**Mucosal vaccines and technology**

**Summary**

There is an urgent and unmet need to develop effective vaccines to reduce the global burden of infectious disease in both animals and humans, and in particular for the majority of pathogens that infect via mucosal sites. Here we summarise the impediments to developing mucosal vaccines and review the new and emerging technologies aimed at overcoming the lack of effective vaccine delivery systems that is the major obstacle to developing new mucosal vaccines.

**Keywords:** bacterial, mucosal, vaccines, viral

**Introduction**

Vaccination is an efficient and cost-effective form of infectious disease prevention that can lead to global eradication, as seen for smallpox (1980) and rinderpest (2011). However, there is an urgent and growing need for the development of new and improved vaccines to further reduce the global burden of infectious disease morbidity and mortality, particularly against those targeting the respiratory and gastrointestinal (GI) tract. The paucity of effective vaccines is also acute in veterinary medicine, which is compounded by increasing multi-drug and antibiotic resistance [1]. Vaccines to combat zoonoses are a particularly urgent priority, as 60% or more pathogens with the potential to harm humans originate in animals [2]. Current vaccines are delivered by injection with associated problems of safety, compliance, morbidity and the high cost of mass immunization, particularly in resource-poor developing countries. Injected vaccines also provide partial or no protection at mucosal sites. Considering that >90% of pathogens gain access to the body via mucosal sites, using mucosal vaccination to generate protective immunity at mucosal sites could overcome the limitations of current injection-based vaccines in providing front-line protection against pathogen invasion and dissemination [3]. However, only a handful of mucosal vaccines are currently licensed. This limited availability of mucosal vaccines is related to the lack of effective delivery systems able to preserve vaccine antigen integrity and strong adjuvanticity, which is compounded by the intrinsic nature of the mucosal immune system to induce tolerance [4].

**Mucosal immunity and vaccine responses**

The majority of mucosal vaccines are administered by the oral and nasal routes with the vaginal, rectal, ocular and sublingual routes being less frequently used. However, not all routes of administration induce an equivalent immune response in terms of potency and longevity, reflecting differences in the organization and cellular make-up of lymphoid structures in different mucosal tissues [5,6]. For example, oral immunization usually stimulates immune responses in the GI tract in addition to the oral mucosa and nasal-associated lymphoid tissues (NALT) and mammary glands. Intranasal delivery effectively induces antibody production in salivary glands, the NALT and the bronchus-associated lymphoid tissue (BALT) of the lower respiratory tract, and in the urogenital tract. Rectal immunization elicits a more pronounced immune and antibody response in nasal secretions, tears and the rectal mucosa. Thus, depending on the mucosal sites targeted by different pathogens, the route of immunization needs to be carefully considered [6–8]. In most cases, mucosal vaccination
is also effective in priming systemic immune responses and generating serum antibodies with neutralizing properties, reflecting the cross-talk between the mucosal and systemic immune systems. Serum immunoglobulin (IgG) responses in vaccinated animals can be a useful correlate of protection, either alone or in combination with secretory mucosa-derived (IgA) antibodies [9].

Size is another important consideration in the design of mucosal vaccines and targeting uptake to inductive immune sites to generate T cell and/or B cell responses. Goblet cell-associated passageways allow the entry of soluble protein antigens, but not inert particles (0.02–2 µm) into the underlying lamina propria [10]. Via endocytosis, enterocytes readily take up nanosized soluble particles of 20–40 nm, whereas M cells are the major route by which larger-sized inert particles of more than 100 nm are taken up [11]. While nanoparticles lead predominantly to T cell responses, larger microparticles are more effective at inducing humoral responses [12,13]. The fate of particles delivered via the intranasal route is influenced by their size with particles smaller than 5 µm being transported across the nasal mucosa for delivery to cells of the BALT. By contrast, larger particles exceeding 10 µm are taken up by alveolar macrophages and dendritic cells (DCs) [14,15].

Current licensed mucosal vaccine formulations

Human use

The majority of currently licensed human mucosal vaccines comprise attenuated strains of pathogenic bacteria or viruses that retain their immunogenicity during transit through the upper GI tract and can target inductive immune sites in the small and large intestine or upper respiratory tract (Table 1). The oral polio vaccine, OPV, is the most successful mucosal vaccine to date [16]. A significant drawback to using attenuated pathogen-based vaccines is the risk of reactogenicity and reversion to a virulent pathogen following vaccination, usually in immunocompromised infants, elderly people or in individuals with a specific immunodeficiency. Although the use of OPV has decreased the number of polio cases by more than 99% since 1988, there are still disease outbreaks of vaccine-associated paralytic polio (VAPP) that arise due to small genetic changes occurring during OPV replication in humans [17,18]. Another concern for live attenuated vaccines is the possibility of retrograde transport to the brain after nasal or intranasal vaccination, as happened with a replication-defective adenovirus vector carrying three proteins from human immunodeficiency virus type

### Table 1. Licensed mucosal vaccines

| Target | Pathogen | Trade name | Delivery route (form) | Formulation (±adjuvant) |
|--------|----------|------------|-----------------------|------------------------|
| Human  | *Vibrio cholerae* | Dukoral® | Oral (liquid) | Inactivated (recombinant cholera toxin subunit B) |
|        |          | ShanChol®, Euvichol® | Oral (liquid) | Inactivated |
|        |          | Vaxchora®, FluMist® | Oral (liquid) | Live attenuated |
|        | Influenza type A and B virus | Biopolio® B1/3, and other oral polio vaccines – OPVs | Oral (liquid) | Live attenuated |
|        | Poliovirus | Rotarix® and RotaTeq® | Oral (liquid) | Live attenuated |
|        | Salmonella typhimurium | Typhi Vivotif® | Oral (capsules) | Live attenuated |
|        | Adenovirus | Not trade name | Oral (tablets) | Live attenuated |
| Animal | Rabies virus | Approved for military use, RabORAL-V-RG | Oral (bait) | Recombinant (Vaccinia virus vector) |
|        | Bovine parainfluenza 3 bovine respiratory syncytiatal virus | Rispoval | Intranasal (spray) | Live attenuated |
|        | Bordetella bronchiseptica | Nobivac® | Intranasal (drops) | Live |
|        | Canine parainfluenza virus | Avinew NeO® | Oral, ocular or nasal (spray, drinking water or drops) | Live attenuated |
1 that was found in the central nervous system of mice after intranasal delivery, which may have reached the brain via olfactory neurones [19].

Apart from live attenuated vaccines, three World Health Organization (WHO) prequalified inactivated oral vaccines are in use for cholera (Dukoral®, Shanchol™ and Euvichol®), which have been shown to provide high levels (60–95%) of long-lived (>2 years) protection in support of the concept that non-living vaccines can be effective for mucosal delivery and vaccination [20]. Although inactivated vaccines are, in general, safe, the process of inactivation (heat and/or formalin treatment) can reduce their immunogenicity and require the addition of adjuvants such as recombinant cholera toxin subunit B, which is included in the Dukoral vaccine [20].

Subunit vaccines comprising synthetic recombinant peptides and proteins, toxoids, DNA or mRNA offer significant safety advantages over attenuated and inactivated vaccines. They are inert and non-infectious, although they can suffer from weak immunogenicity and a requirement for adjuvants. To date, subunit vaccine formulations have failed to confer long-lived protective mucosal immunity in humans [21]. The weak immunogenicity of inert molecules and protein subunit antigens after delivery to mucosal sites is due in large part to their inefficient uptake by the mucosal epithelium and delivery to the delivery to the mucosa-associated lymphoid tissue (MALT) [22,23]. This reflects the significant physical, biochemical and microbial obstacles orally and nasally administered vaccines must overcome in the GI and respiratory tracts in order to access and activate mucosal immune cells. During transit through the GI tract, vaccine antigens are diluted and can be retained or trapped in mucosal secretions and by mucus and be subsequently degraded by non-specific host or microbial enzymes prior to reaching the mucosal immune system. The acidic environment of the upper GI-tract also impacts on the stability and integrity of oral vaccines [24]. In the respiratory tract, physical discharge due to high mucociliary clearance rates, or peristalsis action in the GI tract, also impact upon vaccine integrity and retention time [25].

Veterinary use

Mucosal vaccines have been more successful in the veterinary field, with spray and drinking water vaccines routinely used for mass vaccination in poultry farming. Recent introduction of edible gel-bead-based vaccine systems offer a more stable mucosal delivery, protecting live vaccines against environmental inactivation to improve bioavailability. Use of gel-beads to deliver Eimeria spp. oocysts to day-old chicks offers greater uptake of oocysts than water spray containing Eimeria spp. oocysts, and significantly higher weight gain post-challenge infection [26]. The livestock–wildlife interface consistently poses difficulties in vaccination programmes for animals. Mucosal delivery of vaccines through baiting allows free-ranging animals to voluntarily uptake vaccines, in order to break down interspecies transmission of infectious disease between wild and domesticated animals. Arguably, the most successful bait vaccine is RABORAL V-RG® which, following distribution into wildlife habitats, has aided eradication of wildlife rabies from Belgium, France and Luxembourg [27]. Wildlife bait vaccination has also helped to control other pathogens, including classical swine fever in wild boar (Sus scrofa) in Europe [28] and plague in prairie dogs (Cynomys spp.) in the United States [29,30]. Currently, licensed vaccines for parenteral application could be administered orally where it may not be possible, or feasible, to trap an animal to inject them. Mycobacterium bovis is a causative agent of tuberculosis (TB), and remains one the most difficult diseases of livestock to control, due largely to the prevalence of a wildlife reservoir. Bacillus Calmette–Guérin (BCG) vaccines were developed to protect cattle against bovine tuberculosis with subsequent experimental and field studies, showing that they may be efficacious in the control of M. bovis in wild animals after mucosal administration. White-tailed deer (Odocoileus virginianus) vaccinated with BCG Danish strain 1331 by oral bait or oral liquid had fewer tuberculosis lesions 5 months post-M. bovis challenge than control deer [31]. Badger BCG is a licensed injectable vaccine for European badgers (Meles meles) against TB; however, capturing animals for injection is labour-intensive and stressful. Oral administration of BCG has been shown to reduce M. bovis lesions in badgers [32], with 75% of captured badgers in a further study testing positive for BCG vaccine markers where the vaccine was administered in bait [33]. Dispersing mucosal vaccines in baits into high-risk areas could help to reduce endemic TB in wildlife reservoirs, reducing the risks of devastating TB outbreaks in livestock. Despite promise from field trials, there is still a lack of vaccines licensed for distribution into wildlife. A major drawback is the risk of non-target species consuming the bait; however, with further research into the use of subunit or inactivated mucosal vaccines, instead of live, this threat may be withdrawn.

The need for human mucosal vaccines

Despite many trials, there are no licensed vaccines for many human mucosal-transmitted pathogens (Table 2), or the currently available vaccines generate incomplete protection.

Improving mucosal vaccines

New technologies are being developed with the aim of protecting and preserving antigen structural integrity, as well as increasing bioavailability and induction of local
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Table 2. Infectious diseases in need of mucosal vaccines

| Pathogen                              | Mortality/annum | Morbidity/annum | Ref.       |
|---------------------------------------|-----------------|-----------------|-----------|
| Respiratory tract                     |                 |                 |           |
| Seasonal influenza                    | 470,000         | 4 million       | [34]      |
| RSV-ALRI                              | 128,000         |                 | [35]      |
| Streptococcus pneumonia               | 1.6 million     |                 | [36]      |
| Mycobacterium tuberculosis            | 1.6 million     |                 | [37]      |
| Gastrointestinal tract                |                 |                 |           |
| Rotavirus                             | 215,000         |                 | [38]      |
| Helicobacter pylori                   | 14,500          |                 | [39]      |
| Enterotoxigenic Escherichia coli (ETEC)| 400,000         |                 | [40,41]  |
| Salmonella                            | 32,000 (Africa) | 1.2 million (USA)| [42,43]  |
| Shigella                              | 700,000         | 80 million      | [44]      |
| Clostridium difficile/perfringens     | 14,000          | 500,000         | [45]      |
| Urogenital tract                      |                 |                 |           |
| Syphilis                              | 205,000         |                 | [46]      |
| Gonorrhoea                            |                 | 78 million      | [47]      |
| Herpes simplex virus 2               |                 | 417 million     | [48]      |
| Human papillomavirus (HPV)            | 270,000         |                 | [49]      |
| Hepatitis B                           | 887,000         |                 | [50]      |
| Hepatitis C                           | 399,000         | 71 million      | [51]      |
| HIV                                   | 940,000         | 36.9 million    | [52]      |

and systemic neutralizing immune responses (Table 3). All these delivery vehicles can be modified or complemented with immunostimulatory molecules or coating agents [e.g. polyethylene glycol (PEG), chitosan] to change their charge, adhesive properties, shape, size and/or pH to improve their characteristics, interactions with host cells and targeting sites of inductive immune responses. The incorporation of PEG into polyactide (PLA) nanoparticle vaccine formulations has been shown to be effective for the oral delivery of hepatitis B surface antigens in mice [53]. Chitosan is a biodegradable, biocompatible, muco-adhesive, non-toxic polymer that has been used in a similar way to protect Escherichia coli O157:H7 vaccine formulations during oral delivery [54].

The inclusion of alginate, polyvinyl alcohol, hyaluronan and cellulose to micro- and nanoparticle-based vaccines increases their viscosity and augments the retention time at mucosal surfaces promoting antigen uptake. Molecules that target the carrier or vaccine antigen directly to surface receptors on M cells or antigen-presenting cells [e.g. Toll-like receptors (TLRs)] have also been used [76]. The use of adjuvants includes aluminium hydroxide to facilitate antibody and T helper type 2 (Th2) CD4 T cell responses, or Vibrio cholerae toxin (CT) and heat-labile enterotoxin from E. coli to non-specifically boost cellular and humoral immune responses [77,78]. To date, recombinant cholera toxin subunit B (rCTB) is the only adjuvant accepted for inclusion in licensed mucosal vaccines (i.e. Dukoral® vaccine). rCTB stimulates the production of both anti-bacterial and anti-toxin antibodies without any side effects [79]. Genetically modified enterotoxins are being developed to reduce toxicity without adversely affecting their adjuvanticity.

Plants can be used as bioreactors to produce large quantities of vaccine that are then purified from plant extracts or can be consumed directly as an edible plant vaccine. The plants of choice are rice, lettuce or maize, with an edible rice-based cholera vaccine containing rCTB (MucoRice-CTB) currently in Phase I clinical trials [80]. An experimental lettuce-based hepatitis B virus vaccine has been tested in mice and shown to be effective at inducing neutralizing antibodies after oral administration [81]. Algae are a particularly cost-effective bioreactor option for producing large quantities of recombinant vaccines, and due to their high structural integrity and resilience of their cell walls have the potential to be used intact as vaccine delivery vehicles [74]. Chlamydomonas reinhardtii has been used in experimental Staphylococcus aureus vaccine formulations [82] and Schizochytrium sp. have been used to develop novel zika virus vaccines [83]; in both cases, these algae-based vaccines have been shown to be effective at eliciting both mucosal and systemic humoral immune responses.

Immunostimulatory complex (ISCOM) technology has been incorporated into commercial veterinary vaccines such as Equip F® vaccine against equine influenza for parenteral delivery, although Ghazi et al. have demonstrated protection in mice immunized orally with influenza virus subunit vaccines that incorporate ISCOM [84]. Liposomes and emulsions carriers have been used in experimental vaccines for respiratory virus infections with incorporation of the soybean oil-based emulsion W805EC into influenza
| Delivery system                        | Structure                                                                 | Advantages                                                                 | Limitations                                                                 | Ref.  |
|---------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|-------|
| Liposomes                             | Spherical phospholipid bilayer entrapping an aqueous solution core        | • Ease of incorporating distinct types of antigens                        | • Relatively low intrinsic stability for storage and after administration  | [55]  |
|                                       |                                                                           | • Adaptable physicochemical properties                                    | • Potent toxicity of cationic lipids (dose-dependent)                        |       |
|                                       |                                                                           | • Lepidic compounds with adjuvant properties                               |                                                                           |       |
| Archaeosomes                          | Liposomes composed of Archaea-derived polar lipids                        | • Stable formulations                                                      | • Preparation and purification of Archaea lipids                            | [56]  |
|                                       |                                                                           | • Improved immunogenicity compared with liposomes                          | • Need optimization of production and formulation                           |       |
| Bilosomes                             | Bile salt stabilized vesicles                                             | • Stable in gastric environment                                           | • Relatively low antigen dose                                               | [57]  |
| ISCOM, ISCOMATRIX                     | Cage-like structure comprised of cholesterol, phospholipids and saponin  | • Stable structure                                                         | • Inclusion of antigens into the ISCOM can be difficult                     | [58]  |
| Bacterial outer membrane vesicles (OMV)| OMVs from Gram-negative pathogens containing microbe-associated molecular pattern (MAMPs) and membrane proteins | • In-built adjuvanticity                                                    | • Chemical detoxification required – reduced adjuvanticity                   | [59,60]|
|                                       |                                                                           | • High stability                                                           | • Variable efficacy                                                         |       |
|                                       |                                                                           | • Safe use in children and adults and effective in controlling disease outbreaks | • Strain-specific – limited heterotypical strain protection                 |       |
| Virus-like particles (VLP)            | Natural virus without carrying genetic material                           | • Highly immunogenic without addition of adjuvant                          | • Purification can be a challenge                                           | [61,62]|
|                                       |                                                                           | • Antigens can be chemically conjugated or genetically inserted            | • May have poor quality and consistency                                     |       |
| Gene gun (DNA vaccination)            | DNA-coated colloidal gold particles                                       | • Fast and simple                                                          | • Contamination by host materials                                          | [63]  |
|                                       |                                                                           | • Efficient DNA transduction                                               | • Costly device and reagents                                                |       |
|                                       |                                                                           | • Requires small amounts of DNA (0.1 mg/dose)                              | • Limited to exposed tissues                                                |       |
|                                       |                                                                           | • Can be used to deliver multiple DNAs                                     | • Depth of penetration versus tissue damage                                 |       |
| Emulsions                             | Water-in-oil/oil-in-water                                                 | • Slow release of immunogen                                                | • Preferentially induces T helper type 2 response                            |       |
|                                       | Nanosized droplets                                                        | • Ease of manufacture                                                      | • Multiple factors influence efficacy                                       |       |
|                                       |                                                                           | • Self-adjuvanticity                                                       | • Reactogenicity                                                            | [64,65]|
| Synthetic polymer nanoparticles (e.g. PLA/PLGA) | Polylactide (PLA) or polylactic-co-glycolic acid (PLGA) based nano- and micro particles | • Controlled release of antigens                                           | • Limited stability after administration                                     |       |
|                                       |                                                                           | • Biodegradable and biocompatible biopolymer                               | • Low preservation of antigen structure                                     | [66,67]|
| Natural polymer nanoparticles (e.g. chitosan) | FDA-approved agents Chitosan based nano- and micro particles              |                                                                           | • Sensitivity to harsh gastric environment, low loading capacity            |       |
| Hydrogel (e.g. cCHP nanogel)          | Cationic cholesterol-bearing pullulan nanogel, self-assembles with water due to their amphiphilic polysaccharides | • Biocompatible, biodegradable, mucoadhesive and immunostimulating         | • Irregular distribution, low physical stability                            | [68]  |
|                                       |                                                                           | • Ability to function as an artificial chaperone                            | • Optimization of biodistribution and degradation mechanism                | [69]  |
|                                       |                                                                           | • Prolonged binding to nasal epithelium                                   | • Component toxicity                                                        |       |
virus and inactivated respiratory syncytial virus (RSV) vaccines, both of which are effective at eliciting protective systemic and mucosal antibody immune responses, and RSV vaccine also induces cellular immunity after intranasal administration [85,86]. Similarly, pneumococcal surface protein A (PspA)-based subunit vaccines incorporating a cCHP-based nanogel has been shown to induce both mucosal and systemic neutralizing antibodies in cynomolgus macaques after nasal delivery [87].

Virus-like particles (VLP) are an attractive option as a vaccine delivery vehicle due to their useful properties, such as the ability to induce adaptive immune response and to induce long-term expression of non-self-proteins [88,89]. VLPs from adeno-associated viruses (AAV) have been used to develop novel influenza virus vaccines encoding cameldid-derived anti-influenza antibodies transgenes that when administered intranasally protected mice against lethal influenza A and B challenge [89,90]. Other preclinical studies using AAV as a carrier include norovirus vaccine formulations containing viral protein and RNA, that have shown promise in a Phase I clinical trial [91], and a chimpanzee-derived AAV expressing hepatitis C virus antigens that is currently in Phase II clinical trials [92,93].

Gene gun bombardment is a biolistic system for mucosal DNA vaccination. This needle-free technology is based on propelling DNA-coated colloidal gold microprojectiles at exposed tissue surfaces (e.g. skin, vulva and mouth) and penetrating the cytosol and cell nucleus of cells within deeper layers of the tissue. [94] Epidermal DNA vaccines delivered via a gene gun have been shown to elicit both humoral and cell-mediated mucosal immune responses in experimental animals and cattle [63,95,96]. To improve the potency of immune responses gene to gene immunizations, DNA can be combined with adjuvants such as recombinant protein antigens and plasmids encoding cytokines [94,97]. However, gene gun-mediated delivery has limited or no control over where and how effective DNA transduction is in host cells and is generally ineffective at inducing immune responses of sufficient potency to provide effective and long-lived protection.

The use of genetically modified probiotic strains of bacteria to deliver vaccine antigens has been explored for human papilloma virus (HPV) vaccines. Although a commercial HPV vaccine is available, it does not confer protection to all HPV-related cancers [49]. Generally recognized as safe (GRAS) strains of Lactococcus lacti engineered to express the HPV-16 E6 oncoprotein generated humoral and cellular immune response in mice after oral administration, as well being shown to have an inhibitory effect on tumour growth [98]. There are, however, biosafety and environmental contamination concerns in using genetically modified bacteria [99].

A safer alternative to using viable bacteria as vaccine delivery vehicles are non-viable nanometer-sized lipid-containing microvesicles (outer membrane vesicles; OMVs) that are naturally produced and secreted by most Gram-negative bacteria [59]. Formulations of Neisseria meningitidis OMVs (VA-MENGOC-BC, MenBvac, MeNZB and Bexero) have been successfully used to vaccinate both adults and children and to control outbreaks of meningococcal B infection in several countries [100,101]. OMVs from other Gram-negative pathogens are also promising vaccine candidates, including those from Salmonella [102], Shigella flexneri [103] and V. cholerae [104]. However, their potential for unintended toxicity due to associated toxins is a safety concern and limits their widespread use, although chemical detoxification can overcome this, but at the loss of immunogenicity and adjuvanticity [105]. In principle, these limitations could be overcome by bioengineering the parental bacterium to improve their OMV drug delivery capability [60]. Alternatively, non-pathogenic
commensal bacteria could be used as a source of OMVs to reduce toxicity and improve safety. We are developing an OMV-based drug and biologicals delivery technology platform based on the use of OMVs produced by strains of human commensal *Bacteroides* engineered to express in their OMVs bacterial or viral vaccine antigens or human therapeutic proteins for delivery to the respiratory and GI tracts.

In summary, while mucosal vaccines represent the ideal means of protecting against the majority of infections, there are very few licensed vaccines for either humans or animals. A raft of new technologies and innovations in vaccine antigen encapsulation and delivery are being developed to overcome the obstacles of protecting and preserving antigen structural integrity as well as increasing bioavailability and induction of local and systemic neutralizing immune responses during transit to mucosal inductive immune sites, particularly in the GI tract.

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**Disclosures**

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**References**

1. Vaccines and vaccination. National Office of Animal Health Available at: https://www.noah.co.uk/briefingdocument/antibiotic-resistance-2/ (accessed 26 November 2018).
2. Grace DMF, Ochungo P, Kruska R *et al*. Mapping of poverty and likely zoonoses hotspots. Zoonoses Project 4. Nairobi, Kenya: International Livestock Research Institute (ILRI), 2012.
3. Hoft DF, Brusic V, Sakala IG. Optimizing vaccine development. Cell Microbiol 2011; 13:934–42.
4. Azizi A, Kumar A, Diaz-Mitoma F, Mestecky J. Enhancing oral vaccine potency by targeting intestinal M cells. PLOS Pathog 2010; 6:e1001147.
5. Kantele A, Häkkinen M, Moldoveanu Z *et al*. Differences in immune responses induced by oral and rectal immunizations with *Salmonella typhi* Ty21a: evidence for compartmentalization within the common mucosal immune system in humans. Infect Immun 1998; 66:5630–5.
6. Czerkinsky C, Holmgren J. Mucosal delivery routes for optimal immunization: targeting immunity to the right tissues. In: Kozlowski PA, ed. Mucosal vaccines: modern concepts, strategies, and challenges. Berlin Heidelberg: Springer, 2012:1–18.
7. Fujikuyama Y, Tokuharu D, Kataoka K *et al*. Novel vaccine development strategies for inducing mucosal immunity. Expert Rev Vaccines 2012; 11:367–79.
8. Saroja C, Lakshmi P, Bhaskaran S. Recent trends in vaccine delivery systems: a review. Int J Pharm Invest 2011; 1:64–74.
9. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol 2010; 17:1055–65.
10. McDole JR, Wheeler LW, McDonald KG *et al*. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 2012; 483:345–9.
11. Howe SE, Konjufta VH. Protein-coated nanoparticles are internalized by the epithelial cells of the female reproductive tract and induce systemic and mucosal immune responses. PLOS ONE 2014; 9:e114601.
12. Ogra PL, Faden H, Welliver RC. Vaccination strategies for mucosal immune responses. Clin Microbiol Rev 2001; 14:430–45.
13. Xiang SD, Scholzen A, Minigo G *et al*. Pathogen recognition and development of particulate vaccines: does size matter? Methods 2006; 40:1–9.
14. Thomas C, Gupta V, Ahsan F. Particle size influences the immune response produced by hepatitis B vaccine formulated in inhalable particles. Pharm Res 2010; 27:905–19.
15. Carvalho TC, Peters JI, Williams RO. Influence of particle size on regional lung deposition – what evidence is there? Int J Pharm 2011; 406:1–10.
16. Kim S-H, Jang Y-S. Antigen targeting to M cells for enhancing the efficacy of mucosal vaccines. Exp Mol Med 2014; 46:e85.
17. World Health Organization. Polio vaccines: WHO position paper, January 2014. Wkly Epidemiol Rec 2014; 89:73–92.
18. Jorba J, Diop OM, Iber J *et al*. Update on vaccine-derived polioviruses – worldwide, January 2017–June 2018. Morb Mortal Wkly Rep 2018; 67:1189–94.
19. Lemiale F, Kong WP, Akyurek LM *et al*. Enhanced mucosal immunoglobulin A response of intranasal adenoviral vector human immunodeficiency virus vaccine and localization in the central nervous system. J Virol 2003; 77:10078–87.
20. World Health Organization (L’Organisation Mondiale de la Santé). Cholera vaccines: WHO position paper – August 2017. Wkly Epidemiol Rec 2017; 92:477–98.
21. Mitragotri S. Immunization without needles. Nat Rev Immunol 2005; 5:905–16.
22. Lycke N. Recent progress in mucosal vaccine development: potential and limitations. Nat Rev Immunol 2012; 12:592–605.
23. Kiyono H, Izuhara K. New trends in mucosal immunology and allergy. Allergol Int 2019; 68:1–3.
24. Vela Ramirez JE, Sharpe LA, Peppas NA. Current state and challenges in developing oral vaccines. Adv Drug Deliv Rev 2017; 114:116–31.
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25 Suzuki H, Nagatake T, Nasu A et al. Impaired airway mucociliary function reduces antigen-specific IgA immune response to immunization with a claudin-4-targeting nasal vaccine in mice. Sci Rep 2018; 8:2904.

26 Jenkins MC, Parker C, Kloppe S, O’Brien C, Miska K, Fetterer R. Gel-bead delivery of Eimeria oocysts protects chickens against coccidiosis. Avian Dis 2012; 56:306–9.

27 Maki J, Guiot AL, Aubert M et al. Oral vaccination of wildlife using a vaccinia–rabies–glycoprotein recombinant virus vaccine (RABORAL V-RG(R)): a global review. Vet Res 2017; 48:57.

28 Rossi S, Pol F, Forot B et al. Preventive vaccination contributes to control classical swine fever in wild boar (Sus scrofa sp.). Vet Microbiol 2010; 142:99–107.

29 Rocke TE, Tripp DW, Russell RE et al. Sylvatic plague vaccine partially protects prairie dogs (Cynomys spp.) in field trials. EcoHealth 2017; 14:438–50.

30 Creekmore TE, Rocke TE, Hurley J. A baiting system for delivery of an oral plague vaccine to black-tailed prairie dogs. J Wildl Dis 2002; 38:32–9.

31 Nol P, Palmer MV, Waters WR et al. Efficacy of oral and parenteral routes of Mycobacterium bovis bacille Calmette–Guérin vaccination against experimental bovine tuberculosis in white-tailed deer (Odocoileus virginianus): a feasibility study. J Wildl Dis 2008; 44:247–59.

32 Gormley E, Ni Bhuaichalla D, O’Keeffe J et al. Oral vaccination of free-living badgers (Meles meles) with bacille Calmette–Guérin (BCG) vaccine confers protection against tuberculosis. PLOS ONE 2017; 12:e0168851.

33 Palphramand K, Delahay R, Robertson A et al. Field evaluation of candidate baits for oral delivery of BCG vaccine to European badgers, Meles meles. Vaccine 2017; 35:4402–7.

34 Influenza (seasonal) World Health Organization. 2018. Available at: http://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal) (accessed 7 November 2018).

35 Stein RT, Bont LJ, Zar H et al. Respiratory syncytial virus hospitalization and mortality: systematic review and meta-analysis. Pediatr Pulmonol 2017; 52:556–69.

36 World Health Organization. Pneumococcal disease. Available at: http://www.who.int/ith/diseases/pneumococcal/en/ (accessed 8 October 2018).

37 World Health Organization. Tuberculosis. Available at: http://www.who.int/news-room/fact-sheets/detail/tuberculosis (accessed 8 November 2018).

38 Tate JE, Burton AH, Boschi-Pinto C, Parashar UD. Global, Regional, and National Estimates of Rotavirus Mortality in Children <5 Years of Age, 2000-2013. Clin Infect Dis 2016; 62:596–S105.

39 Kathleen R, Stratton JSD, Lawrence RS. Appendix 7. Helicobacter pylori. Vaccines for the 21st century: a tool for decisionmaking. Washington DC: National Academies Press, 2000.

40 World Health Organization. Weekly Epidemiological Record. No. 11. Geneva: World Health Organization, 2006:81, 97–04.

41 World Health Organization. Future directions for research on enterotoxigenic Escherichia coli vaccines for developing countries. Geneva: World Health Organization, 2006.

42 Centers for Disease Control and Prevention. Salmonella. 2018. Available at: https://www.cdc.gov/salmonella/ (accessed 8 November 2018).

43 World Health Organization. WHO’s first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. Geneva: World Health Organization, 2015.

44 World Health Organization. Guidelines for the control of shigellosis, including epidemics due to Shigella dysenteriae type 1. Geneva: World Health Organization, 2005.

45 Lessa FC, Mu Y, Bamberg WM et al. Burden of Clostridium difficile infection in the United States. N Engl J Med 2015; 372:825–34.

46 World Health Organization. Report on global sexually transmitted infection surveillance 2015. Geneva: World Health Organization, 2015.

47 World Health Organization. WHO guidelines for the treatment of Neisseria gonorrhoeae. Geneva: World Health Organization, 2016.

48 World Health Organization. Innovative approach sheds light on prevalence of STIs and bacterial vaginosis among women in sub-Saharan Africa. Geneva: World Health Organization, 2018.

49 World Health Organization. Human papillomavirus (HPV) and cervical cancer. Geneva: World Health Organization. 2018. Available at: http://who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer (accessed 9 November 2018).

50 World Health Organization. Hepatitis B. Available at: http://www.who.int/news-room/fact-sheets/detail/hepatitis-b (accessed 9 November 2018).

51 World Health Organization. Hepatitis C. Available at: http://www.who.int/news-room/fact-sheets/detail/hepatitis-c (accessed 9 November 2018).

52 World Health Organization. HIV/AIDS. Available at: http://www.who.int/en/news-room/fact-sheets/detail/hiv-aids (accessed 9 November 2018).

53 Jain AK, Goyal AK, Mishra N, Vaidya B, Mangal S, Vyas SP. PEG–PLA–PEG block copolymeric nanoparticles for oral immunization against hepatitis B. Int J Pharm 2010; 387:253–62.

54 Doavi T, Mousavi SL, Kamali M, Amani J, Ramandi MF. Chitosan-based intranasal vaccine against Escherichia coli O157:H7. Iranian Biomed J 2016; 20:97–108.

55 Courtbey B, Bioley G. Lipid-based particles: versatile delivery systems for mucosal vaccination against infection. Front Immunol 2018; 9:431.

56 Jacobsen A-C, Jensen SM, Fricker G, Brandl M, Treusch AH. Archael lipids in oral delivery of therapeutic peptides. Eur J Pharm Sci 2017; 108:101–10.

57 Shukla A, Mishra V, Kesharwani P. Bilosomes in the context of oral immunization: development, challenges and opportunities. Drug Discov Today 2016; 21:888–99.
58 Sun H-X, Xie Y, Ye Y-P. ISCOMs and ISCOMATRIX®. Vaccine 2009; 27:4388–401.
59 Jan AT. Outer membrane vesicles (OMVs) of Gram-negative bacteria: a perspective update. Front Microbiol 2017; 8:1053.
60 Gerritzien MJH, Martens DE, Wijffels RH, van der Pol L, Stork M. Bioengineering bacterial outer membrane vesicles as vaccine platform. Biotech Adv 2017; 35:565–74.
61 Minkner R, Park EY. Purification of virus-like particles (VLPs) expressed in the silkworm Bombyx mori. Biotech Lett 2018; 40:659–66.
62 Steppert P, Burgstaller D, Klausberger M et al. Purification of HIV-1 gag virus-like particles and separation of other extracellular particles. J Chromatogr A 2016; 1455:93–101.
63 Raska M, Turneak J. Chapter 67: DNA vaccines for the induction of immune responses in mucosal tissues. In: Mestecky J, Strober W, Russell MW, Kelsall BL, Cheroutre H, Lambrecht BN, eds. Mucosal immunology, 4th edn. Boston, USA: Academic Press, 2015:1307–35.
64 Petrovsky N. Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. Drug Saf 2015; 38:1059–74.
65 Huang C-H, Huang C-Y, Cheng C-P et al. Degradable emulsion as vaccine adjuvant reshapes antigen-specific immunity and thereby ameliorates vaccine efficacy. Sci Rep 2016; 6:36732.
66 Zhang L, Yang W, Hu C, Wang Q, Wu Y. Properties and applications of nanoparticle/microparticle conveyors with adjuvant characteristics suitable for oral vaccination. Int J Nanomed 2018; 13:2973–87.
67 Silva AL, Soema PC, Slüter B, Ossendorp F, Jiskoot W. PLGA particulate delivery systems for subunit vaccines: linking particle properties to immunogenicity. Hum Vacc Immunother 2016; 12:1056–69.
68 Xing L, Fan Y-T, Zhou T-J et al. Chemical modification of chitosan for efficient vaccine delivery. Molecules (Basel) 2018; 23:229.
69 Azegami T, Yuki Y, Nakahashi R, Itoh H, Kiyono H. Nanogel-based nasal vaccines for infectious and lifestyle-related diseases. Mol Immunol 2018; 98:19–24.
70 Szatraj K, Szczepankowska AK, Chmielewska-Jeznach M. Lactic acid bacteria – promising vaccine vectors: possibilities, limitations, doubts. J Appl Microbiol 2017; 123:325–39.
71 Uddin MJ, Gill HS. Ragweed pollen as an oral vaccine delivery system: mechanistic insights. J Control Release 2017; 268:416–26.
72 Uddin MJ, Gill HS. From allergen to oral vaccine carrier: a new face of ragweed pollen. Int J Pharm 2018; 545:286–94.
73 Concha C, Cañas R, Macuer J et al. Disease prevention: an opportunity to expand edible plant-based Vaccines? Vaccines (Basel) 2017; 5:14.
74 Specht EA, Mayfield SP. Algae-based oral recombinant vaccines. Front Microbiol 2014; 5:60.
75 Ramos-Vega A, Rosales-Mendoza S, Bañuelos-Hernández B, Angulo C. Prospects on the use of Schizochytrium sp. to develop oral vaccines. Front Microbiol 2018; 9:2506.
76 Kim SH, Jang YS. The development of mucosal vaccines for both mucosal and systemic immune induction and the roles played by adjuvants. Clin Exp Vaccine Res 2017; 6:15–21.
77 Norton EB, Lawson LB, Freytag LC, Clements JD. Characterization of a mutant Escherichia coli heat-labile toxin, LT(R192G/L211A), as a safe and effective oral adjuvant. Clin Vaccine Immunol 2011; 18:546–51.
78 Lebens M, Terrinoni M, Karlsson SL et al. Construction and preclinical evaluation of mmCT, a novel mutant cholera toxin adjuvant that can be efficiently produced in genetically manipulated Vibrio cholerae. Vaccine 2016; 34:2121–8.
79 European Medicines Agency. Dukoral Scientific Discussion. 2005. Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/dukoral (accessed 14 November 2018).
80 Kurokawa S, Nakamura R, Meijima M et al. MucoRice–cholera toxin B-subunit, a rice-based oral cholera vaccine, down-regulates the expression of α-amylose/trypsin inhibitor-like protein family as major rice allergens. J Proteome Res 2013; 12:3372–82.
81 Dobrica M-O, Lazar C, Paruch I et al. Oral administration of a chimeric hepatitis B Virus S/preS1 antigen produced in lettuce triggers infection neutralizing antibodies in mice. Vaccine 2018; 36:5789–95.
82 Dreesen IAJ, Hamri GC-E, Fussenegger M. Heat-stable oral alga-based vaccine protects mice from Staphylococcus aureus infection. J Biotechnol 2010; 145:273–80.
83 Márquez-Escobar VA, Bañuelos-Hernández B, Rosales-Mendoza S. Expression of a Zika virus antigen in microalgae: towards mucosal vaccine development. J Biotechnol 2018; 282:86–91.
84 H. O. Ghazi CWP TLSaRJ. Comparative antibody responses and protection in mice immunised by oral or p ISCOMs. J Med Microbiol 1995; 42:53–61.
85 Stanberry LR, Simon JK, Johnson C et al. Safety and immunogenicity of a novel nanoemulsion mucosal adjuvant W805EC combined with approved seasonal influenza antigens. Vaccine 2012; 30:307–16.
86 O’Konek JJ, Makidon PE, Landers JJ et al. Intranasal nanoemulsion-based inactivated respiratory syncytial virus vaccines protect against viral challenge in cotton rats. Hum Vacc Immunother 2015; 11:2904–12.
87 Fukuyama Y, Yuki Y, Katakai Y et al. Nanogel-based pneumococcal surface protein A nasal vaccine induces microRNA-associated Th17 cell responses with neutralizing antibodies against Streptococcus pneumoniae in macaques. Mucosal Immunol 2015; 8:1144–53.
88 Mohsen MO, Gomes AC, Vogel M, Bachmann MF. Interaction of viral capsid-derived virus-like particles (VLPs) with the innate immune system. Vaccines (Basel) 2018; 6:37.
89 Nieto K, Salvetti A. AAV vectors vaccines against infectious diseases. Front Immunol 2014; 5:5.
90 Laursen NS, Friesen RHE, Zhu X et al. Universal protection against influenza infection by a multidomain antibody to influenza hemagglutinin. Science 2018; 362:598–602.
91 Kim L, Liebowitz D, Lin K et al. Safety and immunogenicity of an oral tablet norovirus vaccine, a phase I randomized, placebo-controlled trial. JCI insight 2018; 3:e121077.

92 GlaxoSmithKline. Product pipeline. Available at: https://www.gsk.com/en-gb/investors/product-pipeline/ (accessed 16 November 2018).

93 European Commission. Final Report Summary – PEACHI (Prevention of Hepatitis C Virus (HCV) and HIV-1 coinfections through induction of potent T cell responses using prime-boost viral vector vaccine regimens). Luxembourg: Publications Office of the European Union, 2018.

94 Fuller DH, Loudon P, Schmaljohn C. Preclinical and clinical progress of particle-mediated DNA vaccines for infectious diseases. Methods 2006; 40:86–97.

95 Loudon PT, Yager EJ, Lynch DT et al. GM-CSF increases mucosal and systemic immunogenicity of an H1N1 influenza DNA vaccine administered into the epidermis of non-human primates. PLOS ONE 2010; 5:e11021-e.

96 Fuller DH, Rajakumar PA, Wilson LA et al. Induction of mucosal protection against primary, heterologous simian immunodeficiency virus by a DNA vaccine. J Virol 2002; 76:3309–17.

97 Trimble C, Lin CT, Hung CF et al. Comparison of the CD8+T cell responses and antitumor effects generated by DNA vaccine administered through gene gun, biojector, and syringe. Vaccine 2003; 21:4036–42.

98 Taghinezhad-S S, Mohseni AH, Keyvani H, Razavilar V. Protection against human papillomavirus type 16-induced tumors in C57BL/6 mice by mucosal vaccination with Lactococcus lactis NZ9000 expressing E6 oncoprotein. Microb Pathog 2019; 126:149–56.

99 Wegmann U, Carvalho AL, Stocks M, Carding SR. Use of genetically modified bacteria for drug delivery in humans: revisiting the safety aspect. Sci Rep 2017; 7:2294.

100 de Kleijn ED, de Groot R, Labadie J et al. Immunogenicity and safety of a hexavalent meningococcal outer-membrane-vesicle vaccine in children of 2–3 and 7–8 years of age. Vaccine 2000; 18:1456–66.

101 Sandbu S, Feiring B, Oster P et al. Immunogenicity and safety of a combination of two serogroup B meningococcal outer membrane vesicle vaccines. Clin Vaccine Immunol 2007; 14:1062–9.

102 Howlader DR, Koley H, Sinha R et al. Development of a novel S. Typhi and Paratyphi A outer membrane vesicles based bivalent vaccine against enteric fever. PLOS ONE 2018; 13:e0203631-e.

103 Camacho AI, de Souza J, Sanchez-Gomez S, Pardo-Ros M, Irache JM, Gamazo C. Mucosal immunization with Shigella flexneri outer membrane vesicles induced protection in mice. Vaccine 2011; 29:8222–9.

104 Schild S, Nelson EJ, Camilli A. Immunization with Vibrio cholerae outer membrane vesicles induces protective immunity in mice. Infect Immun 2008; 76:4554–63.

105 van der Ley P, van den Dobbelsteen G. Next-generation outer membrane vesicle vaccines against Neisseria meningitidis based on nontoxic LPS mutants. Hum Vaccin 2011; 7:886–90.