, about 31–37% will shed virus in feces for a mean of 5–7 days. In contrast, about 63–82% of children who were vaccinated with IPV will shed virus in feces for about 7–10 days. A study in Cuba of IPV-vaccinated subjects challenged with OPV compared with unvaccinated subjects demonstrated similar levels of excretion (>90%), but IPV-vaccinated subjects had a modest reduction in viral titer (~0.5 log) [42]. Prior immunization with IPV has been shown to reduce the length of virus excretion in the pharynx and the intestine, especially in the pharynx. Thus, fecal virus shedding is reduced in both OPV and IPV vaccinated individuals compared with non-immune subjects, but to a much larger extent among OPV-vaccinated compared with IPV-vaccinated subjects [43–46].

6. Section 2: immune functions in the neonate, the infant and in early childhood

Mucosal immune responses protect the neonate from disease-producing organisms and environmental macromolecules. Such protection is mediated through activation of innate immunity and development of specific B and T cell responses.

6.1. Innate immunity

The first lines of protection against pathogens in the mucosa are nonspecific. These include mucin and mucous, which may prevent direct contact with mucosal epithelium. Subsequently host-derived effector mechanisms of innate immunity participate in local mucosal defense. These include antimicrobial peptides, macrophages, dendritic cells, neutrophils, complement components, and several cellular and soluble products.

Recent studies have demonstrated impairment of macrophage function in the neonatal period and early infancy. Also, the levels of amylase, lysozyme, lactoferrin, and other soluble factors of innate immunity are significantly reduced at birth, and normal adult levels are frequently attained only after 6–20 weeks of life.

The major effector mechanisms of mucosal innate immunity in the neonate consist of several families of cellular receptors known as the pattern recognition receptors (PRRs), which detect invariant molecular patterns found in most organisms. These unique molecular patterns, integral to the structure of most microorganisms including bacteria, viruses, and parasites, are often referred to as pathogen associated molecular patterns (PAMPs). The role of pattern recognition receptors such as the Toll-like receptors (TLRs) in poliovirus vaccine-induced immunity remains to be determined [47,48].

At birth, the gastrointestinal tract contains no microbiota. The intestinal lumen becomes rapidly populated in the first few months of life.
6.2. Adaptive immunity

Infants have elevated risk of infection due to deficiencies in the antibody response during the first few months of life. Although B cells and T cells are present at birth, the neonate lacks the full ability to mount a T cell-independent B cell response for roughly the first year of life, and full capability is not achieved until 4–5 years of age. The newborn can mount a T cell-dependent B cell response beginning at birth, but the response is often not equal to that of an adult. T cell immunity is more developed than B cell immunity, but the responses may be slower compared to adults. The T cell response appears to be biased toward Th1 type cells rather than Th2, Th17, or T regulatory cells. This may be because neonatal dendritic cells (DCs) are immature and are less able to secrete cytokines such type 1 interferons and IL-12 p70, thus biasing T cell development toward Th1 [49].

Furthermore, levels of circulating CD45+RO+ CD27+ memory T cells are reduced at birth. The neonate also exhibits reduced function of macrophages, dendritic cells, and B-lymphocytes as antigen presenting cells (APCs). The neonate exhibits reduced ability to deliver costimulatory signals to naive T cells, reduced delayed-type hypersensitivity reactions, and reduced intracellular killing of several bacteria, fungi, and viruses. In addition to the impairment of the APC function, the cord blood and neonatal DCs exhibit significant impairment of IL-12 production [50]. In the newborn intestinal mucosal, it is possible to detect B and T lymphocytes but secretory IgA is not detected until the infant is one week old [51]. The appearance of Peyer’s patches is first observed around 10–11 weeks of gestation. After 20–30 weeks of gestation, up 50–90 patches with defined B and T cell zones have been observed.

6.3. Immune responses to poliovirus, IPV, and OPV

The pathogen-specific responses of the cellular and humoral immune systems begin with uptake of the virus into the cell. Uptake occurs at inductive sites of organized lymphoid follicular tissue in the gut associated lymphoid tissue (GALT), nasopharyngeal associated lymphoid tissue (NALT), and broncho–epithelium associated lymphoid tissue (BALT). These areas are rich in antigen reactive B cells, T cells, macrophages, dendritic cells, and other cellular elements involved in the development of specific immune responses [52]. WHO recommends a birth dose based on studies showing that OPV during the neonatal period significantly boosts mucosal and systemic antibody responses [53]. The birth dose confers a protective immune response in up to 50% of infants, according to studies conducted mainly in industrialized countries [33]. However, the above-mentioned recent study conducted in India reported that, after a birth dose of trivalent OPV, the seroconversion to poliovirus types 1, 2, and 3 were only 15%, 25%, and 4%, respectively [16].

A few studies have noted that pre-term infants are capable of mounting mucosal antibody responses to IPV. A study by Adeniyi-Jones et al. found that preterm infants are capable of mounting both nasopharyngeal neutralizing and secretory IgA antibody responses to IPV that are comparable to full-term infants [54]. Limited information is available about the development of function of T cell mediated immune responses to poliovirus after infection or immunization. A study by Klein et al. compared preterm infant T cell responses before and after IPV with those of term infants. Prior to IPV, the researchers found that compared to term infants, preterm infants had fewer circulating CD45+RO+ CD27+ and CD4+CD69 IFN-γ memory T cells in response to infection with staphylococcus enterotoxin B. After IPV immunization, preterm and term infants had about the same number of poliovirus–specific memory T cells. However the proliferation of peripheral blood mononuclear cells (PBMCs) in preterm infants was reduced compared to term infants. Also, serum antibodies to poliovirus type 1 were lower in preterm infants versus term infants [55]. Immunization with OPV during the neonatal period results in high levels of neutralizing antibodies. A study by Vekemans et al. found that neonatal immunization with OPV resulted in production of high levels of serum and mucosal neutralizing antibodies. However, IFN-γ production and T cell proliferation were reduced compared to adults [56]. This finding was consistent with a study in transgenic mice, which found that a Th2 response was required for B-cell activation following OPV [57].

In contrast to these findings, Vekemans’ study of infants did not find any increase in Th2 cytokine production in peripheral blood, suggesting perhaps that OPV vaccination in infancy does not induce a systemic Th2 dominant response. More information is needed to determine if this is a possible mechanism for why OPV may not perform well in infants with insufficient Th2 response.

6.3.1. Immunological memory and poliovirus vaccines

The antibody response induced after natural infection probably provides lifelong immunity against paralytic poliomyelitis.

Both OPV and IPV have been shown to induce long-term immunological memory in the Buffalo study with OPV, IPV and combinations, and in a study in Sweden using three-dose IPV [58].

The longevity of poliovirus–specific protection may be due to one or more of the following factors: the induction of CD80+ memory B cells; the induction of CD45+RO+ CD27+ memory T cells; a relative increase in the frequency of resting naive CD4+ T cells with a prolonged half-life; or a combination of factors. The development of effector CD45+RO+ CD27+ memory T cells producing IFN-γ in the peripheral circulation has been observed after immunization with OPV or IPV in infants as well as in adults who were immunized in childhood with OPV and reimmunized with IPV in later life. These cells have also been shown to express α4β7, the integrin ligand associated with gut homing of sensitized lymphocytes [59].

Debate has focused on whether periodic reexposure to a pathogen is required to maintain immunological memory. Several studies in an experimental mouse model have suggested that antigen is not required for immunological memory in Th1 T cells, Th2 T cells, and in B cells [60,61].

However, it is not known whether repeated exposure to poliovirus is necessary to maintain mucosal immunity as there are few data on the duration of protection in the gastrointestinal tract.

7. Section 3: environmental factors: impacts on induction of effective immunity after OPV

Several oral vaccines, including poliovirus, rotavirus and cholera vaccine, have been shown to induce limited mucosal as well as systemic immunity, possibly due to a common mechanism. The reduced response could be due to the different environmental conditions in developing countries [62]. These include malnourishment, concurrent infections, tropical enteropathy, oral tolerance, maternal factors, and other factors.

7.1. Malnourishment

Infants and children in developing countries often suffer from deficiencies in both protein-calorie nutrition and micronutrients such as vitamin A, vitamin D, zinc, and iron. For example, it has been estimated that over 50% children are zinc-deficient in Bangladesh, either due to lack of zinc-containing food or due to losses during repeated infections.

Iron is needed for humoral responses, and both iron and zinc are needed for cell-mediated responses. Studies indicate that nutrition
is more of a debilitating factor in cell-mediated and innate immunity than in humoral immunity [63]. Studies in animals suggest that malnutrition adversely affects the immune system [64,65]. In humans, providing vitamin A supplements to infants during immunization visits was correlated with an increase in antibodies to poliovirus serotype 1 but not other serotypes [66]. Studies of oral cholera vaccination given together with zinc supplementation have shown to increase immune responses, including complement levels and other innate immune functions. A seroprevalence survey in Morocco found that seroprevalence rate for types 1, 2, and 3 poliovirus was significantly lower among malnourished children [67].

7.2. Concurrent enterovirus infection

Concurrent infection with other enteric viruses has been investigated for the potential to interfere with OPV-induced immunity. Results have been mixed on the question of whether or not simultaneous enterovirus infection has an impact on OPV protection. Previous studies in Mexico and Bangladesh found that viral interference and acute diarrhea negatively affects OPV immunogenicity [68,69]. More research is needed in this area to determine the link between concurrent enteric infections and rates of seroconversion to monovalent or trivalent OPV [70,71].

7.3. Tropical enteropathy

Several intestinal pathologies, collectively referred to as tropical enteropathy (TE), have been observed in tropical countries. These abnormalities include villous atrophy, crypt hyperplasia, increased permeability, and inflammatory cell infiltrates [72]. The mucosal damage is thought to be due to enteric pathogens, including intestinal bacterial, parasitic, and viral agents. Although some infections will have a protective action because they induce immunity, they may also have a damaging effect on the gastrointestinal tract.

It has been proposed that tropical enteropathy may decrease the effectiveness of oral vaccines in infants by disrupting the mucosal immune system. If this is the case, then correction of underlying disorder or treatment of TE could improve vaccine efficacy.

For example, probiotics may restore normal microbial flora and have immune modulating effects, and provide a practical, low cost intervention for tropical developing country settings. One study has examined the effect of administering probiotics on IPV performance. Infants fed formula containing probiotics including *Bifidobacterium longum-infantis* and *Bifidobacterium breve* had higher poliovirus-specific stool IgA titers in a study by Mullié et al. [73]. However, not all bifidobacteria strains provided an immune boost, and other reasons for the higher IgA titers could not be ruled out. In another study, *B. breve* bacteria were shown to have an adjuvant effect on development and functional maturation of Peyer's patches in vitro [74].

Another possible approach to circumvent TE's negative effect on vaccine efficacy may include use of booster immunization with IPV in such patients who have exhibited vaccine failure to previous immunization with OPV.

7.4. Tolerance

The mucosal immune responses may also be pathologic and foster the induction of immunologically mediated diseases and autoimmunity. Such effects may be related to microbial colonization, dietary factors, antibiotics, living conditions, and failure to develop tolerance to dietary macromolecules. It now appears that the development of IgA and other antimicrobial mucosal responses as well as the induction of systemic hyporesponsiveness to dietary antigens (oral tolerance) in the neonatal period and early infancy are essential for maintenance of mucosal homeostasis and prevention of disease later in life [75].

The question arises as to whether repeated mucosal administration of OPV might diminish the induction of systemic cellular and humoral responses, thus diminishing the desired response to the vaccine. It is known that novel antigens, defined as antigens for which the individual has no preexisting immunity, can induce mucosal tolerance (defined as reduction in systemic T cell responses) in humans. However, non-novel antigens, defined as antigens for which individuals have preexisting immune responses, do not result in mucosal tolerance.

Although more research with vaccine antigens is needed, available studies suggest that oral vaccines such as OPV given to persons with preexisting mucosal or systemic immunity are not likely to diminish humoral responses to the vaccine antigen [76].

7.5. Maternal factors

7.5.1. Maternal antibodies

Newborns in developing countries tend to have higher levels of maternally derived antibodies than newborns in the industrialized world. Children with higher maternal antibody levels have lower seroconversion rates following poliovirus vaccination due to interference of maternal poliovirus-specific antibodies that neutralize the vaccine strain and interfere with the development of antibodies [77]. Studies in Puerto Rico and Cuba indicate that IPV is better at inducing seroconversion when given at 2 months, 4 months, and 6 months instead of 6 weeks, 10 weeks, and 14 weeks [78,79]. A later dose during the second year of life has been shown to effectively boost the immune response.

7.5.2. Breastfeeding

Studies have reported mixed results on the connection between breastfeeding and OPV immunogenicity. In one experiment, infants were given a special infant formula containing neutralizing antibodies. When the formula was given two hours prior to vaccination, the infants' OPV responses were diminished. However, formula ingested six or more hours prior to vaccination did not interfere with OPV responses, suggesting that avoiding breastfeeding prior to vaccination may improve vaccine response [80]. Another recent study found that withholding breastfeeding of infants for a short interval prior to and after immunization resulted in a more robust immune response to the oral cholera vaccine, Dukoral®, in Bangladeshi infants [81]. However, other studies in India found no association between breastfeeding and vaccine immunogenicity [82,83], and one study found that breastfeeding boosts the immune response to OPV [84]. On one hand, breast milk may interfere with OPV by neutralizing Sabin virus. However, on the other hand, breast feeding may help infants develop immune responses by stimulating the growth of gastrointestinal flora, including *B. breve* and *B. longum-infantis*, in the infant intestine, which then acts to stimulate GALT and by neutralizing acid in stomach.

7.5.3. Other factors

7.5.3.1. Interference of poliovirus vaccines with other vaccines. A few investigations have examined the impact of poliovirus vaccines on the outcome of concurrent immune responses to other microbial antigens. Simultaneous immunization with OPV and BCG in Guinea-Bissau has been shown to impair development of cell-mediated immunity to BCG. Such immunization resulted in reduced number and size of post BCG scars, reduced skin reactivity to PPD, and impaired synthesis of IFN-γ, IL10, and IL13 [85] while other study in Gambia showed that BCG enhanced antibody response to OPV at time of boosting [86]. Other research has found that OPV interferes with the antibody response to a rotavirus vaccine [87].
These studies have limitations (e.g., small sample size, confounding factors such as maternal infectious diseases, other vaccines received or nutritional status) and clinical significance of such interaction remains to be determined.

7.5.3.2. Timing of vaccination. Vaccination during the rainy season appears to be less effective than vaccination at other times of year. During the warm, wet season, wild-type poliovirus may be able to grow faster than the attenuated OPV viral strains and therefore shed into feces and be transmitted before OPV can induce sufficient mucosal immune responses. It is also possible that the reduced vaccine effectiveness is due to diarrhea or enterovirus infection, both of which are more common during the warm and rainy months.

7.5.3.3. Additional factors. Additional factors that may affect vaccine immunogenicity are inoculum size, exposure, and dose. In addition, the role of the environment such as force of infection must be factored in. For example, the circulating wild-type poliovirus load in poliovirus endemic settings may be so high that the virus can continue to replicate in the mucosa despite the presence of poliovirus-specific antibody.

Another potentially harmful effect on the developing immune system is heavy metal poisoning. Arsenic, lead and cadmium are emerging problems in Bangladesh and the surrounding region. The effect of long-term arsenic exposure on infectious disease and vaccine outcomes needs to be better understood [88].

8. Section 4: possible interventions to overcome focal OPV vaccine failure

Based on the information summarized in the preceding sections of this report, several intervention approaches need to be considered to overcome the OPV failures.

8.1. Reevaluation and modification of use of existing vaccines

One research need is to understand individual mucosal immune responses to vaccination series after specific numbers of doses. Challenge studies that use vaccine strains to simulate WPV exposure could be done following immunization with IPV, OPV, or a combination, to reveal the gut and serum antibody response upon challenge. Mathematical modeling of vaccine performance could help researchers understand where and how OPV fails to protect against infection. Variations in the time from last OPV dose and the amount of challenge virus could reveal optimal protection strategies. Another need is to explore the relative amounts of type 1 and type 3 WPV circulating in the affected communities [89]. More studies are needed to establish the role of IPV in boosting mucosal poliovirus-specific immune responses.

8.2. Novel vaccine preparations and adjuvants

The development of effective mucosal vaccines could be enhanced by innovations in delivery and adjuvant systems. Delivery systems could protect the vaccine from enzymatic degradation, assist the antigen to be taken up by epithelial cells, and target the antigen to antigen-presenting cells (APCs), while adjuvants could stimulate the immune system.

Food-based vaccine delivery systems such as MucoRice may not require cold-chain precautions, would be needle-free, and would offer stability for several years [90]. In other recently developed approaches, cholesterol-based nanoparticles could be used to deliver antigens to the mucosal surface.

Several mucosal adjuvants have shown promise in preclinical systems. These include detoxified cholera toxin, Toll-like receptor (TLR) ligands, IL-1 cytokine, retinoic acid, and other vitamin-based adjuvants. Among others, viral replicon particles (VRPs) were shown to induce mucosal responses after a non-mucosal delivery with different antigens [91].

Another avenue of future research could be the use of immunomodulators to re-direct traffic of dendritic and other immune cells to peripheral lymph nodes to boost production of antibodies on the mucosal surfaces.

8.3. Laboratory approaches to measure and evaluation mucosal immunity against poliovirus

Currently, there are major limitations in studying mucosal immunity against poliovirus, including the lack of a simple laboratory assay. In addition, there is no animal model to assess transmissibility. The gold standard for measuring mucosal immune response to poliovirus vaccine remains the OPV challenge approach, however, challenge study are labor-intensive, time consuming, require stool samples and the use of Sabin live poliovirus. While humoral immune response to poliovirus can be measured through the neutralization assay, these tests do not predict mucosal immunity, especially in IPV-vaccinated individuals. To address this issue, researchers from US CDC and the RIVM in the Netherlands have developed type-specific ELISA assays to measure IgM and IgA in serum. The results from these assays will be compared to OPV challenge studies to determine if they could represent a convenient surrogate for mucosal immunity.

Current methods of measuring intestinal antibody and cytokine responses involve directly analyzing gastrointestinal lavages and tissue biopsies, or measuring immune response indirectly via assays of gut-derived antibody-secreting cells migrating in blood using ELISPOT or antibodies produced ex vivo in short-term cultures peripheral blood mononuclear cells (PBMCs). Levels of these and other markers are not absolute correlates of protection but can provide an indication of which vaccine regimens are most efficacious at generating a mucosal immune response. Current needs include the development of surrogate measures to monitor the impact of multi-dose or boosting regimens as well as improved ways to measure B and T cell memory responses in young children.

9. Summary and conclusions

Current immunization efforts with OPV have resulted in a marked decline in paralytic poliomyelitis throughout the world, including India. Still, the GPEI faces challenges in achieving high population immunity in some tropical areas even after multiple OPV doses. Addressing this issue requires enhanced understanding of the mucosal and systemic responses to wild-type poliovirus and to poliovirus vaccines.

To date, significant progress has been made in the research to identify mechanism of poliovirus infection, including identification of the poliovirus receptor and the virus-host cell interaction, development of mucosal immunity after natural or vaccine-induced exposure to poliovirus. A series of epidemiological and clinical studies have also revealed possible host, vaccine and environmental factors behind the lack of mucosal immune response to the poliovirus vaccine.

In order to translate this progress in research into the actual improvements in vaccine efficacy, research needs to explore further the reasons why oral vaccines perform suboptimally in developing country settings, conduct further experimentation with optimal vaccine schedules and delivery strategies, focus on the development and evaluation of adjuvants, and make substantial improvements in methods for measuring mucosal immunity.
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