Current issues regarding the application of recombinant lactic acid bacteria to mucosal vaccine carriers

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Abstract

Over the past two decades, lactic acid bacteria (LAB) have been intensively studied as potential bacterial carriers for therapeutic materials, such as vaccine antigens, to the mucosal tissues. LAB have several attractive advantages as carriers of mucosal vaccines, and the effectiveness of LAB vaccines has been demonstrated in numerous studies. Research on LAB vaccines to date has focused on whether antigen-specific immunity, particularly antibody responses, can be induced. However, with recent developments in immunology, microbiology, and vaccinology, more detailed analyses of the underlying mechanisms, especially, of the induction of cell-mediated immunity and memory cells, have been required for vaccine development and licensure. In this mini-review, we will discuss the issues, including (i) immune responses other than antibody production, (ii) persistence of LAB vaccine immunity, (iii) comparative evaluation of LAB vaccines with any existing or reference vaccines, (iv) strategies for increasing the effectiveness of LAB vaccines, and (iv) effects of microbiota on the efficacy of LAB vaccines. Although these issues have been rarely studied or discussed to date in relation to LAB vaccine research, further understanding of them is critical for the practical application of LAB vaccine systems.

Keywords Lactic acid bacteria · Mucosal vaccine · Vaccine persistency · Mucosal adjuvant · Microbiota

Introduction

Vaccines are the most cost-effective and suitable means of controlling infectious diseases. Recent vaccine development is focused on subunit vaccines consisting of components specific to pathogens instead of conventional vaccines, such as live or inactivated vaccines composed of whole pathogens. In general, subunit vaccines are thought to be safer than live or inactivated vaccines, as vaccines made of whole pathogenic microbes contain many proteins that are unnecessary for protective immunity but often carry the risk of triggering allergic or autoreactive consequences.

Mucosal routes for vaccine delivery have gained an increased interest in modern vaccinology, as mucosal vaccines can induce mucosal immunity, which is hard to induce with systemic vaccines and as mucosal surfaces represent a major

entry site into the human body for many pathogens (Chen and Cerutti 2010). However, the mucosal administration of subunit vaccines composed of purified antigens normally induces no or only low immune responses, due to their rapid enzymatic degradation on the mucosal surface and/or the induction of mucosal tolerance (Mestecky et al. 2007). Therefore, the development of a carrier is required for the protection of vaccine antigens from the harsh mucosal environment, their delivery to the mucosa-associated lymphoid tissues, and induction of adaptive immune responses rather than tolerogenic immune responses. Currently, there are two major trends in the development of carriers for mucosal vaccines. One is based on physicochemical systems such as emulsions and liposomes (Schwendener 2014; Corthésy and Bioley 2018), and the other uses genetically modified live viruses or bacteria as vectors (da Silva et al. 2014; Lin et al. 2015).

Lactic acid bacteria (LAB) are a group of Gram-positive, non-sporulating bacteria that includes species of *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bifidobacterium*, and several others. LAB is a generic term for bacteria that produce a large amount of lactic acid as the end product of sugar metabolism. From ancient times, dietary LAB have been used to ferment a wide range of raw materials, such as milk for the production of cheeses and yoghurts, as

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part of the food industry. Over the past two decades, LAB have been intensively studied as potential bacterial carriers for therapeutic materials, such as vaccine antigens, to the mucosal tissues, since LAB have several attractive advantages as a mucosal vaccine carrier, such as an excellent safety profile for human use, easy genetic manipulation, ability to be taken up into Peyer's patches, absence of endotoxins, resistance to low pH, intrinsic adjuvanticity, and weak immunogenicity (Wyszyńska et al. 2015).

As several excellent reviews on the development of recombinant LAB as a vaccine candidate against various infectious diseases have been published recently (Wyszyńska et al. 2015; Szatraj et al. 2017; de Castro et al. 2018; LeCureux and Dean 2018), and as they have covered the generation of recombinant LAB vaccine strains, the effects of antigen localization or LAB strains used on their immunogenicity, and the securing of safety in clinical use, here we will discuss the following five issues that have been rarely studied or discussed to date in LAB vaccine research: (1) immune responses other than antibody production, (2) persistence of LAB vaccine immunity, (3) limitations of published comparative evaluation of LAB vaccines, (4) strategies for increasing the immunogenicity of LAB vaccines, and (5) the effects of microbiota on the efficacy of LAB vaccines.

In Table 1 (for virus antigens) and Table 2 (for bacterial or others antigens), we listed the studies on LAB-based vaccine candidates that are discussed in this review or those worthy looking into, as they analyzed various endpoints for evaluation of LAB vaccine efficacy, including T cell and/or dendritic cell (DC) activation, neutralizing activities, and protective effects against challenge of pathogens, on top of the antibody response which is the only endpoint described in most publications. We hope that this article stimulates informative discussion on research issues related to LAB vaccine development, as well as practical ideas for planning future LAB vaccine experiments.

Issues related to the development of LAB vaccines

Immune responses other than antibody production

The majority of the published works on the development of LAB vaccines has focused on whether administration of each recombinant LAB strain can induce antigen-specific antibody responses, not only because measurement of antibody titers is the simplest way to evaluate immune responses, but also because antibodies play a critical role in many approved vaccines, such as influenza, hepatitis, and unconjugated pneumococcal vaccines. As a result, there is a growing number of reports showing that the mucosal administration of recombinant LAB-expressing antigens can induce antigen-specific

mucosal and systemic antibody production. However, for evaluation of vaccine efficacies, it is not always enough to only confirm the increase in antibody titer. Indeed, even if the specific antibody titer rises, such indications are often insufficient for protection against infection (Perez et al. 2005; Sim et al. 2008; Marelli et al. 2011; Berlec et al. 2013). In other words, antibody levels measured by ELISA do not always correlate with the level of protection. Therefore, consideration of the contribution of antibodies to the protective function requires the differentiation of functional antibodies from non-functional, binding antibodies. Measuring of functional antibodies is performed by neutralization assay, opsonophagocytic killing assay, and complement fixation test. In addition to the measurement of the binding and functional antibody titers, confirmation of the protective effect of the vaccine in challenge experiments is important for evaluating the effectiveness of the vaccine. Recently, several reports have suggested that not only CD4⁺ but also CD8⁺ T cell immune responses are induced in mice immunized with *L. lactis* vaccines (Kawana et al. 2014; Chamcha et al. 2015; Zhao et al. 2017). However, these studies did not directly demonstrate a role of the CD8⁺ responses. Based on antibody-dependent depletion experiments, Sebbane and his colleagues found that CD8⁺ T cells had a moderate role in the vaccine effect of *L. lactis*-secreting LcrV, known as an essential component of the type III secretion system of *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*) and as a potential vaccine candidate, although activated CD4⁺ cells were necessary for vaccine-induced protection (Daniel et al. 2016). It would be critical to study whether immune responses other than antibody responses induced by the LAB vaccination, such as cell-mediated immunity, are responsible for the protection.

In recent years, basic research on deciphering the cellular and humoral immune responses has advanced our knowledge of immunology. Along with these advances, the role of cell-mediated immunity is being increasingly recognized as important not just for the prevention of viral infections but also for the prevention of bacterial infections, differentiation of memory cells, and maintenance of antibody production. Indeed, vaccines licensed in the recent years and those close to licensure measure cellular immunology as a part of their endpoints in clinical trials, as recommended by both the WHO and EMA guidelines (World Health Organization 2015; European Medicines Agency 2018). However, the study of cellular immunity remains challenging as the methods used for its assessment are more complex than those for measuring humoral immunity. Therefore, selecting T cell readouts for measurement in pre-clinical vaccine studies to assess vaccine immunogenicity and efficacy requires careful consideration. Currently, in LAB vaccine studies, measurements of IgG subclasses, such as IgG1, IgG2a, and IgG2b, in ELISA are commonly used to assess the Th1/Th2 balance

Table 1 Representative articles describing the protective effects of virus-derived antigen-expressing LAB strains

Pathogen	LAB strain	Antigen	Adjuvant	Expression	Route	Results	Ref.
IVA	<i>L. plantarum</i>	HA2	LTB	Surface	oral	Th1, Th2, Th17, CTL, Challenge	Yang et al. 2017a
IVA	<i>L. plantarum</i>	NP-M1	DCpep	Surface	oral nasal	Serum IgG, Mucosal IgA, Th1, Challenge (nasal > oral)	Yang et al. 2017a
IVA	<i>L. plantarum</i>	M2	–	Surface	oral	DC activation, Th1, CTL, Mucosal IgA, Challenge	Yang et al. 2017b
IVA	<i>L. casei</i>	M2-HA2	CTA	Surface	oral nasal	Serum IgG, Mucosal IgA, Challenge, Persistence of vaccine immunity (7 months)	Li et al. 2015b
IVA	<i>L. casei</i>	M2	CTA	Surface	oral nasal	Serum IgG, Mucosal IgA, CTL (with > without CTA), Challenge	Chowdhury et al. 2014
IVA	<i>L. lactis</i>	NP	CTB	Intracell.	oral	Serum IgG, Mucosal IgA, Th1, Th2, Challenge (only with CTB)	Lei et al. 2015
IVA	<i>L. lactis</i>	NA	–	Surface	oral	Serum IgG, Mucosa IgA, NI titer, Challenge	Lei et al. 2015
IVA	<i>L. lactis</i>	HA1	CTB	Surface	oral	Serum IgG, Mucosal IgA (only with CTB), Splenocyte proliferation, cytokine production (only with CTB), Challenge (only with CTB)	Lei et al. 2011
HIV	<i>L. acidophilus</i>	MPER	–	Surface	oral	Serum IgG, Mucosal IgA, Th1, Th17	Kajikawa et al. 2015
HIV	<i>L. acidophilus</i>	Gag	FliC	Surface	oral	DC activation, IgA+ B cell, Th1	Kajikawa et al. 2012
HIV	<i>L. lactis</i>	Gag	–	Surface	oral nasal rectal	Serum IgG, Mucosal IgA, DC activation, CTL	Chancha et al. 2015
HPV	<i>L. casei</i>	L2	–	Surface	oral	Serum IgG, Mucosal IgG and IgA, NT, Challenge	Yoon et al. 2012
HPV	<i>L. casei</i>	E6	–	Surface	oral	Serum IgG, Mucosal IgA, Th1, Challenge (tumor)	Lee et al. 2010
HPV	<i>L. casei</i>	E7	–	Surface	oral	Mucosal Th1, CTL	Adachi et al. 2010
HPV	<i>L. casei</i>	E7	–	Surface	oral	Serum IgG, Mucosal IgA, Th1, Challenge (tumor)	Poo et al. 2006
Rotavirus	<i>L. lactis</i>	VP8	–	Intracell. Secretion	Surface	Serum IgG, Mucosal IgG and IgA, NT	Marelli et al. 2011
Rotavirus	<i>L. lactis</i>	VP7	–	Intracell. Secretion	Surface	NT	Perez et al. 2005

IVA, influenza virus A; HIV, human immunodeficiency virus; HPV, human papilloma virus; *L. plantarum*, *Lactobacillus plantarum*; *L. casei*, *Lactobacillus casei*; *L. acidophilus*, *Lactobacillus acidophilus*; *L. lactis*, *Lactococcus lactis*; HA, hemagglutinin; NP, nucleoprotein; MPER, membrane proximal external region; LTB, heat-labile enterotoxin B subunit; DCpep, dendritic cell-targeting peptides; CTA, cholera toxin A subunit; CTB, cholera toxin B subunit; Intracell. intracellular; NT neutralizing antibody; NI, neuraminidase inhibition; CTL cytotoxic T lymphocyte

Table 2 Representative articles describing the protective effects of bacteria or other pathogen-derived antigen-expressing LAB strains

Pathogen	LAB strain	Antigen	Adjuvant	Expression	Route	Results	Ref.
ETEC	<i>L. casei</i>	K99 K88	–	Surface	oral	Serum IgG, Mucosal IgA, Th1, TH2, CTL, Challenge	Wen et al. 2012
ETEC	<i>L. casei</i>	F41	–	Surface	oral	Serum IgG (Th2 dominant), Mucosal IgA (remained for > 16 weeks), Challenge	Lin et al. 2017
EHEC	<i>L. acidophilus</i>	espA/Tir-M	–	Secretion	oral	Serum IgG, Mucosal IgA, Serum cytokine, Challenge	Liu et al. 2009
EHEC	<i>L. lactis</i>	EspB	–	cytoplasm	oral	Serum IgG, Mucosal IgA, Th1, Th2, Challenge	Ahmed et al. 2014
<i>S. pneumoniae</i>	<i>L. casei</i>	PspA, PspC	–	Surface	nasal	Serum IgG, Th1, Th17, Complement deposition, Challenge	Ferreira et al. 2009
<i>S. pneumoniae</i>	<i>L. lactis</i>	PspA	–	Intracell.	nasal	Serum IgG (Th2 dominated), Challenge	Hanniffy et al. 2007
<i>S. pyogenes</i>	<i>L. lactis</i>	M protein	–	Surface	nasal	Serum IgG (Th2 dominated), Challenge	Hanniffy et al. 2007
<i>S. pyogenes</i>	<i>L. lactis</i>	Shr	–	Surface	nasal ip	Serum IgG, Challenge	Wozniak et al. 2014
<i>S. agalactiae</i>	<i>L. lactis</i>	Pilus protein	–	Surface	nasal sc ip	Serum IgG, Mucosal IgA, Challenge	Huang et al. 2011
<i>Y. pseudotuberculosis</i>	<i>L. lactis</i>	LcrV	–	secretion	nasal	Serum IgG, CD4+ and CD8+ T cell function, Challenge, Passive immunization assay	Daniel et al. 2016
<i>Y. pseudotuberculosis</i>	<i>L. lactis</i>	LcrV	–	secretion	oral nasal	Serum IgG (IgG1 dominated), Mucosal IgA, Splenocyte cytokine production, Challenge (nasal > oral)	Daniel et al. 2009
<i>L. monocytogenes</i>	<i>L. lactis</i>	LLO	–	Intracell. Secretion	oral ip	CTL, Challenge	Bahey-El-Din et al. 2008
<i>B. abortus</i>	<i>L. lactis</i>	SOD	IL-12	Secretion	oral	Serum IgM and IgG (IgG2a dominated), Mucosal IgA, Challenge	Sáez et al. 2012
<i>C. difficile</i>	<i>L. lactis</i>	TcdA TcdB	–	Unknown	oral	Serum IgG, Mucosal IgA, NT, Challenge	Guo et al. 2015
<i>S. aureus</i>	<i>L. lactis</i>	SEB	–	Secretion	oral	Serum IgG, Mucosal IgA, Challenge	Asensi et al. 2013
<i>B. anthracis</i>	<i>L. acidophilus</i>	PA	–	Surface	oral	Serum IgG, NT, IgA+ B cell, Challenge	Mohammadzadeh et al. 2009
<i>C. tetani</i>	<i>L. lactis</i>	TTFC	–	Intracell.	oral nasal	Serum IgG (IgG1 and IgG2a dominated), Mucosal IgA, Challenge	Robinson et al. 1997
<i>L. major</i>	<i>L. lactis</i>	LACK	–	Intracell.	oral	Mucosal IgA, Th1, Lymphocyte proliferation, Challenge	Hugentobler et al. 2012

ETEC, enterotoxigenic *Escherichia coli*; EHEC, enterohemorrhagic *Escherichia coli*; *Y. pseudotuberculosis*, *Y. pseudotuberculosis*; *L. casei*, *Lactobacillus casei*; *L. acidophilus*, *Lactobacillus acidophilus*; *L. lactis*, *Lactococcus lactis*; Psp, *Pseudomonas* surface protein; LLO, *Listeria* listeriolysin O; SOD, *Staphylococcus aureus* dismutase; SEB, *Staphylococcus aureus* enterotoxin type B; PA, *Proteus* antigen; TTFC, *Tetanus toxin fragment C*; LACK, *Leishmania* homolog of activated C kinase receptor; DCp, dendritic cell-targeting peptides; Intracell, intracellular; NT, neutralizing antibody; CTL, cytotoxic T lymphocyte

of vaccine immunity, as switching to each subclass is controlled under specific cytokine environments. In addition, measurement of IFN- γ - or Th2 cytokines, such as IL-4, -producing CD4⁺ or CD8⁺ T cells in PBMCs (peripheral blood mononuclear cells) or in materials isolated from the systemic or mucosal lymphoid tissues (spleen, mesenteric lymph nodes, Peyer's patches, etc.) by flow cytometry or enzyme-linked immunospot (ELISpot) assay is used to evaluate helper T cell types and cell-mediated immunity. Moreover, methods based on the detection of markers of the degranulation of effector cytotoxic T lymphocytes (CTLs), such as perforin and granzyme B, or markers of apoptosis or necrosis of target cells are used for the measurement of CTL function (Bermúdez-Humarán et al. 2005; Adachi et al. 2010; Chamcha et al. 2015). These indicators are particularly important for the development of vaccines against intracellular bacteria and viruses. The type of immunity induced by LAB vaccine is influenced by the adjuvant effect of the LAB species, summarized in detail by Szatraj et al. (2017). LAB affect cytokine production from intestinal epithelial cells and immune cells and the produced cytokine milieu greatly depend on the LAB strain, resulting in the difference in adjuvant properties for the type of immunity (Th1, Th2, or Th1/Th2 mixed) induced. Thus, for LAB vaccine development, selection of LAB species/strains would be important for induction of the desired immune responses for protection from target pathogens. Or designing LAB to express a certain cytokine would help vaccine development suitable for the purposes as discussed later.

Persistence of vaccine immunity

In general, protective immunity induced by vaccination wanes with time. Insufficient protection due to waning vaccine immunity, known as secondary vaccine failure, has traditionally been more associated with inactivated, subunit, and toxoid vaccines (e.g., pertussis, diphtheria, and tetanus) than live-attenuated vaccines. However, even in tetanus, which is considered to have a relatively short duration of immunity, the half-life of the specific antibody is more than 10 years (Lewis et al. 2014). To date, the duration of the LAB vaccine antibody response has been examined at most up to only a few months. Daniel et al. (2016) reported that in mice orally administered with *L. lactis*-secreting LcrV, the antigen-specific serum antibody titer was maintained at 9 months after the first vaccine administration. However, protective immunity against *Y. pseudotuberculosis* intravenous challenge conferred by *L. lactis*-secreting LcrV decreased slightly over time. To understand the persistency of serum and mucosal antibody responses induced by priming with the oral administration of *L. lactis*-expressing HIV-1 Gag-p24 (LL-Gag) followed by boosting with the intramuscular administration of

recombinant modified vaccinia Ankara (MVA) expressing Gag, Chamcha et al. (2015) tracked Gag-specific IgG in serum and IgA in feces over time. They observed measurable responses until 24 weeks after the MVA boost, demonstrating that LL-Gag/MVA vaccine induces relatively persistent antibody responses in the serum and intestinal secretions. Sim et al. (2008) reported that the serum antibody responses induced by the oral or nasal administration of *L. lactis*-expressing a domain III of E protein (EDIII), which is a major envelope protein of dengue virus and is known as a protective antigen, was sustained for at least 69 days after the last vaccine boost. Li et al. (2015b) reported that immunization of mice with *L. casei*-expressing a fusion protein of influenza virus matrix protein 2 (M2), a well-conserved envelop ion channel protein, hemagglutinin II (HA2) and cholera toxin A (CT-A) subunit, a strong mucosal adjuvant without toxicity, on its surface. Inclusion of CT-A led to the significantly improved antibody levels and protection and allowed to maintain the antibody and protection levels for more than 7 months after immunization. All mentioned studies here suggest that antigen type and immunization regimen, including the route and number and interval of doses, for individual vaccines may affect the duration of persistence. Therefore, it is important to determine the conditions suitable for literally long-lasting protective efficacy by follow-up studies on the immunity levels for a sufficiently long term.

Research on subsets of memory cells and their functions has advanced recently (Todryk 2018). As the induction of memory cells is critical for long-lasting vaccine immunity, we should investigate whether LAB vaccines can induce long-lived memory T and B cells in sufficient numbers and with the correct phenotype capable of clearing pathogens that eventually infect the host at a late time point. Recent studies in mice have suggested that memory T cells that accumulate in tissues, termed tissue-resident memory T (T_{RM}) cells, play a crucial role in maintaining long-term protective immunity to mucosal pathogens (Takamura 2018; Wilk and Mills 2018). However, the role of CD4⁺ T_{RM} cells in protective immunity generated by LAB vaccines has not been investigated. Therefore, the detection and quantification of CD4⁺ T_{RM} cells in tissues in immunized hosts are important for the evaluation LAB vaccine immunity.

Limitations of published comparative evaluation of LAB vaccines

Most studies to date have focused on the development of immunogenic recombinant LAB strains, demonstrating that antigen-specific immunity can be induced by the mucosal administration of LAB vaccines (Wyszyńska et al. 2015). Comparative analyses using standard/reference vaccines play a vital role in the clarification of the toxicity, immunogenicity, and potency of a novel vaccine. However, few studies have

compared the effectiveness/protective efficacy of LAB vaccines with existing or standard vaccines. Although limited examples used as the references to compare with LAB vaccines include inactivated whole pathogen, virus-like particles, or antigen mixed with well-known adjuvant, most of such antigens have been used for induction of humoral immunity. Sim et al. (2008) utilized the intraperitoneal administration of heat-inactivated dengue virus particles as a reference to evaluate the efficacy of oral and nasal vaccination with *L. lactis*-expressing dengue virus EDIII. The binding and neutralizing titers of anti-EDIII IgG antibodies induced by the nasal or oral administration of EDIII-expressing *L. lactis* in BALB/c mice were generally lower than those elicited in mice immunized by intraperitoneal injection of the reference. Yoon et al. (2012) reported injection of the reference. Yoon et al. (2012) reported that oral administration of *Lactobacillus casei*-expressing human papillomavirus (HPV)-16 L2 capsid protein induced serum and vaginal IgG and IgA production, and that the neutralization titers of the serum or vaginal wash from immunized mice were at a level comparable to those from mice subcutaneously immunized with purified HPV-16 L1 virus-like particles. Recently, Wagachchi et al. (2018) compared oral vaccinations with recombinant *L. lactis* expressing a fusion protein of the M1 pilus of *Streptococcus pyogenes* and Ova324–339 peptide (LL-M1p-Ova) and with CT-B-adjuvanted OVA peptides. LL-M1p-Ova induced systemic and mucosal antibody responses at a level similar to that of the OVA peptides/CT-B. Although application of the toxin adjuvants to human vaccine use is limited due to their toxicity (Lycke 2012), the use of candidate antigens adjuvanted with these toxins can be a useful reference for the comparative evaluation of the efficacy of LAB vaccines for the same antigens at least in pre-clinical studies. As the published comparative evaluation of LAB vaccines was dependent mainly on antibody titers, comparison based on protective efficacy is definitely required for future studies.

Strategies for increasing the immunogenicity of LAB vaccines

Many studies have reported that LAB vaccine can induce antigen-specific antibody responses, but antibody titers are often not very high and may be almost none. Therefore, enhancing immunogenicity of LAB vaccines, which is defined by the adjuvanticity of LAB and the immunogenicity of antigen, remains a challenging approach. Several studies have explored strategies for expressing adjuvants, such as cytokines, along with antigens to improve adjuvanticity of LAB. For example, Hugentobler et al. (2012) and Sáez et al. (2012) reported the effectiveness of IL-12 as an adjuvant, and it is possible to skew the immune response to the Th1-type by co-expressing IL-12 with antigens. In addition, there are reports that IL-2 or IL-6 can be used as an adjuvant (Steidler et al. 1998). The use of these

cytokine adjuvants makes it possible not only to enhance vaccine immunogenicity but also to control the type of immune response (for example, Th1 or Th2) to that best suited to protection against the target pathogen infection.

Other approaches are focusing on improvement of the immunogenicity of antigens. For example, pathogen-associated molecular patterns (PAMPs), targeting molecules for M cells and dendritic cells (DCs), and toxins, fused with antigens. With regard to the application of PAMPs to adjuvants, Kajikawa and his colleagues reported a method of expressing FliC (a ligand of TLR5), a component of bacterial flagellin, as a fusion protein with the antigen (Kajikawa et al. 2012). The mucosal adjuvant function of FliC has also been reported in another mucosal vaccine system (Van Maele et al. 2014). We and other groups have reported that the immunogenicity of LAB vaccines can be improved by fusing the antigen with a molecule targeting M cells, a specialized cell for antigen uptake into the Peyer's patches (Li et al. 2015a; Liu et al. 2018; Takahashi et al. 2018). Methods for increasing immunogenicity by fusing the antigen with peptides targeting DCs have also been reported (Mohamadzadeh et al. 2009; Yang et al. 2017). As the toxicity of toxin adjuvants may spoil immune responses enhanced by fusing antigens with toxins (Lei et al. 2011; Chowdhury et al. 2014; Lei et al. 2015; Li et al. 2015b; Jiang et al. 2017), novel mucosal adjuvants, such as the one reported recently (Aoshi 2017), should be examined in the context of LAB vaccines.

Impact of microbiota on vaccine efficacy

Growing evidence suggests that a symbiotic relationship between animals and microbiota is critical for the maintenance of health, including not only the acquisition of nutrients but also the development of the host immune system (Suzuki and Nakajima 2014; Honda and Littman 2016; Belkaid and Harrison 2017). Based on the role of gut microbiota in the development of the immune system, it is logical to expect that the microbiota may influence the efficacy of vaccines, especially mucosal vaccines. Indeed, Kim et al. (2017) recently reviewed the relationship between microbiota and the efficacy of vaccines or mucosal adjuvants. There have been no reports on detailed studies of the effects of bacterial flora and LAB vaccines so far. Early studies have suggested that antigen expression levels and the intestinal persistence of LAB influence the efficacy of LAB vaccines (Grangette et al. 2001; Grangette et al. 2002). Microbiota are likely to affect the metabolism and colonization of the administered LAB, which may alter the effectiveness of LAB vaccines.

In addition to microbial sensing and activation of the innate immune system by host cells, recent studies have clearly shown the importance of bacterial metabolites, such as short-chain fatty acids, in the gut immune responses (Kim et al. 2017; Mariño et al. 2017; Sun et al. 2018). Although

those studies indicated that acetic acid and butyric acid, among the various short chain fatty acids, affect the number and functions of effector and regulatory T cells, lactic acid and its metabolite lactate produced by LAB have been reported to enhance the host intestinal immune system by increasing the antigen up-take of CX3CR1⁺ cells (Morita et al. 2019). Alternatively, LAB may directly affect the microbiota as they have probiotic properties (Patel and Dupont 2015). Accordingly, it is possible that the mutual interaction between the administered LAB and microbiota modulate the host immune system, ultimately affecting the outcome of LAB-based vaccination.

Investigations of the relationship between microbiota and the efficacy of vaccines are at an initial stage, and the data accumulated so far are limited. Therefore, elucidation on the most effective microbiota composition in promoting the efficacy of LAB vaccines will afford important information toward the human application of LAB vaccines.

Conclusions and future perspectives

Previous studies have shown that LAB are attractive and promising candidates as mucosal vaccine carriers. Research on LAB vaccines has been focused to date on whether antigen-specific immunity, particularly antibody responses, can be induced. Indeed, as most approved vaccines, especially subunit vaccines, work through the production of specific antibodies, it is natural to regard antibody production as one of the endpoints of LAB vaccine development. However, with the recent developments in immunology, microbiology, and vaccinology, more detailed analyses of various mechanisms (for example, the induction of cell-mediated immunity and memory cells) are required for the vaccine development and licensure. These components of the immune system are especially important in the development of vaccines against infections that could not be controlled by previous antibody-based vaccine systems. In addition, with the spread of next-generation sequencers, the analysis of the microbiota at the mucosal surface has been greatly advanced, and the relationship between microbiota and host immunity is becoming apparent. As mucosal immunity is considered to be particularly affected by bacterial flora, it is important to investigate the relationship between the efficacy of the mucosal vaccine systems, including the LAB vaccines, and the host bacterial flora. We believe that further understanding of the issues raised in this review is critical for the practical application of this vaccine system.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical statements All animal procedures that we performed and described in this review were approved by the Animal Care and Use Committee of Gifu Pharmaceutical University. Materials obtained from human subjects were not used.

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