Modulation of mucosal antiviral immune response by immunobiotic lactic acid bacteria – Part II: the respiratory mucosa

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Viruses are the most important cause of severe mucosal infections worldwide especially in high risk populations such as in infants, young children, elderly and immunocompromised hosts. Lactic acid bacteria (LAB) are technologically and commercially important and have various beneficial effects on human health. Several studies have demonstrated that certain LAB strains can exert their beneficial effect on the host through their immunomudulatory activity. These strains, termed immunobiotics, have been used for the development of probiotic foods with the ability to stimulate mucosal antiviral immunity. In this review we examine the current scientific literature concerning the advances in our understanding of how probiotic microorganisms are able to modulate respiratory viral immunity and affect the outcome of viral diseases. Moreover, this review explores the recent advances of our laboratories regarding the cellular and molecular interactions between immunobiotics and hosts cells and how this interaction modulate the resistance against respiratory viral infections. Research from the last decade demonstrates that immunobiotic LAB represent a promising resource for the development of prevention strategies against viral infections that could be effective tools for medical application.

Keywords lactic acid bacteria, immunobiotics, respiratory viral infection

1. Modulation of respiratory antiviral immune response by immunobiotic LAB

Respiratory Syncytial Virus (RSV) is a major respiratory pathogen of infants and children and an emerging pathogen of the elderly. Other important viral pathogen is Influenza Virus that is the most common cause of human respiratory infection, and is among the most significant because it causes high morbidity and mortality. Several lines of evidence showed that mucosal administration of immunobiotics is able to increase resistance against respiratory viral infections [1].

The first study demonstrating the beneficial effect of immunobiotics on influenza infection was performed with *Bifidobacterium breve* YIT4064. This strain is able to induce the production of large quantities of IgA in a murine Peyer's Patches (PPs) cell culture method [2]. Moreover, *in vitro* studies demonstrated that the YIT4064 strain enhances the production of anti-influenza virus, anti-rotavirus, and anti-poliovirus antibodies by PPs cells in response to the challenges with the respective viruses [3]. It was shown that the oral administration of *B. breve* YIT4064 protected mice against influenza virus challenge [4]. The authors demonstrated that oral administration of *B. breve* YIT4064 significantly decreased the accumulated symptom rate of influenza virus infection and improved the survival rate. These protective effects were related to augmented levels of anti-influenza virus IgG in serum [4]. Therefore, Yasui et. al demonstrated that *B. breve* YIT4064 protect against respiratory viral infection through an enhancement of humoral immune response. Recently, it was shown that oral administration of heat-killed lactobacilli are able to improve IgA and IgG anti-influenza antibodies in the airway mucosa and lung, and to augment protection against influenza virus infection in mice [5, 6]. Then, immunobiotics are capable to modulate the production of systemic and mucosal antibodies against respiratory viruses (Table 1).

Hori et al. [7] studied the effect of the nasal administration of a non-viable *L. casei* Shirota on respiratory immunity and observed that nasal treatment of adult BALB/c mice with this strain stimulated cellular immunity in the respiratory tract and significantly increased the resistance of mice to influenza virus infection. The authors investigated the production of various cytokines by mediastinal lymphoid nodes cells in mice receiving *L. casei* Shirota intranasally. It was found that the Shirota strain strongly induced production of IL-12 in these cells, which is an important cytokine for cytotoxic T cells and NK cells stimulation and enhancement of Th1 cytokines production and Th1 cells proliferation. Moreover, both IFN-γ and TNF-α levels were improved in mediastinal lymphoid nodes cell cultures from mice administered *L. casei* Shirota intranasally, after influenza virus challenge [7]. This was the first study demonstrating that

nasally administered LAB were able to activate cellular immunity in the respiratory immune system and protected against a respiratory viral infection. Later, the group investigated whether oral administration of *L. casei* Shirota activates the systemic and respiratory immune systems and whether it ameliorates influenza virus infection in the respiratory tract of aged [8] and infant mice [9]. These works showed that orally administered *L. casei* Shirota augmented NK cell activity in splenocytes and lungs and enhanced IFN-γ and TNF-α production of nasal lymphocytes of aged and infant BALB/c mice. These studies also found that viral titers in *L. casei* Shirota groups were significantly lower than that in the control groups [8, 9]. The authors postulated that *L. casei* Shirota was taken up by M cells in the PPs and stimulated Th1 cells and NK cells, which migrated to the mesenteric lymphoid nodes and then, via the thoracic duct and bloodstream migrate to the spleen, lungs, and nasal-associate lymphoid tissue.

Other studies have also emphasized the importance of IFN-γ production and NK cells activation for the protective effect of immunobiotics against influenza infection (Table 1) [10-12]. Recently, Kawase et al. [13] showed that oral administration of heat-killed *L. gasseri* TMC0356 resulted in significantly higher expression of pulmonary IFN-γ in mice when compared to controls. Moreover, authors evaluated the effect of orally administered lyophilized *L. rhamnosus* GG and *L. gasseri* TMC0356 in the outcome of influenza virus infection in mice and found significant differences in pulmonary virus titres between control and lactobacilli-treated mice. In addition, histological tissue examination showed bronchial mucosa epithelium hypertrophy and infiltration of leukocytes in the bronchial submucosa and pulmonary core of infected control mice while similar pathological changes were not observed in lactobacilli-treated mice [13]. Takeda et al. [14] showed that the oral administration of *L. plantarum* 06CC2 is able to increase IFN-γ expression in PPs and lungs. Improved respiratory IFN-γ induced by the 06CC2 strain was associated with augmentation of NK cell activity and correlated with the alleviation of influenza infection in mice. Moreover, authors demonstrated that the immunobiotic treatment differentially modulated the production of cytokines during influenza infection. The levels of IFN-γ, IL-12, and IFN-α in infected mice administered the 06CC2 strain were significantly higher than those in the controls while the level of TNF-α was significantly lower than that in the control mice [14].

Maeda et al. [15] reported that oral administration of heat-killed *Lactobacillus plantarum* L-137 enhances protection against influenza virus infection by stimulation of type I interferon production, demonstrating that orally administered immunobiotics are able to stimulate innate antiviral responses in the respiratory tract. The study showed that all the mice infected with a mouse-adapted virulent strain of influenza virus H1N1 died within 14 days after intranasal challenge. However, the mean survival time was significantly prolonged in *L. plantarum* L-137-treated mice [15]. IFN-β was hardly detected in the serum of control mice on day 1, 2, 3 or 6 after infection, while significant levels of IFN-β were detected in the serum of L-137-treated mice at the early stage days after challenge. This study demonstrated that the increased production of IFN-β induced by the immunobiotic strain contributes to the increased resistance against influenza virus infection; however, detailed studies to investigate the immune mechanisms involved in this effect were not performed.

More recently, Lee et al., [16] investigated whether the sublingual route is useful for the delivery of probiotics against influenza virus infection. The authors demonstrated that sublingual administration of *L. rhamnosus* enhanced protection against influenza virus infection by enhancing of mucosal secretory IgA production and T cell and NK cell activity. Moreover, IL-12 levels in the lungs of *L. rhamnosus*-treated mice increased significantly when compared to controls while IL-6 and TNF-α levels decreased significantly. Considering that IL-6 and TNF-α levels positively correlate with lung inflammation and vascular dysfunction, these results suggest that the decreased levels of pro-inflammatory citokines might also contribute to the protection against influenza virus infection [16].

Collectively, these studies indicate that: a) orally administered immunobiotics can increase respiratory immunity against influenza virus, b) the effect is mediated by the improvement of respiratory tract and systemic levels of IFN-γ and NK cells activity, c) the activation of a Th1 response with IFN-γ+ T cells mobilization from gut to respiratory tract would be the mechanism involved, d) nasal priming with immunobiotic LAB is also an interesting alternative to improve respiratory antiviral defences and, e) immunobiotic LAB have the potential to be successfully used as adjuvants for antiviral vaccines.

The studies described above showed that mucosal administration of immunobiotics had the potential to improve the outcome of influenza virus infection. However, the capacity of immunobiotics to improve protection against pneumoviruses of the family *Paramyxoviridae* such as RSV was not investigated in detail. In an effort to evaluate the capacity of lactobacilli to reduce the pathogenesis of severe pneumovirus infection *in vivo*, Gabryszewski et al., [17] developed a model pneumonia virus of mice (PVM) infection. The authors demonstrated that lactobacilli, when targeted to the respiratory epithelium, are highly effective at suppressing PVM-induced inflammation and protecting against lethal disease. Wild-type mice primed via intranasal inoculation with live or heat-inactivated *Lactobacillus plantarum* or *Lactobacillus reuteri* were completely protected against lethal infection with PVM. Priming with live lactobacilli resulted in diminished granulocyte recruitment, diminished expression of multiple pro-inflammatory cytokines (CXCL10, CXCL1, CCL2, and TNF), and reduced virus recovery. Moreover, Lactobacillus priming also resulted in prolonged survival and protection against the lethal sequelae of PVM infection in MyD88$^{-/-}$ mice, suggesting that the protective mechanisms may be TLR-independent (Table 1).

Our laboratory performed a randomized controlled trial in order to evaluate the effect of a probiotic yogurt containing the immunobiotic strain *L. rhamnosus* CRL1505 on both gut and non-gut related illnesses among children [18]. We demonstrated that administration of *L. rhamnosus* CRL1505 improved mucosal immunity and reduced the incidence and severity of intestinal and respiratory infection in children. Our randomized clinical study demonstrated a significant reduction in occurrence of infectious events associated with consumption of *L. rhamnosus* CRL1505 [18]. We also evaluated the presence or absence of fever during infectious events as well as the need of antibiotic treatment in children who had infections, as indicators of severity. There was a significant decrease in the presence of fever in children who consumed probiotic yogurt as well as a slight decrease in the need for antibiotic treatment, indicating less serious infections in relation to the placebo group [16]. Although we did not evaluate the etiology of respiratory infections in the clinical study, previous evaluations have shown that viral pathogens, such as RSV, human metapneumovirus, influenza A virus, parainfluenza viruses, and rhinoviruses are considered the major viruses that can cause respiratory tract diseases in children [19]. Therefore, the findings of our study suggest that administration of *L. rhamnosus* CRL1505 may provide one of the potential interventions to reduce the burden of common childhood morbidities, especially those associated to viral infections [18].

Later, we evaluated the effect of the oral administration of two *Lactobacillus* strains, *L. rhamnosus* CRL1505 and *L. rhamnosus* CRL1506, on mucosal antiviral immunity. *In vivo* experiments demonstrated that the administration of lactobacilli strains significantly augmented the expression of IFN-γ in PPs compared with the control [20, 21]. Moreover, we found that *L. rhamnosus* CRL1505 was more efficient than CRL1506 strain for increasing the levels of IFN-γ, IL-10 and IL-6 in the intestine. On the contrary, *L. rhamnosus* CRL1506 showed a higher capacity to improve levels of IFN-α, IFN-β and TNF-α in the gut when compared with CRL1505. When we evaluated the levels of serum cytokines we found that *L. rhamnosus* CRL1506 was more efficient than *L. rhamnosus* CRL1505 to increase IFN-α, IFN-β and TNF-α, while serum IFN-γ, IL-10 and IL-6 levels were more efficiently improved by *L. rhamnosus* CRL1505. These changes in the profile of serum cytokines were similar to those found in the intestinal fluid, indicating that levels of serum cytokines are a reflection of intestinal changes [20]. On the contrary, the analysis of respiratory cytokines showed that only *L. rhamnosus* CRL1505 was able to increase the levels of IFN-γ, IL-10 and IL-6 [21]. While these are the same cytokines that were increased by this strain in serum, we cannot attribute a direct correlation between the two increases, as we did not found increased levels of IFN-α, IFN-β or TNF-α in the respiratory tract of *L. rhamnosus* CRL1506 treated mice. Therefore, and taking into account the capacity of *L. rhamnosus* CRL1505 of increasing the number of CD3$^+$CD4$^+$IFN-γ^+ T cells in PPs and the studies mentioned above, we hypothesized that *L. rhamnosus* CRL1505 would be able to induce a mobilization of these cells into the respiratory mucosa. We demonstrated that this hypothesis was true since increased numbers of CD3$^+$CD4$^+$IFN-γ^+ T were found in lungs of *L. rhamnosus* CRL1505 treated mice [21]. Furthermore, we can speculate that the mobilization of CD3$^+$CD4$^+$IFN-γ^+ T cells from the intestine to the airways and the improved production of IFN-γ could be involved in the protective effect against viral infections induced by *L. rhamnosus* CRL1505 that was observed in clinical studies [18].

To mimic the pro-inflammatory and physiopathological consecuences of RNA viral infections in the lung, we used an experimental model of lung inflammation based on the administration of the artificial TLR3/RIG-I ligand and dsRNA analog poly(I:C). Nasal administration of poly(I:C) to BALB/c mice induced a marked impairment of lung function that was accompanied by the production of pro-inflammatory mediators and inflammatory cell recruitment into the airways [21] in accordance with results published by Stowell et al. [61]. Exposure to poly(I:C) induced respiratory epithelial cell death and impaired epithelial barrier function as demonstrated by the increased levels lactate deshidrogenase (LDH) activity and albumin concentration in bronchoalveolar lavages (BAL). Moreover, intranasal administration of three once-daily doses of poly(I:C) resulted in neutrophils and mononuclear cells influx into the lung [60].

In vitro studies have demonstrated that stimulation of lung epithelial cells with poly(I:C) elicited the secretion of multiple cytokines, chemokines, the induction of transcription factors and increased expression of TLRs [22]. In our *in vivo* model increased levels of TNF-α, IL-6, IL-8 and MCP-1 were observed in the respiratory tract, therefore a likely source of cytokines following poly(I:C) administration may be the airway epithelium. In addition, the experimental model used in this work resembles RSV infection since this respiratory virus is able to induce a profile of pro-inflammatory cytokines similar to that observed following *in vivo* poly(I:C) challenge in mice [22, 23]. In fact, natural human RSV infection in children and experimental RSV inoculation in mice result in prominent local secretion of pro-inflammatory cytokines, such as TNF-α, IL-6, and CXC/CC chemokines, including IL-8, MIP-1, RANTES, and MCP-1. The coordinated actions of several of these cytokines strongly promote the recruitment and activation of neutrophils and monocytes/macrophages [24], also observed in our experimental model [21].

During acute viral lung infection, it is imperative that the host's inflammatory response is tightly regulated, enabling pathogen elimination but limiting the detrimental effects of inflammation on the gas exchange. An appropriate balance of anti-inflammatory and pro-inflammatory mediators is essential for a safe and effective antiviral immune response. Thus, an excessive TNF-α/IL-8/MCP-1 response can lead to increased immunopathology, while exuberant IL-10 production can result in delayed pathogen clearance [25]. In this sense, it has been shown that TNF-α contributes to clearance of the virus during the early stages of RSV infection, which is most likely a result of the NK cell response. But continued production of TNF-α exacerbates illness and tissue injuries during the late stages of RSV infection [26].

Interestingly, recent studies demonstrate a role for IL-10 in controlling immunopathology during respiratory viral infections. Sun et al. [27] showed that IL-10 prevents immunopathology and lethal disease during acute influenza virus infection. On the other hand, IL-10 also seems to play a crucial role in controlling disease severity in RSV infection [27, 28]. It was found that IL-10 deficiency during RSV challenge did not affect viral load, but led to markedly increased disease severity with enhanced weight loss, delayed recovery and a greater influx of inflammatory cells into the lung and airways and enhanced release of inflammatory mediators [29]. The preventive administration of *L. rhamnosus* CRL1505 reduced the production of TNF-α, IL-6, IL-8 and MCP-1 in the respiratory tract after the challenge with poly(I:C). Therefore, the reduction of these pro-inflammatory mediators could explain at least partially the reduced lung injuries in the *L. rhamnosus* CRL1505 treated group [21]. Moreover, *L. rhamnosus* CRL1505 treatment prior to poly(I:C) challenge induced a significant increase in IL-10 in lung and serum. Consequently, IL-10 would be valuable for attenuating inflammatory damage and pathophysiological alterations in lungs challenged with the viral pathogen-associated molecular pattern poly(:IC). According to these results, *L. rhamnosus* CRL1505 treatment would beneficially regulate the balance between pro-inflammatory mediators and IL-10, allowing an effective inflammatory response against infection and avoiding tissue damage.

Oral treatment with CRL1505 strain also increased levels of IFN-γ in BAL after poly(I:C) challenge [21]. The higher levels of respiratory IFN-γ after poly(I:C) challenge in *L. rhamnosus* CRL1505 treated mice could be explained by the higher number of CD3$^+$CD4$^+$IFN-γ$^+$ T cells and by an improved activation of these cells by lung DCs. When we analyzed lung DCs in *L. rhamnosus* CRL1505 treated mice after the nasal challenge with poly(I:C) we found increased levels of both CD103$^+$ and CD11b^{high} DCs. Moreover, both DCs populations showed higher expression of MHC-II when compared with controls. However, IL-12 and IFN-γ were increased only in CD103$^+$ DCs [21]. Consistent with our results, it has been demonstrated that CD4$^+$CD62L^{high}DO11.10 T cells, which have been primed with lung CD103$^+$ DCs induced higher frequencies of CD4$^+$ T cells producing IFN-γ than IL-4 [30].

These results clearly indicated that *L. rhamnosus* CRL1505 was a potent inducer of antiviral cytokines and may be useful as a prophylactic agent to control respiratory virus infections as observed in the clinical study. However, further studies were needed in order to conclusively demonstrate the protective effect of *L. rhamnosus* CRL1505. Therefore, we next aimed to investigate whether oral administration of *L. rhamnosus* CRL1505 was able to improve resistance against RSV in infant mice and evaluated the immunological mechanisms involved in the probiotic effect. We have recently demonstrated that oral administration of *L. rhamnosus* CRL1505 to 3-week old BALB/c mice significantly reduce lung viral loads and tissue injuries after the challenge with RSV [31]. Moreover, our study showed that the protective effect achieved by the CRL1505 strain was related to its capacity to differentially modulate respiratory antiviral immune response.

Natural human RSV infection in children and experimental RSV inoculation in mice result in prominent local secretion of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-8, MIP-1, RANTES, and MCP-1 [24]. The excessive TNF-α, IL-8 and MCP-1 response can lead to increased immunopathology. These pro-inflammatory cytokines contribute to clearance of the virus during the early stages of RSV infection, however continued production of these factors exacerbate illness and tissue injuries during the late stages of infection [26]. As mentioned before, an adequate balance of pro-inflammatory and anti-inflammatory factors is essential for a safe and effective antiviral immune response enabling virus elimination but limiting the detrimental effects of inflammation on the lung tissue. We demonstrated that the CRL1505 strain beneficially modulate the balance between pro- and anti-inflammatory cytokines in response to RSV infection [31]. We observed that CRL1505-treated mice were able to early increase the levels of TNF-α and IL-6 in the respiratory tract when compared to controls. The early increase of these cytokines together with the improved levels of IFN-γ should explain the higher capacity of CRL1505-treated mice to reduce viral loads. In addition, orally administered *L. rhamnosus* CRL1505 significantly increased IL-10 levels that would contribute to protection against inflammatory damage [31].

Improved production of IL-10 in CRL1505-treated mice could have other beneficial effects such as the modulation of NK cells activity. Interestingly, it was demonstrated in IL-10 knockout mice that the absence of IL-10 altered the NK cell response in the lung after RSV infection. Authors found a decrease in NK cells numbers and reduced granzyme B expression by NK cells in the lungs and airways after RSV infection [29]. In addition, it was reported that IL-10R blockade during acute murine cytomegalovirus infection resulted in impaired NK cell responsiveness and proposed that IL-10 acts to promote NK cell activation and survival in the lung [32]. Then, considering the reports about the beneficial effect of probiotics against influenza infection through modulation of NK cell activities [33], it would be important to evaluate the effect of orally administered *L. rhamnosus* CRL1505 on lung NK cells and their relationship with IL-10 production which are both interesting topics for future research.

DCs have a central role in the shape of innate and acquired immune responses then, we also examined the effect of immunobiotics on lung DCs activation. We observed that the CRL1505 strain improved the numbers of both CD103$^+$ and CD11b^{high} DCs populations as well as the expression of MHC-II in response to RSV challenge [31]. This effect would have a significant impact in the immune response against RSV since it was reported that both DCs populations are important in the generation of RSV-specific CD4$^+$ and CD8$^+$ T cells. In this regard, Lukens et al. [34], using effectors T cells as a readout system to measure antigen display by MHC-I and MHC-II molecules, found that both migrating CD103$^+$ and CD11b^{high} lung DCs presented RSV-derived antigens to CD4$^+$ and CD8$^+$ T cells. Then,

considering that RSV interaction with lung DCs results in activation and maturation events that play important roles in establishing virus-specific immunity and, that these early events during the initial immune response may determine the quality and durability of host immunity and influence susceptibility to reinfection, we can speculate that *L. rhamnosus* CRL1505 preventive treatment would significantly improve resistance against the infection by beneficially modulating DCs activity for the generation of a protective adaptive immune response.

It was reported that a Th2 immune response is favored during RSV infection, especially in younger hosts. RSV uses multiple mechanisms to induce a Th2 cell response in the host, including RSV G protein–mediated effects [35], increasing IL-4 production from basophils [36] and induction of alternatively activated macrophages [37]. In addition, it was suggested that RSV infection induces Th2-like inflammation in the lung, which promotes a Th2-like effector phenotype in Treg cells and a loss of suppressive function. Then, it is considered that strategies able to improve Th1 responses against RSV would beneficially modulate the outcome of the infection especially in young individuals. It was demonstrated that IFN-γ is able to upregulate the expression of MHC-II and MCH-I molecules in APCs and thereby enhance the cellular immune response to viral infection and suppress the proliferation of Th2-type T cells [38]. Consistent with this notion, we showed that treatment of infant mice with *L. rhamnosus* CRL1505 significantly improved the production of IFN-γ in response to RSV infection and increased the capacity of mice to clear the virus. Then, modulation of respiratory and systemic immunity potentiated by the CRL1505 strain might contribute to an improved Th1 to Th2 shift and thereby favor protective immunity against viral infections such as RSV. We have demonstrated that orally administered *L. rhamnosus* CRL1505 induce a mobilization of CD3$^+$CD4$^+$IFN-γ$^+$ T cells from the gut into the respiratory mucosa and improve local production of IFN-γ [21]. Probably, IFN-γ secreted in response to *L. rhamnosus* CRL1505 stimulation would modulate the pulmonary innate immune microenvironment conducting to the activation of DCs and the generation of a Th1 response with the consequent attenuation of the strong and damaging Th2 reactions associated with the subsequent intranasal RSV challenge [31].

RSV is the chief cause of bronchiolitis and viral pneumonia in younger children. The bronchiolitis and pneumonia induced by RSV infection have been believed to be immunopathological in nature, because a large number of inflammatory cells are accumulated and activated in the lungs after infection. The studies described before showed that immunobiotic administration is able to modulate inflammatory responses induced by pneumoviruses of the *Paramyxoviridae* family in mice, demonstrating that immunobiotics are an interesting alternative to achieve an immunoprotective effect during paramixovirus infections.

Table 1 Effect of immunobiotics on viral respiratory infections.

Strain	Viability	Mice	Route	Challenge	Protective effect	Immunoregulatory effect	Ref.
B. breve YIT4064	Heat-killed	Adult	Oral	Influenza virus H1N1	Reduction of accumulated symptom rate Improvement of survival rate	Improvement of serum IgG	[4]
L. casei Shirota	Heat-killed	Adult	Nasal	Influenza virus H1N1	Reduction of virus titer in nasal wash Improvement of survival rate	Improvement of IL-12, TNF-α and IFN-γ in MLN	[7]
L. casei Shirota	Heat-killed	Aged	Oral	Influenza virus H1N1	Reduction of virus titer in nasal wash	Improvement of NK cell activity in spleen and lung and increase of TNF-α and IFN-γ in nasal lymphocytes	[8]
L. casei Shirota	Viable	Infant	Oral	Influenza virus H1N1	Reduction of virus titer in nasal wash Reduction of accumulated symptom rate	Improvement of NK cell activity in lung and levels of IL-12 in MLN	[9]
L. plantarum L-137	Heat-killed	Adult	Oral	Influenza virus H1N1	Reduction of virus titer in lung Improvement of survival rate	Improvement of serum IFN-β	[15]

L. gasseri TMC0356	Lyophilized	Adult	Oral	Influenza virus H1N1	Reduction of virus titer in lung Reduction of clinical scores Reduction of lung injury	Not studied	[13]
L. pentosus S-PT84	Heat-killed	Adult	Nasal	Influenza virus H1N1	Reduction of virus titer in BALF	Improvement of NK cell activity in lung and levels of IL-12 and IFN-γ in BALF	[10]
L. rhamnosus GG	Lyophilized	Adult	Nasal	Influenza virus H1N1	Improvement of survival rate Reduction of accumulated symptom rate Reduction of lung injury	Improvement of NK cell activity and levels of IL-1β, TNF-α, MCP-1 and IFN-γ in lung	[11]
B. longum BB536	Lyophilized	Adult	Oral	Influenza virus H1N1	Reduction of symptom score Reduction of lung injury Reduction body weigh loss	Improvement of IL-1β, IL-6 and IFN-γ in lung	[12]
L. plantarum 06CC2	Heat-killed	Adult	Oral	Influenza virus H1N1	Reduction of virus titer in lung Reduction body weigh loss	Improvement of NK cell activity in spleen and IFN-α, IFN-β, IFN-γ, TNF-α, IL-12 and IL-6 in BALF Reduction of infiltrated neutrophils	[14]
L. pentosus b240	Heat-killed	Adult	Oral	Influenza virus H1N1	Improvement of survival rate Reduction of virus titer in lung	Improvement of BALF IgA and IgG	[5]
L. plantarum ATCCBAA793	Viable Heat-killed	Adult MyD88-/-	Nasal	Pneumonia virus of mice	Improvement of survival rate Reduction of virus titer in lung	Suppression of virus-induced CXCL10, CCL2, CXCL1, CCL9, TNF and CCL24 in a MyD88–TLR signaling independent manner	[17]
L. plantarum ATCC23272	Viable Heat-killed	Adult MyD88-/-	Nasal	Pneumonia virus of mice	Improvement of survival rate Reduction of virus titer in lung	Suppression of virus-induced CXCL10, CCL2, CXCL1, CCL9, TNF and CCL24 in a MyD88–TLR signaling independent manner	[17]
L. rhamnosus CRL1505	Viable	Adult	Oral	Poly(I:C)	Reduction of lung injury	Improvement of DCs and CD4+IFN-γ+ T cells in lung and levels of IFN-γ, IL-10 and IL-6 in BALF	[21]
Lactobacilli mixture	Formalin treated	Adult	Oral	Influenza virus H1N1	Improvement of survival rate Reduction of lung injury	Improvement of lung IgA Improvement of lung TNF-α and IL-12	[6]

Lactobacilli mixture	Formalin treated	Adult	Nasal	Influenza virus H1N1	Improvement of survival rate Reduction of lung injury	Improvement of lung IgA Improvement of lung TNF-α and IL-12	[6]
L. rhamnosus	Lyophilized	Infant	Sublingual	Influenza virus H1N1	Reduction of virus titer in lung Reduction of lung injury	Improvement of lung IgA, IL-12 and NK cell activity and reduction of IL-6 and TNF-α	[16]
L. rhamnosus CRL1505	Viable	Infant	Oral	Respiratory syncytial virus	Reduction of virus titer in lung Reduction of lung injury	Improvement of DCs and CD4$^+$IFN-γ^+ T cells in lung and levels of IFN-γ, IL-10 and IL-6 in BALF	[1]

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