Vaccines against mucosal infections
Jan Holmgren and Ann-Mari Svennerholm

There remains a great need to develop vaccines against many of the pathogens that infect mucosal tissues or have a mucosal port of entry. Parenteral vaccination may protect in some instances, but usually a mucosal vaccination route is necessary. Mucosal vaccines also have logistic advantages over injectable vaccines by being easier to administer, having less risk of transmitting infections and potentially being easier to manufacture. Still, however, only relatively few vaccines for human use are available: oral vaccines against cholera, typhoid, polio, and rotavirus, and a nasal vaccine against influenza. For polio, typhoid and influenza, in which the pathogens reach the blood stream, there is also an injectable vaccine alternative. A problem with available oral live vaccines is their reduced immunogenicity when used in developing countries; for instance, the efficacy of rotavirus vaccines correlates closely with the national per capita income. Research is needed to define the impact of factors such as malnutrition, aberrant intestinal microflora, concomitant infections, and preexisting immunity as well as of host genetic factors on the immunogenicity of these vaccines.

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Introduction
There remains a great need to develop vaccines against many of the pathogens that infect or take their departure from a mucosal tissue (Table 1). In some cases, such as poliovirus and Salmonella typhi, the pathogens cause disease only after spreading to non-mucosal tissues, but more often they exert their pathogenic effects locally on the mucosal tissue. Collectively, these infections have a serious negative impact on global health by causing more than 3 billion disease episodes and 3 million deaths each year [1]. They also represent a tremendous challenge for vaccine development. Although parenteral immunization in some cases can protect against mucosal pathogens, in most cases a mucosal vaccination route is necessary. In comparison to injectable vaccines, mucosal vaccines would also in general be easier to administer, have less risk of transmitting infections and may also allow simplified manufacturing. Still, however, only a handful of mucosal vaccines for human use are available (Table 2) as compared to more than 30 injectable vaccines.

This review summarizes the properties of currently licensed mucosal vaccines, and when there also exists an injectable vaccine for the same infection compares the mucosal and parenteral alternatives.

Determinants for choice of vaccination route
Mucosal pathogens differ in the way they cause infection and disease. Depending on the pathogen, a vaccine may need to be administered topically or may protect also after parenteral administration. Injectable vaccines of documented efficacy are available alongside with mucosal vaccines against polio, typhoid, and influenza (Table 2).

How parenteral vaccines may protect against selected mucosal infections
The main factors explaining how parenteral vaccination can protect against some but not most mucosal pathogens relate to the site of infection, the invasiveness of the pathogen, and previous natural exposure.

Differential mucosal permeability
Mucosal tissues differ in their permeability for serum-derived antibodies. The lower respiratory tract and the female genital tract are relatively permeable, in contrast to the small intestine that is essentially impermeable to blood proteins unless it is affected by inflammation. Consistent with this, injectable pneumococcal vaccines can in addition to protecting against blood-borne infection, via transudation of serum anti-capsular antibodies also provide some protection against lung pneumonia. By contrast, cholera and enterotoxigenic Escherichia coli (ETEC) infection are examples of non-inflammatory small intestine infections, in which vaccine protection is mediated by locally produced secretory IgA (SIgA) antibodies and normally requires oral-mucosal administration.

Invasion and inflammation
Parenteral vaccination may also work against those enteric pathogens, such as Shigella bacteria, that are translocated across the epithelium through Peyer’s patch ‘M’ cells to infect enterocytes from the basolateral side, where they come in contact with serum-derived antibodies. Likewise,
serum antibodies can effectively attack pathogens that cause disease after inducing inflammation in the submucosal lymphoid tissues (most *Salmonella* spp. and *Campylobacter jejuni*) or, even better, after entering the blood stream, as for *S. typhi* or polio virus.

**Mucosal immunological priming**

Previous exposure to the pathogen, leading to mucosal priming, is another determinant. Although the old injectable whole-cell cholera vaccines never gave rise to impressive or long-lasting immunity, they could induce

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### Table 1

**Examples of important bacterial and viral infections primarily affecting or entering through a mucosal surface**

<table>
<thead>
<tr>
<th>Respiratory tract</th>
<th>Gastrointestinal tract</th>
<th>Urinary tract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumonia</em>, <em>Mycoplasma pneumonia</em>, <em>Mycobacterium tuberculosis</em>, influenza virus, respiratory syncytial virus (RSV), rhinoviruses</td>
<td><em>Helicobacter pylori</em>, <em>Viubio cholerae</em>, Enterotoxigenic <em>Escherichia coli</em> (ETEC), Enteroaggregative <em>E. coli</em> (EAEC), Enteropathogenic <em>E. coli</em> (EPEC), <em>Campylobacter jejuni</em>, <em>Salmonella</em> spp., <em>Shigella</em> spp., <em>Clostridium difficile</em>, <em>Cryptosporidium</em>, rotavirus, calici viruses, polioviruses</td>
<td><em>Escherichia coli</em> (UPEC), <em>Chlamydia trachomatis</em>, <em>Neisseria gonorrhoeae</em>, <em>Herpes simplex</em> virus (HSV), human papilloma viruses (HPV), human immunodeficiency virus (HIV)</td>
</tr>
</tbody>
</table>

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### Table 2

**Internationally licensed vaccines for human use against mucosal infections**

<table>
<thead>
<tr>
<th>Vaccines against</th>
<th>Administration route</th>
<th>Type</th>
<th>Trade name</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholera</strong></td>
<td>Oral</td>
<td>Inactivated <em>V. cholerae</em> bacteria + CTB toxoid (Ca 10^{11} heat-killed or formalin-killed classical and El Tor O1 Inaba and Ogawa bacteria + 1 mg rCTB)</td>
<td>Dukoral™</td>
<td>Crucell-Sweden</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>Inactivated <em>V. cholerae</em> bacteria (Same O1 composition as above+ 5 × 10^{10} formalin-killed O139 bacteria)</td>
<td>ORC-Vax™</td>
<td>VaBiotech (Vietnam)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>Live attenuated <em>V. cholerae</em> O1 bacteria (CVD-HgR, 10^8–10^9 cfu)</td>
<td>Orochol™ or Mutachol™</td>
<td>Shanta Biotechniques (India)</td>
</tr>
<tr>
<td><strong>Typhoid</strong></td>
<td>Oral</td>
<td>Live attenuated <em>S. typhi</em> bacteria (Aro + S. <em>typhi</em> Ty21a)</td>
<td>Vivotif™</td>
<td>Crucell (Switzerland)</td>
</tr>
<tr>
<td></td>
<td>Parenteral</td>
<td>Purified Vi polysaccharide</td>
<td>Typhim Vi™ Typhrix™</td>
<td>Sanofi Pasteur (France)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>Live attenuated, mono-valent rotavirus [RIX4414 human rotavirus strain, specificity G1P [8]] derived from a human rotavirus isolate</td>
<td>Rotarix™</td>
<td>GlaxoSmith Kline (Belgium)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>Live attenuated, penta-valent rotavirus (5 reassorted human-bovine strains expressing G1, G2, G3, G4 and P1 [8])</td>
<td>RotaTeq™</td>
<td>Merck (USA)</td>
</tr>
<tr>
<td><strong>Influenza</strong></td>
<td>Intranasal</td>
<td>Live attenuated, trivalent influenza viruses (cold-adapted A and B viruses adjusted to WHO-identified seasonal needs, e.g. 2009 A[H1N1], A[H3N2] and B, ca 10^6 of each)</td>
<td>FluMist™</td>
<td>MedImmune</td>
</tr>
<tr>
<td></td>
<td>Intranasal</td>
<td>Similar to above</td>
<td>Nasovac™</td>
<td>Inst Exp Med., Russia</td>
</tr>
<tr>
<td><strong>Polio</strong></td>
<td>Oral</td>
<td>Live attenuated, trivalent polio viruses (Sabin strain type 1, type 2 and type 3; ca 10^6 CCID_{50} of each per 0.1 ml dose)</td>
<td>Orlimune™ OPV</td>
<td>Wyeth-Lederle (USA)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>Live attenuated, bivalent polioviruses (Sabin strain, type 1 and type 2, ca 10^6 CCID_{50} of each per 0.1 ml dose)</td>
<td>OPV</td>
<td>Novartis (Italy)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>Live attenuated, monovalent polioviruses (Sabin strain, type 1, at least 10^6 CCID_{50} per 0.1 ml dose)</td>
<td>Poliomyelitis vaccine, Type1 &amp; Type3</td>
<td>BIBC60L (India)</td>
</tr>
<tr>
<td></td>
<td>Parenteral</td>
<td>Inactivated, trivalent polioviruses (formalin-inactivated type 1, type 2 and type 3 wild-type strain derivatives) Same grown in GMK cells</td>
<td>Simpon Polio™ VeroPol</td>
<td>Sanofi Pasteur (France)</td>
</tr>
<tr>
<td></td>
<td>Parenteral</td>
<td>Dukoral</td>
<td>Dukoral</td>
<td>Crucell-Sweden</td>
</tr>
<tr>
<td></td>
<td>Parenteral</td>
<td>Inactivated, trivalent polioviruses (formalin-inactivated type 1, type 2 and type 3 wild-type strain derivatives) Same grown in GMK cells</td>
<td>Imovax Polio™</td>
<td>Sanofi Pasteur-MSD State Serum Institute (DE)</td>
</tr>
<tr>
<td></td>
<td>Parenteral</td>
<td>Dukoral</td>
<td>Dukoral</td>
<td>Crucell-Sweden</td>
</tr>
</tbody>
</table>

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modest protection for a few months in the vaccinated elderly population in cholera endemic settings [2]. Likewise, parenteral polio vaccination protects not only against paralytic disease but may also reduce fecal transmission in polio endemic settings [3]. These effects are explained by the ability of these vaccines to stimulate protective SIgA antibody responses in the gut of individuals previously primed via natural mucosal exposure [4,5].

**Choice of mucosal vaccination route for site-directed immune responses**

It was initially thought that immune responses induced at one mucosal site would be disseminated widely to other mucosal tissues. However, later work has shown that the mucosal immune system has a high degree of anatomic compartmentalization related to the migratory properties of lymphocytes activated at different mucosal sites (reviewed in [6]). This imposes distinct constraints on the choice of mucosal vaccine administration route.

In general, the strongest immune response is obtained at the site of vaccine application and in anatomically adjacent mucosae. Thus, oral vaccination induces immune responses mainly in the upper parts of the intestines, nasal immunization in the upper respiratory tract, rectal immunization in the rectum and lower colon, and vaginal immunization in the genital tract. However, a few notable exceptions have been found that may allow for more practical vaccine administration than would otherwise be possible: for instance, nasal and sublingual immunization can induce immune responses in the genital tract [6]. There are also examples of anatomically distinct but evolutionary linked mucosal tissues, the best known of which is the hormonally influenced gut–mammary gland link in lactating women ensuring that the breast-feeding baby gets epidemiologically ‘updated’ breast-milk SIgA antibodies.

**Licensed vaccines against enteric infections**

Efﬁcacious vaccines are available against three of the most important gastrointestinal pathogens: *V. cholerae*, *S. typhi* and rotavirus. Notably, however, vaccines are still lacking against all other enteric pathogens, including ETEC and *Shigella* that are the two most important causes of bacterial enteric infections in children.

**Oral cholera vaccines**

Almost half of all diarrheas are caused by enterotoxin-producing bacteria. Among these, *V. cholerae* (globally in >98% being of serogroup O1, biotype El Tor) causes the most severe disease and epidemic outbreaks with case fatality rates up to 50%. The 7th cholera pandemic, which started in the early 1960s, now involves almost the entire developing world reﬂecting the spread in three distinct waves of molecularly distinguishable *V. cholerae* from the Bay of Bengal [7]. Immune protection is mediated by locally produced antitoxic and antibacterial SIgA antibodies in the gut, directed against the cell-binding subunit of cholera toxin (CTB), and the cell-wall lipopolysaccharide O antigen, respectively; these antibodies have a synergistic protective effect [2]. The old injectable whole-cell cholera vaccines were abandoned in the 1970s owing to poor efﬁcacy and acceptability. Instead a new generation of oral cholera vaccines (OCVs) with much better capacity to stimulate intestinal SIgA antibody responses have become available and proved to be safe and effective [2].

The as yet only internationally licensed OCV (Dukoral®) consists of recombinantly produced CTB and inactivated *V. cholerae* O1 whole cells of different serotypes (Inaba and Ogawa) and biotypes (El Tor and classical). It is given orally in a bicarbonate buffer in two doses 1–2 weeks apart (in children below age 5 years three oral doses are recommended). The vaccine is safe and stable and has in large phase 3 efﬁcacy and phase 4 effectiveness trials in Bangladesh, Peru and Mozambique (see Table 3) conferred 80–90% vaccine-speciﬁc protection in the ﬁrst year after vaccination (100% protection for the ﬁrst 6 months in children below age 5 years) and 60% protection over three years of follow-up (assuming similar protection in adult males as found in women). Through its CTB component, the vaccine also affords 50–70% cross-protection against LT-producing ETEC for ca 6 months [8–10].

Recently, a second killed OCV has been licensed [11]. This vaccine (ORC-Vax/Shanchol) has the same *V. cholerae* O1 whole-cell composition as Dukoral but lacks the CTB component; instead it additionally contains formalin-killed O139 bacteria. The vaccine is given in two oral doses two weeks apart without a buffer. A recent phase 3 placebo-controlled trial in Kolkata, India found the vaccine to be 66% efﬁcacious over 3 years of follow-up, including good protection for the ﬁrst 2 years also in 1–5 year old children (Table 3) [12]. Both in the Kolkata trial using Shanchol and the Mozambique trial using Dukoral, the high levels of vaccine-speciﬁc protection found were against a new type of apparently more virulent [13] O1 El Tor strains that through gene acquisition produce classical biotype cholera toxin.

Much effort has also been made to develop a live-attenuated OCV. One such vaccine, CVD 103-HgR (Orochol®/Mutachol®), consisting of a genetically manipulated classical *V. cholerae* O1 Inaba strain containing a deletion in the gene encoding cholera toxin, was licensed in several countries for use in travelers. This vaccine, which is administered orally as a single dose in a buffer, was safe, immunogenic and efficacious against experimental cholera challenge when tested in North-American volunteers, yet failed to show protection in a large phase 3 efﬁcacy trial in Indonesia (Table 3) [14]. Production of this vaccine has been suspended since
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2004. Several other live OCVs are in earlier stages of development with the most advanced candidates being in phase 2 clinical testing, for example, the Peru-15 vaccine, which is a toxin-gene deleted, non-motile O1 El Tor Inaba strain (reviewed in [11]).

Recent findings show that OCVs in addition to their direct vaccine-specific protection provide substantial indirect ‘herd’ protection to unvaccinated persons in the community. This results from reduced transmission of cholera in vaccinated populations, is proportional to the rate of vaccination in the community and may approach 80% in settings with high coverage [15]. Mathematical modeling studies indicate that already with a vaccination coverage of 50%, the combined effect of direct and indirect protection would result in a >90% reduction of cholera over several years [16].

WHO now recommends the use of OCVs together with other control strategies in both cholera endemic areas and in selected epidemic situations [17]. The usefulness of OCVs in cholera outbreaks is supported by mathematical modeling studies of recent epidemics. Chao et al. [18] reported that use of OCVs alongside with other interventions in the 2010 cholera outbreak in Haiti would probably have further reduced morbidity and mortality. Likewise, Mukandavire et al. [19] after analyzing the recent severe cholera epidemic in Zimbabwe found that mass vaccination against cholera deployed strategically in Zimbabwe and surrounding regions could prevent future cholera epidemics and eventually eliminate cholera from the region. A study directly testing the effectiveness of reactive use of OCV in a recent outbreak of cholera in Hanoi, Vietnam also found the vaccine intervention effective, providing 76% reduction of cholera [20].

Typhoid fever vaccines

Infection with S. typhi causes 22 million disease episodes and 200 000 deaths per year in Asia, Africa, and Latin America [21]. The organism penetrates the mucosa of both the small and large intestines, and is taken up and multiplies in submucosal macrophages. After rupture of these cells, the bacteria can spread to the blood for further septic dissemination to liver, spleen and lymph nodes. School-aged children is the primary target group [22], but S. typhi infection is also common in younger children [23, 24].

Protective immunity is mediated by mucosal IgA antibodies preventing uptake of the pathogen across the intestinal barrier, and by serum IgG antibodies preventing further spread of the organism; in addition, cell-mediated immunity helps recovery from infection. Consistent with this, two types of safe and effective vaccines are available, an oral live-attenuated vaccine inducing mucosal immunity and a parenteral vaccine inducing protective serum IgG antibodies that can attack bacteria

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results of efficacy and effectiveness trials of licensed oral cholera vaccines (modified from [67])</td>
</tr>
<tr>
<td>Study location (year; [ref])</td>
</tr>
<tr>
<td>Dukoral: Inactivated V. cholerae O1 bacteria + cholera toxin B subunit</td>
</tr>
<tr>
<td>Matlab, Bangladesh (1985–89 [68])</td>
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<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Peru (1994 [69])</td>
</tr>
<tr>
<td>Beira, Mozambique (2003–2004 [70])</td>
</tr>
<tr>
<td>Orochol/Mutachol: Live attenuated V. cholerae O1 (CVD103-HgR) bacteria</td>
</tr>
<tr>
<td>North Jakarta, Indonesia (1993–97 [14])</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Shanchol/Orc-Vax: Inactivated V. cholerae O1 and O139 bacteria</td>
</tr>
<tr>
<td>Kolkata, India (2006–09 [12])</td>
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</tr>
</tbody>
</table>

* Randomized placebo-controlled double-blinded study.
** ITT: Intention-to-treat comprising either one or two-doses recipients; other values are as Per-Protocol.
after invasion. Protective efficacies of these vaccines are surprisingly similar, ca 50–70% for several years (Table 4).

The live attenuated oral vaccine, Ty21a/Vivotif®, was developed already in the 1970s by chemical mutagenesis of a pathogenic S. typhi strain. The current vaccine, which is marketed as enteric-coated capsules containing lyophilized Ty21a organisms, is approved for use in adults and children above 5 years of age, and is given in three doses every other day [22]. It is well tolerated and has, depending on formulation, provided between 47% and 78% protection during 3 years of follow-up and even in one study [25] for up to 7 years after vaccination (Table 4).

The Ty21a vaccine is temperature sensitive requiring a strict cold chain. A modified freeze-drying process that keeps the vaccine stable at 25 °C for up to 12 weeks was recently described [26]. Further, a liquid formulation is now also being tested for use in children who are too young to swallow a capsule; a liquid formulation as was used in early trials may also be more immunogenic than the encapsulated form (Table 4).

The second licensed vaccine against typhoid consists of Vi capsular polysaccharide purified from S. typhi. This vaccine is given intramuscularly in a single dose, and is approved for use in adults and children over two years of age. Vi polysaccharide vaccine is well tolerated and has when tested in high-endemic countries afforded 70% protection against typhoid fever for the first 12–18 months and 55% protection over a 3-year period (reviewed in [22], Table 4). A locally produced Chinese Vi vaccine has afforded similar (70%) protection [27]. The Vi vaccine protects by generating serum antibodies attacking the bacteria after they have entered into the circulation; S. typhi bacteria are highly sensitive to both complement-assisted killing and opsonophagocytic effects via Vi-specific antibodies.

Both types of typhoid vaccines may afford indirect herd protection when vaccine coverage is adequate. This was noted in a recent cluster-randomized effectiveness trial of Vi polysaccharide vaccine in Kolkata, India [28]: a 44% reduction in typhoid among unvaccinated members of the Vi vaccinated clusters was found that significantly contributed to the 61% overall vaccine protection achieved.

Vi polysaccharide has also been conjugated to recombinant mutant Pseudomonas aeruginosa exoprotein (Vi-rEPA) for greater immunogenicity particularly in younger age groups [29]. When tested in children 2–5 years of age, the conjugate vaccine afforded 89% protection during 4 years of follow-up [22,30]. It also gave rise to presumably protective levels of serum anti-Vi antibody when administered to Vietnamese infants in three doses at 2, 4 and 6 months of age [31]. Recently, an alternative Vi-conjugate vaccine (Vi-CRM197) based on conjugation of Vi polysaccharide from Citrobacter to a mutated nontoxic diphtheria toxin carrier protein has also shown excellent immunogenicity when tested in adults [32]; studies are in progress to evaluate this vaccine in small children in South-East Asia.

Rotavirus vaccines

Rotavirus is the most important cause of diarrheal mortality in children below 2 years of age. It is estimated that 453 000 children die from rotavirus diarrhea each year [33] and another two million are hospitalized [34]. Rotavirus may account for 40% of all hospitalized gastroenteritis cases globally [35,36]; disease rates are surprisingly similar in industrialized and developing countries but >85% of deaths occur in Africa and Asia [37].

Two oral attenuated rotavirus vaccines, Rotarix® and RotaTeq®, have recently been introduced in more than 150 countries. These vaccines were preceded by a quadrivalent oral vaccine (RotaShield) based on a Rhesus monkey rotavirus strain equipped with human rotavirus genes [38]. However, this vaccine was soon withdrawn from the market after being implicated to cause intussusception (intestinal invagination) [39].

Knowledge of the mechanisms of disease and immunity in rotavirus infection remains limited. Most of the

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Table 4

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Age group (years)</th>
<th>Number of vaccine doses</th>
<th>Protective efficacy</th>
<th>Length of follow up</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ty21a, Live oral, liquid</td>
<td>5-21</td>
<td>3</td>
<td>74%</td>
<td>3 years</td>
<td>Reviewed in [22,25]</td>
</tr>
<tr>
<td></td>
<td>6-19</td>
<td>3</td>
<td>77%</td>
<td>3 years</td>
<td></td>
</tr>
<tr>
<td>Ty21a, Live-oral Enteric-coated capsules</td>
<td>5-21</td>
<td>3</td>
<td>47%</td>
<td>3 years</td>
<td>Reviewed in [22,25]</td>
</tr>
<tr>
<td></td>
<td>6-19</td>
<td>3</td>
<td>67%</td>
<td>3 years</td>
<td></td>
</tr>
<tr>
<td>Vi polysaccharide parenteral</td>
<td>5-15</td>
<td>1</td>
<td>55%</td>
<td>3 years</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>5-44</td>
<td>1</td>
<td>72%</td>
<td>17 months</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>□2</td>
<td>1</td>
<td>61%</td>
<td>2 years</td>
<td>[28]</td>
</tr>
<tr>
<td>Vi-rEPA-conjugate parenteral</td>
<td>2-5</td>
<td>2</td>
<td>89%</td>
<td>46 months</td>
<td>[73]</td>
</tr>
</tbody>
</table>
mortality results from loss of fluids and electrolytes through vomiting and diarrhea. The infection primarily engages mature intestinal enterocytes leading to cell death. Diarrhea may be caused by malabsorption secondary to the destruction of enterocytes, villus ischemia, intestinal secretion induced by rotavirus enterotoxin (NSP4 protein), and/or through activation of the enteric nervous system by infection of enterochromaffin cells by rotavirus. The latter mechanism may also be the cause of nausea and vomiting [40].

With regard to protective immunity, studies in humans and animals have reported correlations between protection and rotavirus specific IgA antibodies. Cellular immunity also appears to have a role, although the primary role of CD4 T cells may be as helper cells for promoting antibody production and that of CD8 T cells mainly to facilitate the resolution of infection [41].

Most human rotavirus strains belong to one of five serotypes (G1–G4 and G9). The serotype distribution varies by country and year [36] and reinfections are common [42]. RotaTeq and similar vaccine candidates are based on the concept that serotype-specific neutralizing antibody is the primary determinant of protection; hence they contain multiple rotavirus strains representing the major serotypes. The other group of vaccines including Rotarix instead was developed on the assumption that protection does not depend solely on neutralizing antibody; these vaccines are therefore composed of single rotavirus strains [41].

The RotaTeq vaccine contains five human-bovine reassortant rotaviruses, each consisting of the WC3 bovine strain that through reassortment expresses the outer capsid proteins G1, G2, G3, and G4 defining the main human rotavirus serotypes and the attachment protein P1(8). The vaccine is given as 3 oral doses in a buffer at one-month intervals, usually starting at 6 weeks of age. Several Phase III studies have shown that the vaccine provides a very high degree of protection in USA and other affluent countries but has been less efficient in poor countries in the developing world (Table 5).

Rotarix is a live attenuated human rotavirus vaccine containing the RIX4414 strain of G1P(8) specificity. It 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 [43] showing that Rotarix offers sustained high protection (80–90% during two years) against severe rotavirus disease irrespective of serotype in Europe and Latin America but, similar to Rotateq, much lower protection in studies in Africa (Table 5).

In most studies, protection induced by Rotarix or RotaTeq has been assessed against severe rotavirus disease requiring hospitalization, but in some trials the protective effect has also been determined against disease of different severity clearly showing more pronounced protection against severe than against milder forms of disease [35,44]. Both vaccines have shown very good safety with no suggestion of causing intussusception [35,45]. As mentioned, they have afforded excellent protection against severe rotavirus disease in industrialized and middle income countries, but have been less effective when tested in poor populations in the developing world (Table 5). In fact, we find (Figure 1) a strong correlation between the protective efficacy of rotavirus vaccines and the gross national domestic product per capita as tested in different countries. The reasons for this are still unclear but may be due to a multitude of different factors as further discussed below. Since many of these factors may relate to intestinal problems interfering with live oral vaccines [56,63], it has been suggested that a parenteral vaccine could be useful to circumvent the intestinal barrier [46].

### Influenza vaccines

Influenza causes up to 1 billion infections, 3–5 million severe cases, and 300 000–500 000 deaths each year [47]. The highest mortality has been associated with epidemics

| Protective efficacies (PE) of internationally licensed rotavirus vaccines in different countries |
|---|---|---|---|---|---|
| Vaccine | Test countries | PE (%) Year 1 | PE (%) Year2 | Overall 2-year PE (%) | Reference |
| RotaTeq | USA + Finland | 98 | 88 | 93 | [45] |
|  | Bangladesh | 45.7 | 39.3 | 42.7 | [74] |
|  | Vietnam | 72.7 | 64.6 | 63.9 | [74] |
|  | Kenya | 83.4 | 54.0 | 64 | [44] |
|  | Ghana | 65.0 | 29.4 | 55.5 | [44] |
|  | Mali | 1 | 19.2 | 17.6 | [44] |
|  | Nicaragua | 58 | n.t. | | [75] |
| Rotarix | Europe | 96 | 86 | 90 | [76] |
|  | Latin America + Finland | 84.7 | 79 | 81 | [35] |
|  | South Africa | 72.2 | n.t. | | [77] |
|  | Malawi | 49.2 | n.t. | | [77] |
|  | El Salvador | 76.0 | n.t. | | [78] |
caused by influenza virus A (H3N2), but influenza A and influenza B virus epidemics are also common. Therefore, current influenza vaccines are trivalent formulations designed to protect against these three viruses [48].

Common influenza symptoms such as fever, headaches, and fatigue result mainly from proinflammatory cytokines and chemokines produced by influenza-infected cells. In addition, influenza virus infection causes significant tissue damage. The viral hemagglutinin (HA) protein determines which species and where in the respiratory tract an influenza virus strain can infect. Strains that are easily transmitted between people have HA proteins that bind to receptors in the upper part of the respiratory tract. By contrast, the highly lethal H5N1 strain binds to receptors that are mostly found in the lungs. This difference may explain why the H5N1 strain causes severe viral pneumonia in the lungs, but is not easily transmitted by people coughing [49].

The main protective mechanism of parenteral influenza vaccination is the induction of serum antibodies, mainly IgG, which prevent systemic spread of the pathogen and may also through transudation exert a local protective effect at the mucosal surfaces of the lower respiratory tract. The efficacy of injectable influenza vaccines correlates directly to the IgG anti-HA antibody levels after vaccination. In general, HA antibody titers of ≥1:40 provide protection (reviewed in [48]).

Recently, a live influenza vaccine (FluMist) delivered by intranasal spray and comprising cold-adapted, temperature-sensitive attenuated virus reassortant strains (that are being adjusted to the antigenic needs for the actual influenza season) was licensed in USA and other countries [50]. This vaccine induces an immune response that more closely resembles natural immunity than the response elicited by the injectable vaccines. Locally produced SIgA antibodies to virus surface HA and neuraminidase are important for protection of the upper respiratory tract and corresponding serum IgG antibodies for protection of the lower respiratory tract and against viremia. Cell-mediated immunity, mainly against virus matrix and nucleoprotein antigens, facilitates clearance of virus and recovery from illness. The nasal vaccine induces higher local IgA antibodies in nasal washings and local cell-mediated immunity but lower serum antibody titers than the injectable vaccines. Despite these differences in immune responses, the two types of vaccine have similar efficacy, 70–90% in healthy individuals when there is a good antigenic match between vaccine and epidemic virus (reviewed in [51]).

It should also be recalled that live cold-adapted attenuated influenza vaccine given through a nasal spray has been used routinely in Russia since more than 50 years. Current vaccine contains A(H1N1), A(H3N2) and B vaccine strains being annually renewed in accordance to WHO recommendations. The vaccine has proved to be safe, immunogenic and to afford 30–60% protection depending upon the degree of fit between the epidemic and the vaccine strains [52]. Protection has correlated with both serum HA inhibition titers and even more pronounced with the levels of IgA antibodies in nasal swabs and saliva.

**Polio vaccines**

During the past decades, the incidence of paralytic poliomyelitis has declined worldwide. However, although WHO in 1988 resolved to eradicate poliomyelitis still as many as 26 countries in 2011 reported at least one case of clinical infection with wild-type poliovirus, in total 1349 cases [53].

The failure to eradicate polio may partly be ascribed to the fact that the predominantly used oral live poliovirus vaccines (OPVs) carry the risks of both inducing vaccine-associated paralytic poliomyelitis and of being the source of vaccine derived virulent polioviruses. As the global eradication of polio is hopefully approaching, concerns have been raised in most developing countries about the continued use of OPV, and how to financially and logistically make it possible to replace OPV with the safe but more expensive inactivated injectable polio vaccine (IPV).

**Oral polio vaccine (OPV)**

OPV, developed by Sabin in the early 1960s, consists of a mixture of three live attenuated poliovirus strains representing the different serotypes 1, 2, and 3. In addition to its enormous impact in reducing polio in the world, this vaccine has also served as a useful tool for elucidating
fundamental aspects of mucosal immunity in humans [3]. Like IPV, OPV produces antibodies in the blood that will protect against myelitis by preventing the spread of poliovirus to the nervous system. But, superior to IPV, OPV also produces a local SIgA immune response in the intestinal and nasal mucosa, the primary sites for poliovirus entry and multiplication [3]. The intestinal immune response against OPV can rapidly stop person-to-person transmission of wild poliovirus, making mass campaigns with OPV a powerful strategy for the global eradication of polio [54].

Three or more spaced doses of OPV are required to generate adequate levels of seroconversion. Vaccination is recommended at birth, followed by 3 doses at least 4 weeks apart; booster immunizations are also recommended at different intervals. A need for more immunogenic OPVs has been identified, and recent work has supported the use of monovalent OPVs in supplemental immunization activities [55]. The considerably lower seroconversion rates against OPV observed in HIV infected as compared to non-infected African children [56] may pose an obstacle to global polio eradication and indicates the need for improved polio vaccines or vaccination strategies. Still, OPV remains the preferred polio vaccine in most of the world because of its ease of use, that is, oral administration of a few drops on the tongue, low cost and potential to quickly halt transmission [54].

** Injectable polio vaccine (IPV) **
The first licensed polio vaccine, IPV, developed by Salk in the early 1950s, has until recently been used for polio eradication in comparatively few countries. However, because of the OPV-related risk of causing vaccine-associated paralytic poliomyelitis and vaccine-derived virulent polioviruses, it has been proposed that cessation of OPV should be compulsory and be replaced by IPV in order to achieve complete eradication of polio [57]. IPV has been shown to prevent poliovirus outbreaks in many different settings. There is also some evidence of IPV-induced herd protection achieved by reducing the risk of contact with infected individuals [58], which may increase chances of polio eradication even if vaccine coverage is not 100%.

There have been concerns regarding the risk of wild type polio virus escaping from vaccine production when producing IPV. To minimize this problem production of IPV from the attenuated polio virus strains in OPV has been attempted with good results [59]. Further, to reduce costs it was found that IPV could be given intradermally using a 5-fold lower dose than the regular subcutaneous dosage without any loss of immunogenicity [60].

** A need to avoid or overcome ‘tropical barriers’ to mucosal vaccines **
Many oral vaccines, primarily live ones, have shown reduced immunogenicity when used in developing compared to industrialized countries. Reduced immunogenicity of OPV in developing countries is identified as a significant obstacle for polio eradication [3], and, as discussed above, the oral live rotavirus vaccines have had substantially reduced efficacy when tested in poor developing countries, which may limit their impact for the control of rotavirus infections in such settings. Likewise, the licensed oral live cholera vaccine (Orochol) and live oral cholera vaccines in earlier stages of clinical testing have been found to perform less well in developing country settings [14].

The reasons for the reduced immunogenicity of oral live vaccines in developing country settings are not completely understood. It is often attributed to chronic environmental enteropathy (CEE), also called tropical enteropathy, clinically characterized by malabsorption and histologically by intestinal inflammation and blunting of small intestinal villi [61]. Factors that may contribute to CEE include poor sanitation and intestinal flora overgrowth, and metagenomic studies are underway to evaluate the role of the host microbiome for the development of CEE and for immune responsiveness to oral vaccines [62]. In addition, nutrition-related factors, including both protein-calorie and micronutrient malnutrition, may negatively impact on mucosal vaccine immunogenicity as may also be the case with interference from maternal antibodies during breastfeeding, intestinal parasitic infections, intestinal mucosal damage and possibly maternal malnutrition during pregnancy [63]. Host genetic factors may also contribute to the observed differences in responsiveness to mucosal (and other) vaccines in different populations.

Vaccines designed for oral administration will need to be adjusted to these potential problems in order to maximize benefits for all children. Oral vaccines, when given to children in developing countries, may require specific measures to realize their full benefit such as higher doses of vaccine; additional booster doses; nutritional supplements; withdrawal of breast milk before vaccine administration; and deworming medications. A few such strategies have been tried with promising results, including co-administration of vaccines with zinc and vitamin A [64], withdrawal of breast milk for a few hours before oral vaccination [65], and treatment of helminths before vaccination [66].

** Conclusions and perspectives **
For many years, mucosal immunity and mucosal vaccines have attracted less than their due share of research and development. A much improved knowledge about the mucosal immune system in recent years together with methodological improvements for measuring local immune responses, both antibodies and cell-mediated immunity, has led to a rapidly increased interest for mucosal vaccine development. Several new mucosal vaccines are in different stages of clinical testing, including both attempted improved alternatives to existing vaccines and vaccines
against additional mucosal infections, such as, for example, ETEC diarrhea, shigellosis, and calicivirus infections.

However, the development of a broader range of mucosal vaccines, especially subunit vaccines based on purified antigens, will require access to improved antigen delivery systems as well as effective adjuvants. Significant advances have recently been made in both of these fields leading to products that are now in clinical testing [6]. Still, their usefulness in genetically diverse human subjects who also may differ significantly in their intestinal flora, nutritional status and previous immunological experience, all of which are factors that have been found to affect mucosal vaccine efficacy, remains to be defined.

As discussed above, several mucosal vaccines have been found to work less well in developing country settings than in industrialized countries. It is a major challenge to better understand the basis for and find practical means to avoid or overcome this barrier. Further, the pandemic HIV infection problem presents additional challenges with regard to vaccine safety and efficacy, although in this regard at least the killed mucosal vaccines may have advantages over injectable vaccines.

Although mucosal vaccine administration in general is safer than parenteral vaccination, it is notable that two recently developed mucosal vaccines for human use, a first-generation live attenuated oral rotavirus vaccine (RotaShield) and a nasal influenza subunit vaccine given together with (unmodified) E. coli L'T as adjuvant were withdrawn after a short period on the market because of adverse reactions. This underlines the difficult and challenging task for all vaccines to combine vaccine and adjuvant efficacy with safety and public acceptability.

References


