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Vaccines against enteric infections for the developing world

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Since the first licensure of the Sabin oral polio vaccine more than 50 years ago, only eight enteric vaccines have been licensed for four disease indications, and all are given orally. While mucosal vaccines offer programmatically attractive tools for facilitating vaccine deployment, their development remains hampered by several factors:

— limited knowledge regarding the properties of the gut immune system during early life;
— lack of mucosal adjuvants, limiting mucosal vaccine development to live-attenuated or killed whole virus and bacterial vaccines;
— lack of correlates/surrogates of mucosal immune protection; and
— limited knowledge of the factors contributing to oral vaccine underperformance in children from developing countries.

There are now reasons to believe that the development of safe and effective mucosal adjuvants and of programmatically sound intervention strategies could enhance the efficacy of current and next-generation enteric vaccines, especially in lesser developed countries which are often co-endemic for enteric infections and malnutrition. These vaccines must be safe and affordable for the world's poorest, confer long-term protection and herd immunity, and must be able to contain epidemics.

1. Introduction

Enteric infections cause more than a billion disease episodes per year worldwide and claim nearly two million lives each year, mainly in lesser-developed countries (LDCs) [1]. Most enteropathogens could be controlled by vaccines, including *Helicobacter pylori* which has a global prevalence of over 50% and may infect up to 80% of children below 3 years in some of the most impoverished countries [2]. The global burden of enteric infections is mainly weathered by children below the age of 5 years (figure 1). However, enteric infections continue to be important causes of morbidity in higher age groups, where cholera, typhoid fever and shigellosis remain important causes of deaths in LDCs. Currently, licensed oral vaccines for human use are limited to two viral (poliovirus and rotavirus) and two bacterial enteropathogens: *Salmonella* enteric serovar Typhi (*S.* Typhi) and *Vibrio cholerae*. Research efforts are currently intensifying to develop effective vaccines against other major enteric agents, in particular *Shigella*, enterotoxigenic *Escherichia coli* (ETEC), *S.* Paratyphi and noroviruses. Although parenteral immunization may provide protection against invasive mucosal pathogens, and under certain circumstances against colonization in individuals previously exposed mucosally, immunization by a mucosal route is arguably more effective against non-invasive pathogens, especially in young infants and in individuals who have not yet been exposed to the causative agent. Consistent with this notion, there are effective parenteral polio and typhoid vaccines available, in addition to the licensed oral vaccines against cholera, polio, rotavirus and typhoid. In comparison with injectable vaccines, mucosal vaccines are easier to administer and hence do not require trained healthcare personnel, are painless and with lesser risks of transmitting infections, and are in general simpler

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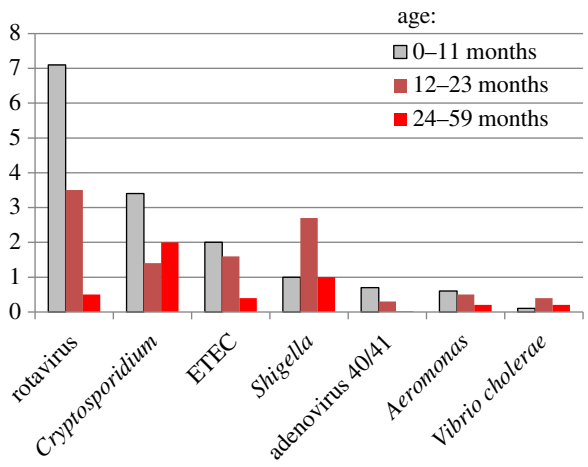

Figure 1. Incidence of enteric infections caused by different pathogens in children below 5 years of age (GEMS study data adapted from Kotloff *et al*. [3] show attributable pathogen-specific incidence per 100 child years). (Online version in colour.)

to manufacture; the latter aspect may facilitate vaccine production by manufacturers from LDCs and their global deployment. In the following, we summarize our views on the main mechanisms involved in immune protection against enteric infections and how this knowledge has guided and also benefited from the development of the currently licensed vaccines. We review the main characteristics of internationally licensed enteric vaccines and draw attention to their reduced efficacy in children from LDCs. Finally, we discuss possible underlying causes and briefly review programmatically pertinent intervention strategies to overcome this problem, including progress achieved in the field of mucosal adjuvants which could accelerate development of a broader range of enteric vaccines.

2. Overview of enteric infections and protective immune mechanisms

Natural infection by most enteric pathogens confers protection against disease caused by subsequent infection with the same pathogen. However, enteric pathogens differ in the way they cause infection and disease, which in turn determines the nature of ensuing immune responses and thus the mechanisms of immune protection involved (table 1). Enteric pathogens and corresponding oral vaccines activate a broad range of innate as well as adaptive immune mechanisms, which vary depending on the nature of the pathogen, including its invasiveness. The innate immune system provides the first line of defence and is activated through interaction between pattern recognition receptors, including Toll-like receptors and multiple yet generally conserved molecules (including toxins produced by pathogens), so-called pathogen-associated molecular patterns. Such interactions lead to production of antimicrobial defensins as well as chemokines and cytokines, which in turn can attract and activate antigen-presenting cells to initiate development of adaptive humoral and T cell-mediated effector immune responses. However, despite marked differences between the various types of enteric pathogens and infections, it may be generalized that protective vaccines should be formulated to: (A) prevent the infectious agent from (i) adhering to and colonizing the mucosal epithelium (non-invasive and invasive pathogens), (ii) translocating through and/or replicating within the gut epithelium and lamina propria (pseudo-invasive and invasive viruses and bacteria) or (iii) reaching the blood stream and spreading to remote tissues (invasive pathogens); and/or (B) neutralize the pharmacological activity or prevent binding of microbial toxins (mainly enterotoxins) to target cells. The main adaptive effector immune mechanisms for specific protection against enteric infections are (i) secretory IgA (SIgA) antibodies that can prevent colonization and local intraepithelial replication of intestinal pathogens and can also neutralize viruses and enterotoxins; (ii) systemic IgG antibodies that can neutralize (e.g. poliovirus) and directionally activate complement-mediated killing and opsonophagocytosis of bacteria (e.g. *S.* Typhi) and (iii) T-cell-mediated and antibody-dependent cytotoxic mechanisms to eliminate target host cells infected by intracellular bacteria (e.g. *S.* Typhi) and viruses (e.g. rotavirus).

(a) Secretory antibodies

SIgA accounts for two-thirds of all immunoglobulin produced in mammals and is produced by plasma cells in the intestinal lamina propria where it is selectively transported into the gut lumen by enterocytes via a unique receptor-mediated mechanism. SIgA provides the first line of defence against bacterial colonization, virus replication and enterotoxin binding to gut epithelial cells [4]. In addition, SIgA is endowed with anti-inflammatory properties [5]. Relatively small amounts of IgG are synthesized in the gastrointestinal tract of healthy individuals, but higher levels are observed in subjects with acute and chronic (inflammatory bowel diseases; coeliac disease) inflammatory conditions mainly through transudation from blood [4,6]. Given the fact that IgG and complement are sensitive to proteolytic degradation in the gut milieu, the protective role of intestinal IgG appears to be limited. Secretory IgM is also found in intestinal secretions at concentrations relatively lower than SIgA, but its concentration is increased in a large proportion of individuals with selective IgA deficiency in which it compensates for lack of SIgA. Low levels of IgE are also produced by gut plasma cells and are thought to protect against intestinal helminths.

(b) Cell-mediated immunity

Intestinal cell-mediated immune responses to enteric pathogens are primarily supported by intraepithelial and lamina propria T lymphocytes. The possible protective role of such responses is being actively explored, especially in patients convalescing from typhoid fever and in subjects immunized with live oral typhoid vaccines [7]. In contrast to intestinal antibody responses, such responses are sustained for years and may explain the long-lasting protection observed in subjects vaccinated with live oral Ty21a typhoid vaccine [8]. Th1 cells producing interferon-gamma and Th17 cells producing IL-17 are often found at high frequencies in the gut lamina propria [7]. Although believed to be major effector T cells against *H. pylori* and intracellular pathogens such as *S.* Typhi and *S.* Paratyphi, their role in immune protection induced by oral vaccines has yet to be established. A significant proportion of the intraepithelial $CD8^+$ T cells express TCR γδ. In humans, these cells account for nearly 10% of the intraepithelial lymphocyte pool. Human TCR γδ T cells recognize non-classical HLA class I molecules, which are upregulated by epithelial cells following adhesion of pathogens such as certain pathotypes of *E. coli* [9,10]. Their significance, if any, in immune protection

Table 1. Pathogenic mechanisms in enteric infections.

mechanism of infection and disease	examples
colonization of the intestinal mucosa without invasion or morphological damage and eliciting watery diarrhoea through the effects of secreted enterotoxins	*V. cholerae* enterotoxigenic *E. coli* (ETEC)
intimate attachment to the mucosa and induction of enterocyte effacement by 'injecting' bacterial proteins	enteropathogenic *E. coli* (EPEC)
induction of enterocyte effacement and release of exotoxins blocking cellular protein synthesis	enterohaemorrhagic *E. coli* (EHEC)
local invasion and intracellular multiplication, inflammation of the intestinal mucosa, and release of enterotoxin with potential effect on the enteric nervous system	rotavirus
local invasion, inflammation, and destruction of the intestinal mucosa	*Shigella* spp.
local invasion of the intestinal mucosa followed by drainage to mesenteric lymph nodes	non-typhoidal *Salmonella*
translocation of the intestinal mucosa into the bloodstream, systemic dissemination to distal organs	*S.* Typhi *S.* Paratyphi poliovirus

remains to be determined. Two main subsets of memory T cell have been described: central/memory T cells (TCMs) and effector memory T cells (TEMs) [11]. Human and mouse studies indicate that TCMs have high proliferative and reconstituting capacity, and are involved in recall responses in secondary lymphoid organs. TEMs are present primarily in peripheral tissues and are endowed with immediate effector function, but limited proliferative properties. Most TCM cells express the chemokine receptor CCR7 and CD45RO, whereas TEM cells express CD45RO in the absence of CCR7 and thus exhibit different migratory properties. The majority of memory T cells residing in the gut lamina propria are TEM cells, whereas TCM cells migrate and patrol between lymphoid organs and appear to be important in the generation of TEM effector cells. Finally, regulatory T cells (T_{reg}) may play a significant role in the gut by maintaining tolerance against environmental antigens and commensal flora as well as controlling inflammatory responses to infectious pathogens [12]. Among the numerous T_{reg} subsets identified, IL-10-secreting CD4 Tr1 cells have been shown to prevent chronic intestinal inflammation; TGF-β-secreting Th3 cells participate in tolerance to dietary antigens; and TGF-β- and IL-10-secreting $Foxp3^+$ $CD4^+$ T cells can prevent auto-reactive responses. The role of different T_{reg} cells in response to oral vaccination and in immune defence against enteric pathogens in humans remains largely unknown.

(c) Memory B cells

Long-lasting immunological memory, mainly through the generation of long-lived B memory (BM) cells in the germinal centres of intestinal Peyer's patches and intestinal lymphoid follicles, may explain how protection after oral vaccination can persist long after the acute IgA response in the intestine has waned. Antigen-specific BM cells have been described in humans after oral vaccination or infection with enteric pathogens, including rotavirus, *V. cholerae* O1, *Shigella* and *S.* Typhi [13,14]. The BM cells can rapidly turn into long-lived high-affinity IgA or IgG antibody-secreting plasma cells, which may then control the infection before it causes disease. For instance, after oral cholera vaccination, functional BM cells for antigen-specific IgA responses have been shown to last for more than 10 years after a primary vaccination [14]. Overall, studies on the development of innate and adaptive mucosal immune responses to mucosal vaccines remain limited to animal systems, and very few studies have been conducted in neonates and infants. Hence, the very limited knowledge regarding the functional characteristics of the evolving gut mucosal immune system poses serious issues regarding the impact of such studies on vaccine development in general and on rational identification of correlates of mucosal protection in particular.

3. Choice of vaccination route for protecting against enteric pathogens

It is likely that most mucosal infections could be controlled by use of effectively designed and administered mucosal vaccines, and, arguably, for many such pathogens a mucosal administration route will be required to induce a protective immune response, especially in subjects who have not previously been naturally exposed to the pathogen as is the case of newborns and young infants. Still, it is clear that there exist injectable vaccines of documented efficacy alongside mucosal vaccines against polio and typhoid. The factors explaining how parenteral vaccines can afford protection against selected mucosal pathogens relate mainly to the invasiveness of the pathogen, the site of infection and previous natural exposure of the host. Different mucosal tissues have different degrees of permeability for serum-derived antibodies. In contrast to the lower respiratory tract and the female genital tract, which are relatively permeable to serum IgG, the upper part of the small intestine is essentially non-permeable to blood proteins as long as it is not affected by inflammation. Accordingly, mucosal vaccination seems to be crucial for inducing protective immunity against non-invasive, non-inflammatory infections at mucosal surfaces that are normally impermeable to serum antibody transudation. Infections (in the small intestine) with *V. cholerae* or ETEC are examples of infections in which locally produced SIgA mediates vaccine-induced protection mainly, if not exclusively. However, as further discussed below, a parenteral vaccine

Table 2. Compartmentalization of the mucosal antibody response after different routes of immunization (adapted from [19]).

	immunization route					
expression site	nasal	sublingual	oral	rectal	vaginal	transcutaneous
upper respiratory	+++	+++	−	−	−	+++
stomach	−	+/+++[a]	+/+++[a]	−	−	?
small intestine	−	+++	+++	−	−	+
colon	−	?	+	++	+	+
rectum	−	?	(+)	+++	−	?
blood	+++	+++	+	++	+	+++

[a]Strong response was seen only in *H. pylori* infected individuals.

can also elicit a mucosal immune response in individuals who have been already primed through natural (mucosal) exposure to the pathogen; this may explain the modest protective effect achieved with the previous generation of injectable cholera vaccines in older individuals in cholera endemic countries. It may also form a basis for possible combined prime–boost routes of immunization currently discussed, as described later in this article, for e.g. polio vaccination. A parenteral route of vaccination may also be efficient against enteric infections in which the pathogen is first transported across the epithelial barrier (by specialized intestinal epithelial M cells) and then infects the basolateral side where it can readily be attacked by serum-derived antibodies. Bacillary dysentery caused by *Shigella* sp. is an example of such infections. Serum antibodies can also attack pathogens that cause disease by inducing inflammation in the submucosal tissue (e.g. *Shigella* sp., most *Salmonella* spp. and *Campylobacter jejuni*) and, even more effectively, when pathogens spread from the mucosa through the blood stream and infect remote tissues, such as is the case for *S.* Typhi bacteria or poliovirus. Previous mucosal exposure to the pathogen, leading to mucosal priming, is an important determinant of whether parenteral immunization can induce protection even when a parenteral route is ineffective in previously unexposed or 'naive' individuals. The long abandoned injectable whole-cell cholera vaccines never gave rise to very impressive or long-lasting immunity, yet induced modest protection for a few months in adults from cholera endemic settings [15]. Likewise, parenteral polio vaccination could protect not only against paralytic disease by means of serum neutralizing antibodies, but could also, although less efficiently than the oral polio vaccine, reduce faecal virus transmission in older children and adults from polio endemic settings, but not in younger children [16]. Similarly, the injectable cholera and polio vaccines were reported to elicit strong specific SIgA responses in breast milk of Pakistani women with serological evidence of having been naturally exposed to *V. cholerae* bacteria and poliovirus, whereas such responses were not seen in Swedish women [17,18]. It is tempting to speculate that the superior clinical efficacy of a novel parenteral *Shigella sonnei* protein–polysaccharide conjugate vaccine in adult Israeli soldiers when compared with young toddlers from the same area may also be related to differences in prior natural exposure to *S. sonnei*. These effects may reflect the ability of parenteral vaccines to recruit migrating antigen-experienced memory B cells and/or central memory T cells with gut homing properties to promote secondary intestinal SIgA immune responses.

4. Alternative routes of mucosal vaccine administration

Although, as discussed above, parenteral vaccination in some instances can provide protection against mucosal infections, in most cases and especially in naive subjects such as newborns and young infants, a mucosal route is needed for effective immunization. In the early days of mucosal immunology, it was assumed that immune responses initiated at one mucosal site could disseminate widely to multiple mucosal tissues. However, as recently reviewed [19], further work has shown that the mucosal humoral immune system exhibits a fair degree of anatomic compartmentalization related to the migratory patterns of antibody-producing plasma blasts generated at different mucosal sites. This imposes constraints on the choice of mucosal vaccine administration route. The selective localization of IgA-secreting B cells to specific tissue sites is governed by specific 'homing' molecules together with chemokine receptors on IgA-committed mucosal B cells with affinity for and interacting with tissue-specific 'addressins' and mucosal epithelial cell-derived chemokines that are differentially expressed at various mucosal 'effector' sites. Similar mechanisms also govern the migration of mucosal T cells to different mucosal compartments [4,20]. Therefore, the mucosal immune responses are highly compartmentalized, not only between separate mucosal organs but also between regions from the same mucosal organ such as the gut. In general, the strongest immune response is obtained at the site of vaccine application and in anatomically adjacent or evolutionary linked sites; an example of the latter is the intestine–mammary gland link whereby B cells activated in the gut can migrate to the mammary glands and produce breast-milk SIgA antibodies which allow mothers to protect their offspring against enteric pathogens. However, a few notable exceptions have been found that may allow for more practical and tissue-targeted vaccine administration than would otherwise be possible [19]. Traditional routes of mucosal immunization include the oral and nasal routes. As outlined in table 2, oral immunization elicits immune responses mainly in the upper digestive tract and specifically linked glandular systems, such as the salivary glands and the lactating mammary glands; systemic immune

Table 3. Internationally licensed vaccines for human use against enteric infections.

disease target	route	type	trade name	manufacturer
cholera	oral	inactivated *V. cholerae* bacteria + CTB toxoid (heat- or formalin-killed classical and El Tor 01 Inaba and Ogawa bacteria + rCTB)	Dukoral®	Crucell-Sweden
	oral	inactivated *V. cholerae* bacteria (Same 01 composition as above + formalin-killed 0139 bacteria)	Orc-Vax® Shanchol®	VaBiotech (Vietnam) Shanta Biotechniques (India)
	oral	live attenuated *V. cholerae* 01 bacteria (CVD-HgR, 108–109 cfu)	Orochol® or Mutachol®	Berna/Crucell (Switzerland)—not produced after 2004
typhoid fever	oral	live attenuated *S.* Typhi bacteria (Aro- *S.* Typhi Ty21a)	Vivotif®	Crucell (Switzerland)
	parenteral	purified Vi polysaccharide	Typhim Vi® Typhrix®	Sanofi Pasteur (France) GlaxoSmithKline (Belgium)
rotavirus	oral	live attenuated, mono-valent rotavirus (RIX4414 human rotavirus strain, specificity G1P[8] derived from a human rotavirus isolate)	Rotarix®	GlaxoSmithKline (Belgium)
	oral	live attenuated, pentavalent rotaviruses (5 reassorted human-bovine strains expressing G1, G2, G3, G4 and P1[8])	RotaTeq®	Merck (USA)
polio	oral	live-attenuated, trivalent polio viruses (Sabin strain type 1, type 2 and type 3)	Orimune® OPV OPV OPV	Wyeth-Lederle (USA) Novartis (Italy) BIBCOL (India) BioFarma (Indonesia) etc.
	Oral	live attenuated, bivalent polio viruses (Sabin strain, type 1 and type 2)	poliomyelitis vaccine, type 1 and type 3	Sanofi Pasteur (France)
	Oral	Live attenuated, monovalent polio viruses (Sabin strain, type 1)	poliomyelitis vaccine, type 1	Sanofi Pasteur Novartis GlaxoSmithKline
	parenteral	inactivated, trivalent polio viruses (formalin-inactivated type 1, type 2 and type 3 wild-type strain derivatives) same grown in GMK cells	Imovax Polio® (viruses grown in Vero cells) Ipol® (viruses grown in GMK cells)	Sanofi Sanofi Netherlands Vaccine Institute (NL); Staten Serum Institute (DE) etc

responses after oral immunization are usually modest. Nasal administration induces mucosal immune responses mainly in the respiratory and reproductive tract mucosae and is often efficient for inducing systemic antibody responses, but does not efficiently induce intestinal immune responses in humans [19]. Other, yet less explored, routes for inducing intestinal immunity include the rectal, sublingual and transcutaneous immunization routes. Rectal vaccination can elicit strong immune responses in the large intestine, but not in the stomach, small intestine or other remote mucosal tissues. Sublingual immunization is a rather new approach for inducing mucosal and systemic T cell and antibody responses with an exceptionally broad dissemination to different mucosae, including the gastrointestinal and respiratory tracts and the genital mucosa, and can also give rise to strong systemic immune responses in mice (table 3). For instance, sublingual vaccination evokes protective gut IgA and T cell responses against *H. pylori* infection in mice [19]. Sublingual administration of an adjuvanted *Shigella* common protein-based subunit vaccine also induced intestinal IgA responses in mice, and a systemic booster [21] further expanded these responses. Similarly, small doses of adjuvanted inactivated polio vaccine formulated in a thermoresponsive gel were able to evoke systemic and intestinal antibody responses in mice [22]. Clinical studies are in progress to determine to what extent these promising results will translate to humans. Transcutaneous vaccination, based on the application onto the skin of adhesive patches containing antigens and adjuvants (preferentially cholera toxin or *E. coli* heat-labile enterotoxin, LT), has shown great promise in animal studies for eliciting specific

cellular and humoral immune responses both systemically and, more surprisingly, in mucosal tissues. When tested in humans, transcutaneous administration of *E. coli* heat-labile toxin, a potent immunogen and adjuvant, was found to induce faecal IgA antibodies [23]. The ability of transcutaneous immunization to elicit intestinal antibody responses is intriguing and may relate to previous exposure of the vaccines to LT (which is ubiquitous), which would have primed the gut mucosa for subsequent responses to skin-applied vaccine.

5. Licensed vaccines against enteric infections

As mentioned earlier, effective vaccines are now available against three of the most important gastrointestinal pathogens—*V. cholerae*, *S.* Typhi and rotavirus—and the properties and impact of these vaccines as well as that of vaccines against polio (table 3) will be discussed below. Notably, however, vaccines are still lacking against other major enteric pathogens, including the two most important causes of bacterial enteric infections in children, i.e. ETEC and *Shigella*, and against norovirus, another major enteropathogen. Similarly, no vaccine is yet in sight against *H. pylori*, which causes the overall highest numbers of both infected and clinically ill individuals globally. This review will primarily focus on the current internationally licensed enteric vaccines and on the relative performance of these vaccines in LDCs when compared with industrialized countries.

6. Oral cholera vaccines

Almost half of all laboratory confirmed bacterial diarrhoeas are the result of enterotoxin-producing bacteria. Among these, *V. cholerae* causes the most severe disease, including epidemic outbreaks with case fatality rates up to 50%. The current seventh cholera pandemic, which started in the early 1960s, now involves almost the entire developing world. It represents an important global challenge with more than three million cases and 100 000 deaths annually. Serogroup O1 *V. cholerae* causes more than 98% of cases worldwide, whereas up to 5% of cholera cases in parts of southeast Asia is caused by serogroup O139 [15]. The O1 serogroup, which is further divided into Inaba and Ogawa serotypes, comprises two biotypes—'classical' which is assumed to have caused the six previous cholera pandemics, and 'El Tor' which is currently the prevailing biotype; a recent study showed that this organism has spread across the world in three distinct waves during the current pandemic [24]. Notably, a new variant ('hybrid') strain of O1 El Tor, which first emerged during the mid-1990s and produces the classical biotype toxin, appears to cause a more severe illness than the previously encountered O1 El Tor and O139 strains [25] and this 'hybrid' variant has rapidly become the major cause of cholera globally. Immune protection against cholera is mediated by locally produced antitoxic and antibacterial SIgA in the gut. These protective antibodies are directed against cholera B subunit (CTB), i.e. the cell-binding part of cholera toxin, and the cell wall O1 oligosaccharide antigen moiety of LPS, respectively, and the antitoxic and antibacterial antibodies cooperate synergistically in their protective function [15]. Previously used injectable cholera vaccines, which did not induce significant gut mucosal immune responses, afforded poor protection and have long since been abandoned. As discussed above, they could, however, induce some stimulation of mucosal immunity and protection in naturally primed older individuals from cholera endemic areas. Instead, a new generation of oral cholera vaccines with strong ability to stimulate intestinal IgA responses and with proven efficacy and effectiveness has become available [26]. Importantly, while the acute mucosal IgA response is also of relatively short duration, less than 1 year, mucosal immunological memory induced by clinical infection or oral vaccination lasts for many years. The most widely used of these vaccines (Dukoral®) consists of recombinantly produced CTB and inactivated *V. cholerae* O1 whole cells covering different serotypes and biotypes (table 3). This vaccine, which was developed in Sweden by the authors and their associates, is given orally in a bicarbonate buffer in two doses one to two weeks apart (in children below age 5 years, three oral doses are recommended). The vaccine has proven to be safe and effective in large phase III and phase IV clinical trials involving more than 150 000 children and adults in Bangladesh, Peru and Mozambique (table 3), conferring 80–90% protection against cholera for the first year after vaccination and 100% protection for the first six months in young children (age 2–5 years) [15]. Through its B subunit component, the Dukoral vaccine also has been found to afford significant (more than 50%) cross-protection for approximately six months against ETEC-producing LT, a cholera toxin analogue, both in children living in developing countries and among travellers to such countries. Initially supported through technology transfer from Sweden, a second oral killed whole-cell cholera vaccine has been produced, tested and licensed, first for local use in Vietnam and more recently also in India and other countries [26]. The vaccine, which is almost identical to Dukoral but lacks the CTB component, is given without a buffer. A recently completed phase 3 placebo-controlled trial in 67 000 children and adults in India found the vaccine to be 66% efficacious during 3 years of follow-up, including a high level of protection for 2 years in children below 5 (table 3) [27]. Based on these findings, the vaccine was licensed in India under the trade name Shanchol® and recently received WHO prequalification allowing its purchase by international agencies. Protective efficacies of the Dukoral and Shanchol oral cholera vaccines in two pivotal large field trials are illustrated in figure 2. New inactivated oral cholera vaccines are also under development, e.g. a formalin-inactivated single strain *V. cholerae* O1 vaccine genetically engineered to stably express both the

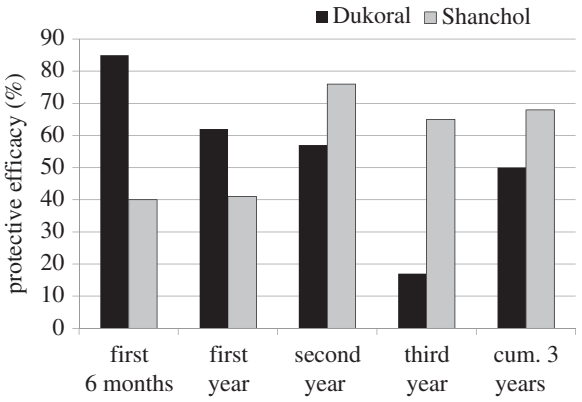

Figure 2. Summary of protective efficacies of Dukoral and Shanchol in large field trials in Bangladesh 1985–1988 (Dukoral) and India 2006–2009 (Shanchol). Data adapted from [27,28] (PEs for Dukoral are based on whole population assuming same PE in adult males as in women).

Table 4. Protective efficacy of different typhoid vaccines tested in various age groups (adapted from [37–44]).

vaccine	age group (years)	vaccine doses (*n*)	protective efficacy (%)	length of follow up
Ty21a, live oral, liquid	5–21	3	74	3 years
	6–19		77	3 years
			78	5 years
Ty 21a, enteric-coated capsules	5–21	3	47	3 years
	6–19		67	3 years
			62	7 years
Vi polysaccharide, parenteral	5–15	1	55	3 years
	5–44	1	72	17 months
	>2	1	61	2 years
Vi-rEPA-conjugate, parenteral	2–5	2	89	46 months

Ogawa and Inaba serotype O1 LPS antigens on the cell surface has been constructed [29]. It is known that convalescents from clinical cholera disease are significantly protected against new disease episodes for several years. Recurrent episodes are usually restricted to youngest children and, for unknown reasons, a first episode with the Inaba serotype was found to provide more long-lasting immunity also against the Ogawa serotype than vice versa [15]. Based on this, efforts have for a long time been made to develop a live oral cholera vaccine with the hope that this could enable effective vaccination through a single-dose regimen. The first of such oral single dose vaccines, CVD 103 HgR (Orochol® or Mutachol®), consisting of a genetically manipulated classical *V. cholerae* O1 Inaba strain deleted of the cholera toxin gene, was licensed for use in travellers but failed to protect in a large phase 3 trial in Indonesia, and its production was suspended in 2004 [30]. Several other live oral cholera vaccines are at earlier stages of development, with the most advanced candidate, Peru-15 oral live vaccine, based on a toxin-deleted, non-motile El Tor Inaba strain, undergoing phase 2 clinical testing (reviewed in [26]). An important finding with available killed oral cholera vaccines is that they provide substantial indirect protection, so-called herd immunity, to unvaccinated persons in the community. Such herd protection most likely results from reduced transmission of cholera by vaccinated subjects, and overall efficacy—direct efficacy and herd protection—can be quite substantial (more than 80%) depending on vaccination coverage [31]. Estimates from mathematical modelling studies indicate that with vaccination coverage of 50%, the combined effect resulting from direct protection and herd protection in a community could exceed 90% protection against cholera over several years [32]. Thus, effective control of disease can be expected from use of the available licensed killed oral cholera vaccines in cholera endemic settings. WHO now recommends the use of oral cholera vaccines together with other preventive strategies in cholera endemic areas, and in epidemic situations where conditions are favourable for vaccine deployment. In this respect, the effectiveness of reactive use of oral cholera vaccine was documented during a recent outbreak of cholera in Vietnam, providing 76% reduction of cholera in this setting [33]. The usefulness of cholera vaccines in epidemic situations has recently gained further support from mathematical modelling studies on the recent cholera outbreaks in Haiti and Zimbabwe. These studies support the build-up of a global cholera vaccine stockpile to ensure timely supply of vaccine in emergency situations.

7. Typhoid fever vaccines

Typhoid fever remains a major cause of morbidity and mortality with an estimated incidence of 22 million cases and 200 000 deaths per year, mainly in LDCs [34]. *S.* Typhi causes disease after penetrating the mucosa of both the small and large intestine, primarily by invading and replicating in the M cells of the Peyer's patches of the lower small intestine, followed by uptake by underlying macrophages in which the bacteria proliferate. After rupture of these cells, the bacteria can spread to adjacent epithelial cells and reach lymphoid tissue in the lamina propria, and then enter the bloodstream to disseminate the infection to the liver, spleen and lymph nodes. School-aged children are the primary target group for infection and thus also for preventive vaccination, but typhoid infection has been found to be an important cause of disease also in younger children [35]. Protective immunity can be mediated by SIgA preventing uptake of the pathogen across the intestinal barrier, and/or by serum antibodies preventing systemic spread of the organism. T-cell-mediated immunity may play a role, especially during recovery from disease [36]. Two types of safe and effective vaccines are available internationally, one being an oral live-attenuated vaccine inducing mucosal immunity and the other a parenteral vaccine consisting of purified Vi capsular polysaccharide inducing protective serum IgG antibodies against a major surface antigen on the bacterium after invasion (table 3). The live-attenuated oral vaccine, Vivotif®, developed in the early 1970s, requires cold storage and at least three doses for optimal protection, and is supplied as gelatin capsules containing lyophilized Ty21a, a mutant strain of *Salmonella enterica* serovar Typhi (*S.* Typhi). This vaccine is approved for use in children above 5 years of age [36]. The vaccine is well tolerated and has provided 50–70% protection, depending on formulation, during 3 years of follow-up in school children and adults in several vaccine trials (table 4). Studies in Chile have shown that Ty21a vaccine may even provide protection for up to 7 years after vaccination [36]. The extent to which protection by Vivotif is mediated by local mucosal immunity in the gut or by systemic immunity, and the relative roles of antibodies and cell-mediated immune mechanisms remain to be established amid strong B- and T-cell responses observed in peripheral blood after vaccination with this oral vaccine [37]. Because typhoid fever also affects younger children, there is a need

to develop a paediatric formulation of Vivotif for use in infants and toddlers. Studies are ongoing in Bangladesh to evaluate the safety and immunogenicity of a liquid formulation of this vaccine in children aged 2–5 years. The second licensed typhoid vaccine is an injectable formulation consisting of Vi capsular polysaccharide purified from a *S. enterica* serovar Typhi strain. Of note, Vi is not expressed by *S.* Typhi Ty21a nor by *S.* Paratyphi B, explaining why the vaccine fails to cross-protect against paratyphoid B. The Vi vaccine is given intramuscularly in a single dose, and is approved for use in children over 2 years of age. Vi polysaccharide vaccine is well tolerated and, when tested in typhoid endemic countries, has afforded *ca* 70% protection against typhoid fever during the first 12–18 months and 55% protection over a 3-year study period (reviewed in [38]; table 4). The Vi capsular vaccine is believed to protect by way of generating serum bactericidal IgM, preventing the spread of the pathogen from the gut into the circulation; *S.* Typhi bacteria are highly sensitive to both complement-mediated killing and opsono-phagocytosis directed by specific antibodies. Corresponding serological assays have been developed and provide correlates of protection to support vaccine licensure. Both injectable and oral typhoid vaccines can provide herd protection when vaccine coverage is adequate [38–40]. Because Vi polysaccharide alone, as for other carbohydrate vaccines, is not effective in inducing T-cell-dependent memory responses in young infants, a conjugate vaccine composed of Vi capsular polysaccharide covalently linked to a mutant from *Pseudomonas aeruginosa* exoprotein A (Vi-rEPA) was developed [41]. This conjugate vaccine was shown to be highly protective (nearly 90%) in children 2–5 years, conferring long-lasting (more than 3 years) immunity [42]. A follow-up study conducted in Vietnamese infants receiving three doses at 2, 4 and 6 months of age showed that the Vi-rEPA vaccine induced substantial serum IgG anti-Vi responses [43]. Recently, an alternative Vi-conjugate vaccine based on conjugation of Vi polysaccharide from *Citrobacter freundii* to CRM197, a mutated non-toxic diphtheria toxin carrier protein, was shown to be safe in adult volunteers and to induce serum antibody responses to Vi antigen comparable to those induced by a 20-fold higher dose of monovalent Vi polysaccharide vaccine [44]. However, in spite of the excellent safety and efficacy profile of the Vi-rEPA vaccine in young children, only one Vi conjugate vaccine has been licensed and in India only. Despite the global burden of typhoid fever, the availability of two types of affordable licensed vaccines, and evidence that use of these vaccines would be cost-effective, very few countries have as yet included typhoid vaccination in their national immunization programmes.

8. Rotavirus vaccines

Rotavirus is the most important cause of diarrhoeal mortality in infants and children below 2 years. It has recently been estimated that 450 000 children die from rotavirus diarrhoea each year, and another two million are hospitalized owing to rotavirus disease [45]. Rotavirus has been reported to account for nearly 40% of all cases of hospitalized gastroenteritis [46]. Although the incidence of rotavirus disease is similar in developed and developing countries, more than 85% of deaths occur in Africa and Asia [46,47]. Two oral live-attenuated rotavirus vaccines, Rotarix® and RotaTeq® [48,49], have been introduced in more than 150 countries and incorporated into national children immunization programmes in a large number of countries. These vaccines were preceded by a quadrivalent oral vaccine (RotaShield), which was based on a Rhesus monkey rotavirus strain equipped with human rotavirus genes. However, this vaccine after having been licensed for a short time was withdrawn from the market owing to rare but serious cases of intussusception. Despite the clinical importance and the development of effective rotavirus vaccines, knowledge of the pathophysiological as well the protective immune mechanisms in rotavirus infection remains limited. Most of the mortality from rotavirus infection results from excessive loss of fluids and electrolytes. Diarrhoea may be caused by several mechanisms, including malabsorption secondary to destruction of enterocytes, villus ischaemia, intestinal hypersecretion stimulated by the rotavirus non-structural enterotoxin protein NSP4, and activation of vagal afferent sensory neurons which transmit information to the hindbrain, leading to nausea and vomiting [50]. With regard to protective immunity, studies in humans and animals have reported correlations between rotavirus antibody levels and protection, the most consistent of which has been with rotavirus-specific serum IgA antibodies. IgA in neonatal blood appears to be of both maternal and fetal origin [51]. This observation suggests that rotavirus-specific IgA found in the serum of vaccinated infants represents a spillover of mucosal/intestinal (polymeric) IgA and that measurement of virus-specific polymeric IgA antibodies should provide a more accurate and discriminative surrogate marker of vaccine efficacy. Cellular immunity comprising both $CD8^+$ and $CD4^+$ T cells also appears to have a role in protection induced by prior rotavirus infection in mice [52]. Most rotavirus strains belong to one of five serotypes, i.e. G1–G4 and G9. However, there is a diversity of rotavirus G and P types across continents, and their distribution differs by country and year. Levels of reinfections with rotavirus are high, in particular in locations with a high viral diversity. Development of live rotavirus vaccines has been highly influenced by views regarding the importance of serotype-specific antibodies. Development of several candidate vaccines, including the widely licensed RotaTeq vaccine, is based on the concept that neutralizing antibodies are the primary determinant of protection. These vaccines are composed of multiple rotavirus strains representing the major human rotavirus serotypes. The other group of vaccines has been developed based on the theory that protection is not solely dependent on neutralizing antibody. These candidates are composed of single rotavirus strains as in the case of Rotarix. RotaTeq is a pentavalent vaccine containing five human–bovine reassortant rotaviruses, four of which consist of the WC3 bovine strain expressing either of the outer capsid proteins VP7 of the G1, G2, G3 and G4 human rotavirus serotypes or the attachment protein VP4 (type P7) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein VP4 (type P1A) from the human rotavirus parent strain and the outer capsid protein VP7 (serotype G6) from the bovine rotavirus parent strain. The vaccine is given as three oral doses in a buffer at one-month intervals, usually starting at 6 weeks of age. As shown in several phase III studies, the vaccine has provided a very high degree of protection in the USA, but has been less efficient in developing countries (table 5). Rotarix is a

Table 5. Protective efficacies (PEs) of internationally licensed rotavirus vaccines (adapted from [48,49,53,54]).

vaccine	test countries	PE (%) year 1	PE (%) year 2	overall 2-year PE (%)
Rotateq	USA + Finland	98	88	93
	Bangladesh	45.7	39.3	42.7
	Vietnam	72.7	64.6	63.9
	Kenya	83.4	−54.0	64
	Ghana	65.0	29.4	55.5
	Mali	1	19.2	17.6
	Nicaragua	58	n.t.	
Rotarix	Europe	96	86	90
	Latin America + Finland	84.7	79	81
	South Africa	72.2	n.t.	
	Malawi	49.2	n.t.	
	El Salvador	76.0	n.t.	

live-attenuated human rotavirus vaccine containing the RIX4414 strain of G1P [9] specificity and was developed from a parent clinical isolate (strain 89-12). The vaccine is administered in two oral doses at least one month apart to children aged 6–24 weeks. More than 30 clinical trials have been undertaken with Rotarix. Results of phase III studies have shown that Rotarix offers sustained high protection (80–90% during 2 years) against severe rotavirus disease for each of the five most prevalent rotavirus types in Europe and Latin America but, similar to Rotateq, induced substantially lower protection in Africa (table 5). In most efficacy studies, protection induced by Rotarix or RotaTeq has been assessed against severe rotavirus disease requiring hospitalization, but in some clinical trials the protective effect has also been determined against disease of any severity; in the latter type of studies, protection has been more pronounced against severe forms of disease. The levels of protection induced by either the monovalent or the pentavalent vaccine are surprisingly similar when tested in comparable socioeconomic settings, and suggest that protective immune responses are directed to conserved rather than type-specific antigens. Both RotaTeq and Rotarix have shown good safety profiles and have been associated with a low risk of intussusception, far outweighed by their efficacy [55]. The two vaccines have induced comparable frequencies of seroconversion (serum IgA responses) in a majority of vaccinees. There are only few studies reporting on human mucosal antibody responses against rotavirus vaccines, a problem that may relate to difficulties in collecting suitable samples from newborns and young infants. Very recently, an Indian vaccine, Rotavac®, based on a naturally reassorted rotavirus strain isolated from an asymptomatic Indian neonate in 1986, was developed. This strain has the VP4 of bovine rotavirus but all other segments of human rotavirus origin, and showed exceptional promise, because infants infected with this strain manifested strong immunity against subsequent infections caused by other strains. Following a phase 3 study showing comparable safety and efficacy to Rotateq and Rotarix in India, the vaccine has been recommended for introduction by the Government of India and launched in the private market in India in 2015 at a substantially lower price than the two internationally licensed vaccines. Other live oral rotavirus vaccines are undergoing advanced clinical development and include a human neonatal P[6]G3 strain, RV3, developed in Australia, and a human bovine reassortant vaccine developed by the US National Institutes of Health. As mentioned, the available licensed rotavirus vaccines have afforded excellent protection against severe rotavirus disease with substantial herd effects [56–58] in industrialized and middle income countries, but have been less effective in low-income developing countries [53,54] where rotavirus vaccines are most needed. It remains to be determined to which extent herd protection induced by rotavirus vaccination may increase the overall effectiveness of these oral vaccines in LDCs if vaccine coverage is sufficiently high.

9. Polio vaccines

During the past decades, the incidence of paralytic poliomyelitis has markedly (by more than 99% since 1988) declined worldwide, and India, which was formerly a stronghold of paralytic polio, was formally declared a polio-free country in 2014 [59]. Type 2 wild poliovirus was eradicated in 1999, and cases of type 3 poliovirus are down to their lowest-ever numbers. Although WHO 25 years ago resolved to eradicate poliomyelitis, three countries (Afghanistan, Nigeria and Pakistan) remain polio-endemic (down from more than 125 in 1988) and outbreaks are feared in civil war areas (Ukraine, Syria), owing to lower vaccine coverage associated with civil insecurity, weakened health systems and poor sanitation. However, failure to eradicate polio from these last remaining strongholds may result in as many as 200 000 new cases every year worldwide, within 10 years [59]. The failure to eradicate polio may partly be ascribed to the fact that the predominantly used oral live poliovirus vaccines (OPV) carry the risk of shedding viruses that have reverted to virulence, so-called circulating vaccine-derived poliovirus (cVDPV), and inducing vaccine-derived paralytic poliomyelitis [60]. Because of these risks, most industrialized countries have now replaced the use of OPV with inactivated injectable polio vaccine (IPV) in their vaccination programmes. As the global eradication of polio is hopefully nearing, concerns have been raised in most developing countries about the continued use of OPV, and how to financially and logistically make it possible in these

countries to replace OPV with safe but significantly more expensive IPV. Additional efforts are also deployed to develop poliovirus with stable attenuation properties for improving the safety of future generation oral polio vaccines.

(a) Oral polio vaccine

OPV, developed by Albert Sabin in the early 1960s, is the first widely used oral–mucosal vaccine. OPV consists of a mixture of live-attenuated poliovirus strains of each of the three serotypes, selected by their ability to mimic the immune response following infection with wild polioviruses, but with a significantly reduced incidence of spreading to the central nervous system. Three or four doses of OPV are required during the first year of life to generate adequate levels of seroprotection (serum-neutralizing antibodies), but frequent boosters are required to maintain a sufficient level of mucosal protection in children and adolescents from polio endemic regions. In addition to its enormous impact in reducing polio worldwide, this vaccine has also served as a useful tool for elucidating fundamental aspects of mucosal immunity in humans [61]. Like IPV, OPV induces antibodies in the blood that will protect against paralytic disease by preventing the spread of poliovirus to the nervous system. But, superior to IPV, at least in newborns and young infants, OPV also stimulates SIgA immune response in the intestinal and nasopharyngeal mucosae, the primary tissues for poliovirus entry and replication. The intestinal immune response against OPV can halt person-to-person transmission of wild poliovirus, making mass campaigns with OPV a powerful strategy for the global eradication of polio. Most OPVs are trivalent and contain three attenuated poliovirus strains representing the three different serotypes. Because type 2 poliovirus has been eliminated for more than 15 years, bivalent OPVs comprising type 1 and 3 poliovirus are being used in several countries. Furthermore, such bivalent vaccines are more effective than trivalent OPV and nearly as effective as the monovalent type 1 (mOPV1) and type 3 mOPV3) vaccine formulations, especially in youngest children being immunized for the first time. Thus, mOPV1 (or mOPV3) provides a much stronger immunity to type 1 (or type 3) poliovirus and vaccinated children excrete less viruses and for a shorter period of time compared with trivalent OPV. Vaccination is recommended at birth, followed by two to three doses at least four weeks apart and yearly or bi-annual booster immunizations are also recommended during childhood in polio-risk areas. Still, OPV remains the preferred polio vaccine in most of the world because of its ease of use in mass immunization campaigns, low production costs and potential to quickly halt transmission. Despite the tremendous impact of OPV vaccines in global polio eradication, an increasing number of polio-free countries are using IPV, because the risk of paralytic polio associated with routine use of OPV is deemed greater than the risk of importing wild virus. However, as IPV does not induce intestinal immunity and hence does not stop transmission of the virus in young infants who received this vaccine for the first time, OPV is used wherever a polio outbreak occurs. Once polio has been eradicated, use of the oral polio vaccine will need to be stopped to prevent re-establishment of transmission owing to vaccine-derived polioviruses. Switching to IPV is one option for this post-OPV era.

(b) Injectable polio vaccine

The inactivated trivalent polio vaccine that was initially developed by Jonas Salk during the early 1950s has until recently been used for polio eradication in comparatively few countries. IPV has been shown to prevent poliovirus outbreaks in multiple different settings. There is also some evidence of IPV-induced herd protection, which is achieved by reducing the risk of contact with infected individuals; such herd protection may increase chances of polio eradication even if vaccine coverage is not 100%. The experience of using IPV is extensive. Thus, more than 100 clinical trials have been undertaken with immune responses evaluated in more than 100 000 subjects. These studies have revealed that three injections are superior to two and that primary immunizations should ideally not be started before 2 months of age to avoid interference with transplacentally acquired maternal antibodies. The studies have also indicated that a prolonged interval between two consecutive doses of vaccine is favourable and that an initial series of vaccinations during the initial six months of life followed by a booster immunization between 12 and 18 months provides seroprotection for at least 7 years. Both the strong immunogenicity and excellent safety profile of IPV support that it could replace OPV and be given together with other EPI (WHO Expanded Program on Immunization) vaccines. The higher production and logistical delivery (injection devices and trained healthcare persons) costs have also to be considered when planning future polio eradication strategies based on IPV. Attempts to reduce some of these costs have been successful, indicating that the IPV could be given with comparable safety and immunogenicity intradermally using a fivefold lower dose than the regular subcutaneous dose [62]. Importantly, two recent studies demonstrate that a single IPV dose given in Indian children after three or more OPV doses enhanced not only seroprotection compared with a single OPV booster but also intestinal immunity as indicated by reduced and shortened faecal excretion of vaccine-derived poliovirus following challenge with bivalent OPV [63,64]. Such protection correlated with enhanced circulating mucosal antibody-secreting cell responses to type 3 poliovirus and these responses were markedly superior to the responses seen after an OPV boost (Dey *et al.*, unpublished data). Taken together, these results demonstrate that IPV can efficiently boost waned intestinal mucosal immunity in subjects previously vaccinated with OPV, and provide a rational basis for WHO recommendation of using a supplemental dose of IPV in OPV-using countries to reduce the risk of epidemic transmission of poliovirus in these countries [65].

10. Underperformance of oral live vaccines in developing countries

Many oral vaccines, primarily live ones, have shown reduced immunogenicity and efficacy when used in developing compared with industrialized countries. Reduced immunogenicity and performance of OPV in developing countries is well recognized and identified as a significant obstacle for the eradication of polio by vaccination [65,66], and the experience with OPV has been found to extend to other oral live vaccines, both viral and bacterial ones. For instance, as discussed above, the oral live rotavirus vaccines, whether pentavalent or monovalent, have also been found to have substantially reduced immunogenicity and protective efficacy when tested in low-income developing countries. The licensed oral live cholera vaccine (Orochol) and several other live oral cholera vaccines, which had provided good levels of protection against challenge in vaccinated US volunteers, failed to be sufficiently immunogenic or to protect South Asian vaccinees. Similarly, the

reasons for the comparatively poor protective efficacies of Rotarix and RotaTeq in low-income developing countries are still unclear. The reasons for the reduced immunogenicity and efficacy of oral live vaccines in developing country settings are not well understood [67]. It appears that nutrition-related factors—including both protein-calorie and micronutrient deficiencies, alterations of the gut microbiota, interferences on vaccine take by maternal breast milk and/or placental antibodies, inflammation owing to concurrent infections including intestinal parasitic infections and maternal undernutrition during pregnancy—may be the main factors or cofactors leading to lower immunogenicity and efficacy of oral vaccines in resource-poor countries. In this regard, chronic environmental enteropathy (CEE) or 'tropical enteropathy'—a reversible but rather ill-defined subclinical inflammatory condition of the gut associated with villous blunting and impaired intestinal barrier function—occurs in people living under poor sanitation and hygiene, and is suspected to be a leading cause of oral vaccine failure [68]. CEE is thought to result from overt exposure to enteropathogens and could be implicated in growth faltering and impaired child development. Undernourished children living under extreme poverty are especially vulnerable to this condition. Two major clinical studies of CEE—the Mal-ED study of Malnutrition and Enteric Diseases and the PROVIDE study on the impact of CEE on oral poliovirus and rotavirus vaccine failure in children from developing countries—are attempting to define biomarkers of CEE and to evaluate more precisely the impact of CEE on immune responsiveness to enteric vaccines. Interim results from the PROVIDE study, which are presented elsewhere in this issue [69] indicate that CEE is associated with delayed maturation of the gut microbiota, alterations of intestinal barrier function, upregulation of pro-inflammatory cytokines and chemokines, and hyporesponsiveness to oral polio and rotavirus vaccines. Thus, intervention strategies to improve vaccine performance in these children may include the use of antibiotics, anti-inflammatory agents, probiotics or drugs that modulate intestinal permeability. Because maternal nutritional status is a determinant of child development, interventions aimed at improving the nutritional status of the mother could also be effective preventing measures. Other practical measures may be needed in order to maximize the benefits of oral vaccines for all children. Oral vaccines, when given to children in developing countries, may require being administered at higher doses and/or additional booster doses; macro- and micro-nutrient (zinc; vitamins (A and D) supplementation; withdrawal of breast milk before vaccine administration to avoid the inhibitory effect of maternally derived breast milk antibodies on vaccine take; deworming medications; antibiotics; and other programmatically acceptable measures, to realize their full benefit [70]. Several such strategies have been tested in developing country settings using the internationally licensed oral killed cholera vaccine (Dukoral) with initial promising results. These options may be of even greater importance for use with various live oral vaccines, such as rotavirus vaccines and OPV.

11. Conclusion and perspectives

For many years, mucosal immunity and mucosal vaccines have attracted less than their due share of research and development, especially when considering that most infections take place at a mucosal surface or have a mucosal portal of entry and often can only be prevented by vaccination by a mucosal route. Mucosally administered vaccines are in general also easier to administer, carry less risk of transmitting infections and may allow simplified manufacturing compared with injectable vaccines. The latter aspect increases the potential for local vaccine production in low- and middle-income countries, which may in turn facilitate both availability and affordability of newer generations of vaccines for use in low- and middle-income countries. It is also notable that the mucosal immune system develops earlier in life than systemic immune responsiveness, suggesting a comparative advantage for use of mucosal vaccination in infants. In recent years, methodological advances allowing more intense study of both the humoral and cellular arms of mucosal immune responses have led to a growing interest to better define the specific features of mucosal when compared with systemic immune responses, as well as to develop mucosal vaccines for preventing globally important infections. Methods for improved monitoring of mucosal immune responses in humans, including infants and young children, have been developed, primarily for measuring IgA responses. Still, however, practical assays for assessing mucosal T-cell responses in clinical and field settings are scarce, as are methods for predicting the efficacy of candidate mucosal vaccines in humans. Although a few effective oral–mucosal vaccines for human use are available, it is increasingly recognized that the development of a broader range of mucosal vaccines for prevention of infectious diseases, especially subunit vaccines based on purified antigens, will require access to antigen delivery systems that can help present the relevant protective antigens efficiently to the mucosal immune system as well as effective adjuvants to promote and direct the mucosal immune response towards the desired effect. Significant advances have recently been made in the development of improved mucosal vaccine delivery systems, and promising novel mucosal adjuvants are undergoing clinical testing [12]. As of today, all vaccines but one (oral polio vaccine) recommended by the EPI are administered by injections. Live oral rotavirus vaccines are also included in many countries' EPI schedules. As discussed in the foregoing, parenteral vaccines induce immunity in blood and in peripheral tissues but are relatively inefficient for eliciting immune responses in the gastrointestinal tract, especially in young infants. On the other hand, mucosal administration of a number of experimental as well as few licensed enteric vaccines has been shown to be more efficient for inducing intestinal immunity in animals and in humans. Modern biotechnology has yielded an abundance of vaccine candidates against enteric infections, but few vaccines have been registered for human use. If these candidates are to reach those in need in developing countries, then several lessons from field research done with currently licensed oral vaccines tested in these settings must be considered. These include the need to develop vaccines that avoid storage in a cold chain and can be administered without needles or support from trained heath care workers, and the need to formulate innovative intervention strategies to improve the performance of these vaccines in underprivileged children. Ultimately, these vaccines must reach the world's poorest, confer long-term protection and herd immunity, and must be able to contain epidemics following complex emergencies.

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