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LACTIC ACID BACTERIA AS DELIVERY VECTOR FOR MUCOSAL VACCINATION

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ABSTRACT

The development of efficient mucosal vaccines against infectious diseases and cancer is one of the major concerns in modern vaccinology. Mucosal immunization is considered as safe, inexpensive and easy. Food-grade Lactic Acid Bacteria (LAB) such as lactococci and lactobacilli are excellent candidates as delivery vectors for mucosal immunization. Many recombinant LAB vaccines have been tested successfully for their prophylactic and therapeutic effects in animal models. Herein, this review summarizes the use of LAB-based mucosal vaccines against cancer and infectious diseases. These findings indicate the potential value of using LAB to develop novel mucosal vaccines.

KEYWORDS: Lactic Acid Bacteria, vaccine, mucosal immunization, *Lactobacillus*, *Lactococcus.*

INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore-forming bacteria found in milk products and produce lactic acid as the major metabolic end product of carbohydrate fermentation. They are ubiquitous and found in plants, wine, milk, meat, fermented food (yogurt, cheese, pickles, etc.), as well as in the oral cavities, gastrointestinal tracts (GIT), and vaginas of humans and animals. There is evidence that some strains of LAB have a favorable influence on physiologic and pathological processes of the host due to their specific health-promoting probiotic characteristics that relate to modulation of the immune system.[1]

In the past decade, genetically modified or live-attenuated pathogenic bacteria, such as *Salmonella*, *Shigella* and *Listeria* species are used as delivery vectors for vaccine candidates.[2,3,4] There is always a risk of reversion to virulence in these vectors. Recently, lactic acid bacteria (LAB) are used as an attractive delivery system for mucosal immunization[12,25,32,35,36] because LAB are considered to be generally regarded as safe (GRAS) organisms, inexpensive, they exhibit probiotic and adjuvant properties, and they are weakly immunogenic.[7-11] In addition, antigens expressed on the surfaces of LAB are better recognized by the immune system.[5,6] Genetically modified LAB have been effective in delivering antigen to the mucosal immune system and inducing a local immune response.[12] Mucosal immunization provides many advantages such as reduction of side-effects, possibility to modulate both systemic and mucosal immune responses and easy administration.[13,14] Many lactic acid bacteria (LAB) are acid and bile resistant and thus are well adapted to oral delivery. Therefore, lactic acid bacteria are excellent candidates for the development of safe mucosal vaccine delivery vehicles.

In this review, we discussed about different lactic acid bacterial vectors expressing antigens from bacteria, virus and parasites.

Recombinant lactic acid bacterial (LAB) vaccines against bacterial infections

Lactococci and lactobacilli are excellent candidates as delivery vectors in the development of new vaccines. Recombinant *Lactococcus lactis* strain expressing tetanus toxin fragment C (TTFC) induced systemic and mucosal immune responses when administered orally or intranasally to C57 BL/6 mice.[12,15,16] *Lactobacillus plantarum* intracellularly expressing TTFC also induced significant levels of circulating TTFC-specific immunoglobulin G (IgG) following nasal or oral delivery.[17,18] Mucosal vaccination in mice model with *Lactococcus lactis* that expresses the conserved C-repeat region (CRR) of M protein from *Streptococcus pyogenes* serotype 6 produced CRR-specific salivary IgA and serum IgG, prevented pharyngeal infection with *S. pyogenes*, and promoted survival.[19]

Recombinant *Lactobacillus*-based live oral vaccine protected mice from tick-transmitted *Borrelia burgdorferi* infection (lyme disease). Recombinant *Lactobacillus plantarum* expressing *B. burgdorferi* outer surface protein A (OspA) induces both systemic and mucosal immunity after oral administration.[20] Even though lactic acid bacteria (LAB) are used for treating and preventing diarrhea, several LAB-based vaccines have been developed against diarrhea. Recombinant *Lactobacillus casei* 525 strain stably expressing a fusion protein

comprising poly-γ-glutamate synthetase A (PgsA) and fimbrial protein of enterotoxigenic *Escherichia coli* F41 (ETEC) elicited a protective immune response against F41 infection in vivo.[21,22] Specific mucosal and systemic antibody responses were induced by oral immunization of mice with the *Lactococcus lactis* expressing enterohemorrhagic *Escherichia coli* (EHEC) EspB antigen. Moreover, immunized mice exhibited significant protection against *E. coli* O157:H7 colonization.[23] *Helicobacter pylori* adhesin Hp0410- producing *Lactobacillus acidophilus* GIM 1.208 strain exhibited significant protection against gastric *Helicobacter* infection following a challenge with *H. pylori* Sydney strain 1 (SS1). The recombinant live bacterial vaccine elicited mucosal secretory IgA antibodies and serum IgG antibodies.[24]

Currently, two typhoid vaccines are commercially available: attenuated *S. typhi strain Ty21a* and the purified capsular polysaccharide of *S. typhi* antigen Vi. Live oral vaccine Ty21a is well-tolerated, but modestly immunogenic, requiring 3–4 consecutive doses to achieve moderate levels of protection. Intramuscular vaccine Vi is protective but commonly associated with injection site reactions. Thus, although Ty21a is licensed in 56 countries and Vi is licensed in more than 92 countries, neither has been widely adopted in public health programs in countries where typhoid and non-typhoid fevers are endemic. Recombinant flagellin (FliC) protein has been reported as a protective antigen against *Salmonella enterica serovar Typhi* infection in mice.[25] Thus, this antigen in combination with lactic acid bacteria was developed as a vaccine candidate. Recombinant *Lactobacillus casei* expressing a flagellar (FliC) antigen from *Salmonella enterica serovar Enteritidis* conferred protective immunity against *Salmonella* infection in mice.[6,26,27]

L. plantarum NCDO1193 and *L. helveticus* ATCC15009 expressing pneumococcal surface antigen A (PsaA) of *Streptococcus pneumonia* elicited IgA and IgG antibody responses and decreased the *S. pneumoniae* recovery from nasal mucosa, after nasal inoculation in C57Bl/6 mice.[28]

Recombinant lactic acid bacterial (LAB) vaccines against viral infections

Safe and effective mucosal vaccines have been developed against viral infections. Mucosal immunization with recombinant *L. casei* expressing cholera toxin subunit A1 (CTA1)-conjugated to influenza A virus consensus matrix protein 2 (sM2) protein on its surface induced potent mucosal, humoral and cell-mediated immune responses after oral and nasal inoculation in BALB/c mice. This immunization gave protection against challenges with

divergent influenza virus subtypes containing heterologous sM2 sequence.[29] Mice immunized with *Lactococcus lactis* expressing the avian influenza (H5N1) virus hemagglutinin (HA1) antigens on its surface and recombinant cholera toxin subunit B (CTB) protein were completely protected from lethal challenge of the H5N1 influenza virus.[30]

Severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV), belongs to the Coronaviridae, is transmitted through the mucosal surfaces of the upper respiratory or gastrointestinal tracts in human. Mucosal immunization with recombinant *L. casei* expressing SARS-associated coronavirus S protein on its surface elicited protective immune response against the virus in C57 BL/6 mice.[31]

Currently, no effective HIV vaccine exists. Various HIV vaccines have been tested in clinical trials almost since the discovery of HIV. Mucosal LAB vaccines are under development. Oral immunization with *L. lactis* IL1403 strain expressing the envelope protein (Env) of HIV on its cell surface induced both mucosal and humoral immune responses in BALB/c mice. Moreover, this vaccine elicited a protective cellular immune response against HIV.[32]

Recombinant lactic acid bacterial (LAB) vaccines against cancer

Development of cervical cancer is associated with the infection caused by human papillomavirus type 16 (HPV-16). E7 antigen of HPV-16 is considered to be a good antigen candidate for the development of new vaccines against cervical cancer. *Lactococcus lactis* expressing inducible cell-wall-anchored form of HPV-16 E7 protein induced antigen-specific cellular response after intranasal administration in C57 BL/6 mice.[33,34,35]

Recombinant lactic acid bacterial (LAB) vaccines against parasite infections

Drug resistance is a major problem in malaria treatment. Prevention of malaria parasite by vaccination is the easiest way to eradicate the infection compared to treatment. Oral immunization with recombinant *L. lactis* expressing C-terminal 19-ku fragments of *Plasmodium yoelii* merozoite surface protein 1 ($MSP\text{-}1_{19}$) antigen protected BALB/c and C57BL/6 mice against malaria parasites challenge.[36] *Lactococcus lactis* expressing a fragment of the *Plasmodium falciparum* (Pf) glutamate-rich protein (GLURP; amino acids 79-1500) fused in frame to a correctly-folded fragment of the sexual stage Pfs48/45 antigen (amino acids 475-1284) induced antibody responses against the parasite in rodents.[37]

There is no human vaccine currently available against *Leishmania* infections. Alanine racemase deficient strain of *Lactococcus lactis* (PH3960) co-expressing mouse interleukin-12 and *Leishmania* homologue of activated C kinase with Usp45 secretion signal in its N-terminus (secLACK) significantly reduced the parasite burden and induced an antigen-specific mucosal immune response and a LACK-specific T(H)1 immune response in splenocytes and mesenteric lymph node cells in orally immunized BALB/c mice. This vaccination strategy protected the mice against *Leishmania* major infection.[38]

CONCLUSIONS AND FUTURE ASPECTS

Even though several mucosal vaccines have been studied for many years, no live recombinant LAB vaccine is available commercially. Cervical cancer vaccine consisting of *Lactobacillus casei* expressing the human papillomavirus virus (HPV) type 16 E7 antigen, BLS_ILS_E710c, entered phase I/II clinical trial (NCT02195089). Lactic acid bacteria are non-pathogenic, non-invasive and food-grade bacterial vectors capable of delivering antigen to the mucosal and systemic immune systems generating specific antibody and cell-mediated immune responses in serum and mucosal secretions. LAB vaccines exhibit probiotic and adjuvant properties along with the prophylactic and therapeutic properties. Most commercial vaccines available today are delivered by injection, with problems of safety, patient acceptability and morbidity. Administration of LAB vaccines is very easy and a normal person can take the vaccine without the need for trained personnel. *Lactococcus lactis* is the most widely used LAB as a delivery vehicle for mucosal immunization followed by *Lactobacillus casei* and *Lactobacillus plantarum*. Altogether, these studies have reinforced the use of recombinant lactic acid bacteria as delivery vehicles for mucosal immunization.

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