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REVIEW ARTICLE

Probiotics and Paraprobiotics in Viral Infection: Clinical Application and Effects on the Innate and Acquired Immune Systems

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Abstract: Recently, the risk of viral infection has dramatically increased owing to changes in human ecology such as global warming and an increased geographical movement of people and goods. However, the efficacy of vaccines and remedies for infectious diseases is limited by the high mutation rates of viruses, especially, RNA viruses. Here, we comprehensively review the effectiveness of several probiotics and paraprobiotics (sterilized probiotics) for the prevention or treatment of virally-induced infectious diseases. We discuss the unique roles of these agents in modulating the cross-talk between commensal bacteria and the mucosal immune system. In addition, we provide an overview of the unique mechanism by which viruses are eliminated through the stimulation of type 1 interferon production by probiotics and paraprobiotics via the activation of dendritic cells. Although further detailed research is necessary in the future, probiotics and/or paraprobiotics are expected to be among the rational adjunctive options for the treatment of various viral diseases.

Keywords: Probiotics, paraprobiotics, virus infection, interferon, plasmacytoid dendritic cell, vaccines.

1. INRODUCTION

Various strategies, such as those using vaccines and antibiotics, have been exploited for the prevention and treatment of infectious diseases, but infection control has not yet been achieved at a sufficient level. In addition to avian influenza [1], severe acute respiratory syndrome [2], and Ebola hemorrhagic fever [3], many problematic diseases of tropical origin remain poorly controlled, such as dengue fever and Zika virus infection [4].

As climate change including global warming and the increased geographical movement of people and goods have emerged, the numbers of pathogenic virus species and affected areas have increased [5]. Therefore, the risk of viral infection has now become a critical issue. A representative pathogenic virus, influenza virus, sometimes undergoes a process of discontinuous mutation. As a result, the efficacy of vaccines against influenza virus may become disrupted, and this phenomenon has sometimes caused pandemics [6]. In Japan, the first case of Zika virus infection was reported in 2016 [7]. Some cases infected with dengue virus were reported in 2014 after the virus had been absent for 70 years previously [8]. In addition, influenza and Norovirus infections often occur seasonally.

With the progress of recent immunological research, the innate immune response and the subsequently activated acquired immune response for the recognition and elimination of viruses have been unveiled [9]. It has been reported that the process of viral elimination largely depends on the induction of type 1 interferons (IFNs) [10], and that the regulation of inflammatory cytokines is mediated via pattern recognition receptors such as Toll-like receptors [9, 11] and retinoic-acid-inducible gene I [12]. Studies have begun to elucidate the immunostimulatory effects of lactic acid bacteria and have reported their capacity to contribute to the prevention of viral infections including influenza [13] as well as the treatment of *Helicobacter pylori* infection [14]. As discussed in the following sections, type 1 IFNs are considered to be pivotal in mediating the protective effect of lactic acid bacteria against these infections.

In this review, we first summarize the recent preventive and therapeutic strategies against infectious diseases with special emphasis on the viral infection and then outline the possible applications of several probiotics and paraprobiotics as prophylactics or therapies against (viral) infectious diseases on the basis of published clinical trial data. Since it is very difficult to cite all studies regarding the effects of paraprobiotics or probiotics on virus infectious disease, we showed representative studies below with two criteria; 1) Single probiotics or paraprobiotics study in human clinical trial (excluded mixture of strains and animal studies), 2) Evaluation of efficacy on (viral) infectious disease.

Finally, we introduce the unique topic of immune defense mechanisms against viral infections that are induced by probiotics and paraprobiotics.

2. PREVENTIVE AND THERAPEUTIC STRATEGIES AGAINST VIRAL INFECTIONS

Infectious diseases, especially nosocomial infections (i.e. influenza, pneumonia, methicillin-resistant *Staphylococcus aureus*, Norovirus, severe acute respiratory syndrome corona virus, *etc.*), have periodically caused widespread outbreaks and are considered to represent serious threats to public health [15, 16]. In this review, we outline strategies for the prevention and treatment of infections of the upper respiratory and gastrointestinal tracts as representative infectious diseases. It is well known that influenza virus, rhinovirus, and respiratory syncytial virus play major roles in Upper Respiratory Tract Infections (URTIs), while norovirus and rotavirus are also important causes of gastrointestinal diseases.

It is generally acknowledged that the main prophylactic measures against these infectious diseases are vaccinations and everyday

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hygienic behaviors such as gargling and hand-washing. Nagatake *et al*. outlined that gargling with a povidone-iodine solution was effective to reduce the incidence of episodes of acute respiratory infections, since colonized bacteria were destroyed by gargling [17]. Heijne *et al*. reported that enhanced hygienic measures including proper hand cleaning using soap and disposable paper towels effectively limited the transmission of norovirus during an outbreak [18].

Although vaccines are promising prophylactics against influenza infection, their efficacy is limited by the frequent and fast mutation of RNA viruses including the influenza virus [19]. Popular influenza treatment regimens include neuraminidase inhibitors and M2 ion channel protein inhibitors, which are both actively prescribed in clinical settings [20]. Inhibitors of hemagglutinin, sialidase, nucleocapsid protein, or RNA-dependent RNA polymerase are also currently used [19, 21]. However, the development of acquired resistance against current anti-influenza drugs and emerging mutations of the influenza virus continue to present major challenges for the effective treatment of influenza.

Norovirus and rotavirus are representative gastrointestinal pathogens that are highly infectious and cause severe diarrhea [22]. In the present situation, without an effective vaccine or medicine, the prevention of viral transmission must rely mainly on basic measures including quarantine and thorough hand washing after physical contact. In particular, rotavirus is resistant to chlorine and alcohol, although infection can be prevented by wiping contaminated sites with a 79% ethanol solution containing 0.1% *o*-phenylphenol [23]. Vaccination against neonates is also being conducted and has shown a good efficacy [24]. *Norovirus* is also highly contagious and resistant to alcohol. For contaminated sites, it is recommended to wipe with bleach containing more than 500 ppm of chlorine [23]. Vaccines for norovirus are currently under development. There are currently no effective treatments for either of these viral infections. Thus, the treatment that is generally recommended is to prevent the dehydration caused by diarrhea and vomiting by maintaining a sufficient fluid intake and wait for the virus to be eliminated from the gastrointestinal tract.

3. PROPHYLAXIS AND MEDICAL TREATMENT OF INFECTIOUS DISEASES BY PROBIOTICS AND PARAPROBIOTICS

With the progress of research on the relationship between the microbiota and diseases in recent years, commensal intestinal bacteria have been investigated for their ability to modulate the host immune system, not only in healthy individuals but also in those who are suffering from a wide range of diseases [25]. It has been revealed that commensal bacteria also regulate regulatory T cells, type 3 innate lymphoid cells, and T helper 17 cells through the recognition of the bacteria themselves or their metabolites/products by the immune cells and greatly affect mucosal immunity [25-29].

Probiotics act on both the innate and acquired immune systems and have the potency to decrease the severity of infections in the gastrointestinal [30] and upper respiratory tracts [13, 31]. Probiotics are defined as live microorganisms that have health benefits for the host [32], and they are generally consumed as a component of fermented foods such as yoghurt, cheese, and pickles or as supplements. Recently, "ghost probiotics" or paraprobiotics were reported to retain their immunomodulatory potency beyond their viability [33]. Probiotics contain immunostimulatory substances such as lipoteichoic acid, peptidoglycan, and nucleic acid, which are Toll-like receptor (TLR) ligands, and muramyl dipeptide, which is a Nod-like receptor ligand [34]. In the following sections, we focus on the effects of probiotics including paraprobiotics on viral infections. Particularly, we discuss representative probiotics that have shown unique preventive or therapeutic potencies against viral diseases in clinical studies (Table **1**).

3.1. *Lactobacillus casei* Shirota (LcS)

LcS reduced plasma cytomegalovirus and Epstein–Barr virus antibody titers in highly physically active people (university athletes) [35]. However, there was no significant difference in the incidence of norovirus-induced gastroenteritis between the LcS and control groups in long-stay elderly people at a health service facility [36]. Although the effectiveness of LcS remains controversial, a potential mechanism was reported wherein LcS was found to modulate the activity of natural killer (NK) cells, which are one of the first-line defense mechanisms against viral infection [37].

3.2. *Lactobacillus rhamnosus* (LGG)

The preventive effects of LGG on experimental rhinovirus infections in healthy volunteers were evaluated. After the ingestion of LGG for 6 months, subjects were intranasally inoculated with rhinovirus. The infection rate, occurrence, and severity of cold symptoms were evaluated. The frequency and severity of cold symptoms and the number of subjects with rhinovirus infection in the LGG group were lower than those of the control group, although the difference between the groups did not reach significance [38]. When LGG was administered for 4 weeks to children with gastroenteritis who were positive for either rotavirus or *Cryptosporidium* species in stool, a significant increase in serum immunoglobulin (Ig)G levels post-intervention was observed in children with rotavirus-induced diarrhea who received LGG. Among the children with cryptosporidial diarrhea, those receiving LGG showed a significant improvement in intestinal permeability [39]. The mechanisms by which probiotics exert immunomodulatory effects are not completely understood. However, LGG was demonstrated to modulate the innate and adaptive immune responses, particularly those against gastrointestinal pathogens, resulting in increased levels of serum IgG and secretory IgA targeting enteric pathogens including rotavirus [39].

3.3. *Lactobacillus delbrueckii* ssp. bulgaricus OLL1073R-1 (R-1)

The study demonstrated that the consumption of yoghurt fermented with R-1 augmented NK cell activity and reduced the risk of catching the common cold in elderly individuals [40]. Other studies showed that R-1 and its secreted polysaccharides improved immune system functions accompanied by the activation of NK cells. Thus, R-1 or its products might contribute to the prevention of respiratory infections caused by respiratory or influenza viruses [41, 42].

3.4. *Lactobacillus paracasei* ssp. paracasei (*L. casei* 431)

L. casei 431 was reported to have the potency to shorten the duration of upper respiratory symptoms, although it showed no effect on the immune response to influenza vaccination in healthy adults [43]. In another study, *L. casei* 431 was demonstrated to modulate the immune system using a vaccination model in healthy subjects. Increase from baseline in the titers of vaccine-specific IgG, IgG1, and IgG3 in plasma as well as that of vaccine-specific secretory IgA in saliva were significantly greater in both probiotic groups, as compared with the control group [44]. Although more detailed studies are needed to explore the comprehensive mechanisms underlying the immunomodulatory and anti-infectious effects of *L. casei* 431, it is considered that this probiotic stimulates the innate viral defense mechanisms and reduces inflammation in the host.

3.5. *Lactobacillus paracasei* MCC1849 (MCC1849)

Non-viable MCC1849 did not show significant effects on immune parameters involved in the response to influenza vaccination in elderly people with immunosenescence [45].

Table 1. Clinical efficacy of various major lactic acid bacteria for infectious diseases.

Strain	Efficacy			Refs.
	Target Disease (Virus)	Subjects	Outcome	
Lactobacillus casei (Yakult)	Upper respiratory tract infection Epstein–Barr virus (EBV) Cytomegalovirus (CMV)	Healthy athletes	Reduced plasma CMV and EBV antibody titers	[34]
	Upper respiratory tract infection	Elderly people	No significant difference in the incidence of respiratory symptoms and influenza-vaccination immune response	[35]
	Norovirus gastroenteritis	Elderly people	No significant difference in the incidence of Norovirus infection in elderly people	[36]
Lactobacillus rhamnosus GG	Experimentally induced Rhinovirus infection	Healthy volunteers with intranasal inoculation of Rhinovirus (type 39)	Decrease in the occurrence and severity of cold symptoms and number of subjects with Rhinovirus infection, but not significant	[37]
	Acute gastroenteritis (positive for Rotavirus or Cryptosporidium)	6M to 5Y children with acute gastroenteritis positive for Rotavirus or Cryptosporidium	Significant decrease in repeated episodes of Rotavirus diarrhea. Improvement in intestinal function in children with rotavirus and cryptosporidial gastroenteritis	[38]
Lactobacillus delbrueckii ssp. bulgaricus OLL1073R-1	Common cold symptoms	Elderly people (meta-analysis of two independent cohorts)	Significant increase of natural killer cell activity and reduced risk of catching the common cold	[39]
Lactobacillus paracasei ssp. paracasei, *L. casei* 431	Response to influenza vaccination	Healthy adults with influenza vaccination	Significant reduction of the duration of upper respiratory symptoms No significant difference in immune responses to influenza vaccination and incidence or severity.	[42]
	Response to influenza vaccination	Healthy adults with influenza vaccination	Significant increases of vaccine-specific IgG, IgG1, and IgG3 in plasma as well as vaccine-specific secretory IgA in saliva in both probiotic-treated groups	[43]
Lactobacillus paracasei MCC1849 (Morinaga)	Antibody response against vaccination	Eldery people with influenza vaccination	No significant effect of non-viable *L. paracasei* MCC1849	[44]
Lactobacillus casei (DN-114 001)	Incidence of acute diarrhea	Children aged 6–24 months	Significant reduction in the incidence and frequency of diarrhea.	[45]
	Incidence of common infectious diseases	Children aged 3–6 years	Significantly lower incidence rate of common infectious diseases in DN-114 group	[46]
Lactobacillus plantarum L-137	Upper respiratory tract infection	Healthy adults with high psycological stress	Significant decrease in the incidence of upper respiratory tract infections	[49]
Enterococcus faecalis FK-23	Hepatitis C virus	Adult with anti-HCV antidodies positive	Significant decrease of ananie aminotransferase No significant change in viral load	[51]
Saccharomyces boulardii	Acute rotavirus diarrhea	Children (1-23 months) hospitalized for acute diarrhea by rotavirus	Significant decrease in duration period of diarrhea and fever	[52]
Bifidobacterium animalis (Bb12)	Intestinal antibody responses to polio- and rota-virus in infants	Healthy 6 week full-term infants (prospective study)	Bb12 significantly increased fecal anti-poliovirus specific IgA, and increased anti-rotavirus specific IgA.	[53]

(Table 1) Contd....

Strain	Efficacy			Refs.
	Target Disease (Virus)	**Subjects**	**Outcome**	
Bifidobacterium lactis B94	Acute rotavirus diarrhea	Children (5 months to 5 years) hospitalized for diarrhea by rotavirus	Sgnificantly decrease in duration period of diarrhea	[54]
Lactococcus lactis JCM5805 (*L. lactis* plasma)	pDCs acitivity among PBMCs and symptoms of common cold	Healthy adults	*L. lactis* JCM 5805 activated pDCs among PBMCs and significantly reduced the risk of morbidity from the common cold	[55]
	Influenza-like illness and immunological response to influenza virus	Healthy adults	Significant decrease in the cumulative incidence days of "cough" and "feverishness". Significant increase in IFN-α-inducible antiviral factor, interferon-stimulated gene 15	[56]
	Influenza-like illness and immunological response to influenza virus	Healthy adults	Significant decrease in the cumulative incidence days of "sore throat" and "cough". Significant increase in IFN-α mRNA in PBMCs	[57]
	Anti-viral immune response and physical condition	Healthy adults	Significantly increased pDC activation and increased mRNA expression of ISG15 Significant decrease in the cumulative incidence days of cold-like symptoms	[58]
	Influenza Infection	School children	Significant decreases in both the incidence rate and the cumulative incidence rate of influenza	[59]
	Anti-viral immune response to influenza virus	Healthy adults	Significant increase in secretary IgA in saliva Significant prevention of decrease in phagocytic activity of neutrophil during common cold season	[60]

3.6. *Lactobacillus casei* strain DN-114 001 (DN-114)

Pedone *et al*. found that the administration of DN-114 reduced the incidence of acute diarrhea in healthy children aged 6–24 months. The incidence and frequency of diarrhea were significantly reduced by supplementation with DN-114 as compared with the control group [46]. Merenstein *et al*. reported that DN-114 001 could reduce the incidence of common infectious diseases including diarrhea in children aged 3–6 years who were attending daycare or school, although the detailed mechanism is still unclear [47].

3.7. *Lactobacillus plantarum* L-137 (HK L-137)

Previously the immunomodulatory effects of heat-killed HK L-137 were evaluated and the results showed that HK L-137 augmented the innate and acquired immune responses in mice and human subjects, especially in view of the production of type 1 IFNs and interleukin (IL)-12 [48, 49]. Hirose *et al*. assessed the effects of HK L-137 intake for 12 weeks on URTI symptoms and immune functions in human subjects who were experiencing high levels of psychological stress. The incidence of URTIs was significantly lower in the HK L-137-treated group than in the control group. In addition to the incidence of URTIs, the severity and duration of medication showed significant negative correlations with the duration of HK L-137 intake. The percentage change from baseline of the concanavalin A-induced proliferation of peripheral blood mononuclear cells (PBMCs) was significantly greater in the HK L-137-treated group than in the control group, although serum IFN-β production was not significantly different between these groups [50].

3.8. *Enterococcus faecalis* FK-23

Oo *et al*. reported that the paraprobiotic FK-23 (*Enterococcus faecalis* strain FK-23) significantly reduced alanine aminotransferase (ALT) levels in adult HCV-positive subjects but did not decrease viral load. Although detailed mechanism was still unclear, they suggested that FK-23 might change the microbiota in HCV patients then it played a role in decrease in ALT level [51].

3.9. *Saccharomyces boulardii*

Oral administration of *Saccharomyces boulardii* and rehydration significantly shortened duration of diarrhea in acute rotavirus gastroenteritis children in Bolivia, compared with control rehydration alone. Detailed mechanism was not available [52].

3.10. *Bifidobacterium animalis* Bb12

To investigate the effect of infant starter formula containing the probiotic *Bifidobacterium animalis* subspecies lactis (Bb12) on intestinal immunity and inflammation. Six-week-old healthy, full-term infants were enrolled in a prospective study (Control formula and Control + Bb12 (10^6CFU / g / head)) for 6 weeks. Anti-poliovirus-specific IgA and anti-rotavirus-specific IgA were assessed.

Bb12 significantly increased anti-poliovirus-specific IgA, but not anti-rotavirus-specific IgA, although it showed the tendency of increase (P =0.056) [53].

3.11. *Bifidobacterium lactis* B94

In Turkey, Erdoğan *et al*. reported that *Bifidobacterium lactis* B94 with oral rehydration treatment significantly shortened diarrheal period in acute rotavirus gastroenteritis children (5 months to 5 years old), compared with control oral rehydration alone [54].

3.12. *Lactococcus lactis* subsp. Lactis JCM 5805 (*L. lactis* JCM 5805)

Plasmacytoid dendritic cells (pDCs) play a crucial role in antiviral immunity through the production of large amounts of IFNs. *L.*

lactis JCM5805 was found to activate human pDCs among PBMCs from healthy volunteers, especially in a subgroup of volunteers who originally showed a low pDCs activity, and it also significantly attenuated cumulative common cold symptoms [55].

The prophylactic effects of *L. lactis* JCM5805 on influenza-like illness in healthy volunteers during the winter season were reported based on the results of a double-blinded trial [56, 57]. The administration of *L. lactis* JCM5805 resulted in a significant decrease in the cumulative number of days of incidence of "cough" and "feverishness", which were defined as the major symptoms of an influenza-like illness, as compared with the control group. Furthermore, when PBMCs from the volunteers treated with *L. lactis* JCM 5805 were cultured with inactivated human influenza virus A/H1N1 (A/PR/8/34), the expression of IFN-α showed a higher tendency and that of interferon-stimulated gene 15 (*ISG15*) was significantly elevated as compared with the control group. These results suggest that the intake of *L. lactis* JCM5805 can prevent the pathogenesis of an influenza-like illness via the enhancement of an IFN-α-mediated response to the influenza virus [56]. In two separate studies, the expression of IFN-α and the mRNA level of *ISG15* in PBMCs were significantly higher in groups treated with *L. lactis* JCM 5805 as compared to the corresponding control groups [57, 58]. In addition, Sakata *et al*. reported that the intake of yogurt containing *L. lactis* JCM 5805 significantly reduced the cumulative incidence rate of influenza among schoolchildren in a rural area of Japan [59]. Finally Fujii *et al*. showed the effects of oral *L. lactis* JCM 5805 on systemic and mucosal immunological parameters of healthy volounteers in winter (common cold season in Japan). After 4 continuous weeks administration, *L. lactis* JCM 5805 significantly reduced cumulative days of symptom of sore throat, compared with control. In *L. lactis* JCM 5805 group, change in secretory IgA levels in saliva and phagocytic activity of neutrophil were significantly lower than those of initial level, but not in the placebo group, although they could not reach statistical differences between groups at endpoint [60]. The proposed mechanism of action for *L. lactis* JCM 5805 is discussed in detail in the next section.

4. MECHANISM OF *L. LACTIS* JCM 5805-MEDIATED INHIBITION OF VIRAL INFECTIONS

4.1. Role of pDCs and Type 1 IFNs in Viral Infection

As dendritic cells (DCs) are well known to be a pivotal immune cell subset that links the innate and acquired immune responses by recognizing pathogenic and endogenous inflammatory signals [61]. Dendritic cells are subdivided into pDCs, myeloid DCs (mDCs), and $CD8^+$ dendritic cells. Among them, pDCs are a rare and critical subset that acts as a "control tower" during viral infections [62, 63].

To detect the presence of bacteria and viruses, pDCs utilize certain TLRs. Especially, they use TLR9 for the recognition of microbial nucleic acids via detecting unmethylated CpG motifs of DNA and TLR7 for the recognition of microbial RNA or synthetic guanosine analogs. The activation of pDCs by TLR ligand binding leads to the production of type 1 IFNs [64]. The type 1 IFN family includes IFN-α and IFN-β, which serve as components of the first-line defense against infection by blocking viral replication [65]. The induction of type 1 IFNs is mostly associated with viral infections and it is well known that pathogenic bacteria stimulate IFN-α production [66]. However, nonpathogenic bacteria including probiotics used in food preparation have been less intensively studied regarding their ability to stimulate DC-mediated IFNs induction [67]. As described separately in detail, we previously screened various lactic acid bacteria for their ability to stimulate IFN-α production by pDCs and found that *L. lactis* JCM 5805 was the most potent stimulator of type 1 IFN production [67].

4.2. Direct Activation of pDC by *L. Lactis* JCM 5805

The stimulatory effects of *L. lactis* JCM 5805 on type 1 IFNs production by pDCs and mDCs were evaluated using DCs derived from Flt-3L-stimulated murine bone marrow obtained from several types of TLR-knockout mice. As a result of this direct activation of DCs by *L. lactis* JCM 5805, the major type 1 and type 3 IFNs (i.e., IFN-α, -β, and -λ) were found to be induced efficiently. However, IFN-α production was completely abolished in dendritic cells obtained from TLR9 or MyD88 knockout mice. Thus, these data strongly suggested that *L. lactis* JCM 5805 stimulated IFN-α production via TLR9/MyD88 signaling. Furthermore, we examined whether IFN-α production was induced by CpG DNA, which is a known TLR9 agonist, or DNA extracted from *L. lactis* JCM 5805. Both CpG DNA and the DNA extracted from L. lactis JCM 5805 strongly induced IFN production. In addition, *L. lactis* JCM 5805 was observed to be specifically taken up by pDC, suggesting that its phagocytosis played an important role in activating pDCs and consequently inducing the production of IFNs [67].

IFN-α production by pDCs was synergistically elevated by *L. lactis* JCM 5805 treatment when they were co-cultured with mDCs. Therefore, cross-talk or direct contact between mDCs and pDCs was considered to be necessary for the effective induction of IFN-α production by *L. lactis* JCM 5805. In addition, *L. lactis* JCM 5805 stimulated the expression of immunoregulatory receptors such as ICOS-L and PD-L1 on pDCs and accordingly reinforced the induction of $CD4^+CD25^+FoxP3^+$ regulatory T cells [67].

4.3. The Mechanism of Action of *L. Lactis* JCM 5805 on the Immune System in Infectious Disease Models

When a lethal dose of parainfluenza virus (mPIV1) was inoculated intranasally to mice fed with *L. lactis* JCM5805, the survival rate was significantly higher than that of the control group (69% survival in *L. lactis* JCM 5805 and 0% survival in the control group on day 15 after inoculation). In the intestinal pDCs from the mice treated with *L. lactis* JCM 5805, type 1 IFN expression was significantly elevated, while a remarkable preventive effect against the infiltration of neutrophils into the lung tissue was observed. A significant increase in the expression of genes with antiviral activities including IFN-inducible mRNAs such as *Isg15*, *Oasl2* (2'-5' oligoadenylate synthetase-like 2), and *Rsad2* (radical S-adenosyl methionine domain containing 2) was observed upon treatment with *L. lactis* JCM 5805. Although lung is distantly located from the contact site (intestinal Peyer's patches) and *L. lactis* JCM 5805 cannot stimulate local pDCs in the lung directly, oral *L. lactis* JCM 5805 treatment resulted in a strong resistance against parainfluenza virus infection *in vivo* [68].

As is well known, IFN-α plays an important role in the antiviral immune response by inducing the cytotoxic activity of NK cells, which contributes to the host defense against viral infections [69, 70]. Indeed, Suzuki *et al*. reported that *L. lactis* JCM5805 activated NK cells both *in vitro* and *in vivo* [71]. Furthermore, the effect of *L. lactis* JCM5805 on NK cells was dependent upon the activity of dendritic cells [71]. Among a number of activation factors for NK cells including IFN-α, IL-2, IL-15, and IL-18, IFN-α is regarded as one of the most efficient NK cell activation factors [72]. In addition, it was reported that IFN-α production by virus-stimulated pDCs markedly increased the cytotoxic activity of NK cells [73] (Fig. **1**).

Although we discussed the direct activation of pDCs by *L. lactis* JCM 5805 as the most plausible mechanism for the inhibition of viral infection, other underlying mechanisms have not been ruled out. Indeed, Salminen *et al*. reported that specific probiotic bacteria could bind and inactivate rotaviruses [74]. Besides this direct interaction with viruses, it is also possible that *L. lactis* JCM 5805 competes for viral receptors on the surface of target cells, produces antimicrobial and potentially antiviral substances, and stimulates host-cell immune defense systems. Thus, in the future, it is necessary to test whether *L. lactis* JCM 5805 can also directly bind and inactivate viruses, since the resulting data could provide a basis for

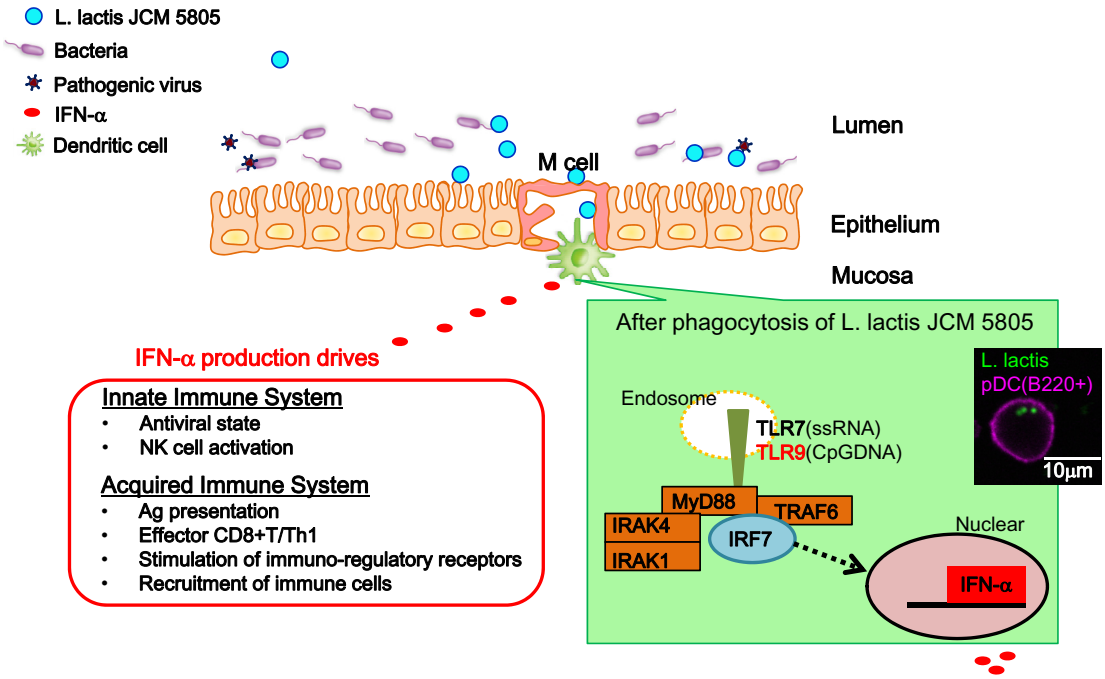

Fig. (1). Possible mechanism for the antiviral effects of *L. lactis JCM 5805* via activation of plasmacytoid dendritic cells (pDCs).
To detect the presence of bacteria and viruses, pDCs utilize certain Toll-like receptor (TLR) families. Especially, they use TLR9 for the recognition of microbial nucleic acids by detecting unmethylated CpG motifs of DNA and TLR7 for the recognition of microbial RNA or synthetic guanosine analogs. The activation of pDCs by ligand binding to TLRs leads to the production of type 1 IFNs. *L. lactis* JCM 5805 was specifically taken up by pDCs and its DNA extracts strongly induced IFN production. These observations suggest that the phagocytosis of *L. lactis* JCM 5805 by pDCs plays an important role in activating pDCs and stimulating IFN production via TLR9/MyD88 signaling.
IFN-α plays an important role in mediating the antiviral immune response by inducing the cytotoxic activity of natural killer cells, which contributes to the host defense against viral infection.
Microscopic observations of human pDCs stimulated by *L. lactis* JCM5805 are also shown (Inset). The pDCs stained with anti-B220 (purple) are in the process of phagocytosing fluorescein isothiocyanate-stained *L. lactis* JCM5805 (green) [55]. (The color version of the figure is available in the electronic copy of the article).

novel approaches to inactivate viruses and reduce the risk of mucosa-associated viral infections.

CONCLUSION

We described the efficacy of probiotics and paraprobiotics for the prevention or treatment of infectious diseases, which have been increasing in incidence in recent years. Although the benefits of vaccines and antiviral drugs for the prevention and treatment of infectious diseases are obvious, their effectiveness is hindered by the large number of viral species and their subtypes as well as the high mutation rate of viruses. Since viruses evolve constantly and produce a serologically diverse viral population, it is challenging to establish an effective means of protecting humans from viral infections. There have been several clinical reports regarding the use of probiotics or paraprobiotics for the prophylaxis or treatment of infectious diseases. Here, we reviewed the literature regarding several probiotic or paraprobiotic agents based on the papers which described single paraprobiotics / probiotics in clinical trial to avoid the crosstalk or mutual interference between probiotics. Such agents are considered to be safe, affordable and easy to consume because of their long history of use in foods.

The state of knowledge regarding the immunomodulatory effects of probiotics has recently advanced and various studies have especially focused on the interactions between commensal bacteria and the mucosal immune system. Furthermore, the role of type 1 IFNs in the elimination of pathogenic viruses, which involves the concerted activities of the innate and acquired immune systems, has been widely studied.

In this review, we discussed the anti-influenza activity of *L. lactis* JCM 5805, which has the potency to directly stimulate pDCs via TLR9 and thereby promote viral control. Although further detailed research is necessary, probiotics and/or paraprobiotics are expected to be among the rational adjunctive options for the treatment and prophylaxis of viral infections.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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