**Immunology Research and Perspectives**

https://irp.cultechpub.com/irp

Vol. 2, No. 2, June 2026

Cultech Publishing

*Review*

# Mucosal Vaccine Platforms for Respiratory and Enteric Pathogens: Current Advances, Key Barriers, and Translational Opportunities

**Shoaib Manzoor***

Department of Microbiology, University of Jhang, Punjab, Pakistan

*Corresponding author: shoaibmanzoor064@gmail.com

## Abstract

Respiratory and enteric pathogens cause significant global morbidity and mortality, transmitting efficiently across mucosal surfaces. Conventional injectable vaccines primarily induce systemic immunity, offering limited protection at these entry sites. Mucosal vaccination is a promising strategy to elicit local and systemic immunity, particularly secretory immunoglobulin A (IgA) and tissue-resident memory T cells, which block infection and reduce transmission. Licensed mucosal vaccines, including intranasal influenza (FluMist), oral rotavirus (Rotarix/RotaTeq), oral polio, and oral cholera (Dukoral) vaccines, demonstrate the feasibility of this approach. This review summarizes the immunological basis of mucosal immunity and evaluates delivery routes like intranasal and oral administration. A systematic search of PubMed, Scopus, and Web of Science (Jan 2020-Mar 2026) was conducted. Major vaccine platforms–live attenuated vectors, protein subunits, and nucleic acid-based systems–are discussed alongside adjuvants, nanoparticles, and bioadhesive formulations. Applications to key respiratory (influenza, SARS-CoV-2, RSV, Mycobacterium tuberculosis) and enteric (Shigella, ETEC, Campylobacter, rotavirus) pathogens are highlighted. Critical barriers include mucosal tolerance, antigen degradation, and manufacturing complexity. Emerging innovations in mRNA technology, synthetic biology, and artificial intelligence offer new opportunities to overcome these obstacles, enhancing herd immunity and global control of infections.

## Keywords

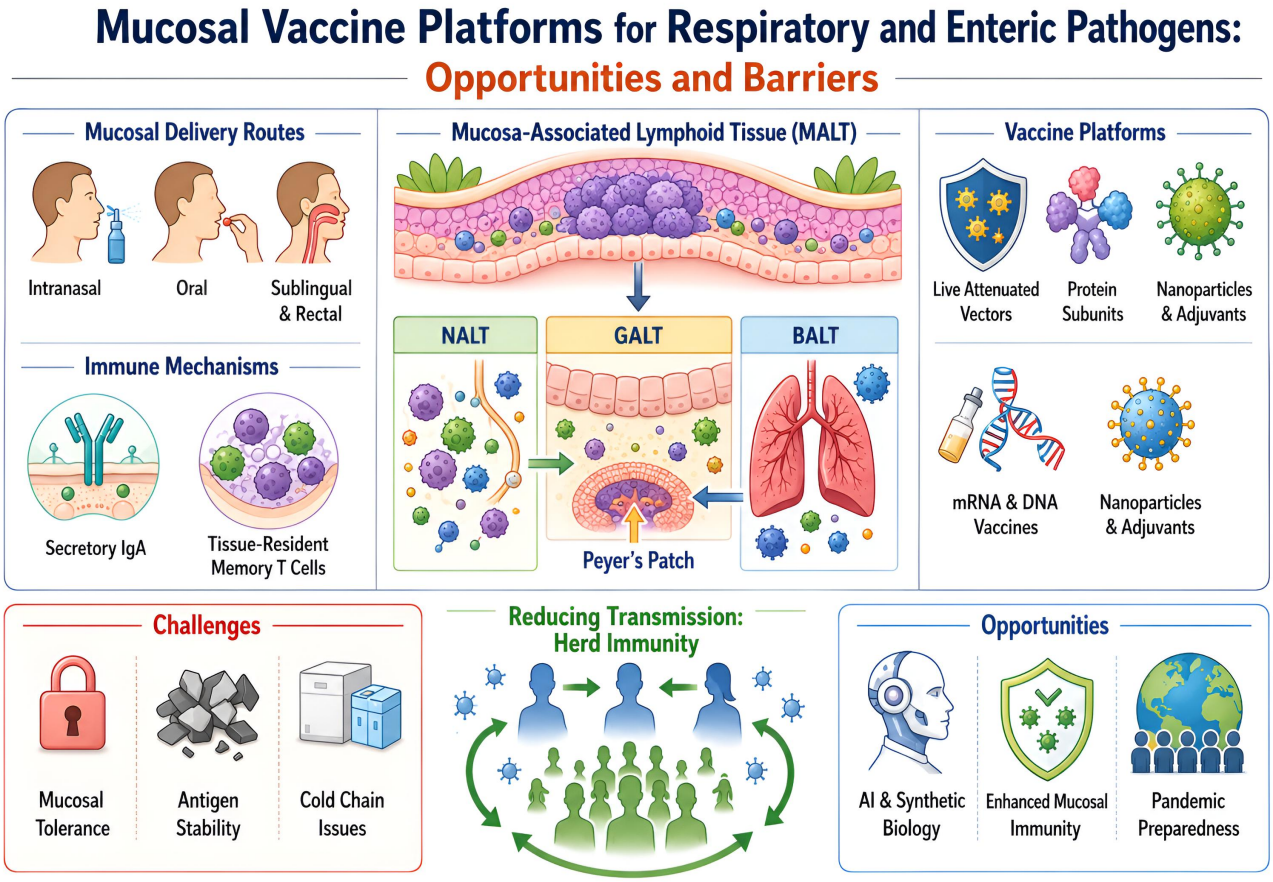
Mucosal vaccines, Respiratory pathogens, Enteric pathogens, Mucosal immunity, Vaccine delivery systems, Clinical translation, Intranasal vaccines, Oral vaccines

## Article History

Received: 22 February 2026 Revised: 27 March 2026

Accepted: 19 May 2026 Available Online: 16 June 2026

**Graphical Abstract**




## 1. Introduction

Respiratory and enteric infections impose a substantial global health burden, contributing to high morbidity and mortality rates worldwide. Lower respiratory infections remain a leading cause of death, particularly among older adults, immunocompromised individuals, and those with chronic conditions, with diseases such as influenza, respiratory syncytial virus (RSV), and tuberculosis causing significant health challenges [1,2]. Similarly, enteric viral and bacterial infections continue to affect both human and animal populations, leading to considerable health and economic impacts [3].

Although injectable vaccines have been pivotal in reducing disease incidence and severity, they face limitations in preventing transmission at mucosal surfaces where initial infection commonly occurs. These vaccines predominantly induce systemic immunity but often fail to adequately stimulate mucosal immune defenses, resulting in continued pathogen spread despite vaccination efforts [4]. However, several successful mucosal vaccines have already demonstrated the power of this approach. The oral polio vaccine (OPV), a live attenuated vaccine, has been instrumental in interrupting poliovirus transmission in endemic regions through induction of intestinal immunity [5]. Oral rotavirus vaccines (Rotarix and RotaTeq) have reduced severe rotavirus diarrhea by 80-90% in high-income countries and substantially decreased childhood mortality in low-resource settings [6]. The intranasal live attenuated influenza vaccine (FluMist) provides protection at the respiratory portal of entry by inducing mucosal IgA and local cellular immunity [7]. Oral cholera vaccines (Dukoral, Shanchol) have proven effective in outbreak control through stimulation of intestinal mucosal immunity [8].

To address remaining challenges and expand upon these successes, mucosal vaccination has emerged as a promising strategy capable of inducing both local mucosal and systemic immunity. Mucosal vaccines delivered via routes such as nasal or oral administration directly target the mucosal immune system, which serves as the first line of defense against inhaled or ingested pathogens. This dual immune induction holds the potential to more effectively block pathogen entry and subsequent transmission, thereby enhancing protection beyond what injectable vaccines alone can achieve [9,10]. Moreover, inducing robust mucosal immunity is especially critical in combating diseases like pulmonary tuberculosis and COVID-19, where respiratory mucosal immunity plays a vital role in protection [4,11]. This review aims to provide a comprehensive overview of the current landscape and advances in mucosal vaccination strategies targeting respiratory and enteric infections. It will explore the underlying mucosal immune mechanisms, evaluate existing vaccines and novel developments focusing on mucosal delivery, and critically assess the challenges and future directions in this field.

## 2. Methods

A systematic literature search was conducted to identify relevant studies on mucosal vaccine platforms for respiratory and enteric pathogens. The search was performed in three electronic databases: PubMed, Scopus, and Web of Science, covering the period from January 2020 to March 2026. This timeframe was selected to capture the most recent advances, including COVID-19 pandemic-related developments in mucosal vaccine technology. The search strategy employed key terms and Boolean operators including combinations of "mucosal vaccine," "intranasal vaccine," "oral vaccine," "sublingual vaccine," "buccal vaccine" with "respiratory pathogen," "influenza," "SARS-CoV-2," "COVID-19," "RSV," "*Mycobacterium tuberculosis*," "enteric pathogen*," "rotavirus," "*Shigella*," "ETEC," "*Campylobacter**," "mucosal immunity," "secretory IgA," "tissue-resident memory T cells (TRM)," "clinical trial," "preclinical," and "licensed vaccine."

The review included original research articles, systematic reviews, and meta-analyses that evaluated mucosal vaccine platforms, delivery systems, or adjuvants and reported immunological outcomes including mucosal IgA, TRM cells, or systemic responses. Clinical trials from Phase I through III and licensed vaccine data were prioritized. Articles were excluded if they were editorials, commentaries, or conference abstracts without original data, focused exclusively on parenteral vaccination without mucosal components, or did not report primary immunological or clinical outcomes.

The initial search yielded 1,847 articles across the three databases. After removing 389 duplicates, 1,458 articles were screened by title and abstract, leading to the exclusion of 982 articles deemed irrelevant to mucosal vaccines. Full-text review of 476 articles was conducted, resulting in 312 articles meeting inclusion criteria. Additional references were identified through manual searching of reference lists and inclusion of key historical papers on licensed mucosal vaccines. The final bibliography comprises 164 references, with priority given to publications from 2023–2026 to ensure currency.

## 3. Immunological Basis of Mucosal Vaccination

### 3.1 Mucosal Immune System and Immune Induction Sites

The mucosal immune system is a specialized network of tissues and cells that protect mucosal surfaces, such as those lining the respiratory, gastrointestinal, and genitourinary tracts, which are primary entry points for many pathogens. This system consists predominantly of mucosa-associated lymphoid tissue (MALT), which are organized lymphoid structures embedded in mucosal surfaces that initiate immune responses. These structures function to initiate and regulate immune responses to encountered antigens at mucosal sites [12]. The major components of MALT include nasopharynx-associated lymphoid tissue (NALT), gut-associated lymphoid tissue (GALT), and organized structures such as Peyer's patches within GALT. Each of these tissues comprises inductive sites for the initiation of antigen-specific immunity and effector sites where immune responses are executed.

NALT, located in the nasopharynx, plays a crucial role in respiratory mucosal immunity. It is characterized by lymphoid follicles, interfollicular regions, and follicle-associated epithelium that supports the generation of antigen-specific T helper cells and IgA-producing B cells, facilitating both mucosal and systemic protective responses following intranasal antigen exposure. GALT, which includes Peyer's patches embedded in the small intestinal wall, serves as an important inductive site in the gut. Peyer's patches contain follicles rich in B cells, T-cell zones, and specialized follicle-associated epithelium, notable for the presence of microfold (M) cells, which are specialized epithelial cells that sample and transport luminal antigens to underlying immune cells. These cells are crucial for antigen sampling from the intestinal lumen [12-14].

Bronchus-associated lymphoid tissue (BALT) is an inducible lymphoid structure that develops in the lower respiratory tract following antigenic stimulation or inflammation. Unlike NALT and GALT, which are present constitutively, BALT formation is dynamic and depends on microbial exposure and inflammatory signals. BALT serves as an important inductive site for local immune responses in the lungs, facilitating the generation of respiratory mucosal immunity and contributing to protection against pulmonary pathogens such as influenza virus and *Mycobacterium tuberculosis*. The inducible nature of BALT highlights the plasticity of the mucosal immune system and its ability to adapt to environmental challenges [15,16]. BALT functions in coordination with regional lymph nodes to orchestrate both local and systemic immune responses, with lymphocytes primed in BALT acquiring homing receptors that direct them to respiratory effector sites [16].

Antigen sampling at mucosal surfaces is uniquely facilitated by specialized epithelial cells termed M cells within the follicle-associated epithelium of mucosal lymphoid tissues such as Peyer's patches and NALT. M cells possess the ability to transcytose luminal antigens, including bacteria and particulate matter, efficiently delivering them to underlying antigen-presenting cells (APCs) such as dendritic cells and macrophages [13,17]. This antigen uptake is critical for initiating adaptive immune responses by the activation of T and B lymphocytes within the organized lymphoid follicles. Dendritic cells in the subepithelial dome region process and present these antigens to naive lymphocytes, leading to the differentiation and proliferation of antigen-specific effector cells including IgA-secreting plasma cells [17,18]. Additionally, other layers provide innate defenses, including a mucus layer that acts as a physical

and biochemical barrier, epithelial tight junctions, intraepithelial lymphocytes, and antimicrobial peptides produced by epithelial cells, all of which contribute to pathogen sensing and elimination [18].

In summary, the mucosal immune system is organized around MALT structures such as NALT and GALT, with Peyer's patches serving as pivotal inductive sites. Antigen sampling is predominantly mediated by M cells which transport luminal antigens to APCs, triggering both local mucosal and systemic immune responses essential for protection against infections at mucosal surfaces. The dynamic nature of BALT adds another layer of complexity and adaptability to respiratory mucosal immunity. This anatomical and functional compartmentalization underscores the importance of targeting these sites for effective mucosal vaccination strategies.

### 3.2 Role of Secretory IgA and Tissue-Resident Memory Cells

Secretory immunoglobulin A (IgA), TRM, and innate immune components collectively form essential protective mechanisms at mucosal surfaces against respiratory and enteric pathogens.

Secretory IgA is the predominant antibody isotype at mucosal sites, playing a critical role in immune defense by neutralizing pathogens and preventing their adherence and invasion at epithelial surfaces. IgA is produced locally by plasma cells within mucosal tissues and transported across the epithelium into the lumen via the polymeric Ig receptor. This localized secretion allows IgA to effectively intercept respiratory viruses and enteric pathogens at the portals of entry, thereby hindering infection and transmission. Studies in pulmonary models demonstrate that mucosal (intranasal) immunization induces lung-resident IgA-secreting B cells that provide superior protection compared to systemic antibodies alone, highlighting the importance of secretory IgA in respiratory mucosal immunity [19].

TRM are a distinct subset of memory T cells localized permanently in peripheral mucosal tissues such as the lungs and intestines. Unlike circulating memory T cells, TRM cells reside at the frontline entry sites of pathogens, enabling rapid recognition and response upon re-exposure. Respiratory TRM cells, including CD4+ and CD8+ subsets, have been shown to mediate effective adaptive immunity against various respiratory pathogens such as *Bordetella pertussis*, influenza virus, and RSV. These cells not only directly contribute to pathogen clearance through cytokine secretion and cytotoxicity but also orchestrate broader immune responses. Their presence correlates with long-lasting, cross-protective immunity and early containment of infection. However, exuberant TRM activity can also contribute to chronic inflammatory pathology, which underscores the need for balanced immune regulation [20-24].

Recent human studies have provided critical insights into the protective role of TRM cells. In tuberculosis, bronchoalveolar lavage samples from *Mycobacterium tuberculosis*-exposed household contacts revealed that donor-unrestricted mucosal-associated invariant T (MAIT) cells and $CD8^+$ TRM cells correlate with resistance to infection [25]. A 2023 study demonstrated that individuals with latent tuberculosis infection who remain disease-free exhibit higher frequencies of lung-resident *Mycobacterium*-specific $CD4^+$ TRM cells producing IFN-γ and TNF-α [26]. For COVID-19, analysis of autopsy specimens and bronchoscopy samples from convalescent individuals showed persistent SARS-CoV-2-specific $CD8^+$ TRM cells in the lungs for up to 10 months post-infection, correlating with protection against reinfection [27]. A 2024 clinical trial of an intranasal COVID-19 booster in previously vaccinated adults demonstrated significant expansion of lung-resident spike-specific $CD8^+$ TRM cells, which correlated with reduced viral shedding upon subsequent breakthrough infection [28]. Influenza studies have documented that nasal-associated lymphoid tissue contains hemagglutinin-specific CD4+ TRM cells that persist for years and correlate with reduced severity of seasonal influenza [29].

Together, the compartmentalized action of secretory IgA, localized TRM, and innate immune defenses are vital for robust protection against respiratory and enteric pathogens at mucosal surfaces. Their synergistic functions not only prevent pathogen establishment and transmission but also contribute to long-term mucosal immune memory, making them critical targets for vaccine development aimed at enhancing mucosal immunity.

A summary of established and emerging correlates of protection for major respiratory and enteric pathogens is presented in Table 1.

**Table 1.** Correlates of protection for mucosal vaccines against respiratory and enteric pathogens.

| Pathogen | Mucosal IgA | Systemic IgG | TRM Cells | Clinical Evidence (2020-2026) | References |
|---|---|---|---|---|---|
| SARS-CoV-2 | Nasal IgA neutralizes virus at entry; correlates with reduced transmission | Serum IgG prevents systemic spread; wanes over time | Lung-resident CD4+/CD8+ TRM provide durable protection against variants | Intranasal boosters expand TRM and reduce breakthrough infections (2024 trial) | [27,28,30] |
| Influenza | Nasal IgA correlates with cross-protection against drifted strains | Systemic IgG prevents severe disease | Lung TRM enable heterosubtypic immunity; persist for years in NALT | Nasal TRM correlate with reduced severity in seasonal epidemics | [29,31,32] |
| RSV | Nasal IgA reduces initial infection risk | Serum neutralizing antibodies standard correlate | Lung TRM essential for clearance upon re-exposure | Phase III nirsevimab trials; mucosal vaccines in development | [33,34] |
| TB | Mucosal IgA may limit early bacterial adherence | Th1 IgG responses contribute | Lung CD4+ TRM producing IFN-γ/TNF correlate with resistance | Household contact studies show TRM associated with non-infection | [25,26,35] |
| Rotavirus | Intestinal IgA correlates with protection; used as correlate in vaccine trials | Serum IgA used as surrogate | Gut CD8+ TRM contribute to viral clearance | Rotarix efficacy correlates with intestinal IgA in infants | [36,37] |
| ETEC | Intestinal IgA against colonization factors | Serum antibodies to LT/CFs | Gut TRM under investigation | dmLT adjuvant enhances IgA responses in Phase II | [38,39] |
| Shigella | Fecal IgA correlates with protection in controlled human infection models | Serum LPS IgG correlates with reduced severity | Gut TRM may contribute to long-term immunity | CPS-specific IgA correlates with protection in vaccinees | [40,41] |

As shown in Table 1, mucosal IgA correlates with protection for most pathogens, while TRM cells have emerged as key correlates for respiratory viruses including SARS-CoV-2, influenza, RSV, and tuberculosis. For enteric pathogens such as rotavirus and *Shigella*, intestinal or fecal IgA remains the most established correlate, though gut TRM cells are under active investigation.

Innate immune components of the mucosa provide the first line of defense and include physical barriers such as mucus and epithelial tight junctions, antimicrobial peptides, and pathogen recognition receptors including Toll-like receptors (TLR), which are proteins that recognize pathogen-associated molecules and activate innate immune responses. These components act rapidly to sense and eliminate invading pathogens prior to the activation of adaptive immunity. Furthermore, TRM cells communicate with neighboring epithelial and innate immune cells, enhancing the local antiviral state—for example, $CD8^+$ TRM cells induce interferon-gamma-dependent antiviral gene expression in epithelial cells during herpes simplex virus infection, promoting cell-intrinsic resistance and containment of reactivation. Such coordination between innate and adaptive mucosal immunity amplifies protection against repeated infections [42].

## 4. Mucosal Vaccine Delivery Routes

### 4.1 Intranasal Vaccines

Intranasal vaccine delivery for respiratory pathogens offers a promising non-invasive immunization route that directly targets the respiratory mucosa, the primary site of infection for many airborne pathogens. The nasal mucosa houses NALT, a key inductive site capable of initiating robust mucosal and systemic immune responses upon antigen exposure. Intranasal vaccines enable antigen uptake through the mucosal epithelium, notably via specialized M cells in the follicle-associated epithelium, and facilitate antigen delivery to underlying dendritic cells and other APCs in the NALT, fostering immune activation [43,44].

Mechanistically, antigen uptake at the nasal mucosa can be enhanced by novel vaccine formulations, including bioadhesive hydrogels that prolong antigen residence time and facilitate internalization and cross-presentation by dendritic cells, leading to efficient maturation of immune responses in NALT and recruitment of immune cells [45]. Additionally, targeting the neonatal Fc receptor (FcRn), a receptor that transports immunoglobulins across epithelial barriers, for receptor-mediated transcytosis has been utilized to improve antigen delivery and immune stimulation in the respiratory tract, resulting in potent neutralizing antibody production and the generation of lung-resident memory T cells [46].

Intranasal vaccination induces both local mucosal immunity and systemic immune responses. Importantly, it promotes the generation of secretory IgA antibodies within the respiratory mucosa, which are critical for neutralizing pathogens at the site of entry. It also establishes TRM cells in the lungs, offering rapid and enduring cell-mediated immunity against respiratory viruses such as influenza and SARS-CoV-2 [19,47]. Moreover, intranasal boosters following parenteral priming have been shown to convert circulating immunity into robust mucosal IgA responses and local cellular immunity without the need for additional adjuvants, highlighting the versatility and efficiency of this route [48].

The advantages of intranasal vaccination over traditional parenteral vaccination are multifold. It avoids needle-associated risks and improves patient compliance due to its non-invasive and painless administration. By directly stimulating mucosal immunity, intranasal vaccines can prevent early pathogen invasion, replication, and transmission more effectively than systemic vaccines, which often elicit insufficient mucosal responses [43]. They also exploit the common mucosal immune system to provide broader protection at other mucosal sites beyond the respiratory tract. Furthermore, intranasal vaccines can induce a strong cytotoxic T cell response, beneficial for therapeutic vaccination needs [43]. Efficacy studies demonstrate that intranasal vaccines, including live-attenuated and subunit formulations, provide improved protection in animal models and clinical settings against influenza, tuberculosis, and SARS-CoV-2, often outperforming or complementing parenteral vaccines [47,49,50]. FluMist (MedImmune/AstraZeneca), a live attenuated influenza vaccine administered intranasally that was first licensed in the US in 2003 and is approved for individuals aged 2-49 years, induces mucosal IgA and systemic immunity and demonstrates efficacy against matched and drifted strains, though efficacy varies by season and age group [7].

In summary, intranasal vaccine delivery leverages mucosal immune mechanisms to induce potent local and systemic immunity, with enhanced antigen uptake through the nasal mucosa and robust induction of secretory IgA and TRM cells. Its non-invasive nature, ability to block transmission, and superior protection at the infectious entry point make it a highly advantageous alternative to parenteral vaccination for respiratory pathogens.

### 4.2 Oral Vaccines

Oral vaccine delivery for enteric pathogens is a strategic method aimed at inducing protective immunity specifically within GALT, the largest lymphoid organ in the human body responsible for immune surveillance of the intestinal mucosa [51,52]. Enteric pathogens such as *Salmonella* and *Shigella* initiate infections by invading the intestinal epithelium, causing localized inflammation or systemic disease, highlighting the need for effective mucosal immunization to prevent these infections. Oral vaccines are designed to evoke immune responses at the site of pathogen entry, offering the advantage of stimulating both mucosal and systemic immunity through the common mucosal immune system.

A primary challenge for oral vaccines is ensuring antigen stability throughout the gastrointestinal tract's harsh environment that includes low pH in the stomach and proteolytic enzymes in the intestines, which can degrade vaccine antigens before they reach inductive sites such as Peyer's patches and other GALT components [53]. To overcome this, oral vaccine formulations often utilize protective delivery systems like microparticles, nanoparticles, or plant-based vehicles such as rice seed-based platforms that shield the antigen from enzymatic degradation and allow sustained release within the gut [52]. These delivery vehicles enhance antigen uptake by intestinal M cells located within follicle-associated epithelium, which specialize in sampling luminal antigens and transcytosing them to underlying dendritic cells and immune compartments within GALT [54,55].

Following uptake, antigens are presented to mucosal immune cells, leading to robust induction of intestinal secretory IgA, which is critical for neutralizing enteric pathogens at mucosal surfaces. Secretory IgA acts by blocking pathogen adherence and invasion, thus preventing infection and transmission [51]. Furthermore, oral vaccines stimulate local cellular immunity and generate memory responses within GALT, supporting long-lasting mucosal protection [52]. Reverse transcytosis, the process of transporting substances from the basolateral to the apical surface of epithelial cells, of secretory IgA-antigen complexes via receptors such as Dectin-1 on M cells also promotes antigen delivery and modulates immune responses, a mechanism that can be exploited for designing targeted mucosal vaccines [55].

Licensed oral vaccines have demonstrated the feasibility of this approach. OPV, a live attenuated vaccine containing three serotypes, has been the cornerstone of the Global Polio Eradication Initiative by inducing intestinal immunity that interrupts fecal-oral transmission, leading to polio elimination in most of the world, although rare cases of vaccine-derived poliovirus circulation remain a challenge [5,56]. Rotavirus vaccines (Rotarix and RotaTeq), live attenuated oral vaccines, have reduced severe rotavirus gastroenteritis by 80-90% in high-income countries, though efficacy is lower at 50-70% in low-resource settings due to factors including maternal antibodies, malnutrition, and co-infections [6,36]. Oral cholera vaccines (Dukoral, Shanchol, and Euvichol), which are killed whole-cell vaccines with or without cholera toxin B (CTB) subunit, provide 60-80% protection for 2-3 years and are used in outbreak control and endemic settings [8,57].

Despite these advances, challenges remain in overcoming oral vaccine underperformance, particularly in developing countries, due to factors such as immature gut immune systems in infants, the presence of tolerogenic gut environments, and the lack of effective mucosal adjuvants [58]. Nonetheless, oral vaccination remains programmatically advantageous due to ease of administration, potential for mass immunization without needles, and induction of mucosal immunity that systemic vaccines poorly elicit [52,53].

In summary, oral vaccine delivery for enteric pathogens relies on protecting vaccine antigens through the gastrointestinal tract to enable effective uptake by M cells and antigen presentation within GALT. This approach induces robust local secretory IgA and cellular immunity critical for combating enteric infections, making it a vital strategy for preventing gastrointestinal infectious diseases.

### 4.3 Alternative Mucosal Routes

Sublingual, buccal, and rectal mucosal delivery routes offer alternative, noninvasive approaches for drug and vaccine administration with distinct immunological relevance due to their unique anatomical and immunological properties.

The sublingual route delivers substances under the tongue, facilitating rapid absorption directly into the systemic circulation while bypassing first-pass metabolism and gastrointestinal degradation. Immunologically, sublingual administration has been shown to induce both systemic and mucosal immune responses, including T cell and antibody responses that broadly disseminate to various mucosal sites such as the gastrointestinal, respiratory, and genital mucosae. This broad mucosal immune activation occurs through the common mucosal immune system, where lymphocytes activated at one mucosal site can home to other mucosal tissues. Notably, sublingual vaccines have been effective in inducing systemic immunity with lower adverse risks compared to nasal routes, as sublingual administration does not direct antigens to the brain. Clinical studies have demonstrated that sublingual immunization can induce humoral immune responses, although sometimes with lower magnitude compared to intramuscular injection, suggesting a need for improved delivery systems and adjuvants to enhance efficacy [59-61].

The buccal route involves administration through the inner cheek mucosa, which is highly vascularized and allows both local and systemic drug delivery. Buccal mucosa benefits from avoidance of first-pass metabolism and provides a relatively permeable membrane for drug absorption. Immunologically, the buccal mucosa is an attractive site for vaccine delivery because it harbors immunologically active cells capable of inducing localized and systemic immune responses, leveraging the common mucosal immune system. Advances in formulations such as mucoadhesive films, patches, and nanoparticulate delivery systems have improved drug retention and controlled antigen release at the buccal site, enhancing immunogenicity while minimizing injection use. Buccal vaccines can induce both antibody-mediated and cell-mediated immunity and offer high patient compliance due to painless administration and easy self-application [62-64].

The rectal mucosal route targets the rectal mucosa, which is part of GALT and capable of mounting robust mucosal immune responses. Rectal administration is especially useful for inducing immunity at mucosal surfaces because it can stimulate local and systemic immune responses, including in the genital and intestinal tracts. It also bypasses the stomach's harsh environment, preserving antigen integrity. Rectal delivery of vaccines and drugs can induce protective mucosal immunity targeting pathogens that enter via mucosal surfaces. However, clinical use may be limited by acceptability and formulation challenges. The immunological relevance of the rectal route lies in its capacity to stimulate mucosal immune effectors, including secretory IgA and T-cell responses, through activation of specialized immune inductive sites in the rectal mucosa [59,65].

Overall, these mucosal routes are promising for immunization strategies because they exploit the common mucosal immune system, allowing vaccination to induce protection at multiple mucosal sites where many infections initiate. They provide benefits such as noninvasiveness, improved patient compliance, elimination of needles, and potential for self-administration. However, challenges like formulation stability, permeation barriers, and variable absorption need ongoing innovation in delivery technologies including nanoparticulates, mucoadhesive systems, and permeation enhancers to optimize immunogenicity and clinical efficacy [64,66,67].

Table 2 summarizes the key immunological characteristics, licensed examples, advantages, and limitations of each mucosal delivery route.

**Table 2.** Comparison of mucosal vaccine delivery routes and their immunological characteristics [7,8,36,43,51,59,64,68].

| Route | Primary Target Site | Key Immune Inductive Tissue | Main Immune Responses | Licensed Examples (Year) | Advantages | Limitations |
|---|---|---|---|---|---|---|
| Intranasal | Respiratory mucosa | NALT | Secretory IgA, systemic IgG, lung TRM cells | FluMist (2003, US); iNCOVACC (2022, India) | Non-invasive; blocks infection at entry site; reduces transmission; good for respiratory pathogens | Risk of mucociliary clearance; formulation stability; safety concerns near CNS; variable efficacy by season |
| Oral | Intestinal mucosa | GALT, Peyer's patches | Intestinal secretory IgA, mucosal T cells, systemic immunity | OPV (1961); Rotarix (2008); RotaTeq (2006); Dukoral (1991) | Needle-free; ideal for enteric pathogens; suitable for mass vaccination; induces intestinal immunity | Antigen degradation (acid, enzymes); immune tolerance; lower efficacy in developing countries; vaccine-derived virus risk (OPV) |
| Sublingual | Under tongue (oral mucosa) | Oral mucosal lymphoid tissue | Systemic IgG, mucosal IgA, T-cell responses | None licensed | Avoids GI degradation and CNS exposure; rapid absorption; good safety profile | Lower immunogenicity; requires potent adjuvants and delivery systems |
| Buccal | Inner cheek mucosa | Oral mucosal immune cells | Local and systemic antibody and T-cell responses | None licensed | Painless; high patient compliance; controlled release via films/patches | Limited permeability; formulation challenges |
| Rectal | Rectal mucosa | GALT (distal gut) | Secretory IgA, mucosal and systemic T-cell immunity | None licensed | Bypasses stomach acidity; induces genital and intestinal immunity | Low acceptability; variable absorption; formulation issues |

The comparative analysis in Table 2 highlights that while intranasal and oral routes have yielded licensed vaccines, sublingual, buccal, and rectal routes remain experimental but offer unique advantages for specific applications. Intranasal vaccination is particularly effective for respiratory pathogens due to direct NALT targeting, whereas oral vaccines are optimal for enteric pathogens but face efficacy challenges in low-resource settings.

## 5. Vaccine Platforms for Mucosal Immunization

### 5.1 Live Attenuated and Recombinant Vectors

Live attenuated and recombinant bacterial or viral vectors represent powerful platforms for mucosal vaccination due to their ability to mimic natural infections and efficiently stimulate mucosal as well as systemic immunity.

Live attenuated bacterial vectors are genetically weakened forms of pathogenic bacteria designed to be safe yet immunogenic. These vectors can deliver heterologous antigens by colonizing or transiently replicating in the host mucosa, closely resembling the infection pathways of natural pathogens. This mimicking of natural infection facilitates engagement of the host's mucosal, humoral, and cellular immune compartments, leading to robust and broad immune responses including mucosal IgA, systemic antibodies, and cell-mediated immunity. Commonly studied live bacterial vectors include attenuated strains of *Salmonella*, *Shigella*, *Vibrio cholerae*, and *Listeria*. These organisms carry intrinsic immunostimulatory molecules such as lipopolysaccharides and flagella, which act as natural adjuvants enhancing immune activation. Because live bacteria replicate in vivo, they provide sustained antigen exposure that can amplify immune responses. However, the challenge involves balancing attenuation to maintain safety without overly diminishing immunogenicity. Several recombinant *Salmonella* vaccines are in clinical trials for mucosal immunization. In addition, non-pathogenic lactic acid bacteria have been explored as antigen carriers, offering safer alternatives but typically with less natural infection mimicry [69-73].

Live attenuated bacterial vectors allow mucosal administration routes such as oral, intranasal, or inhalational delivery, promoting localized mucosal immunity at pathogen entry points while inducing systemic immune protection. Their ability to target professional APCs at mucosal inductive sites enhances antigen presentation and immune priming. Moreover, these bacteria can be engineered to deliver DNA vaccines or cytokines, further broadening their immunotherapeutic utility [71,74].

Recombinant viral vectors, particularly poxviruses such as the modified vaccinia virus Ankara (MVA), are also widely employed for mucosal vaccination. These vectors express foreign antigens and induce both mucosal and systemic immune responses by replicating or transiently infecting mucosal epithelial cells, thus closely simulating natural viral infections. Recombinant MVA delivered via mucosal routes has been effective in eliciting protective immunity against respiratory viruses like influenza and RSV, and HIV, with robust immune responses at genital and rectal mucosae observed in animal models. The ability of these viral vectors to stimulate T-cell and antibody responses in both mucosal and peripheral tissues renders them promising candidates for combating mucosal pathogens. Recombinant viral vectors can also be employed in prime-boost vaccination strategies to improve immunogenicity against complex pathogens such as *M. tuberculosis* [75].

Licensed examples of live attenuated vaccines include OPV, a live attenuated viral vaccine used globally for polio eradication [5]; rotavirus vaccines (Rotarix and RotaTeq), which are live attenuated viral vaccines administered orally [36]; and FluMist, a live attenuated influenza vaccine administered intranasally [7]. These vaccines demonstrate the feasibility and public health impact of live attenuated mucosal immunization.

Overall, live attenuated and recombinant bacterial or viral vectors reproduce many features of natural infections, including mucosal colonization or infection, antigen presentation in relevant immune inductive sites, and intrinsic stimulatory signals that act as self-adjuvants. This allows them to induce strong, broad, and durable mucosal and systemic immune responses essential for protection against pathogens entering through mucosal surfaces. However, ensuring safety, stable antigen expression, balanced attenuation, and robust immunogenicity remain key challenges for their development and clinical use.

### 5.2 Protein Subunit and Inactivated Vaccines

Nanoparticle-based delivery systems represent another vital strategy, as they protect protein and inactivated antigens from degradation and clearance, and enhance uptake by APCs via size and surface property optimization. Polymeric nanoparticles such as poly(lactic-co-glycolic acid) (PLGA) and fatty acid-mimetic micelles not only improve antigen stability but also co-deliver immunostimulatory molecules such as TLR agonists to boost dendritic cell activation and cross-presentation, eliciting strong cellular and humoral immune responses after mucosal immunization.

Additionally, the use of particulate formulations like immune response-stimulating complexes (ISCOMs) as antigen carriers can improve the induction of protective Th1-type mucosal immunity as demonstrated in vaccines targeting pathogens like *Chlamydia trachomatis* [76]. Similarly, coadministration of inactivated viruses can function as mucosal adjuvants, enhancing immune responses to co-delivered antigens by promoting antibody production, T cell activation, and cytotoxic responses [77].

Licensed examples of inactivated and subunit-based mucosal vaccines include oral cholera vaccine (Dukoral), which consists of killed whole-cell *V. cholerae* with recombinant CTB and is administered orally [8], and inactivated polio vaccine (IPV), which is parenteral but has informed newer formulations exploring mucosal delivery.

Overall, formulation strategies for protein and inactivated mucosal vaccines focus on protecting the antigen, enhancing uptake by mucosal immune cells, prolonging antigen retention, and incorporating adjuvants to activate innate immunity. These approaches are necessary to achieve strong, durable, and protective immune responses at mucosal surfaces where many infections are first established [78-80].

### 5.3 Nucleic Acid-Based Mucosal Vaccines

mRNA and DNA vaccines adapted for mucosal delivery leverage nucleic acid technology to induce antigen expression locally at mucosal surfaces, thereby stimulating both mucosal and systemic immunity. However, direct delivery of these genetic materials faces significant challenges such as enzymatic degradation, inefficient cellular uptake, and limited local expression efficiency in the harsh mucosal environment.

Encapsulation strategies are essential to protect mRNA and DNA from degradation, facilitate cellular uptake, and optimize antigen expression. For mRNA vaccines, lipid nanoparticles (LNPs) are the most advanced and widely used delivery vehicles. LNPs encapsulate mRNA, shielding it from nucleases while promoting efficient endocytosis and endosomal escape upon uptake by target cells. The composition of LNPs, including PEGylated lipids, critically influences their colloidal stability during aerosolization for inhalation or nasal delivery, as well as in vivo transfection efficiency. For example, increasing PEG-lipid content enhances aerosol stability but may reduce cellular transfection, and the choice of administration route (nasal versus pulmonary) affects the intensity and duration of protein expression in respiratory mucosa [81,82].

Similarly, plasmid DNA vaccines benefit from encapsulation in LNPs, though they face additional hurdles like acute inflammatory responses triggered by innate DNA sensing pathways such as cGAS-STING. Innovative LNP formulations incorporating endogenous anti-inflammatory lipids such as nitro-oleic acid have been shown to reduce inflammation and sustain longer-term transgene expression, exceeding that of mRNA-LNPs in animal models. Optimization of LNP formulations through iterative screening can greatly enhance transgene expression *in vitro* and *in vivo* [83].

Beyond lipid-based systems, emerging encapsulation methods include polymers, coacervates, and peptide carriers designed to protect nucleic acids and enhance mucosal delivery efficiency. Complex coacervates, for example, form macromolecular-rich phases that provide strong affinity to mRNA, protecting it from enzymatic degradation, facilitating cellular uptake, and enabling sustained gene expression in target tissues. Despite their promise, coacervate-based mRNA delivery requires further exploration for optimization of encapsulation, release kinetics, and biocompatibility [84].

Mucosal delivery of nucleic acid vaccines also confronts immunological and physiological challenges, including mucosal tolerance mechanisms, rapid antigen dilution, physical mucus barriers, and competition with commensal microbiota. Formulation strategies must therefore incorporate targeting ligands to mucosal APCs or M cells, include mucosal adjuvants to activate innate immunity, and ensure stability in variable mucosal microenvironments. The integration of these elements can significantly improve local antigen expression, durable immune responses, and clinical efficacy of mucosally delivered DNA and mRNA vaccines [84-86].

While no mucosal mRNA vaccines are currently licensed, intranasal mRNA vaccines against COVID-19 are in Phase I/II trials by Pfizer/BioNTech and Moderna exploring mucosal formulations. The inhaled adenoviral vaccine iNCOVACC (CanSino Biologics) was approved in China and India in 2022, representing a significant step for respiratory mucosal delivery of genetic vaccines [87].

In summary, successful mucosal delivery of mRNA and DNA vaccines depends heavily on advanced encapsulation technologies – primarily LNPs and emerging polymer/coacervate systems – that protect nucleic acids, promote cellular uptake, and modulate local immune activation as explained in Figure 1. Challenges related to mucosal barriers, local expression efficiency, and immune tolerance necessitate carefully engineered formulations and targeting strategies to realize the full potential of these next-generation vaccines for mucosal protection.

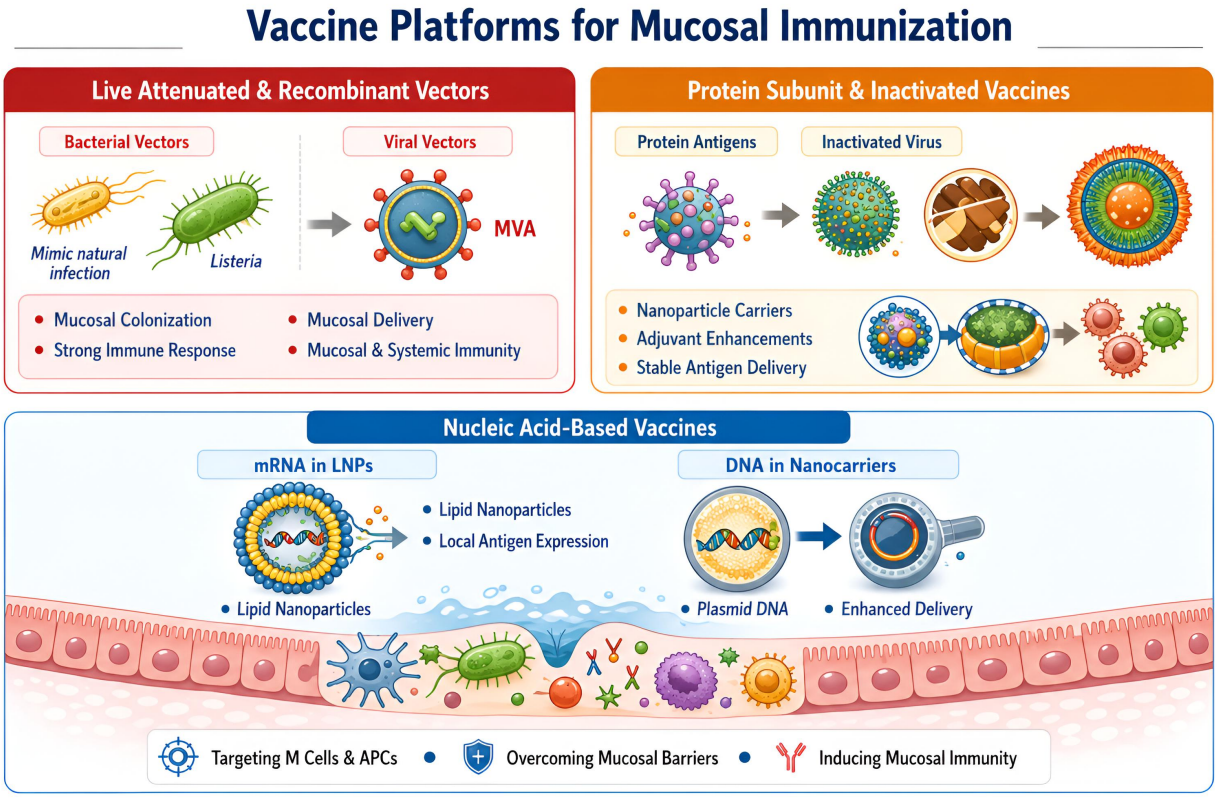



**Figure 1.** Vaccine Platforms for Mucosal Immunization. Schematic overview of live attenuated vectors, protein subunit vaccines with nanoparticle carriers, and nucleic acid-based vaccines utilizing LNPs, highlighting targeting of M cells and APCs to overcome mucus barriers and induce mucosal immunity.

## 6. Mucosal Adjuvants and Immune Modulators

### 6.1 Classical and Novel Mucosal Adjuvants

Classical mucosal adjuvants, such as cholera toxin (CT) derivatives, have been widely studied for their potent ability to enhance immune responses at mucosal sites. CTB subunit, for instance, binds GM1-ganglioside receptors on mucosal epithelial and immune cells, facilitating efficient antigen uptake and presentation. However, the full toxin's inherent toxicity limits its clinical use, leading to the development of genetically detoxified derivatives that retain adjuvanticity while reducing toxicity. These classical adjuvants act primarily by promoting mucosal antigen delivery and stimulating local immune activation, thereby enhancing both mucosal IgA and systemic antibody responses.

Emerging adjuvant systems have increasingly focused on innate immune receptor agonists, particularly TLR agonists, which mimic pathogen-associated molecular patterns to potently activate dendritic cells and other APCs. Examples include non-protein microbial components such as lipopolysaccharides (TLR4 agonists), CpG oligodeoxynucleotides (TLR9 agonists), and small-molecule compounds targeting TLR7/8. These TLR agonists induce APC maturation, pro-inflammatory cytokine production, and robust adaptive immune responses. Their protein nature allows modification and fusion with vaccine antigens, facilitating co-delivery to enhance immunogenicity while minimizing systemic toxicity. Novel, stable TLR2 and TLR8 agonists have been developed that efficiently promote antigen cross-presentation and cytotoxic T-cell activation with reduced inflammatory side effects. Synthetic peptides mimicking TLR4 agonists derived from bacterial lipopolysaccharide are also under investigation to elicit localized immune activation similar to natural adjuvants [88-94].

Nanoparticle platforms incorporating combinations of TLR agonists, such as saponin-based nanoparticles with TLR1/2, TLR4, and TLR7/8 ligands, have been formulated to induce distinct cytokine signatures and tailored T helper responses, enhancing potency against diverse viral vaccines including SARS-CoV-2 and HIV. Co-delivery of dual or multiple TLR agonists in particulate systems has been shown to increase the magnitude and quality of immune responses, promoting durable humoral and cellular immunity. Hyperbranched polymers conjugated with high-density TLR7/8 agonists potentiate type I interferon responses and foster rapid antibody isotype switching towards Th1-biased immunity, desirable against intracellular pathogens [95,96]. These advances illustrate the modular and customizable potential of TLR agonist adjuvants to fine-tune vaccine-induced immunity.

Cytokine-based adjuvants complement these strategies by directly modulating immune cell recruitment, differentiation, and activation at mucosal sites to further amplify vaccine efficacy. Although less detailed in the current context, cytokine adjuvants are recognized for steering immune responses toward desired profiles such as Th1, Th2, or regulatory phenotypes, critical for effective prophylactic and therapeutic vaccination.

A major regulatory concern for intranasal adjuvants is the potential for retrograde transport to the central nervous system (CNS) via the olfactory nerve pathway. This issue gained prominence following reports of Bell's palsy associated with an intranasal inactivated influenza vaccine containing an *Escherichia coli* heat-labile toxin adjuvant (LT) in Switzerland in 2000-2001, which led to withdrawal of the vaccine [97]. Subsequent studies confirmed that certain LT formulations could migrate along olfactory nerves to the olfactory bulb and cause transient facial nerve paralysis. Modern delivery technologies aim to mitigate these risks through nanoparticle encapsulation that prevents direct contact of adjuvants with olfactory epithelium and limits CNS translocation [98]; mucoadhesive formulations that prolong retention at nasal mucosa while restricting diffusion to olfactory neurons [99]; receptor-targeted delivery that directs antigens and adjuvants specifically to M cells or dendritic cells, minimizing off-target exposure [100]; and structure-based detoxification that engineers LT and CT derivatives to retain adjuvanticity while eliminating neurotoxicity, such as dmLT which has shown safety in clinical trials [101]. dmLT (double-mutant heat-labile toxin from *E. coli*) has been evaluated in Phase II trials as an oral adjuvant for ETEC vaccines, demonstrating safety and enhanced mucosal IgA responses [38,39] and is also being explored for intradermal and intranasal delivery with careful safety monitoring.

In summary, classical mucosal adjuvants like CT derivatives enhance mucosal immune responses primarily through targeted antigen delivery and local immune activation but face safety limitations. Emerging adjuvant systems leverage TLR agonists and cytokine-based molecules to safely and specifically potentiate innate and adaptive immunity through well-characterized receptor signaling pathways. Integration of these adjuvants into modern vaccine formulations, often within nanoparticle delivery platforms, with careful attention to safety profiles, offers significant promise for developing potent, safe, and tailored mucosal vaccines.

### 6.2 Nanoparticles and Bioadhesive Systems

Nanoparticles, liposomes, and mucoadhesive polymers enhance antigen stability, targeting, and immune activation at mucosal sites through multiple complementary mechanisms.

Nanoparticles improve antigen stability by protecting antigens from enzymatic degradation and physiological barriers present at mucosal surfaces. Their ability to encapsulate and sustain release of antigens minimizes premature degradation, facilitating prolonged antigen presence in mucosal tissues. Moreover, nanoparticles can mimic the size and surface properties of pathogens, enhancing uptake and processing by APCs, which boosts immune activation. Nanoparticles also promote targeted delivery of antigens and co-encapsulated molecular adjuvants, such as TLR agonists, to critical immune cells, resulting in more effective innate and adaptive immune responses both systemically and at mucosal sites [102,103].

Liposomes, which are phospholipid vesicles formulated at the nanoscale, provide a biocompatible and versatile delivery platform. Due to their structural similarity to cellular membranes, liposomes efficiently encapsulate antigens, enhancing antigen stability against mucosal enzymatic and pH-related degradation. Liposomal nanoparticles can target APCs by size-dependent uptake and surface modifications, promoting processing and presentation of antigens that lead to both humoral and cell-mediated immune responses. Additionally, liposomes can be engineered with ligands for mucosal targeting and can facilitate various administration routes, thereby improving mucosal immune activation [104,105].

Mucoadhesive polymers, such as chitosan, play a critical role in enhancing antigen retention and absorption at mucosal surfaces by adhering to the mucus layer. This adhesion prolongs the residence time of the antigen delivery system at the mucosa, overcoming rapid clearance mechanisms such as mucociliary clearance in the nasal cavity. Furthermore, mucoadhesive polymers protect antigens from degradation, increase local antigen concentration near epithelial and immune cells, and improve permeation across mucosal barriers. By controlling antigen release and facilitating interaction with APCs, these polymers effectively stimulate both mucosal and systemic immune responses. Biodegradable mucoadhesive polymers allow for sustained antigen presentation and provide opportunities for ligand-mediated targeting to epithelial receptors, thereby enhancing uptake and immune activation [106-109].

Clinical examples include chitosan-based nasal vaccines, with several candidates in clinical development for influenza and COVID-19 that leverage mucoadhesive properties to enhance retention [110], and PLGA nanoparticles, which are used in preclinical and early clinical mucosal vaccine studies for sustained antigen release [111].

In summary, nanoparticles and liposomes improve antigen stability by encapsulating and protecting antigens from degradation, enhance targeting by mimicking pathogens and allowing surface functionalization for receptor-mediated uptake, and boost immune activation through efficient delivery to APCs and co-delivery of immunostimulatory agents. Mucoadhesive polymers augment these effects at mucosal sites by prolonging antigen residence time, enhancing mucosal permeation, and enabling controlled release, collectively resulting in robust mucosal and systemic immunity. These biomaterial strategies address critical challenges faced by mucosal vaccines, such as enzymatic degradation, rapid clearance, and inefficient antigen delivery, thus greatly advancing mucosal immunization approaches.

Table 3 provides an overview of mucosal adjuvants and delivery systems, their mechanisms, immunological effects, and clinical status.

**Table 3.** Mucosal adjuvants and delivery systems: Mechanisms, immunological effects, and clinical status [38,39,88,93,97,98,101,110].

| Category | Examples | Primary Function | Mechanism of Action | Immunological Outcome | Clinical Status / Limitations |
|---|---|---|---|---|---|
| Classical mucosal adjuvants | CT, CTB derivatives, dmLT | Enhance antigen uptake and presentation | Bind GM1 receptors on epithelial/immune cells; promote antigen transport to APCs | Increased mucosal IgA and systemic IgG responses | dmLT in Phase II trials (ETEC); CT/CTB not licensed due to toxicity; LT-associated Bell's palsy historical concern |
| TLR agonist adjuvants | CpG (TLR9), LPS mimetics (TLR4), TLR7/8 ligands (imiquimod, resiquimod) | Activate innate immunity | Stimulate dendritic cell maturation and cytokine production via TLR signaling | Strong humoral and cellular immunity (Th1/CTL responses) | Multiple in preclinical/early clinical; TLR4 agonists in licensed parenteral vaccines (e.g., AS04); intranasal safety under evaluation |
| Nanoparticle-based adjuvants | PLGA nanoparticles, saponin–TLR ligand particles | Protect and co-deliver antigen and adjuvant | Encapsulation, sustained release, pathogen-size mimicry for APC uptake | Enhanced antigen stability, potent mucosal and systemic immunity | Preclinical and early clinical; manufacturing complexity; scale-up challenges |
| Cytokine-based adjuvants | IL-12, GM-CSF, IL-1β (experimental) | Modulate immune polarization | Recruit and activate immune cells at mucosal sites; direct Th1/Th2/regulatory responses | Directed immune profiles | Limited clinical translation; safety concerns for systemic cytokine exposure |
| Liposomes | Phospholipid vesicles (e.g., virosomes) | Improve antigen stability and targeting | Membrane fusion and size-dependent uptake by APCs | Balanced antibody and T-cell immunity | Licensed parenteral virosome vaccines (Epaxal, Inflexal V); mucosal formulations in development |
| Mucoadhesive polymers | Chitosan, alginate, carbopol, starch | Prolong mucosal residence and enhance permeation | Adhere to mucus layer; protect antigen from degradation; open tight junctions | Increased mucosal IgA and systemic responses | Chitosan-based nasal vaccines in clinical trials; safety profile favorable; efficacy variable |

As detailed in Table 3, classical mucosal adjuvants such as CT derivatives and dmLT have advanced to clinical trials but face toxicity concerns. TLR agonist adjuvants offer targeted innate immune activation and are being incorporated into nanoparticle formulations. Mucoadhesive polymers like chitosan have entered clinical trials for nasal vaccines, while cytokine-based adjuvants remain largely experimental due to safety concerns.

## 7. Applications to Respiratory Pathogens

### 7.1 Influenza

Mucosal vaccine strategies for influenza have been informed by decades of experience with live attenuated intranasal vaccines. FluMist, licensed in 2003, induces mucosal IgA and systemic IgG, along with lung-resident TRM cells that provide heterosubtypic protection [7,29]. However, efficacy varies by season and age group, with reduced effectiveness against certain pandemic strains and in older adults [112]. Current research focuses on improving formulation stability, broadening strain coverage, and developing universal influenza vaccines targeting conserved epitopes such as hemagglutinin stalk, M2e, and neuraminidase delivered via intranasal or sublingual routes [31,32]. Recent advances include nanoparticle-based hemagglutinin vaccines that induce cross-reactive mucosal immunity in preclinical models [49].

### 7.2 SARS-CoV-2 and COVID-19

The COVID-19 pandemic accelerated mucosal vaccine development, with multiple candidates advancing to clinical trials. Mucosal vaccines administered intranasally or orally aim to generate mucosal IgA and TRM cells in the respiratory tract, the primary site of infection, to enhance protective immunity beyond systemic vaccination.

In preclinical studies, an intramuscularly primed/intranasally boosted vaccine with spike protein subunits and adjuvants including IL-15 and TLR agonists in a rhesus macaque model elicited mucosal IgA and interferon-alpha responses and achieved full protection against SARS-CoV-2 replication in upper and lower respiratory tracts, highlighting the complementarity of mucosal boosting to systemic priming [113]. Intranasal vaccines based on live attenuated virus vectors, such as influenza virus vectors expressing the SARS-CoV-2 receptor-binding domain (RBD), demonstrated rapid and broad protection in preclinical hamster models, inducing local innate immunity, mucosal IgA and IgG, and lung-resident T cell responses, and also showed cross-protection against influenza viruses [114,115]. Adenoviral vector-based intranasal vaccines expressing multivalent SARS-CoV-2 antigens elicited superior mucosal TRM cells, mucosal trained innate immunity, and systemic antibodies compared to intramuscular vaccines, protecting against ancestral and variant strains including B.1.1.7 and B.1.351 [116]. Combination mucosal vaccines targeting both SARS-CoV-2 and influenza also induced potent mucosal and systemic humoral and cellular immunity and showed efficacy in preclinical models, suggesting potential for broader protection.

Licensed mucosal COVID-19 vaccines include iNCOVACC (CanSino Biologics), an inhaled adenovirus type 5 vectored vaccine approved in China in September 2022 and in India in 2022 for use as a booster, which induces mucosal IgA and systemic immunity. RBD-Hawkeye (Beijing Wantai Biological Pharmacy), an intranasal live attenuated influenza virus vectored vaccine expressing SARS-CoV-2 RBD, was approved in China in 2022. Multiple mucosal COVID-19 vaccines are in Phase I-III trials globally, including intranasal adenoviral vectors, live attenuated SARS-CoV-2, and mRNA formulations exploring mucosal delivery.

### 7.3 Respiratory Syncytial Virus

RSV is a leading cause of lower respiratory tract infection in infants and older adults, with no licensed mucosal vaccine to date. However, significant progress has been made in understanding mucosal immune correlates, with lung TRM cells essential for clearance upon re-exposure and nasal IgA correlating with reduced infection risk [33,34].

Clinical development of mucosal RSV vaccines includes several candidates advancing to Phase III, such as live attenuated intranasal vaccines like Sanofi/GSK's RSVt vaccine, which showed efficacy in preventing RSV-associated lower respiratory tract disease in Phase IIb trials, and adenoviral vector-based formulations [117]. While maternal immunization with parenteral RSV vaccines such as Pfizer's Abrysvo protects infants via transplacental antibody transfer, mucosal vaccines could provide direct infant protection [118]. The history of enhanced respiratory disease with formalin-inactivated RSV vaccines in the 1960s necessitates careful safety evaluation for mucosal formulations [119].

### 7.4 Tuberculosis

Mucosal immunization approaches for tuberculosis focus on developing vaccines capable of inducing robust mucosal immunity at the portal of entry. Tuberculosis vaccines delivered mucosally aim to stimulate mucosal IgA, TRM cells, and trained innate immunity to prevent *Mycobacterium tuberculosis* infection or progression. Immune correlates of protection for tuberculosis include mucosal TRM CD4+ and CD8+ T cells producing IFN-γ and TNF, mucosal IgA antibodies, and trained innate responses that confer long-term protection at lung mucosa. These correlates guide the design of mucosal vaccine candidates employing live attenuated mycobacteria, viral vectors, or protein subunits combined with adjuvants to induce both mucosal and systemic immunity [25,26,35].

Clinical examples include MTBVAC, a live attenuated *M. tuberculosis* vaccine in Phase II trials exploring aerosol delivery [120]; ChAdOx1 85A/MVA85A, viral vectored vaccines administered via aerosol in Phase I trials showing safety and immunogenicity [121]; and VPM1002, a recombinant BCG vaccine in Phase III trials with potential for mucosal administration [122].

For emerging respiratory pathogens, mucosal vaccines aim to mimic natural infection routes to induce local neutralizing antibodies and T cell responses that limit early viral replication and transmission. Correlates of protection include antigen-specific mucosal IgA, local memory T cells capable of rapid effector function, and trained innate immunity that augments antigen presentation and cytokine milieu. Mucosal delivery platforms such as adenoviral vectors, live attenuated vaccines, and nanoparticle-based formulations are being investigated preclinically for these properties, with the goal to achieve broad-spectrum and durable airway immune protection.

In summary, mucosal vaccine strategies for influenza and SARS-CoV-2 have advanced through preclinical and clinical development, demonstrating the ability to induce protective mucosal and systemic immunity. Lessons from these efforts inform tuberculosis and emerging respiratory pathogen vaccine designs that focus on mucosal immune correlates of protection, notably mucosal IgA, TRM cells, and trained innate responses, to achieve effective local defense at respiratory mucosal sites. Continued clinical evaluation will clarify the translational potential of mucosal vaccines for controlling respiratory infectious diseases globally.

## 8. Applications to Enteric Pathogens

### 8.1 Viral Enteric Pathogens

Rotavirus remains the leading cause of severe gastroenteritis in children under 5 years worldwide. Two live attenuated oral vaccines, Rotarix (monovalent, GSK) and RotaTeq (pentavalent, Merck), were licensed in 2006-2008 and have dramatically reduced rotavirus hospitalizations and deaths in high-income countries [6,36]. However, efficacy is lower at 50-70% in low- and middle-income countries (LMICs) due to factors including maternal antibodies, breast milk interference, malnutrition, co-infections, and differences in gut microbiota [123]. Rotarix efficacy correlates with intestinal IgA responses, which are lower in infants in LMICs [37]. Next-generation rotavirus vaccines aim to improve thermostability through vaccines such as Rotavac and Rotasiil licensed in India and explore neonatal administration to bypass maternal antibody interference [124].

Enteric coronaviruses, while SARS-CoV-2 primarily causes respiratory disease, can also infect the gastrointestinal tract with viral RNA detected in stools and potential for fecal-oral transmission [125]. Mucosal vaccines inducing intestinal immunity could theoretically reduce enteric replication and transmission, though data are limited. Porcine epidemic diarrhea virus (PEDV), a coronavirus causing severe enteric disease in pigs, has been targeted with oral vaccines, providing proof-of-concept for enteric coronavirus vaccines [126].

Norovirus has no licensed vaccine, but virus-like particle vaccines administered intranasally or orally are in clinical development, showing induction of systemic and mucosal antibodies [127].

### 8.2 Bacterial Enteric Pathogens

Mucosal vaccine platforms targeting major bacterial enteric pathogens employ diverse strategies to induce protective immune responses at the intestinal mucosa, the primary site of pathogen entry. These platforms include live attenuated vaccines, inactivated/killed vaccines, recombinant protein subunits, bacterial vector vaccines, and novel delivery systems such as spores and multiepitope fusion antigens. They aim to overcome the challenges posed by rapid antigen degradation in the gastrointestinal tract, mucosal barrier permeability, and pathogen heterogeneity.

Effectiveness of mucosal vaccines against enteric pathogens hinges on their ability to elicit local secretory IgA, systemic IgG, and robust cellular immunity. Live attenuated vaccines are particularly promising as they mimic natural infection, promoting mucosal immune responses; however, safety and reactogenicity remain concerns. Inactivated and subunit vaccines provide better safety profiles but often require potent mucosal adjuvants to enhance immunogenicity. Delivery platforms such as spores and bacterial vectors can act as both antigen carriers and adjuvants, augmenting mucosal immunity. The main limitations across platforms include variable efficacy in young children, complexity of inducing broad protective immunity against heterogeneous pathogen strains, logistical challenges of oral vaccine delivery, and lack of well-defined immune correlates of protection for many enteric diseases [128-130].

#### 8.2.1 *Shigella*

*Shigella* is a major cause of diarrheal disease in children in LMICs and a leading etiology of moderate-to-severe diarrhea. Vaccine development has extensively investigated live attenuated strains engineered to delete virulence-associated genes, yielding several candidates with promising immunogenicity and safety data in human trials. Advances in lambda red recombineering have enabled precise genomic editing of *Shigella* to create stable, attenuated vaccine strains [131]. Novel approaches include engineering *Shigella* strains to express ETEC antigens, thereby providing multivalent protection against both major enteric pathogens, with the goal to generate vaccines that induce broad

mucosal immunity targeting both pathogens simultaneously. Clinical candidates include CVD 1208S, a live attenuated *S. flexneri* vaccine evaluated in Phase II trials [132]; InvaplexAR, a detoxified *Shigella* subunit vaccine delivered intranasally in Phase I/II [133]; and Shigella-ETEC combination vaccines in preclinical development.

### 8.2.2 Enterotoxigenic *E. coli* (ETEC)

ETEC is a leading cause of travelers' diarrhea and childhood diarrhea in LMICs. Vaccine efforts focus on stimulating protective immunity against bacterial colonization factors and enterotoxins including heat-labile toxin LT and heat-stable toxin ST. Vaccines include parenterally administered recombinant antigens combined with TLR agonist adjuvants, such as the TLR4 agonist SLA-SE, which can also induce functional mucosal antibodies and intestinal IgA despite systemic delivery. Traditional mucosal adjuvants like dmLT enhance mucosal immune responses in oral vaccines, highlighting the importance of adjuvant selection [38,39]. The limited availability of licensed vaccines for ETEC underscores the need for improved mucosal immunization platforms. Multi-antigen vaccines targeting both colonization and toxin antigens seek to synergistically enhance protective efficacy. Clinical candidates include ETVAX, an oral inactivated whole-cell vaccine with dmLT adjuvant in Phase II/III trials showing safety and immunogenicity in infants and adults; ACE527, a live attenuated *E. coli* expressing ETEC antigens evaluated in Phase I/II [134]; and dmLT adjuvant used in multiple ETEC vaccine trials to enhance mucosal IgA [39].

### 8.2.3 *Campylobacter*

*Campylobacter* is a leading cause of bacterial gastroenteritis worldwide and associated with post-infectious sequelae including Guillain-Barré syndrome. Vaccine development has been slower, partly due to limited understanding of immune correlates and pathogen variability. Novel mucosal delivery strategies are exploring the use of biodegradable spores, such as *Bacillus subtilis* spores conjugated with *Campylobacter* antigens, that enable antigen presentation at mucosal surfaces and function as adjuvants [135]. Intranasal and oral spore-based vaccines have induced antigen-specific systemic and mucosal IgA responses and shown bactericidal activity in animal models, indicating potential for prophylactic immunity. The development of multivalent vaccines combining major antigens like leukotoxin and outer membrane proteins aims to provide broad protection. Despite promising preclinical data, *Campylobacter* mucosal vaccines face challenges in translation to clinical efficacy. Clinical candidates include CJCV1, an inactivated whole-cell *C. jejuni* vaccine with dmLT adjuvant in Phase I trials [136], and spore-based vaccines in preclinical development [135].

In summary, mucosal vaccine platforms for enteric pathogens such as rotavirus, *Shigella*, ETEC, and *Campylobacter* actively pursue approaches that enhance mucosal antigen delivery, stability, and immune activation. While live attenuated vaccines demonstrate strong immunogenicity, safety concerns and strain heterogeneity drive development of multivalent, subunit, and vector-based vaccines incorporating innovative adjuvants and delivery systems like spores. Effectiveness varies across platforms, with limitations including variable pediatric efficacy, incomplete protection across diverse strains, and challenges in inducing durable mucosal immunity. Continued advances in biotechnology, immunology, and genetic engineering hold promise for improving efficacy and expanding availability of mucosal vaccines against these major enteric pathogens.

## 9. Barriers and Challenges in Mucosal Vaccine Development

Mucosal vaccines face significant scientific and practical challenges that must be addressed to enhance their effectiveness and facilitate widespread adoption.

### 9.1 Immunological Barriers

Mucosal tolerance presents a major immunological challenge, as the mucosal immune system is designed to maintain homeostasis by tolerating a vast array of commensal microbes and environmental antigens, which can dampen the immune activation elicited by vaccine antigens delivered to mucosal surfaces. Overcoming this requires potent mucosal adjuvants and delivery systems that specifically target APCs like dendritic cells and M cells to effectively prime immune responses without inducing adverse inflammation. Mechanistically, mucosal tolerance is maintained through regulatory T cell (Treg) responses, where oral antigen exposure induces FoxP3+ Tregs that suppress effector T cell responses via IL-10 and TGF-β [137]; tolerogenic dendritic cells, particularly intestinal CD103+ dendritic cells that promote Treg differentiation and are conditioned by epithelial cell-derived factors such as TGF-β and retinoic acid [138]; the cytokine milieu, with high local concentrations of IL-10 and TGF-β in the gut mucosa favoring regulatory over inflammatory responses [139]; and oral tolerance mechanisms, where feeding of antigens leads to systemic unresponsiveness mediated by Tregs and anergy, which vaccines must bypass [140].

Enzymatic degradation of vaccine antigens by proteases and acidic pH in mucosal secretions compromises antigen stability and bioavailability, limiting immune stimulation, so formulations must therefore protect antigens via encapsulation or stabilization strategies to preserve immunogenicity [141,142]. Variability in individual mucosal immune responses, influenced by factors such as age, microbiota composition, nutritional status, and mucosal tissue heterogeneity, complicates dose optimization and consistency of protection, and determining effective dosing regimens

remains challenging as the relationship between antigen dose, mucosal immunogenicity, and systemic responses is not fully elucidated [142,143].

### 9.2 Practical and Manufacturing Challenges

Vaccine stability is another critical concern, as many mucosal vaccines, especially those using live attenuated or protein subunit components, require stringent cold-chain storage typically at 2-8°C to preserve antigen integrity, posing logistical barriers in resource-limited settings. Advances in thermostable formulations employing excipients, nanoencapsulation, and drying technologies such as lyophilization have improved storage resilience, enabling ambient temperature stability and reducing cold-chain dependency, developments that are crucial for global immunization efforts particularly in low-income regions [144,145].

Large-scale manufacturing of mucosal vaccines poses complexities due to the need for specialized production processes, stringent quality control of biological components, and challenges in scaling nanoparticle or vector-based delivery systems with consistent efficacy. Supply chain constraints, including sourcing quality raw materials and production capacity stresses observed during COVID-19 vaccine development, further underscore these challenges [143].

### 9.3 Safety Concerns

From a safety perspective, intranasal vaccines face concerns about potential neuroinvasion, as mucosal delivery vectors or antigens may theoretically access the CNS via the olfactory epithelium. However, studies with intranasal replication-defective adenoviral vectors show minimal dissemination to brain regions, suggesting low neuroinvasion risk, though careful evaluation remains essential for regulatory approval [146]. The historical association of LT-adjuvanted intranasal influenza vaccine with Bell's palsy underscores the need for rigorous preclinical neurotoxicity assessment [97].

Regulatory challenges for COVID-19 mucosal vaccines during their rapid development and conditional approval, such as iNCOVACC in China and India, highlighted pathways for novel delivery routes. These challenges included the lack of established correlates of mucosal protection, requiring regulators to accept demonstration of both systemic and mucosal immune responses without validated surrogate markers [87]; manufacturing consistency for novel platforms, with inhaled powder formulations requiring new quality control standards; and post-marketing safety surveillance to monitor for rare adverse events such as pulmonary toxicity or Bell's palsy in large populations post-approval.

Regulatory hurdles for mucosal vaccines are notable, as the novelty of mucosal delivery routes necessitates comprehensive safety and efficacy data including characterization of local and systemic immune responses, potential off-target effects, and long-term outcomes. Regulators require well-defined correlates of mucosal protection, which are often lacking or incomplete, complicating the approval process, and manufacturing consistency, cold-chain logistics, and device compatibility must meet stringent standards to gain licensure [141,143].

### 9.4 The Translational Gap: Preclinical Models to Human Trials

One of the most significant challenges in mucosal vaccine development is the gap between successful preclinical results and limited success in human clinical trials. Key factors contributing to this gap include differences between animal and human mucosal immune systems, as murine models lack NALT constitutively and have different M cell distribution and TLR expression patterns compared to humans [147], while non-human primates are more predictive but expensive and limited by genetic diversity and husbandry conditions [148]. Human intestinal organoids represent emerging models that better recapitulate human mucosal physiology but lack full immune complexity [149].

Variability in mucosal microbiota influences baseline immune activation and vaccine responses, with differences in microbiota composition between animal facilities and human populations contributing to variable outcomes [150]. Mucus barrier properties differ as human mucus has distinct thickness, composition, and turnover rates compared to animal models, affecting antigen penetration and vaccine retention [151]. Limitations of murine models for predicting efficacy are exemplified by vaccines that worked in mice but failed in humans, including several oral ETEC and *Shigella* candidates, highlighting the need for better predictive models [130,152].

Table 4 summarizes selected clinical outcomes of mucosal vaccine candidates, highlighting successes and failures across different pathogens.

**Table 4.** Summary of successful vs. unsuccessful clinical mucosal vaccine candidates.

| Pathogen | Candidate | Platform | Route | Clinical Stage | Outcome/Limitation | References |
|---|---|---|---|---|---|---|
| *Influenza* | FluMist | Live attenuated | Intranasal | Licensed (2003) | Effective in children; variable efficacy in adults; reduced effectiveness against pandemic strains | [7,112] |
| *Rotavirus* | Rotarix/RotaTeq | Live attenuated | Oral | Licensed (2006-2008) | 80-90% efficacy in high-income countries; 50-70% in LMICs | [6,36,123] |
| *Cholera* | Dukoral | Killed whole-cell + CTB | Oral | Licensed (1991) | 60-80% protection for 2-3 years; requires buffer; limited use in young children | [8,57] |
| *Polio* | OPV | Live attenuated | Oral | Licensed (1961) | Induces intestinal immunity; risk of VDPV; replaced by IPV in many countries | [5,56] |
| *COVID-19* | iNCOVACC | Adenoviral vector | Inhaled | Licensed (2022, China/India) | Safe and immunogenic as booster; efficacy data limited post-licensure | [87] |
| *ETEC* | ETVAX | Inactivated whole-cell + dmLT | Oral | Phase II/III | Safe, immunogenic; efficacy data pending | _ |
| *Shigella* | CVD 1208S | Live attenuated | Oral | Phase II | Immunogenic but reactogenicity concerns | [132] |
| *RSV* | RSVt vaccine | Live attenuated | Intranasal | Phase IIb | Reduced LRTD in infants; advanced to Phase III | [117] |
| *Tuberculosis* | MVA85A | Viral vector | Aerosol | Phase IIb | Failed to show efficacy in infants despite immunogenicity | [148] |
| *HIV* | Various | Multiple | Oral/intranasal | Multiple failed | No efficacy in human trials; mucosal challenges underestimated | [149] |

The data in Table 4 illustrate the translational gap in mucosal vaccine development. While licensed vaccines exist for influenza (FluMist), rotavirus (Rotarix/RotaTeq), cholera (Dukoral), polio (OPV), and COVID-19 (iNCOVACC), several promising candidates (e.g., MVA85A for tuberculosis, various HIV mucosal vaccines) have failed in human trials despite preclinical success. ETVAX (ETEC) and CVD 1208S (*Shigella*) are progressing through clinical stages but face efficacy and reactogenicity challenges, respectively.

In summary, mucosal vaccines must overcome immunological barriers such as mucosal tolerance and enzymatic degradation while addressing variability in mucosal responses. Practical challenges include enhancing antigen stability, optimizing dosing, reducing cold-chain dependence through thermostable formulations, and scaling up manufacturing processes. Safety concerns, particularly neuroinvasion risks for intranasal vaccines, necessitate thorough investigation. The translational gap between preclinical success and human efficacy demands improved animal models and better understanding of human mucosal immunology. Finally, regulatory pathways require extensive data to ensure safety, efficacy, and manufacturing quality, demanding focused efforts to streamline mucosal vaccine development and approval for broader public health impact.

## 10. Opportunities for Innovation and Integration

### 10.1 mRNA Technology

mRNA vaccines provide a flexible and rapid platform to generate potent immune responses by encoding pathogen-specific antigens for in situ protein expression. Their adaptability allows rapid redesign against emerging variants and pathogens, as exemplified during the COVID-19 pandemic. However, mucosal delivery of mRNA vaccines remains challenging due to the need for optimized formulations that protect mRNA from degradation and enable efficient uptake at mucosal surfaces [153-155].

Recent advances from 2023 to 2026 include LNPs optimized for aerosol delivery through PEG-lipid content modulation that enhances stability during nebulization while maintaining transfection efficiency [81]; self-amplifying mRNA

(saRNA) platforms that require lower doses and induce longer-lasting expression, now being explored for intranasal delivery [156,157]; and thermostable mRNA formulations using lyophilized mRNA vaccines stable at ambient temperature for months, facilitating distribution in LMICs.

### 10.2 Synthetic Biology

Synthetic biology advances facilitate precise engineering of vaccine components and delivery systems. Platforms like bacteriophage T4-based nanoparticles allow high-density antigen display and intrinsic adjuvant properties, enabling needle-free, intranasal delivery that induces robust humoral, cellular, and mucosal immunity including sterilizing mucosal protection, while supporting scalable and stable vaccine manufacturing accessible to low- and middle-income countries [158,159]. Moreover, modular nanoparticle approaches such as layer-by-layer antigen presentation engineered through synthetic biology allow multivalent mucosal vaccines that stimulate broad and durable protective immunity.

### 10.3 Artificial Intelligence-Driven Vaccine Design

Artificial intelligence (AI) enhances mucosal vaccine design by optimizing epitope prediction, antigen selection, and vaccine construct design through deep learning models integrating multi-omics data. AI accelerates RNA secondary structure prediction, delivery vehicle formulation, and stability assessments, increasing efficacy and reducing development time.

AI applications in mucosal vaccine development from 2023 to 2026 include epitope prediction for mucosal antigens using models such as NetMHCpan and MARIA that predict MHC class I and II epitopes for respiratory and enteric pathogens, enabling design of multiepitope vaccines targeting conserved regions; mucosal antigen stability prediction through deep learning algorithms that predict proteolytic cleavage sites in gastrointestinal and respiratory environments, guiding antigen engineering to enhance stability; LNP formulation optimization using machine learning that screens lipid compositions for mucosal mRNA delivery, predicting transfection efficiency and toxicity; structure-based antigen design with AlphaFold and RoseTTAFold predicting protein structures for rational design of stable vaccine antigens such as SARS-CoV-2 RBD ferritin nanoparticles; and personalized vaccine design where AI integrates host genomics, microbiome data, and immune profiles to tailor mucosal vaccines for populations or individuals [160,161].

Although AI models face challenges of explainability and biological interpretation, hybrid AI-traditional experimental workflows promise to refine vaccine personalization and population-level effectiveness [162,163].

### 10.4 Impact on Transmission and Herd Immunity

Mucosal vaccines uniquely reduce pathogen transmission by inducing local immune effectors such as secretory IgA and TRM cells at initial points of infection – respiratory, gastrointestinal, or urogenital mucosae – thereby neutralizing pathogens before systemic dissemination or shedding. This local interception decreases viral or bacterial load, reducing spread within communities. Consequently, mucosal immunization can substantially contribute to herd immunity by lowering the basic reproduction number (R0) and interrupting transmission chains, critical in controlling pandemics. Achieving sterilizing immunity at mucosal surfaces may prevent asymptomatic carriage and breakthrough infections observed with systemic vaccines.

Real-world evidence demonstrates that OPV interrupted transmission in endemic regions through intestinal immunity, whereas IPV (parenteral) prevents disease but not shedding [5]; intranasal influenza vaccines reduce shedding in animal models and human challenge studies [164]; and iNCOVACC (inhaled COVID-19 booster) reduced breakthrough infections compared to intramuscular booster in observational studies. Next-generation mucosal vaccines, enabled by mRNA, synthetic biology, and AI, are poised to provide rapid, scalable, and durable mucosal immunity needed to suppress future pandemics at their portal of entry.

In summary, integration of mRNA technology with synthetic biology platforms and AI-driven computational design accelerates the development of safe, effective, and scalable mucosal vaccines. By fostering potent mucosal immune responses that block transmission, these vaccines hold promise for enhancing herd immunity and pandemic preparedness, ultimately transforming infectious disease control worldwide.

## 11. Future Directions and Translational Outlook

### 11.1 Research Priorities

Future research priorities in mucosal vaccine development focus on several key areas to address current limitations and maximize global health impact. The development of universal mucosal vaccines that provide broad and durable protection against diverse strains or species of pathogens, particularly respiratory and enteric viruses and bacteria, can be achieved by exploiting conserved antigens such as influenza hemagglutinin stalk, SARS-CoV-2 RBD conserved regions, and bacterial surface proteins, and by designing multivalent formulations capable of stimulating both mucosal and systemic immunity to enhance cross-protection and reduce the need for frequent reformulation.

Combined respiratory-enteric vaccines represent another important area given the epidemiological overlap and co-infection burden of respiratory and enteric infections, as such combination vaccines could improve compliance, reduce costs, and strengthen population immunity through coordinated induction of protective mucosal responses across multiple portals of pathogen entry. A real-world example includes a combined COVID-19 plus influenza mucosal vaccine in preclinical development, aiming to induce both respiratory mucosal immunity against SARS-CoV-2 and influenza while potentially providing enteric protection against gastrointestinal manifestations, and combination oral vaccines targeting multiple enteric pathogens such as Shigella-ETEC are advancing.

Personalized mucosal immunization represents an emerging frontier leveraging individual variations in mucosal immunity, microbiota, and genetic factors to tailor vaccine formulations, dosing, and delivery routes for enhanced efficacy, with advances in systems biology and immunoprofiling guiding development of individualized vaccines that maximize protective responses while minimizing adverse events [9].

### 11.2 Translational Pathways

The translational potential of mucosal vaccine platforms is strong, fueled by advances in antigen design, delivery technologies, and adjuvants that enable more effective mucosal targeting and immune stimulation. Recent successes with inhaled adenoviral vectors for COVID-19 vaccines and novel adenoviral tuberculosis vaccines demonstrating superior protection via mucosal administration exemplify this progress, and noninvasive administration modalities also improve vaccine acceptance and access globally [11]. Key translational steps include improved animal models such as humanized mice, non-human primates, and organ-on-chip systems that better predict human responses [147,149]; controlled human infection models (CHIMs) for enteric pathogens such as ETEC, *Shigella*, and *Campylobacter* and for respiratory viruses including influenza, RSV, and SARS-CoV-2, which accelerate efficacy testing and correlate identification [35]; and biomarker and correlate discovery through systems biology approaches that identify transcriptional and proteomic signatures of protective mucosal immunity.

### 11.3 Global Health Impact

The global health impact of mucosal vaccines can be profound, particularly in low- and middle-income countries burdened by respiratory and enteric infectious diseases. Mucosal vaccines directly protect at the pathogen entry sites, potentially halting transmission and disease establishment more efficiently than systemic vaccines alone. Their needle-free delivery and reduced cold chain requirements can facilitate mass immunization programs with improved compliance and equity. As mucosal vaccines advance, integration into global immunization schedules could substantially reduce morbidity, mortality, and economic costs associated with infectious diseases worldwide [9]. Priority targets for LMICs include improved oral rotavirus vaccines with higher efficacy, first licensed vaccines for ETEC and *Shigella*, mucosal boosters for BCG-primed populations against tuberculosis, and affordable intranasal or inhaled COVID-19 boosters for global access.

In conclusion, future research should prioritize universal and combination mucosal vaccines, personalized vaccination approaches, and innovative delivery and adjuvant technologies that enhance mucosal immunogenicity and safety. These efforts will accelerate translational pathways and realize the transformative potential of mucosal vaccine platforms to improve infectious disease control and global health equity.

## 12. Conclusion

Mucosal vaccine platforms offer a transformative approach to controlling respiratory and enteric infections by generating protective immunity precisely at the sites of pathogen entry, inducing secretory IgA, TRM cells, and coordinated systemic responses that conventional injectable vaccines rarely achieve effectively. Needle-free delivery routes, combined with advanced nanoparticle, liposomal, and bioadhesive technologies, improve patient acceptance, enable mass immunization, and support rapid deployment during outbreaks and pandemics.

Despite these advantages, development faces persistent hurdles including antigen instability and rapid clearance in mucosal environments, limited availability of safe and potent adjuvants, inter-individual immunological variability, incomplete definition of mucosal correlates of protection, manufacturing complexity, thermostability challenges, and equitable global distribution barriers. The translational gap between preclinical success and human efficacy demands improved animal models, better understanding of human mucosal immunology, and rigorous clinical evaluation.

The defining strength of mucosal vaccination lies in its unique capacity to interrupt pathogen transmission at the portal of entry, thereby reducing viral and bacterial shedding, limiting spread, strengthening herd immunity, and providing a foundation for controlling both seasonal epidemics and future pandemics more effectively than systemic immunity alone. Licensed mucosal vaccines – including oral polio, rotavirus, and cholera vaccines, and intranasal influenza and COVID-19 vaccines – have already demonstrated feasibility and impact, paving the way for next-generation platforms.

To realize this potential, the field should prioritize discovery and optimization of safe, potent mucosal adjuvants and delivery systems such as nanoparticles, liposomes, hydrogels, and mucoadhesives to enhance antigen stability, targeting, and immunogenicity. Deeper elucidation of mucosal immune mechanisms and reliable correlates of protection is needed

to guide rational, predictive vaccine design. Integration of mRNA, synthetic biology, and multivalent platforms can create thermostable, broadly protective, and rapidly adaptable mucosal vaccines. Formulation refinement must overcome mucosal barriers, improve bioavailability, and ensure durable responses. Rigorous safety profiling, especially for intranasal routes, is essential to address risks such as neuroinvasion or excessive inflammation. Investment in scalable, thermostable manufacturing and equitable global supply chains will ensure access in low- and middle-income settings. Leveraging AI and computational design for epitope prediction, antigen engineering, and formulation optimization will accelerate development.

Ultimately, mucosal vaccines represent one of the most promising frontiers in modern vaccinology. Overcoming current immunological, technological, and logistical obstacles through sustained multidisciplinary collaboration and strategic support will unlock their capacity to substantially reduce the global burden of respiratory and enteric infectious diseases and markedly strengthen pandemic preparedness and health equity worldwide.

## Conflict of Interest

The author declares no conflict of interest.

## Generative AI Statement

The authors declare that no generative artificial intelligence (AI) tools were used in the writing, analysis, or preparation of this manuscript.

## References


[1] Ecarnot F, Maggi S. Vaccination against respiratory infections in the immunosenescent older adult population: Challenges and opportunities. Seminars in Respiratory and Critical Care Medicine, 2025, 46(1), 53-62. DOI: 10.1055/a-2500-2121

[2] Torres A, Cilloniz C, Aldea M, Mena G, Miró JM, Trilla A, et al. Adult vaccinations against respiratory infections. Expert Review of Anti-infective Therapy, 2025, 23(2-4), 135-147. DOI: 10.1080/14787210.2025.2457464

[3] Chattha KS, Roth JA, Saif LJ. Strategies for design and application of enteric viral vaccines. Annual Review of Animal Biosciences, 2014, 3(1), 375-395. DOI: 10.1146/annurev-animal-022114-111038

[4] Sunagar R, Singh A, Kumar S. SARS-CoV-2: Immunity, challenges with current vaccines, and a novel perspective on mucosal vaccines. Vaccines, 2023, 11(4), 849. DOI: 10.3390/vaccines11040849

[5] Sutter RW, Kew OM, Cochi SL. Poliovirus vaccine – live. Elsevier, 2018, 866-917. DOI: 10.1016/B978-0-323-35761-6.00048-1

[6] Burnett E, Parashar UD, Tate JE. Rotavirus vaccines: Effectiveness, safety, and future directions. Pediatric Drugs, 2018, 20(3), 223-233. DOI: 10.1007/s40272-018-0283-3

[7] Carter NJ, Curran MP. Live attenuated influenza vaccine (FluMist®; Fluenz™): A review of its use in the prevention of seasonal influenza in children and adults. Drugs, 2011, 71(12), 1591-622. DOI: 10.2165/11206860-000000000-00000

[8] Clemens JD, Sack DA, Harris JR, Van Loon F, Chakraborty J, Ahmed F, et al. Field trial of oral cholera vaccines in Bangladesh: Results from three-year follow-up. Lancet, 1990, 335(8684), 270-273. DOI: 10.1016/0140-6736(90)90080-O

[9] Anggraeni R, Ana ID, Wihadmadyatami H. Development of mucosal vaccine delivery: An overview on the mucosal vaccines and their adjuvants. Clinical and Experimental Vaccine Research, 2022, 11(3), 235-248. DOI: 10.7774/cevr.2022.11.3.235

[10] Nian X, Zhang J, Huang S, Duan K, Li X, Yang X. Development of nasal vaccines and the associated challenges. Pharmaceutics, 2022, 14(10), 1983. DOI: 10.3390/pharmaceutics14101983

[11] Wang J, Thorson L, Stokes RW, Santosuosso M, Huygen K, Zganiacz A, et al. Single mucosal, but not parenteral, immunization with recombinant adenoviral-based vaccine provides potent protection from pulmonary tuberculosis. Journal of Immunology, 2004, 173(10), 6357-6365. DOI: 10.4049/jimmunol.173.10.6357

[12] Cesta MF. Normal structure, function, and histology of mucosa-associated lymphoid tissue. Toxicologic Pathology, 2006, 34(5), 599-608. DOI: 10.1080/01926230600865531

[13] Jang MH, Kweon M-N, Iwatani K, Yamamoto M, Terahara K, Sasakawa C, et al. Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proceedings of the National Academy of Sciences, 2004, 101(16), 6110-6115. DOI: 10.1073/pnas.0400969101

[14] Kuper CF. Histopathology of mucosa-associated lymphoid tissue. Toxicologic Pathology, 2006, 34(5), 609-615. DOI: 10.1080/01926230600867735

[15] Tschernig T, Pabst R. Bronchus-associated lymphoid tissue (BALT) is not present in the normal adult lung but in different diseases. Pathobiology, 2000, 68(1), 1-8. DOI: 10.1159/000028109

[16] Randall TD. Bronchus-associated lymphoid tissue (BALT) structure and function. Advances in Immunology, 2010, 107, 187-241. DOI: 10.1016/B978-0-12-381300-8.00007-1

[17] Yuki Y, Kiyono H. New generation of mucosal adjuvants for the induction of protective immunity. Reviews in Medical Virology, 2003, 13(5), 293-310. DOI: 10.1002/rmv.398

[18] Nochi T, Kiyono H. Innate immunity in the mucosal immune system. Current Pharmaceutical Design, 2006, 12(32), 4203-4213. DOI: 10.2174/138161206778743457

[19] Oh JE, Song E, Moriyama M, Wong P, Zhang S, Jiang R, et al. Intranasal priming induces local lung-resident B cell populations that secrete protective mucosal antiviral IgA. Science Immunology, 2021, 6(66), eabj5129. DOI: 10.1126/sciimmunol.abj5129

[20] Cheon IS, Son YM, Sun J. Tissue-resident memory T cells and lung immunopathology. Immunological Reviews, 2023, 316(1), 63-83. DOI: 10.1111/imr.13201

[21] Luangrath MA, Schmidt ME, Hartwig SM, Varga SM. Tissue-resident memory T cells in the lungs protect against acute respiratory syncytial virus infection. ImmunoHorizons, 2021, 5(2), 59-69. DOI: 10.4049/immunohorizons.2000067
[22] Wilk MM, Misiak A, Mcmanus RM, Allen AC, Lynch MA, Mills KHG. Lung CD4 tissue-resident memory T cells mediate adaptive immunity induced by previous infection of mice with Bordetella pertussis. Journal of Immunology, 2017, 199(1), 233-243. DOI: 10.4049/jimmunol.1602051
[23] Xie D, Lu G, Mai G, Guo Q, Xu G. Tissue-resident memory T cells in diseases and therapeutic strategies. MedComm, 2025, 6(1), e70053. DOI: 10.1002/mco2.70053
[24] Zens KD, Chen JK, Farber DL. Vaccine-generated lung tissue-resident memory T cells provide heterosubtypic protection to influenza infection. JCI Insight, 2016, 1(10), e85832. DOI: 10.1172/jci.insight.85832
[25] Nathan A, Asgari S, Ishigaki K, Valencia C, Amariuta T, Luo Y, et al. Single cell eQTL models reveal dynamic T cell state dependence of disease loci. Nature, 2022, 606(7912), 120-128. DOI: 10.1038/s41586-022-04713-1
[26] Ogongo P, Porterfield JZ, Leslie A. Lung tissue resident memory T cells in the immune response to Mycobacterium tuberculosis. Frontiers in Immunology, 2019, 10, 992. DOI: 10.3389/fimmu.2019.00992
[27] Poon MML, Rybkina K, Kato Y, Kubota M, Matsumoto R, Bloom NI, et al. SARS-CoV-2 infection generates tissue-localized immunological memory in humans. Science Immunology, 2021, 6(65), eabl9105. DOI: 10.1126/sciimmunol.abl9105
[28] Afkhami S, D'Agostino MR, Zhang A, Stacey HD, Marzok A, Kang A, et al. Respiratory mucosal delivery of next-generation COVID-19 vaccine provides robust protection against both ancestral and variant strains of SARS-CoV-2. Cell, 2022, 185(5), 896-915.e19. DOI: 10.1016/j.cell.2022.02.005
[29] Pizzolla A, Nguyen TH, Sant S, Jaffar J, Loudovaris T, Mannering SI, et al. Influenza specific lung resident memory T cells are proliferative and polyfunctional and maintain diverse TCR profiles. Journal of Clinical Investigation, 2018, 128(2), 721-733. DOI: 10.1172/JCI96957
[30] Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell, 2021, 184(4), 861-880. DOI: 10.1016/j.cell.2021.01.007
[31] Nachbagauer R, Krammer F. Universal influenza virus vaccines and therapeutic antibodies. Clinical Microbiology and Infection, 2017, 23(4), 222-228. DOI: 10.1016/j.cmi.2017.02.009
[32] Krammer F. The role of vaccines in the COVID-19 pandemic: what have we learned? Seminars in Immunopathology, 2024, 45(4-6), 451-468. DOI: 10.1007/s00281-023-00996-2
[33] McCool RS, Musayev M, Bush SM, Derrien-Colemyn A, Acreman CM, Wrapp D, et al. Vaccination with prefusion-stabilized respiratory syncytial virus fusion protein elicits antibodies targeting a membrane-proximal epitope. Journal of Virology, 2023, 97(10), e0092923. DOI: 10.1128/jvi.00929-23
[34] Mazur NI, Terstappen J, Baral R, Bardají A, Beutels P, Buchholz UJ, et al. Respiratory syncytial virus prevention within reach: The vaccine and monoclonal antibody landscape. Lancet Infectious Diseases, 2023, 23(1), e2-e21. DOI: 10.1016/S1473-3099(22)00291-2
[35] Andersen P, Scriba TJ. Moving tuberculosis vaccines from theory to practice. Nature Reviews Immunology, 2023, 23(8), 511-525. DOI: 10.1038/s41577-023-00854-5
[36] Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. The New England Journal of Medicine, 2006, 354(1), 11-22. DOI: 10.1056/NEJMoa052434
[37] Patel M, Shane AL, Parashar UD, Jiang B, Gentsch JR, Glass RI. Oral rotavirus vaccines: How well will they work where they are needed most? Journal of Infectious Diseases, 2009, 200(Suppl 1), S39-S48. DOI: 10.1086/605035
[38] Svennerholm AM, Qadri F, Lundgren A, Kaim J, Rahman Bhuiyan T, Akhtar M, et al. Induction of mucosal and systemic immune responses against the common O78 antigen of an oral inactivated ETEC vaccine in Bangladeshi children and infants. Vaccine, 2022, 40(2), 380-389. DOI: 10.1016/j.vaccine.2021.10.056
[39] 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. Clinical and Vaccine Immunology, 2011, 18(4), 546-551. DOI: 10.1128/CVI.00538-10
[40] Talaat KR, Alaimo C, Martin P, Bourgeois AL, Dreyer AM, Kaminski RW, et al. Human challenge study with a Shigella bioconjugate vaccine: Analyses of clinical efficacy and correlate of protection. EBioMedicine, 2021, 66, 103310. DOI: 10.1016/j.ebiom.2021.103310
[41] Cohen D, Ashkenazi S, Green MS, Gdalevich M, Robin G, Slepon R, et al. Double blind vaccine controlled randomised efficacy trial of an investigational Shigella sonnei conjugate vaccine in young adults. Lancet, 1997, 349(9046), 155-159. DOI: 10.1016/S0140-6736(96)06255-1
[42] Peng T, Phasouk K, Sodroski CN, Sun S, Hwangbo Y, Layton ED, et al. Tissue resident memory CD8+ T cells bridge innate immune responses in neighboring epithelial cells to control human genital herpes. Frontiers in Immunology, 2021, 12, 735643. DOI: 10.3389/fimmu.2021.735643
[43] Hellfritzsch M, Scherließ R. Mucosal vaccination via the respiratory tract. Pharmaceutics, 2019, 11(8), 375. DOI: 10.3390/pharmaceutics11080375
[44] Xu Y, Yuen PW, Lam JK. Intranasal DNA vaccine for protection against respiratory infectious diseases: The delivery perspectives. Pharmaceutics, 2014, 6(3), 378-415. DOI: 10.3390/pharmaceutics6030378
[45] Fu W, Guo M, Zhou X, Wang Z, Sun J, An Y, et al. Injectable hydrogel mucosal vaccine elicits protective immunity against respiratory viruses. ACS Nano, 2024, 18(17), 11200-11216. DOI: 10.1021/acsnano.4c00155
[46] Ochsner SP, Li W, Rajendrakumar AM, Palaniyandi S, Acharya G, Liu X, et al. FcRn Targeted mucosal vaccination against influenza virus infection. Journal of Immunology, 2021, 207(5), 1310-1321. DOI: 10.4049/jimmunol.2100297
[47] Lei H, Hong W, Yang J, He C, Zhou Y, Zhang Y, et al. Intranasal delivery of a subunit protein vaccine provides protective immunity against JN.1 and XBB lineage variants. Signal Transduction and Targeted Therapy, 2024, 9(1), 311. DOI: 10.1038/s41392-024-02025-6
[48] Kwon DI, Mao T, Israelow B, Santos Guedes de Sá K, Dong H, Iwasaki A. Mucosal unadjuvanted booster vaccines elicit local IgA responses by conversion of pre existing immunity in mice. Nature Immunology, 2025, 26(6), 908-919. DOI: 10.1038/s41590-025-02156-0

[49] Moriyama M, Rodrigues G, Wang J, Jayewickreme R, Hudak A, Dong H, et al. Intranasal hemagglutinin protein boosters induce protective mucosal immunity against influenza A viruses in mice. Proceedings of the National Academy of Sciences USA, 2025, 122(39), e2422171122. DOI: 10.1073/pnas.2422171122
[50] Santosuosso M, McCormick S, Zhang X, Zganiacz A, Xing Z. Intranasal boosting with an adenovirus vectored vaccine markedly enhances protection by parenteral Mycobacterium bovis BCG immunization against pulmonary tuberculosis. Infection and Immunity, 2006, 74(8), 4634-4643. DOI: 10.1128/IAI.00517-06
[51] Holmgren J. Mucosal immunity and vaccination. FEMS Immunology and Medical Microbiology, 1991, 4(1), 1-9. DOI: 10.1111/j.1574-6968.1991.tb04964.x
[52] Takaiwa F, Wakasa Y, Takagi H, Hiroi T. Rice seed for delivery of vaccines to gut mucosal immune tissues. Plant Biotechnology Journal, 2015, 13(8), 1041-1055. DOI: 10.1111/pbi.12423
[53] Van Der Weken H, Cox E, Devriendt B. Advances in oral subunit vaccine design. Vaccines (Basel), 2020, 9(1), 1. DOI: 10.3390/vaccines9010001
[54] Azizi A, Kumar A, Diaz Mitoma F, Mestecky J. Enhancing oral vaccine potency by targeting intestinal M cells. PLOS Pathogens, 2010, 6(11), e1001147. DOI: 10.1371/journal.ppat.1001147
[55] Rochereau N, Drocourt D, Perouzel E, Pavot V, Redelinghuys P, Brown GD, et al. Dectin 1 is essential for reverse transcytosis of glycosylated SIgA antigen complexes by intestinal M cells. PLOS Biology, 2013, 11(9), e1001658. DOI: 10.1371/journal.pbio.1001658
[56] Platt LR, Estívariz CF, Sutter RW. Vaccine associated paralytic poliomyelitis: A review of the epidemiology and estimation of the global burden. Journal of Infectious Diseases, 2014, 210(Suppl 1), S380-S389. DOI: 10.1093/infdis/jiu184
[57] Bi Q, Ferreras E, Pezzoli L, Legros D, Ivers LC, Date K, et al. Protection against cholera from killed whole cell oral cholera vaccines: A systematic review and meta analysis. Lancet Infectious Diseases, 2017, 17(10), 1080-1088. DOI: 10.1016/S1473-3099(17)30359-6
[58] Czerkinsky C, Holmgren J. Mucosal delivery routes for optimal immunization: Targeting immunity to the right tissues. Current Topics in Microbiology and Immunology, 2010, 354, 1-18. DOI: 10.1007/82_2010_112
[59] Huo Z, Bissett SL, Giemza R, Beddows S, Oeser C, Lewis DJM. Systemic and mucosal immune responses to sublingual or intramuscular human papilloma virus antigens in healthy female volunteers. PLoS ONE, 2012, 7(3), e33736. DOI: 10.1371/journal.pone.0033736
[60] Czerkinsky C, Çuburu N, Kweon MN, Anjuere F, Holmgren J. Sublingual vaccination. Human Vaccines, 2011, 7(1), 110-114. DOI: 10.4161/hv.7.1.13739
[61] Jacob S, Nair AB, Boddu SHS, Gorain B, Sreeharsha N, Shah J. An updated overview of the emerging role of patch and film based buccal delivery systems. Pharmaceutics, 2021, 13(8), 1206. DOI: 10.3390/pharmaceutics13081206
[62] Mokabari K, Iriti M, Varoni EM. Mucoadhesive vaccine delivery systems for the oral mucosa. Journal of Dental Research, 2023, 102(7), 709-718. DOI: 10.1177/00220345231164111
[63] Trincado V, Gala RP, Morales JO. Buccal and sublingual vaccines: A review on oral mucosal immunization and delivery systems. Vaccines (Basel), 2021, 9(10), 1177. DOI: 10.3390/vaccines9101177
[64] Brako F, Boateng J. Transmucosal drug delivery: Prospects, challenges, advances, and future directions. Expert Opinion on Drug Delivery, 2025, 22(4), 525-553. DOI: 10.1080/17425247.2025.2470224
[65] Hua S. Advances in nanoparticulate drug delivery approaches for sublingual and buccal administration. Frontiers in Pharmacology, 2019, 10, 1328. DOI: 10.3389/fphar.2019.01328
[66] Sohi H, Ahuja A, Ahmad FJ, Khar RK. Critical evaluation of permeation enhancers for oral mucosal drug delivery. Drug Development and Industrial Pharmacy, 2010, 36(3), 254-282. DOI: 10.3109/03639040903117348
[67] Detmer A, Glenting J. Live bacterial vaccines – a review and identification of potential hazards. Microbial Cell Factories, 2006, 5, 23. DOI: 10.1186/1475-2859-5-23
[68] Garmory HS, Leary SE, Griffin KF, Williamson ED, Brown KA, Titball RW. The use of live attenuated bacteria as a delivery system for heterologous antigens. Journal of Drug Targeting, 2003, 11(8-10), 471-479. DOI: 10.1080/10611860410001670008
[69] Kotton CN, Hohmann EL. Enteric pathogens as vaccine vectors for foreign antigen delivery. Infection and Immunity, 2004, 72(10), 5535-5547. DOI: 10.1128/IAI.72.10.5535-5547.2004
[70] Lin I, Van T, Smooker P. Live attenuated bacterial vectors: Tools for vaccine and therapeutic agent delivery. Vaccines (Basel), 2015, 3(4), 940-972. DOI: 10.3390/vaccines3040940
[71] Tarahomjoo S. Development of vaccine delivery vehicles based on lactic acid bacteria. Molecular Biotechnology, 2011, 51(2), 183-199. DOI: 10.1007/s12033-011-9450-2
[72] Daudel D, Weidinger G, Spreng S. Use of attenuated bacteria as delivery vectors for DNA vaccines. Expert Review of Vaccines, 2007, 6(1), 97-110. DOI: 10.1586/14760584.6.1.97
[73] Gherardi MM, Esteban M. Recombinant poxviruses as mucosal vaccine vectors. Journal of General Virology, 2005, 86(11), 2925-2936. DOI: 10.1099/vir.0.81181-0
[74] Galen JE, Pasetti MF, Tennant S, Ruiz-Olvera P, Sztein MB, Levine MM. Salmonella enterica serovar Typhi live vector vaccines finally come of age. Immunology & Cell Biology, 2009, 87(5), 400-412. DOI: 10.1038/icb.2009.31
[75] Ranasinghe C. New advances in mucosal vaccination. Immunology Letters, 2014, 161(2), 204-206. DOI: 10.1016/j.imlet.2014.01.006
[76] Koirala P, Shalash AO, Chen SR, Faruck MO, Wang J, Hussein WM, et al. Polymeric nanoparticles as oral and intranasal peptide vaccine delivery systems: The role of shape and conjugation. Vaccines (Basel), 2024, 12(2), 198. DOI: 10.3390/vaccines12020198
[77] Vajdy M, Srivastava I, Polo J, Donnelly J, O'Hagan D, Singh M. Mucosal adjuvants and delivery systems for protein, DNA and RNA based vaccines. Immunology & Cell Biology, 2004, 82(6), 617-627. DOI: 10.1111/j.1440-1711.2004.01288.x
[78] Wang S, Liu H, Zhang X, Qian F. Intranasal and oral vaccination with protein based antigens: Advantages, challenges and formulation strategies. Protein & Cell, 2015, 6(7), 480-503. DOI: 10.1007/s13238-015-0164-2
[79] Ongun M, Lokras AG, Baghel S, Shi Z, Schmidt ST, Franzyk H, et al. Lipid nanoparticles for local delivery of mRNA to the respiratory tract: Effect of PEG lipid content and administration route. European Journal of Pharmaceutics and Biopharmaceutics, 2024, 198, 114266. DOI: 10.1016/j.ejpb.2024.114266

[80] Zeng C, Zhang C, Walker PG, Dong Y. Formulation and delivery technologies for mRNA vaccines. Current Topics in Microbiology and Immunology, 2022, 440, 71-110. DOI: 10.1007/82_2020_217
[81] Omo-Lamai S, Wang Y, Patel MN, Milosavljevic A, Zuschlag D, Poddar S, et al. Limiting endosomal damage sensing reduces inflammation triggered by lipid nanoparticle endosomal escape. Nature Nanotechnology, 2025, 20(9), 1285-1297. DOI: 10.1038/s41565-025-01974-5
[82] Forenzo C, Larsen J. Complex coacervates as a promising vehicle for mRNA delivery: A comprehensive review of recent advances and challenges. Molecular Pharmacology, 2023, 20(9), 4387-4403. DOI: 10.1021/acs.molpharmaceut.3c00439
[83] Chadwick S, Kriegel C, Amiji M. Delivery strategies to enhance mucosal vaccination. Expert Opinion on Biological Therapy, 2009, 9(4), 427-440. DOI: 10.1517/14712590902849224
[84] Hameed SA, Paul S, Dellosa GKY, Jaraquemada D, Bello MB. Towards the future exploration of mucosal mRNA vaccines against emerging viral diseases; lessons from existing next generation mucosal vaccine strategies. NPJ Vaccines, 2022, 7(1), 1-15. DOI: 10.1038/s41541-022-00485-x
[85] Longet S, Lundahl MLE, Lavelle EC. Targeted strategies for mucosal vaccination. Bioconjugate Chemistry, 2018, 29(3), 613-623. DOI: 10.1021/acs.bioconjchem.7b00738
[86] Baldridge JR, McGowan P, Evans JT, Cluff C, Mossman S, Johnson D, et al. Taking a Toll on human disease: Toll like receptor 4 agonists as vaccine adjuvants and monotherapeutic agents. Expert Opinion on Biological Therapy, 2004, 4(7), 1129-1138. DOI: 10.1517/14712598.4.7.1129
[87] Ebrahimian M, Hashemi M, Maleki M, Hashemitabar G, Abnous K, Ramezani M, et al. Co delivery of dual Toll like receptor agonists and antigen in poly(Lactic Co Glycolic) acid/polyethylenimine cationic hybrid nanoparticles promote efficient *in vivo* immune responses. Frontiers in Immunology, 2017, 8, 1077. DOI: 10.3389/fimmu.2017.01077
[88] Guo X, Wu N, Shang Y, Liu X, Wu T, Zhou Y, et al. The novel Toll like receptor 2 agonist SUP3 enhances antigen presentation and T cell activation by dendritic cells. Frontiers in Immunology, 2017, 8, 158. DOI: 10.3389/fimmu.2017.00158
[89] Kayesh MEH, Kohara M, Tsukiyama Kohara K. TLR agonists as vaccine adjuvants in the prevention of viral infections: An overview. Frontiers in Microbiology, 2023, 14, 1249718. DOI: 10.3389/fmicb.2023.1249718
[90] Kokatla HP, Sil D, Tanji H, Ohto U, Malladi SS, Fox LM, et al. Structure based design of novel human Toll like receptor 8 agonists. ChemMedChem, 2014, 9(4), 719-723. DOI: 10.1002/cmdc.201300573
[91] Kumar S, Sunagar R, Gosselin E. Bacterial protein toll like receptor agonists: A novel perspective on vaccine adjuvants. Frontiers in Immunology, 2019, 10, 1144. DOI: 10.3389/fimmu.2019.01144
[92] Shanmugam A, Rajoria S, George AL, Mittelman A, Suriano R, Tiwari RK. Synthetic Toll like receptor 4 (TLR 4) agonist peptides as a novel class of adjuvants. PLoS ONE, 2012, 7(2), e30839. DOI: 10.1371/journal.pone.0030839
[93] Liong CS, Smith AAA, Mann JL, Roth GA, Gale EC, Maikawa C, et al. Enhanced humoral immune response by high density TLR agonist presentation on hyperbranched polymers. Advances in Therapy, 2021, 4(4), 2000081. DOI: 10.1002/adtp.202000081
[94] Ou BS, Baillet J, Filsinger Interrante MV, Adamska JZ, Zhou X, Saouaf OM, et al. Saponin nanoparticle adjuvants incorporating Toll like receptor agonists drive distinct immune signatures and potent vaccine responses. Science Advances, 2024, 10(32), eadn7187. DOI: 10.1126/sciadv.adn7187
[95] Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, et al. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. The New England Journal of Medicine, 2004, 350(9), 896-903. DOI: 10.1056/NEJMoa030595
[96] Lewis DJ, Huo Z, Barnett S, Kromann I, Giemza R, Galiza E, et al. Transient facial nerve paralysis (Bell's palsy) following intranasal delivery of a genetically detoxified mutant of Escherichia coli heat labile toxin. PLoS ONE, 2009, 4(9), e6999. DOI: 10.1371/journal.pone.0006999
[97] Illum L. Nasal drug delivery – recent developments and future prospects. Journal of Controlled Release, 2012, 161(2), 254-263. DOI: 10.1016/j.jconrel.2012.01.024
[98] Kim SH, Jang YS. Antigen targeting to M cells for enhancing the efficacy of mucosal vaccines. Experimental & Molecular Medicine, 2014, 46(3), e85. DOI: 10.1038/emm.2013.165
[99] Clements JD, Norton EB. The mucosal vaccine adjuvant LT(R192G/L211A) or dmLT. mSphere, 2018, 3(4), e00215-18. DOI: 10.1128/mSphere.00215-18
[100] Bolhassani A, Javanzad S, Saleh T, Hashemi M, Aghasadeghi MR, Sadat SM. Polymeric nanoparticles. Human Vaccines & Immunotherapeutics, 2013, 10(2), 321-332. DOI: 10.4161/hv.26796
[101] Ghaffar K, Giddam A, Zaman M, Skwarczynski M, Toth I. Liposomes as nanovaccine delivery systems. Current Topics in Medicinal Chemistry, 2014, 14(9), 1194-1208. DOI: 10.2174/1568026614666140329232757
[102] Musacchio T, Torchilin VP. Recent developments in lipid based pharmaceutical nanocarriers. Frontiers in Bioscience, 2011, 16(4), 1388-1412. DOI: 10.2741/3795
[103] Cho CS, Hwang SK, Gu MJ, Kim CG, Kim SK, Ju DB et al. Mucosal vaccine delivery using mucoadhesive polymer particulate systems. Tissue Engineering and Regenerative Medicine, 2021, 18(5), 693-712. DOI: 10.1007/s13770-021-00373-w
[104] Garg NK, Mangal S, Khambete H, Tyagi RK. Mucosal delivery of vaccines: Role of mucoadhesive/biodegradable polymers. Recent Advances in Drug Delivery and Formulation, 2010, 4(2), 114-128. DOI: 10.2174/187221110791185015
[105] Mishra N, Goyal AK, Tiwari S, Paliwal R, Paliwal SR, Vaidya B, et al. Recent advances in mucosal delivery of vaccines: Role of mucoadhesive/biodegradable polymeric carriers. Expert Opinion on Therapeutic Patents, 2010, 20(5), 661-679. DOI: 10.1517/13543771003730425
[106] Shim S, Yoo HS. The application of mucoadhesive chitosan nanoparticles in nasal drug delivery. Marine Drugs, 2020, 18(12), 605. DOI: 10.3390/md18120605
[107] Smith A, Perelman M, Hinchcliffe M. Chitosan: A promising safe and immune-enhancing adjuvant for intranasal vaccines. Human Vaccines & Immunotherapeutics, 2014, 10(3), 797-807. DOI: 10.4161/hv.27449
[108] Ambrose CS, Levin MJ, Belshe RB. The relative efficacy of live attenuated and inactivated influenza vaccines in children and adults. Influenza and Other Respiratory Viruses, 2011, 5(2), 67-75. DOI: 10.1111/j.1750-2659.2010.00183.x
[109] Sui Y, Li J, Zhang R, Prabhu SK, Andersen H, Venzon D, et al. Protection against SARS CoV 2 infection by a mucosal vaccine in rhesus macaques. JCI Insight, 2021, 6(10), e148494. DOI: 10.1172/jci.insight.148494

[110] Chen J, Wang P, Yuan L, Zhang L, Zhang L, Zhao H, et al. A live attenuated virus based intranasal COVID 19 vaccine provides rapid, prolonged, and broad protection against SARS CoV 2. Science Bulletin, 2022, 67(13), 1372-1387. DOI: 10.1016/j.scib.2022.05.018
[111] Zhang L, Jiang Y, He J, Chen J, Qi R, Yuan L, et al. Intranasal influenza vectored COVID 19 vaccine restrains the SARS CoV 2 inflammatory response in hamsters. Nature Communications, 2023, 14(1), 4117. DOI: 10.1038/s41467-023-39560-9
[112] Afkhami S, D'Agostino MR, Zhang A, Stacey HD, Marzok A, Kang A, et al. Respiratory mucosal delivery of next generation COVID 19 vaccine provides robust protection against both ancestral and variant strains of SARS CoV 2. Cell, 2022, 185(5), 896-915.e19. DOI: 10.1016/j.cell.2022.02.005
[113] Xing M, Hu G, Wang X, Wang YH, He FR, Dai WQ, et al. An intranasal combination vaccine induces systemic and mucosal immunity against COVID 19 and influenza. NPJ Vaccines, 2024, 9(1), 64. DOI: 10.1038/s41541-024-00857-5
[114] Kampmann B, Madhi SA, Munjal I, Simões EAF, Pahud BA, Llapur C, et al. Bivalent prefusion F vaccine in pregnancy to prevent RSV illness in infants. The New England Journal of Medicine, 2023, 388(16), 1451-1464. DOI: 10.1056/NEJMoa2216480
[115] Acosta PL, Caballero MT, Polack FP. Brief history and characterization of enhanced respiratory syncytial virus disease. Clinical and Vaccine Immunology, 2015, 23(3), 189-195. DOI: 10.1128/CVI.00609-15
[116] Tameris M, Mearns H, Penn Nicholson A, Gregg Y, Bilek N, Mabwe B, et al. Live attenuated Mycobacterium tuberculosis vaccine MTBVAC versus BCG in adults and neonates: a randomised controlled, double blind dose escalation trial. The Lancet Respiratory Medicine, 2019, 7(9), 1-14. DOI: 10.1016/S2213-2600(19)30251-6
[117] Satti I, Meyer J, Harris SA, Thomas ZRM, Griffiths K, Antrobus RD, et al. Safety and immunogenicity of a candidate tuberculosis vaccine MVA85A delivered by aerosol in BCG-vaccinated healthy adults: A phase 1, double-blind, randomised controlled trial. The Lancet Infectious Diseases, 2014, 14, 939-946.
[118] Loxton AG, Knaul JK, Grode L, Gutschmidt A, Meller C, Eisele B, et al. Safety and immunogenicity of the recombinant Mycobacterium bovis BCG vaccine VPM1002 in HIV unexposed newborn infants in South Africa. Clinical and Vaccine Immunology, 2017, 24(2), e00439-16. DOI: 10.1128/CVI.00439-16
[119] Parker EP, Ramani S, Lopman BA, Church JA, Iturriza-Gómara M, Prendergast AJ, et al. Causes of impaired oral vaccine efficacy in developing countries. Future Microbiology, 2018, 13(1), 97-118. DOI: 10.2217/fmb-2017-0128
[120] Bhandari N, Rongsen-Chandola T, Bavdekar A, John J, Antony K, Taneja S, et al. Efficacy of a monovalent human bovine (116E) rotavirus vaccine in Indian infants: A randomised, double blind, placebo controlled trial. Lancet, 2014, 383(9935), 2136-2143. DOI: 10.1016/S0140-6736(13)62630-6
[121] Guo M, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. Nature Reviews Gastroenterology & Hepatology, 2021, 18(4), 269-283. DOI: 10.1038/s41575-021-00416-6
[122] Song D, Moon H, Kang B. Porcine epidemic diarrhea: A review of current epidemiology and available vaccines. Clinical and Experimental Vaccine Research, 2015, 4(2), 166-176. DOI: 10.7774/cevr.2015.4.2.166
[123] Atmar RL, Estes MK. Norovirus vaccine development: Next steps. Expert Review of Vaccines, 2012, 11(9), 1023-1025. DOI: 10.1586/erv.12.78
[124] O'Ryan M, Vidal R, Del Canto F, Salazar JC, Montero D. Vaccines for viral and bacterial pathogens causing acute gastroenteritis: Part II: Vaccines for Shigella, Salmonella, enterotoxigenic E. coli (ETEC) enterohemorragic E. coli (EHEC) and Campylobacter jejuni. Human Vaccines & Immunotherapeutics, 2015, 11(3), 601-619. DOI: 10.1080/21645515.2015.1011578
[125] Seo H, Duan Q, Zhang W. Vaccines against gastroenteritis, current progress and challenges. Gut Microbes, 2020, 11(6), 1486-1517. DOI: 10.1080/19490976.2020.1770666
[126] Woodrow KA, Bennett KM, Lo DD. Mucosal vaccine design and delivery. Annual Review of Biomedical Engineering, 2012, 14(1), 17-46. DOI: 10.1146/annurev-bioeng-071811-150054
[127] Ranallo RT, Barnoy S, Thakkar S, Urick T, Venkatesan MM. Developing live Shigella vaccines using λ Red recombineering. FEMS Immunology and Medical Microbiology, 2006, 47(3), 462-469. DOI: 10.1111/j.1574-695X.2006.00118.x
[128] Barry EM, Levine MM. A tale of two bacterial enteropathogens and one multivalent vaccine. Cellular Microbiology, 2019, 21(11), e13067. DOI: 10.1111/cmi.13067
[129] Turbyfill KR, Oaks EV, Kaminski RW, et al. A randomized, placebo controlled, phase 1 study to evaluate the safety and immunogenicity of an intranasal Shigella flexneri 2a InvaplexAR vaccine in healthy adults. Vaccine, 2024, 42(5), 1123-1134. DOI: 10.1016/j.vaccine.2024.01.045
[130] Liang H, Poncet D, Seydoux E, Rintala N, Maciel M, Ruiz S, et al. The TLR4 agonist adjuvant SLA SE promotes functional mucosal antibodies against a parenterally delivered ETEC vaccine. NPJ Vaccines, 2019, 4(1), 1-8. DOI: 10.1038/s41541-019-0116-6
[131] Uddin MS, Kaldis A, Menassa R, Ortiz Guluarte J, Barreda DR, Guan LL, et al. Mucosal immunization with spore based vaccines against Mannheimia Haemolytica enhances antigen specific immunity. Vaccines (Basel), 2024, 12(4), 375. DOI: 10.3390/vaccines12040375
[132] Poly F, Noll AJ, Riddle MS, Porter CK. Update on Campylobacter vaccine development. Human Vaccines & Immunotherapeutics, 2019, 15(6), 1389-1400. DOI: 10.1080/21645515.2018.1528410
[133] Pabst O, Mowat AM. Oral tolerance to food protein. Mucosal Immunology, 2022, 15(4), 678-688. DOI: 10.1038/s41385-022-00534-1
[134] Scott CL, Aumeunier AM, Mowat AM. Intestinal CD103$^{+}$ dendritic cells: Master regulators of tolerance? Trends in Immunology, 2011, 32(9), 412-419. DOI: 10.1016/j.it.2011.06.003
[135] Iweala OI, Nagler CR. The microbiome and food allergy. Annual Review of Immunology, 2019, 37, 377-403. DOI: 10.1146/annurev-immunol-042718-041621
[136] Rezende RM, Weiner HL. Oral tolerance: An updated review. Immunological Reviews, 2022, 245, 29-37. DOI: 10.1016/j.imlet.2022.03.007
[137] Fujkuyama Y, Tokuhara D, Kataoka K, Gilbert RS, McGhee JR, Yuki Y, et al. Novel vaccine development strategies for inducing mucosal immunity. Expert Review of Vaccines, 2012, 11(3), 367-379. DOI: 10.1586/erv.11.196
[138] Gebril A, Alsaadi M, Acevedo R, Mullen AB, Ferro VA. Optimizing efficacy of mucosal vaccines. Expert Review of Vaccines, 2012, 11(9), 1139-1155. DOI: 10.1586/erv.12.81

[139] Rele S. COVID 19 vaccine development during pandemic: Gap analysis, opportunities, and impact on future emerging infectious disease development strategies. Human Vaccines & Immunotherapeutics, 2020, 17(4), 1122-1127. DOI: 10.1080/21645515.2020.1822136
[140] Das S, Chatterjee A, Manna J, Das Mandal SK, Maiti TK, Bhattacharyya TK. Systems integrated thermostable vaccine delivery: Converging cold chain free design, AI augmented formulation, and climate resilient infrastructure. Molecular Pharmacology, 2025, 22(12), 7285-7312. DOI: 10.1021/acs.molpharmaceut.5c01296
[141] Raut R, Shrestha R, Adhikari A, Fatima A, Naeem M. Revolutionizing veterinary vaccines: Overcoming cold chain barriers through thermostable and novel delivery technologies. Applied Microbiology, 2025, 5(3), 83. DOI: 10.3390/applmicrobiol5030083
[142] Damjanovic D, Zhang X, Mu J, Fe Medina M, Xing Z. Organ distribution of transgene expression following intranasal mucosal delivery of recombinant replication defective adenovirus gene transfer vector. Genetic Vaccines and Therapy, 2008, 6, 5. DOI: 10.1186/1479-0556-6-5
[143] Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. Journal of Immunology, 2004, 172(5), 2731-2738. DOI: 10.4049/jimmunol.172.5.2731
[144] Bartfeld S, Clevers H. Stem cell derived organoids and their application for medical research and patient treatment. Journal of Molecular Medicine, 2017, 95(7), 729-738. DOI: 10.1007/s00109-017-1531-7
[145] Lynn DJ, Benson SC, Lynn MA, Pulendran B. Modulation of immune responses to vaccination by the microbiota: Implications and potential mechanisms. Nature Reviews Immunology, 2022, 22(1), 33-46. DOI: 10.1038/s41577-021-00554-7
[146] Cone RA. Barrier properties of mucus. Advanced Drug Delivery Reviews, 2009, 61(2), 75-85. DOI: 10.1016/j.addr.2008.09.008
[147] Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: Lessons from a live cholera vaccine. BMC Biology, 2010, 8, 129. DOI: 10.1186/1741-7007-8-129
[148] Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: A randomised, placebo controlled phase 2b trial. Lancet, 2013, 381(9871), 1021-1028. DOI: 10.1016/S0140-6736(13)60177-4
[149] Haynes BF, Burton DR. Developing an HIV vaccine. Science, 2017, 355(6330), 1129-1130. DOI: 10.1126/science.aan0662
[150] Cai X, Li JJ, Liu T, Brian O, Li J. Infectious disease mRNA vaccines and a review on epitope prediction for vaccine design. Briefings in Functional Genomics, 2021, 20(5), 289-303. DOI: 10.1093/bfgp/elab027
[151] Rzymski P, Szuster Ciesielska A, Dzieciątkowski T, Gwenzi W, Fal A. mRNA vaccines: The future of prevention of viral infections? Journal of Medical Virology, 2023, 95(2), e28572. DOI: 10.1002/jmv.28572
[152] Zhou W, Jiang L, Liao S, Wu F, Yang G, Hou L, et al. Vaccines' new wra RNA vaccine. Viruses, 2023, 15(8), 1760. DOI: 10.3390/v15081760
[153] Xu Y, Masuda K, Groso C, Hassan R, Zhou Z, Broderick K, et al. Microfluidic synthesis of scalable layer by layer multiple antigen nano delivery platform for SARS CoV 2 vaccines. Vaccines (Basel), 2024, 12(3), 339. DOI: 10.3390/vaccines12030339
[154] Pulendran B, Davis MM. The science and medicine of human immunology. Science, 2020, 369(6511), eaay4014. DOI: 10.1126/science.aay4014
[155] Imani S, Li X, Chen K, Maghsoudloo M, Jabbarzadeh Kaboli P, Hashemi M, et al. Computational biology and artificial intelligence in mRNA vaccine design for cancer immunotherapy. Frontiers in Cellular and Infection Microbiology, 2025, 14, 1501010. DOI: 10.3389/fcimb.2024.1501010
[156] Bloom K, van den Berg F, Arbuthnot P. Self amplifying RNA vaccines for infectious diseases. Gene Therapy, 2021, 28(3-4), 117-129. DOI: 10.1038/s41434-020-00204-y
[157] Zhao Y, Wang H. Artificial intelligence driven circRNA vaccine development: Multimodal collaborative optimization and a new paradigm for biomedical applications. Briefings in Bioinformatics, 2025, 26(3), bbaf263. DOI: 10.1093/bib/bbaf263
[158] Zhu J, Ananthaswamy N, Jain S, Batra H, Tang WC, Lewry DA, et al. A universal bacteriophage T4 nanoparticle platform to design multiplex SARS-CoV-2 vaccine candidates by CRISPR engineering. Science Advances, 2021, 7(37), eabh1547. DOI: 10.1126/sciadv.abh1547
[159] Lavelle EC, Ward RW. Mucosal vaccines – fortifying the frontiers. Nature Reviews Immunology, 2021, 22(4), 236-250. DOI: 10.1038/s41577-021-00583-2
[160] Yuki Y, Kiyono H. Mucosal vaccines: Novel advances in technology and delivery. Expert Review of Vaccines, 2009, 8(8), 1083-1097. DOI: 10.1586/erv.09.61
[161] Dotiwala F, Upadhyay AK. Next generation mucosal vaccine strategy for respiratory pathogens. Vaccines (Basel), 2023, 11(10), 1585. DOI: 10.3390/vaccines11101585
[162] Azegami T, Yuki Y, Kiyono H. Challenges in mucosal vaccines for the control of infectious diseases. International Immunopharmacology, 2014, 26(9), 517-528. DOI: 10.1093/intimm/dxu063
[163] Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. Nature Medicine, 2005, 11(4 Suppl), S45-S53. DOI: 10.1038/nm1213
[164] Lycke N. Recent progress in mucosal vaccine development: Potential and limitations. Nature Reviews Immunology, 2012, 12(8), 592-605. DOI: 10.1038/nri3251