Recent development of oral vaccines (Review)

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Abstract. Oral immunization can elicit an effective immune response and immune tolerance to specific antigens. When compared with the traditional injection route, delivering antigens via the gastrointestinal mucosa offers superior immune effects and compliance, as well as simplicity and convenience, making it a more optimal route for immunization. At present, various oral vaccine delivery systems exist. Certain modified bacteria, such as *Salmonella*, *Escherichia coli* and particularly *Lactobacillus*, are considered promising carriers for oral vaccines. These carriers can significantly enhance immunization efficiency by actively replicating in the intestinal tract following oral administration. The present review provided a discussion of the main mechanisms of oral immunity and the research progress made in the field of oral vaccines. Additionally, it introduced the advantages and disadvantages of the currently more commonly administered injectable COVID-19 vaccines, alongside the latest advancements in this area. Furthermore, recent developments in oral vaccines are summarized, and their potential benefits and side effects are discussed.

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Contents

1. Introduction

The first oral vaccine was introduced in the 1960s, and the oral polio vaccine (OPV) was the first oral vaccine proven to be effective. The OPV remains widely used today in numerous countries to prevent polio infections (1). Oral vaccines are administered via the gastrointestinal mucosa for the delivery of antigens, and this pathway generates a similar immune response to that of traditional injectable immunizations (2). However, the capacity of the gastrointestinal mucosa to induce immunity through antigen presentation is limited (3). Furthermore, the high quantity of antigen required for oral immunization poses a challenge to its widespread use. Nevertheless, following advancements in medicine and molecular biology, significant improvements in enhancing the mucosal antigen presentation of oral vaccines have been made, thereby improving immune responses and immune tolerance to the antigens (4).

The aim of oral vaccines is to stimulate immune responses in the mucosal tissues lining the gastrointestinal tract. To achieve this, the antigens in oral vaccines undergo a series of processes that involve their destruction and subsequent presentation to the immune system (2). Upon ingestion, oral vaccines encounter a number of challenges within the digestive system, including exposure to low pH levels in the stomach and the presence of various proteolytic enzymes in the gastrointestinal tract (5). These hostile conditions pose the risk of antigens being fully degraded before they reach the target immune cells. To overcome these challenges, a number of strategies have been devised to protect the antigens

while ensuring their destruction in a controlled manner. One common approach involves encapsulating the antigen within specialized vehicles or carriers, providing protection during transit through the digestive system (6). These carriers are typically composed of materials such as liposomes, microspheres or protein-based nanoparticles. Additionally, oral vaccine antigens have been engineered to be more resistant to degradation by proteolytic enzymes. By modifying the structure or incorporating stabilizing agents, vaccine antigens can withstand enzymatic breakdown, to a certain extent, allowing them to reach the desired sites of immune stimulation (7). Once the antigen-carrier complex reaches the mucosal surfaces of the intestine, it encounters specialized immune cells such as dendritic cells. These cells possess mechanisms to capture, process and present antigens to immune cells, effectively initiating an immune response (8). The differences between classical injectable vaccination and oral vaccination are presented in Table I.

Despite the number of challenges faced by oral immunization, the existence of multiple licensed formulations indicates that oral immunization is feasible. In the United States, vaccines that target enteric pathogens such as rotavirus, enterotoxigenic *Escherichia coli, Vibrio cholerae* and *Shigella* (*S. flexneri, S. sonnei*, *S. boydii* and *S. dysenteriae*) have been approved (9). These vaccines are also effective against pathogens that enter the body through the intestinal mucosa, leading to systemic diseases such as *Salmonella enterica serovar* typhi and poliovirus (2). Rapid advancements in technology, especially in relation to the global impact of COVID-19, have led to significant developments in the field of vaccines. This paper will provide a more up-to-date overview of the latest advancements in vaccine development.

2. Mechanisms of oral immunity

Factors influencing immune response to oral immunization. The immune response to oral immunization depends on the dose of vaccine, the frequency of administration, the form of spacer antigen and the metabolism of the individual. The degree of immune response or immune tolerance differs in local and/or systemic mucosa (10).

Mechanisms of oral immunization. The immunogenicity of antigenic proteins administered via oral mucosal inoculation alone is weak and is typically enhanced by the addition of specific adjuvants or vectors to achieve the desired effect. Different adjuvants, and even the same adjuvant with modified subunits, elicit different immune responses (11). Numerous adjuvants and vectors possess molecular structures that are recognized and targeted by innate immunity, such as lipopolysaccharides, the acidic components of membranes, basic peptidoglycan structures and non-methylated CpG structures (12). These molecular structures are recognized by gastrointestinal macrophages, dendritic cells and Toll-like receptors, which facilitate antigen recognition, presentation and subsequent T cell activation and differentiation through a series of signal transductions and cytokine secretion (13). The dendritic cell subtype and the corresponding cytokines play a crucial role in determining the proportion of subsequently activated $CD4^+$ T cells (14). When an antigen is presented to the cell, T helper (Th)1 cells differentiate and release cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, to mediate cellular immunity and induce production of the neutralizing antibody, IgG2a, by B cells (15). Antigens induce Th2 cell differentiation and increase the secretion of IL-4, 5, 10 and 13, which assist the production of neutralizing antibodies, IgE, IgA and IgG (16). Liposomes, immune stimulatory complexes, biodegradable microparticles and naked DNA can present exogenous antigens via endogenous pathways to stimulate the $CD8^+$ T cell response and mediate cytotoxic immune responses (17). The most characteristic aspect of the immune response produced by oral mucosal immunity is the production of secreted IgA (sIgA) (18). Following oral inoculation, the antigen is taken up by M cells to activate dendritic cells and T cell subsets, which then release a large number of cytokines and chemokines (19). Expression of major histocompatibility complex (MHC) class I and II antigens eventually leads to the activation of B cells, specific integrin expression and phenotypic conversion, especially to IgA (20). In addition to the local mucosa where the antigen makes contact, the corresponding sIgA can also be detected in the mucosal tissues of distant effector organs. The migration of IgA-producing cells is associated with the simultaneous expression of specific adhesion molecules in the endothelium of these tissues. As aforementioned, both Th1 and Th2 cells, along with their cytokines, are involved in B cell activation and sIgA production (21). However, studies suggest that transcriptional growth factor (TGF)-β promotes the generation of surface IgA^+ B cells (22,23). Fig. 1 shows the mechanism of oral immunization (Fig. 1).

Mechanisms of oral immune tolerance and antigen-specific immunosuppression. Oral immune tolerance is a process within the mucosal immune response aimed at managing immunized antigens, which operates through two primary mechanisms, namely clone inactivation and active inhibition (24). A previous study revealed that a solitary high dose of antigen can trigger apoptosis of antigen-specific $CD4^+$ T cells *in vivo* (25). This apoptosis is considered to be mediated by the p55 TNF receptor and is closely associated with C-C motif chemokine ligand 2 (CCL2) and its corresponding receptor, CCR2. By contrast, active inhibition arises from repetitive stimulation by low-dose antigens. This induces the activation of Th3 cells, which secrete TGF-β to initiate bystander inhibition. The bystander suppression caused by TGF-β-secreting Th3 cells can broadly inhibit both cell-mediated and humoral immune responses (26). However, it is worth noting that TGF-β also promotes the generation of IgA^+ B cells, thereby reducing the production of other antibodies while augmenting the synthesis and secretion of IgA (27).

Not all oral tolerance processes exhibit elevated levels of inhibitory factors. Several animal studies have discovered that peripheral tolerance (such as the suppression of delayed-type hypersensitivity) is accompanied by a notable increase in IFN-γ levels, without any alteration in the inhibitory factor (28,29). It is hypothesized that oral antigens induce the expression of α4β7 and its interaction with mucosal addressin cell adhesion molecule-1, which is expressed in the intestinal epithelium, thus inducing the secretion of IFN-γ and enhancing the local cellular immune response with the synergistic effects of

Table I. Differences between classical injectable vaccination and oral vaccination.

Type of Vaccination	Classic injectable vaccination	Oral vaccination
Method of Administration	Injection with a needle and syringe	Oral administration through the gastrointestinal tract
Delivery of Antigens	Injected into muscle or subcutaneous tissue	Delivered to gastrointestinal mucosa
Immune response	Stimulates systemic immunity	Stimulates both systemic and mucosal immunity
Immune pathway	Generates immune response through antigen presentation to circulating cells	Generates immune response through antigen presentation to mucosal-associated lymphoid tissue
Dosage	Requires lower quantities of antigen per dose	Requires higher quantities of antigen per dose
Stability	Vaccine is usually stable at room temperature or refrigerated	Vaccine may require specific storage temperature and conditions
Adjuvants	Often requires adjuvants to enhance immune response	May not require adjuvants
Cost	Involves additional costs associated with the use of needles, syringes and trained personnel	May reduce costs associated with the use of needles, syringes and trained personnel
Safety	Possibility of adverse reactions such as injection site reactions	Possibility of adverse reactions such as gastrointestinal distress

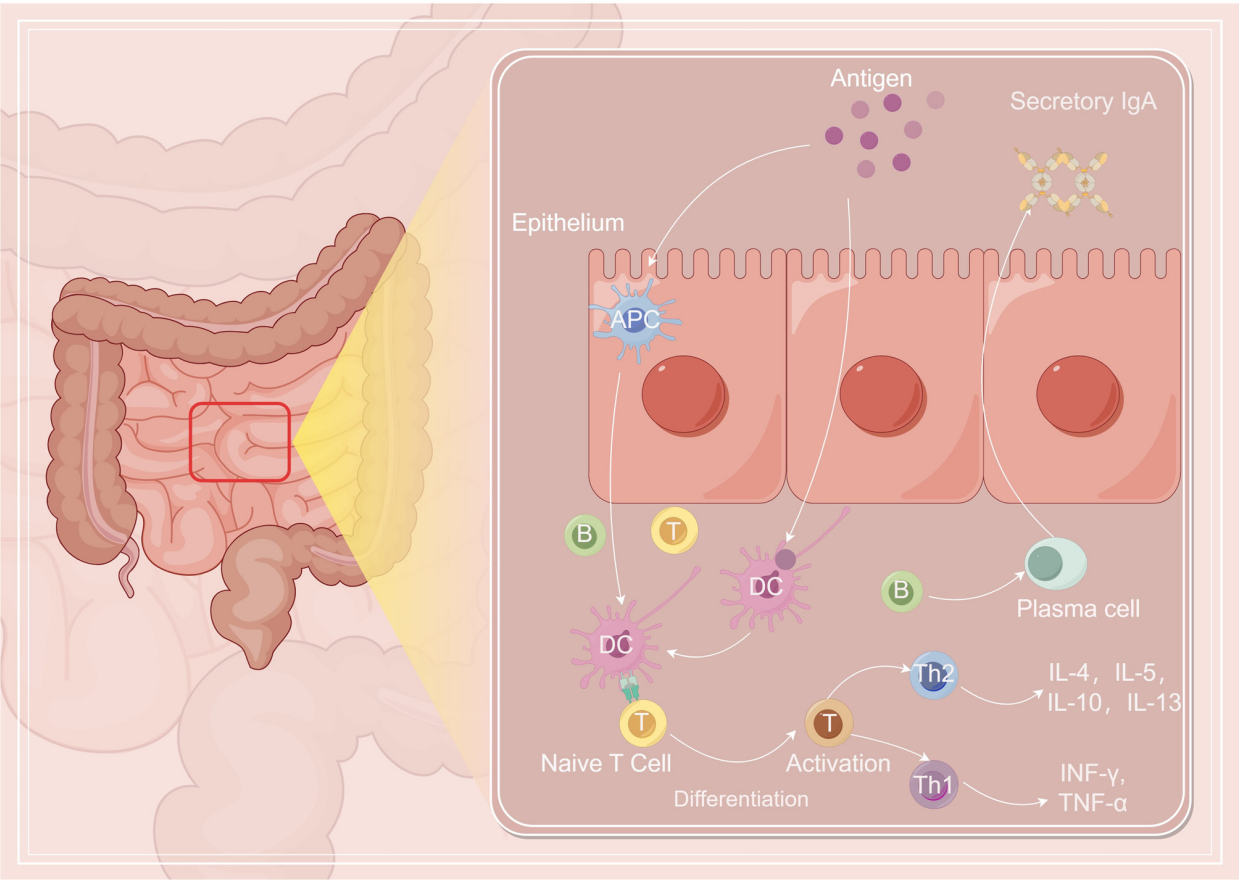

Figure 1. Mechanism of oral immunization. In the intestine, immune responses can be summarized as follows: At the inductive site, M cells transfer antigens to antigen-presenting cells. DCs, as antigen-presenting cells, stimulate the differentiation of naive T cells into Th cells. Th1 cells differentiate and produce cytokines such as IFN-γ and TNF-α, which mediate cellular immunity. Allergens induce the differentiation of Th2 cells, which produce cytokines like IL-4, IL-5, IL-10 and IL-13, assisting in the production of different types of neutralizing antibodies. Stimulated B cells leave the lymph nodes and enter the circulatory system. Eventually, B cells migrate to effector sites where they differentiate into plasma cells. These plasma cells produce specific sIgA. Figure created using Figdraw (https://www.figdraw.com/static/index.html#/). Th, T helper; IFN-γ, interferon-γ; DC, dendritic cell; TNF-α, tumor necrosis factor α; sIgA, secretory IgA; B, B cell; T, T cell.

intestinal lumen bacteria (30). The interaction with bacteria in the intestinal lumen synergistically enhances the immune response. Simultaneously, the downregulation of α4β1 and p-selectin ligand reduces the migration of memory T cells

to peripheral tissues and suppresses the peripheral immune response (31). Additionally, T lymphocyte activation relies on antigen-presenting cells (APCs), and when presenting antigens to T cells, APCs must express appropriate co-stimulators. Quiescent APCs do not express the corresponding co-stimulator or instead exhibit a very low expression level during antigen presentation, thereby causing a loss of tolerance effect and the auxiliary function of T cells (32). Regulatory and non-reactive cells can directly mediate inhibition by producing inhibitory cytokines, as well as indirectly through competition for growth factors, MHC-peptide complexes or co-stimulatory molecules on APCs (33).

Mucosal tolerance is linked to the active function of T cell subsets expressing γδ T cell receptor (TCR) in Peyer's patch (PP) nodes and the epithelium of the small intestine. Mucosal tolerance serves as a fundamental regulator of mucosal immune tolerance and IgA production, which is primarily mediated by the immune modulation of IL-4 and IL-10 (34).

Mechanisms and factors in oral immune tolerance induction. Immune tolerance can be induced by small doses of antigen. T cells expressing γδTCR can inhibit the specific response of traditional antigen-specific αβT cells, leading to an 'immune non-response' to antigen stimulation. The immune tolerance induced by ovalbumin (OVA) can be blocked by the anti-γδTCR monoclonal antibody (35). Furthermore, deficiency in γδT cells also results in downregulation of the synthesis and activation of IgA^+ B cells. However, immune tolerance to OVA can still be induced in γδTCR-knockout mice, suggesting that multiple factors are involved in the development of oral immune tolerance (36).

The PP junction plays a crucial role in the immune tolerance induced by oral proteins, while hapten tolerance is primarily induced by the small intestinal epithelial barrier (37). Additionally, the generation of oral immune tolerance is closely associated with the normal flora of the gastrointestinal tract (38). It is generally considered that the failure of intestinal bacteria to induce oral tolerance is due to a significant decrease in associated T lymphocytes in the PP node.

3. COVID-19 vaccine development

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) (39), is a disease that has infected nearly 780 million individuals and resulted in nearly 7 million mortalities to date (2023) worldwide according to the World Health Organization. Given the lack of effective medications, the development and utilization of COVID-19 vaccines have become crucial in controlling the COVID-19 disease outbreak (40). SARS-COV-2 is an enveloped, positive sense, single-stranded RNA virus (41). The spike (S) protein (42) and the receptor binding domain (43) of the S protein are the primary targets for currently available COVID-19 vaccines. The first two vaccines approved for clinical use were an inactivated vaccine from China (Sinovac) and an mRNA-based vaccine from the USA (Pfizer-BioNTech). As of December 2021, there were six different vaccine platforms, including an inactivated virus vaccine, DNA and mRNA vaccines, an adenovirus vector vaccine, a subunit vaccine, a virus-like vaccine and a lentivirus vaccine. Moreover, >194 candidate vaccines have been approved for clinical trials worldwide (44). However, due to the various development platforms of COVID-19 vaccines, concerns regarding safety, effectiveness and stability during transportation and storage have arisen (45). Although the safety of COVID-19 vaccines in phase III clinical trials has been reported to be excellent, vaccine safety remains a significant concern (46). For example, live attenuated vaccines require replication within the body and therefore entail the potential risk of virulent atavism (47) or viral transmission (48). DNA vaccines carry the risk of oncogene activation (49) and chromosome instability (50) due to the integration of foreign DNA into the host genome. The synthetic components and encapsulating materials utilized in mRNA vaccine synthesis may exhibit toxicity and provoke apoptosis of surrounding host cells (51). According to the World Health Organization, a minimum protective efficacy of 50% is required for the introduction of COVID-19 vaccines (52). Inactivated, adenovirus vector and mRNA vaccines currently possess protective efficacies of 79.34% (53), 62-90% (54) and <90% (55), respectively, all meeting the aforementioned requirements. Nonetheless, evaluating or comparing the clinical efficacy of different vaccines for COVID-19 has proven challenging due to variations in their clinical schemes. Consequently, the effectiveness of vaccine protection still necessitates extensive verification through subsequent large-scale phase IV clinical trials.

The different types of injectable vaccines possess their own merits and faults and, to the best of our knowledge, different vaccines may have differences in effectiveness, safety and suitability for different populations, so there is no one vaccine that is considered to be universally optimal for all situations.

Inactivated virus vaccines employ either heat or chemical methods to render the virus obtained from culture inactive (56). As such, inactivated viruses lose their pathogenic virulence while retaining the primary antigenic properties of the viral shell, thus stimulating a specific immune response within the human body (57). The development process for an inactivated virus vaccine is straightforward and does not require any conceptual design or validation as it simply necessitates finding the appropriate means to inactivate the virus, which significantly enhances vaccine preparation time (58). However, inactivated vaccines can lead to severe adverse reactions (59). For example, an inactivated vaccine for respiratory syncytial virus was tested in clinical trials during the mid-1960s, and it instead exacerbated disease progression (60). Therefore, despite achieving certain successes in clinical trials related to the SARS virus, caution should still be exercised in the use of inactivated virus vaccines for COVID-19.

In the design of live attenuated vaccines, a less virulent strain is selected from the offspring and the process is repeated until the pathogenicity of the strain is eliminated (61). Live attenuated vaccines provide stronger immunity and have a longer duration of action compared with inactivated virus vaccines (62). However, there are certain disadvantages to live attenuated vaccines. The screening process in the early stages of development is time-consuming (63), making it challenging to develop early products within a short timeframe.

Recombinant protein vaccines involve transferring the gene sequence capable of expressing the viral surface antigen into prokaryotes via genetic engineering (64). This method allows for large-scale expression of the antigen protein. The

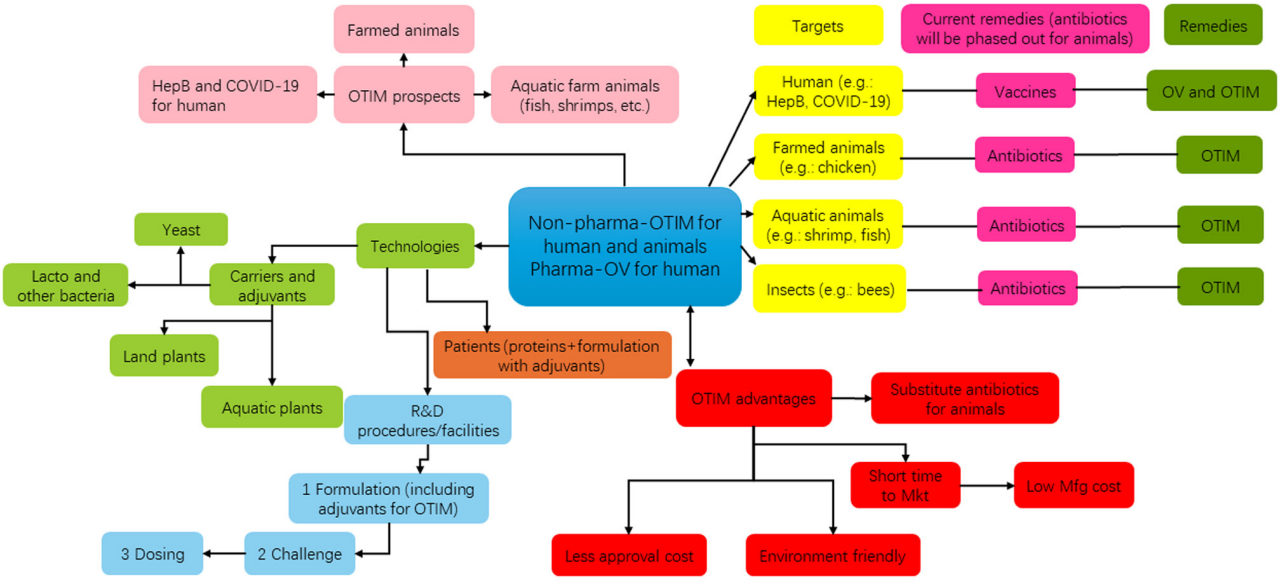

Figure 2. Oral vaccine and oral targeted immunomodulator platform. HepB, hepatitis B; OTIM, oral targeted immunomodulator; OV, oral vaccine; Mkt, market; Mfg, manufacturing.

recombinantly expressed antigen protein is then extracted and purified for inoculation into individuals. Recombinant protein vaccines have been extensively utilized in clinical practice (65). For example, hepatitis B surface antigen (HBsAg) is used in a commonly administered recombinant protein vaccine for hepatitis B (66). One significant benefit of this vaccine type is that the enriched or modified recombinant antigen protein exhibits a high level of immunogenicity, and the production process has reached a relatively advanced stage of development. However, the development of recombinant protein vaccines is hindered by factors such as the induction of non-specific immune responses in the body (67).

Viral vector, DNA and mRNA vaccines share a similar biological mechanism, as they all involve encoding the gene sequence of the antigen protein in the human body (68). The utilization of host cells for the production of viral antigen stimulates a specific immune response. Both DNA and mRNA vaccines are primarily delivered through non-biological methods, such as nanomaterial delivery (69). However, the development of viral vector vaccines is a complex process that involves not only the screening of suitable antigens but also the selection of appropriate vector viruses. Some studies have indicated that DNA remains unmetabolized in the human body for up to 2 years (39,70). The presence of foreign genetic information in the nucleus poses a risk of integration into the host genome, which can result in mutations and potentially cancer (71).

By contrast, mRNA is easily degraded, thus avoiding issues related to gene recombination. However, certain patients in mRNA vaccine clinical trials have experienced varying degrees of adverse reactions, which may relate to a proinflammatory action of the lipid nanoparticles used or the delivered mRNA (i.e., the vaccine formulation), as well as to the unique nature, expression pattern, binding profile and proinflammatory effects of the produced antigens, S protein and/or its subunits/peptide fragments, in human tissues or organs (72), which hinder the widespread use of mRNA vaccines. In addition, when considering the storage and transportation of COVID-19 vaccines, mRNA vaccines are unstable and prone to degradation, necessitating strict storage conditions (73). The mRNA vaccine (Pfizer-BioNTech COVID-19 mRNA vaccine), jointly developed by scientists in the USA and Germany, requires storage at -70˚C, and once thawed the vaccine vials can only be stored at 2-8˚C for a maximum of 5 days. Additionally, there is another mRNA vaccine (Moderna COVID-19 mRNA vaccine) that remains stable at temperatures of 2-8˚C for a duration of 30 days. However, this particular vaccine must be stored at -20˚C (73).

4. Feasibility and challenges of oral vaccines

Oral vaccines provide a more feasible approach for preventing contracting COVID-19. The mechanism of mucosal absorption of oral vaccines has been comprehensively described in previous studies (74-81). Fig. 2 shows the oral vaccine and oral targeted immunomodulator platform. Oral vaccines have gained significant attention in vaccine research and development in recent years, as they offer several advantages over traditional systemic vaccines. Oral vaccines primarily target gut-associated lymphoid tissue (GALT) upon delivery into the gut. This strategy capitalizes on the inherent immune properties of GALT, which play a crucial role in the development and regulation of intestinal immune responses (82). GALT is capable of inducing strong and specific mucosal immune responses, including sIgA, antibody-secreting cells and B and T cell memory cells (83). These responses provide protection at the mucosal surface and prevent the spread of infectious material. In addition to targeting specific immune responses, oral vaccines offer several advantages for individuals with compromised immune systems. These benefits may include reduced risk of infection, no need for medical personnel, non-invasive administration and thermal stability (84). Unlike systemic vaccines, oral vaccines do not affect the blood vessels or the circulatory system, reducing the risk of adverse events (85).

The efficacy of oral vaccines is influenced by the absorption of the vaccine by the gastrointestinal mucosa and the efficiency of local mucosal APCs in presenting the antigen (86). The immunogenicity of pure antigenic proteins is significantly low. The key to a successful oral vaccine lies in enhancing oral antigen presentation in the gastrointestinal mucosa and inducing effective mucosal and systemic immune responses. A well-designed adjuvant and antigen carrier system can reduce the antigen dose required for inducing an optimal immune response and immune tolerance (11). It is also important to note that, in the development of oral vaccines, it is crucial to consider their ability to withstand the various pH levels encountered throughout the gastrointestinal tract and the presence of proteolytic enzymes that can degrade antigen proteins (2). These challenges require a number of strategies, such as the use of stabilizing agents and protective encapsulation systems, to ensure the preservation of vaccine integrity and its effective delivery to the immune system (87). Addressing these factors is essential for the successful development of oral vaccines to elicit robust immune responses (88).

The term 'recombinant vaccine' refers to the purified antigenic protein or subunit that is produced *in vitro* using recombinant technology. This process involves removing the original virulence and infectivity of the pathogen while retaining its immunogenicity (89). However, when these recombinant antigens are administered alone, there are challenges involving uptake via the mucosal route (90). Therefore, these antigens are often combined with various adjuvants such as chitosan/aluminum, glucan and squalene-based adjuvants or carriers (91). Recombinant vaccines against HBsAg (92), tetanus toxin (93), diphtheria toxin (94) and pertussis toxin (95) have been developed and can be produced on a large scale. Furthermore, research into the development of oral forms of these recombinant vaccines is being conducted.

The production of oral attenuated live vaccines involves eradicating the virulence of pathogenic microorganisms, while also exhibiting self-replication ability and natural adjuvant activity (63). The effectiveness of their adjuvant activity in preventing reinfection, generating serum and mucosal immune responses and establishing long-lasting immune memory is noteworthy (96). However, further understanding and control of the toxicity of oral attenuated live vaccines is imperative, as numerous studies are currently only focused on animal experimentation (97,98).

Oral DNA vaccines are effective, but they require an appropriate delivery system as naked DNA vaccines administered orally are inefficient (2). To enhance the efficacy of an oral DNA vaccine, recombinant herpes simplex virus DNA can be used as a vector for *Salmonella typhimurium*, allowing localization to both the mucosal and systemic regions such as the spleen, ileal lymph nodes and PP node (99). Oral vaccines are less effective in eliciting humoral immune responses compared with intramuscular injections, but they are more effective in inducing local cell-mediated immune responses (100).

Mucosal adjuvants, such as cholera toxin, heat-stable protein of *E. coli*, phospholipase A, phosphatidylglycerol, bacterial DNA CpG motifs, immune-stimulating complexes, actin and cytokines, can significantly enhance the immunogenicity of oral antigens (101). The use of adjuvants alone can result in inadequate or suboptimal immune responses (12). However, when adjuvants are mixed or combined with other antigens, they can effectively stimulate both the humoral and cellular immune response (102). Among these adjuvants, cholera toxin and heat-stable protein of *E. coli* are particularly noteworthy (103). Research on cholera toxin has shown that its adjuvant properties are primarily exerted through the GM1 ganglioside receptor. The cholera toxin enhances the expression of co-stimulatory molecules on the surface of dendritic cells, thereby augmenting their ability to present antigens to antigen-specific T cells (96). This results in the increased expression of gangliosides on dendritic cells, facilitating the recognition of proteins from non-self bacterial enterotoxins (104). Current research is focused on the generation of recombinant weak strains or subunits of cholera toxin via site-directed mutagenesis (105). This approach significantly reduces toxicity while maintaining strong adjuvant activity.

Microspheres are a type of biodegradable, micron-structured material with a uniform particle size, and include poly lactide-glycolide microspheres, polylactic acid microspheres, polypropylene microspheres, starch microspheres and alginate microspheres (106). Most microspheres are natural and non-toxic, and have an adhesive effect during the transportation of antigens. This effect helps antigens pass through the intestinal mucosa epithelial cell layer. When using microspheres as a carrier system, immune tolerance or the immune response can be selectively induced through a single low dose of antigen administered orally. Due to their small diameter, microspheres can carry antigens and selectively deliver them to PP nodes and the systemic lymphatic system (107). Microspheres also release antigens slowly and in controlled amounts, resulting in a significant reduction in the required antigen dose (108). The immune response triggered by microspheres is closely related to their diameter, and as delivery systems, they hold the promise of being comparatively safe (109).

At present, the main bacterial carriers include *Salmonella enterica (serovars Typhi* and *Typhimurium)*, *E. coli*, *Lactobacillus*, recombinant *Mycobacterium bovis*, *Streptococcus gotelli* and *Vibrio cholera* (110). *Salmonella enterica serovars Typhi* was the first bacterial carrier to be studied. Live attenuated *Salmonella enterica serovars Typhi* is considered an ideal vector for mucosal immunity and as such, is one of the most commonly used live attenuated vaccine vectors (111). *Salmonella* is an organism of the gut that can therefore infect and multiply in the gut when administered orally (112). Live *Salmonella enterica serovars Typhimurium* can be ingested by small intestinal M cells and cross the intestinal epithelial barrier as its antigens are also presented to immune cells by APC cells, and thus it can act as an adjuvant to prevent the development of immune tolerance via oral administration (113). There are multiple mutant strains of *Salmonella* (such as strains containing one or more aroA or missing aroB, aroC and aroD) in the typhoid vaccine vector. Oral vaccines against typhoid bacteria with carriers such as herpes simplex virus, mycoplasma, *Bordetella pertussis*, tetanus granulosus and *Leishmania tarentolae* have also been successfully used in mouse animal models (114).

Lactobacillus spp. is one of the commensal bacteria residing in the human intestine (115). Certain studies have demonstrated that *Lactobacillus casei* can serve as an effective carrier, capable of inducing the immune response and immune

tolerance (113,116,117). Research has revealed that plasmids containing the non-toxic C fragment of *Tetanus bacillus* can express the non-toxic C fragment protein within *Lactobacillus plantarum*, leading to the production of a non-toxic C fragment specific IgG following oral administration of the vaccine in mice (118). However, the expression of non-toxic C fragment protein on the surface of *Lactobacillus plantarum* fails to elicit effective antibody production due to the presence of an additional plasmid carrying an anchor-protein fragment (119). Furthermore, a *Lactococcus lactis* strain carrying the same recombinant plasmid produced significantly lower levels of specific IgG compared with *L. plantarum* (120). These findings suggested that the ability of an oral vaccine to stimulate an immune response or immune tolerance is closely associated with the site of antigen protein expression, the plasmid carrier and the strain used as the carrier.

Significant advancements have also been made in the study of viral agents as vectors. Particularly noteworthy advancements include mucosal vaccines that utilize poliovirus and adenovirus as live vectors. Research has demonstrated that a poliovirus vector carrying an antigen can activate $CD4^+$ T cells, thereby regulating the activity of IgA-associated B cells and generating specific cytotoxic T lymphocytes (121). An oral vaccine for human immunodeficiency virus (HIV) employs poliovirus as a carrier. The poliovirus envelope gene is replaced with the pol and gag genes from HIV, and the resulting recombinant virus expresses the P1 virus sheath protein (122). An oral vaccine for measles utilizes a recombinant defective adenovirus as a vector. This vector contains a mutated form of the cytomegalovirus promoter that is missing a portion of the E1 region and successfully induces a T cell immune response in mice through expression of the measles virus H protein (123).

Oral vaccines present challenges in clinical trials. Unlike injectable vaccines, which are typically administered in one or multiple doses depending on the vaccine type, individual age, weight and immune system, oral vaccines require additional testing to study their tolerance to the acidic environment of the stomach and their ability to remain intact and activate an immune response during the digestive process (124).

5. Promising transgenic plant-based oral vaccines

The concept of producing oral vaccines using edible plants was initially proposed by Dominic Lam and subsequently implemented in the early 1990s (125). Plant-based oral vaccines refer to vaccines that are generated from genetically modified plants; the immunity provided by plant-based oral vaccines in human trials is achieved by ingesting plant tissue containing the vaccine. Plant-based vaccines have gained significant attention in the field of biotechnology and notable advancements have been made in this area. Thus far, the antigen genes expressed in transgenic plants include hepatitis B virus surface antigen (74,5), tuberculosis virus secretory protein, MPT64 (126) and measles virus hemagglutinin glycoprotein (79) gene. The plants used include tobacco (127), potato (128), *Arabidopsis* (129), soybean (130), peanut (131), lettuce (132), carrot (133), tomato (134), white clover (135), alfalfa (136), corn (137), kelp (138) and lupine (139). Plant-based oral vaccines do not require processing, purification or cryopreservation, making them easy to use and promote.

Transgenic plants offer a novel platform for developing recombinant proteins, with a number of advantages. Through genetic modification, plants can be engineered to produce proteins for pharmaceutical, industrial or agricultural use, offering benefits such as low production costs, scalability and increased safety (140). Transgenic plants have been shown to be the most effective form of oral vaccine due to their ability to facilitate easy administration, reduce production and storage costs, and improve accessibility, especially in areas with limited healthcare infrastructure (141). As a result, in addition to bacteria and viruses, plants have also been successfully utilized to express and present vaccine antigens (142). Transgenic tobacco, potato, tomato and other plants have been found to be capable of expressing various human pathogen antigens, including heat-labile enterotoxin subunit B (LTB) subunit, hepatitis B surface antigen, rotavirus and virus-like particles (143,144). These expressed antigens can stimulate a specific immune response without the need for adjuvants (11). Some transgenic plants can also induce protective immune responses against certain allergens, such as bacterial outer membrane vesicles from *Pseudomonas syringae* and *P. fluorescens* activate plant immune responses that protect against bacterial and oomycete pathogens (145). However, the expression level of antigens in transgenic plants is relatively low. Furthermore, although they can enhance the immunogenicity of presented antigens, transgenic plants also pose the risk of compromising the body's tolerance to food such as inducing new allergic reactions to foods that were previously non-allergenic, or exacerbating existing allergies by increasing the immune system's sensitivity to certain antigens found in foods. Therefore, further research is needed to explore the development of plant-based oral vaccines (146).

Due to their taste, lack of toxic ingredients and high nutrient content, most vegetables are considered suitable for use as receptors for plant-based oral vaccines. Among these vegetables, potatoes have emerged as the primary plant model for developing plant-based oral vaccines (147). However, since potatoes are not edible in their raw state, they must be cooked before consumption, which limits the applicability of this receptor. Tomatoes have also emerged as a promising expression system and have successfully been utilized for the transfer of various genes such as the hepatitis B virus surface antigen gene, HIV gag and gp genes (148) and the rabies virus coat protein (149). In addition, significant advancements have been achieved in the tissue culture and genetic transformation of carrots, making them another ideal candidate for studying plant-based oral vaccines (150). For example, carrots have been used to express the measles virus hemagglutinin, which exhibits both antigenicity and immunogenicity. This engineered protein is capable of stimulating a Th2 immune response (151), indicating its ability to activate both humoral and cellular immunity. Additionally, the structural protein, VP1, of the foot-and-mouth disease virus has been effectively expressed in carrot leaves (152). Subsequent ELISA results indicate that the expressed antigen exhibits specific and active binding to the corresponding antibody (153). Therefore, the utilization of genetically modified vegetables in the production of orally-administered vaccines, particularly for

COVID-19, holds potential. Nevertheless, certain challenges were addressed in a study (154). One such obstacle is that most vegetables do not possess a high protein content, which could impede the expression of antigens (112).

Research is currently being conducted to explore fruits as a viable option for edible oral vaccines. For example, vaccines synthesized in bananas using fruit-specific promoters could be utilized for disease prevention through their consumption (155). Papaya, a widely available tropical and subtropical fruit, can be consumed in its raw form (156). Thus, the papaya transformation and regeneration system has been well-established, and is currently regarded as an optimal candidate for oral vaccine production (157). Transformation of the *Mycobacterium tuberculosis* secreted protein, early secreted antigenic target 6 kDa, in papaya is still undergoing follow-up experiments (158). For the production of oral vaccines, seed plants that contain substantial amounts of soluble protein and that can maintain their quality under storage conditions are typically regarded as more appropriate candidates.. Cereals, such as corn and rice, are also particularly well suited due to the abundance of soluble proteins in the endosperm, which can be separated from the rest of the seed, thereby increasing antigen concentration and reducing the required dosage (159). Currently, antigen gene expression has been successfully achieved in corn (160) and rice (161). In addition, with the establishment of industrial algae production, research into the use of transgenic algae as bioreactors for the production of exogenous proteins has begun (162). At present, the genetic transformation of algae has been successful in Cyanobacteria (163) and *Arthrospira platensis* (*Spirulina)* (164). Furthermore, hepatitis B virus surface antigen has been successfully expressed in cyanobacteria (165). Using algae as a bioreactor to produce oral vaccines may solve a number of problems such as high production costs, risk of contamination with human pathogens, complex purification processes, and cold chain storage and distribution requirements that are difficult to overcome with other organisms.

6. Innovative vaccine delivery systems: Harnessing *Bacillus subtilis*, yeast and nanoparticle vectors

In the development of future vaccines, promising candidates for eliciting the necessary immune response and enabling oral administration include *Bacillus subtilis*, yeast and nanoparticles (166). These innovative vaccine vectors offer unique advantages, such as their inherent immunogenicity and ability to traverse the gastrointestinal tract unharmed (53). *B. subtilis*, a versatile bacterium, can deliver antigens effectively and stimulate both the mucosal and systemic immune responses (167). Yeast-based vaccine platforms have also demonstrated their potential in inducing strong humoral and cellular immune responses (168). Mucosal surfaces are the first line of defense against most infectious diseases, and oral immunization can stimulate cellular and humoral immune responses at both systemic and mucosal levels, thereby inducing broad-spectrum and long-lasting immunity (169). However, successful oral vaccines need to overcome the harsh gastrointestinal environment, including extremely low pH, proteolytic enzymes, bile salts, low permeability, and low immunogenicity (170).

Over recent years, innovative delivery systems utilizing nanoparticles and microparticles have been meticulously engineered to enhance the administration and efficacy of oral vaccines. The incorporation of these particles into vaccine formulations has been demonstrated to bolster antigen stability, increase antigen availability and augment adjuvanticity. Furthermore, they possess an enhanced capacity to stimulate the immune system, ensure targeted delivery and facilitate controlled release of the vaccine components (170). The use of these vaccine vectors holds great promise for the future of oral vaccine development, offering new avenues for achieving the desired immune response and improving vaccine accessibility. Recombinant vaccines against hepatitis B virus surface antigen (171), tetanus toxin (172), diphtheria toxin (173) and pertussis toxin (174) have been developed and can be mass-produced. Live attenuated oral vaccines not only eliminate pathogen toxicity as the carrier but also possess self-replication ability and natural adjuvant activity. As such, they are highly effective in preventing reinfection, establishing an immune response between serum and the mucous membrane, and maintaining lasting immune memory. Therefore, live attenuated vaccines hold significant application value as oral vaccines. However, the control of their toxicity requires further improvement, and a number of studies are still in the animal experimentation stage (175,176).

7. Conclusion

Research on oral vaccines in the past 20 years suggests that this approach could be highly beneficial for mass administration of vaccinations worldwide. The safety, efficacy, convenience and cost-effectiveness of oral vaccines make them an excellent option for disease prevention. We plan to further explore this approach by developing oral vaccines for other human infectious diseases such as hepatitis B, as well as for infectious diseases in animals such as shrimp and chickens (Fig. 2).

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Author's contributions

YL, DL and ML were responsible for writing the manuscript and investigation of the subject, such as conducting literature

reviews to understand the current state of research. WZ and HA edited, conceptualized and supervised the study. All authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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