Oral Administration of Lactobacillus pentosus Strain S-PT84 Enhances Anti-Influenza Virus-Specific IgG Production in Plasma after Limited Dose of Influenza Virus Vaccination in Mice

Keywords: Influenza vaccine; Lactobacillus pentosus strain S-PT84; Adjuvant effect; IgG; IgA

Abstract

Background: It has been reported that various Lactobacillus species enhance antigen-specific antibody production after viral infection and/or vaccination in animals and humans. In this study, the effect of oral administration of Lactobacillus pentosus strain S-PT84 on subcutaneous administration of limited dose of 2.5 ng of influenza A virus (IAV) [H1N1] split hemagglutinin (HA) vaccine was studied in mice.

Methods: Seven-week-old BALB/c female mice were fed the ALN-93M diet with or without 0.186% S-PT84 for 6 weeks ad libitum and then given subcutaneous injection of IAV/California/7/2009 [H1N1] HA vaccine at doses of 2.5–15.0 ng/mice and boost on day 28. Two weeks after the last vaccination, the mice were sacrificed under anesthesia, and the amount of anti-HA-specific IgG in plasma was measured by ELISA.

Results: The levels of anti-HA-specific IgG in plasma were significantly higher in the S-PT84 group than in the control group without S-PT84 treatment. Anti-HA-specific IgA levels in nasal washes were under the detection level in both groups.

Conclusion: Oral administration of S-PT84 enhanced the production of HA-specific IgG antibody in plasma after subcutaneous vaccination, even at the limited dose of 2.5 ng of IAV [H1N1] HA in mice, suggesting that S-PT84 has a potent adjuvant activity against IAV.

Introduction

Influenza is an acute viral infection that results in high morbidity and significant mortality, particularly in infants and the elderly populations [1]. They sometimes have a high risk of fatal influenza pneumonia and thus vaccination is recommended for prevention. The World Health Organization has recommended use of oseltamivir (Tamiflu®, Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) and The World Health Organization has recommended use of oseltamivir (Tamiflu®, Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) for the treatment of influenza. These antiviral neuraminidase inhibitors are useful options for seasonal influenza infections in the world [2]. However, Shinahara et al. recently reported that administration of these inhibitors decreases anti-IAV HA-specific IgG and IgA production in plasma and nasopharyngeal secretions, probably because of inhibition of viral replication and limited production of viral antigens [3]. The limited viral antigens do not induce sufficient acquired immunity in patients treated with anti-viral neuraminidase inhibitors, resulting in a high frequency of influenza virus re-infection in the subsequent year [3].

Lactic acid bacteria (LAB) are widely used as a health food ingredient and could modulate mucosal and systemic immune responses [4,5]. Oral administration of live Lactobacillus casei Shirota significantly increases the survival rate of neonatal and infant mice infected with IAV by stimulating IL-12 production and NK activity in the lung [6]. Oral administration of heat-killed Lactobacillus plantrum L-137 [7] and Lactobacillus pentosus strain b240 [8] enhances type 1 interferon production and increases the production of anti-IAV IgG in plasma and IgA in bronchoalveolar lavage fluid, respectively, and prolongs the survival period of mice infected with IAV. These studies showed that oral administration of live and nonviable Lactobacillus species enhances the protective effects against airway infection of IAV.

It has recently been reported that oral administration of probiotic LAB enhances vaccination-induced antibody production. Lactobacillus acidophilus enhances rotavirus-specific antibody after vaccination of rotavirus in the neonatal gnotobiote pig [9]. Davidson et al. reported that volunteers receiving Lactobacillus GG show higher protective titers after administration of live attenuated IAV/H3N2 vaccine compared to a placebo group after vaccination [10]. Moreover, Boge et al. reported that daily consumption of a probiotic drink increases relevant specific antibody responses to influenza...
vaccination in healthy volunteers over 70 years of age [11]. Therefore oral administration of *Lactobacillus* species might be effective in preventing influenza virus infection by stimulating antibody production.

We previously reported that *Lactobacillus pentosus* strain S-PT84 enhances splenic natural killer (NK) activity and exhibits anti-allergic effects by modulating T-helper1/T-helper2 (Th1/Th2) balance [12]. Moreover, it has been reported that oral administration of S-PT84 enhances interferon-a production from plasmacytoid dendritic cells against an insufficient dose of hemagglutinating virus of Japan (HJV) infection [13], and that intranasal administration of S-PT84 protects against influenza infection [14]. However, the effects of S-PT84 ingestion on limited dose of IAV HA vaccination and HA-specific antibody induction have not yet been examined. In this study, the effect of oral administration of S-PT84 on the induction of IAV HA-specific antibody in mice immunized by IAV HA vaccine in the nanogram range was investigated.

Materials and Methods

Animals

BALB/c female mice, 7 weeks old, were obtained from Japan SLC, Inc. (Shizuoka, Japan). Mice were housed and fed a commercial diet (CE-2) and tap water ad libitum for 1 week at 25±1 °C and 60% ± 5% humidity under a 12 h light-dark cycle before experimentation. Experiments were performed according to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1996).

Bacterial strains and bacteria preparation

S-PT84 was isolated from Kyoto pickles “SHIBAZUKE” [12] and was cultivated in a medium containing glucose and yeast extract (Aromild™, SK yeast extract Hi-K) at 37 °C for 24 hours. Cultured cells were collected by centrifugation at 9,190 x g for 5 min, washed twice with sterile saline and once with distilled water, and heat-killed at 95 °C for 5 min. Heat-killed S-PT84 was lyophilized for use in the experiments.

Vaccine

The influenza antigen used in the present studies, IAV/California/7/2009(H1N1) virus processed for HA vaccine (split-product, 0.636 μg protein/0.341 μg HA/mL), was purchased from DENKA SEIKEN Co., Ltd. (Tokyo, Japan).

Procedure and sample collection

BALB/c mice were fed on a commercial diet (AIN-93M, Oriental Yeast Co. Ltd., Tokyo, Japan) with or without 0.186% S-PT84 and tap water ad libitum for 6 weeks. Mice were given subcutaneous vaccinations (2.5–15.0 ng HA/100 μL) and boosted on day 28. Two subcutaneous injections of IAV HA at 2.5 ng alone, a small amount of antigen-specific IgG antibody production was detected in plasma. The S-PT84 group of mice treated with continuous feeding of the AIN-93M diet with 0.186% S-PT84 during the experimental period also received two subcutaneous injections of IAV HA at 2.5 ng. HA-specific IgG antibody levels in plasma were higher in the S-PT84 group than in the control group (Figure 2A). On the other hand, HA-specific IgA in nasal washes was under detection levels in both groups (data not shown). The induced HA-specific IgG titers in plasma were significantly higher in the S-PT84 group than in the control group (p<0.007) (Figure 2B).

Discussion

In this study, oral administration of S-PT84 enhanced HA-specific IgG induction in plasma, but not HA-specific IgA induction in nasal wash, after subcutaneous administration of limited dose of IAV (H1N1) HA in mice. The findings suggest that S-PT84 has an adjuvant effect against IAV HA on plasma IgG induction, even at minimal dose. Nomaka et al. reported that S-PT84 induces cytokine
production from peritoneal macrophages in vitro and enhances the activity of NK cells in vivo [12]. Koizumi et al. also reported that S-PT84 stimulates IFN-γ and IL-12 production through Toll-like receptor (TLR)-2 and TLR-4 on dendritic cells [16]. Moreover, Izumo et al. reported that Salmonella-specific antibody is enhanced by S-PT84 ingestion in Salmonella typhimurium-infected mice [17]. Accordingly, we thought that antigen-presenting cells were activated by ingestion of S-PT84 in this experiment, and production of HA-specific IgG in plasma may be enhanced, as in a previous report [17]. It is well known that influenza vaccines administered intramuscularly or subcutaneously induce a predominantly IgG-mediated protection in the systemic immune compartment, but this systemic immunization offers inadequate induction of IgA in airway mucosa [18]. Therefore, oral administration of S-PT84 might affect HA antigen-induced dendritic cell-mediated systemic immunity and induce HA-specific IgG in plasma, but not affect mucosal immunity and HA-specific IgA production.

The present data suggest that S-PT84 enhances antibody production as an adjuvant with limited dose of influenza viral antigen. Hui-Tsu et al. reported that the induction of HA-specific antibody in plasma was enhanced by IAV HA (H1N1) vaccination at 10 ng dose in mice, and the survival rate was also significantly improved by this vaccination [19]. The optimal density (OD) level of HA-specific IgG was similar compared with our present study. Moreover, the strain of IAV/California/7/2009 (H1N1) was same in both experiments. Therefore, we speculate that HA-specific antibody production level by the killed bacterial adjuvant S-PT84 might be sufficient for the viral protection and then an ample titer was obtained for influenza virus exclusion. It suggests that S-PT84 may decrease the risk of influenza virus re-infection treated with anti-viral neuraminidase inhibitors.

As a next step, it should be to investigate whether oral administration of S-PT84 together with the vaccination on limited dose of HA antigen can protect IAV infection, and/or whether oral administration of S-PT84 can induce sufficiently HA-specific IgG antibody treated with anti-viral neuraminidase inhibitors after IAV infection.
Japan is becoming a super-aging society more rapidly than other countries. Elderly persons have a high risk of influenza-associated pneumonia, which is sometimes fatal. Thus, management of influenza outbreaks is important for them. Indeed, seroconversion and seroprotection are lower in elderly individuals than in adults after influenza vaccination, and their immune functions are weaker than in adults [20]. It is important that preventive action be taken to enhance immune function or provide vaccination for the elderly. In this regard, S-PT84 may have a potential to improve their immune response.

References


