Full Paper

Consumption of yogurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 augments serum antibody titers against seasonal influenza vaccine in healthy adults

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Seasonal influenza is a major upper respiratory tract infection occurring in winter. Vaccination is the best method for preventing this infection. We conducted two randomized, double-blind, placebo-controlled trials to examine whether consumption of yogurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1, which has been reported to reduce the risk of catching the common cold, augments serum antibody titers against seasonal influenza vaccines. In the first trial, which included university students, serum antibody titers against influenza A (H3N2) and B viruses were significantly higher in the yogurt group than in the placebo group. According to the guidelines established by the European Medicines Agency (EMA) for the assessment of vaccines, the seroconversion rate and mean geometric increase of influenza A (H3N2) and seroprotection of influenza B met the criteria only in the yogurt group. In the second trial, which included healthy adults, serum antibody titers against influenza A (H1N1) and B viruses were significantly higher in the yogurt group than in the placebo group. The seroconversion rate and mean geometric increase of influenza B met the EMA criteria only in the yogurt group. Furthermore, the cumulative days of ill health, such as throat complaints, upper respiratory inflammation, and cold, were significantly lower in the yogurt group than in the placebo group. Therefore, daily intake of yogurt fermented with *L. bulgaricus* OLL1073R-1 could reduce the duration of symptoms caused by respiratory infections and act as a mucosal adjuvant enhancing acquired immune responses against vaccines, leading to the improvement of public health.

Key words: adjuvant effect, influenza vaccine, *Lactobacillus bulgaricus*, OLL1073R-1, yogurt, clinical trials

INTRODUCTION

Seasonal influenza is a major upper respiratory infection occurring in winter, and it has symptoms such as a high fever, cough, sore throat, and headache. It takes at least a week for patients to recover from influenza. It can cause severe illness, as well as complications leading to hospitalization and even death, in high-risk individuals [1, 2]. In addition to seasonal influenza, human societies are experiencing serious public health issues related to coronavirus disease 19 (COVID-19). It causes serious pneumonia that requires intensive medical care and has become an enormous burden on healthcare workers. Since the

clinical symptoms of COVID-19 are similar to those of seasonal influenza [3], co-circulation of influenza would place a greater burden on healthcare workers. In addition to public health concerns, seasonal influenza is estimated to be responsible for a substantial economic burden [4]. Therefore, the prevention of seasonal influenza would contribute to both public health and the economy.

Vaccination is the best method to protect against seasonal influenza. However, its effectiveness is estimated to be only about 70% [5]. One way to improve the effectiveness is to augment antibody production, which is affected by the state of the vaccinee. For instance, senescence lowers the antibody production in elderly individuals due to their reduced immune function [6, 7]. The gender of the vaccinee also affects the efficacy of vaccines, as antibody production is lower in men than in women [8]. Other factors, including stress, lack of sleep, and an irregular circadian rhythm, may affect immune function, resulting in a reduced production of antibodies [9, 10]. The microbiota has also been reported to affect the host immune state and the efficacy of vaccines [11, 12].

Yogurt is fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. Several strains of *L. bulgaricus* have been reported to stimulate the immune system [13, 14]. *L. bulgaricus* OLL1073R-1 (OLL1073R-1) has also been reported to possess immunomodulatory properties. We previously demonstrated that the consumption of yogurt fermented with OLL1073R-1 enhanced natural killer cell activity and reduced the risk of catching the common cold in elderly people [15]. Moreover, the administration of yogurt augmented virus-specific immunoglobulin (Ig) A and IgG1 titers in bronchoalveolar lavage fluid of mice infected with influenza, reduced virus titers in lungs, and improved survival rate [16]. Therefore, the consumption of yogurt fermented with OLL1073R-1 may have the potential to augment antibody production.

To investigate whether the consumption of yogurt fermented with OLL1073R-1 augments antibody production, two randomized controlled trials were conducted. The subjects of these trials were male university students living in a dormitory and healthy 25- to 59-year-old adults. The subjects consumed yogurt fermented with OLL1073R-1 or placebo and were vaccinated against seasonal influenza. Serum antibody titers were measured and evaluated according to the guidelines on the requirements for seasonal influenza vaccines defined by the European Medicines Agency (EMA) [17].

MATERIALS AND METHODS

First trial

Subjects

Healthy male students attending Juntendo University School of Medicine and living in a dormitory were recruited. Subjects who met the following criteria were included: >18 years of age, scheduled to receive seasonal influenza vaccination conducted annually by the university, and provided written informed consent. Subjects who met at least one of the following conditions were excluded: infected with influenza in the previous 6 months, had a systemic disease, had lactose intolerance, had food allergies, and had participated in another clinical trial within the past 30 days.

Study design and vaccine

The first trial was a randomized, double-blind, placebo-controlled trial conducted during the 2012/2013 influenza season. Subjects who provided informed consent were randomly assigned to one of two groups, which were matched for age, height, weight, and body mass index (BMI). They consumed 112 mL of the test food or placebo once a day for about 10 weeks, from 3 weeks before until 10 weeks after vaccination, excluding 3 weeks of winter vacation (Fig. 1A). They were subcutaneously vaccinated at 0 weeks (0 wk), and blood samples were collected at −3, 1, 5, 8, and 10 wk. The vaccine used in this trial was the influenza hemagglutinin (HA) vaccine (Denka Co., Ltd., Tokyo, Japan), which contained A/California/7/2009pdm9 (X179-A), A/Victoria/361/2011 (IVR-165), and B/Wisconsin/1/2010 (BX-41A).
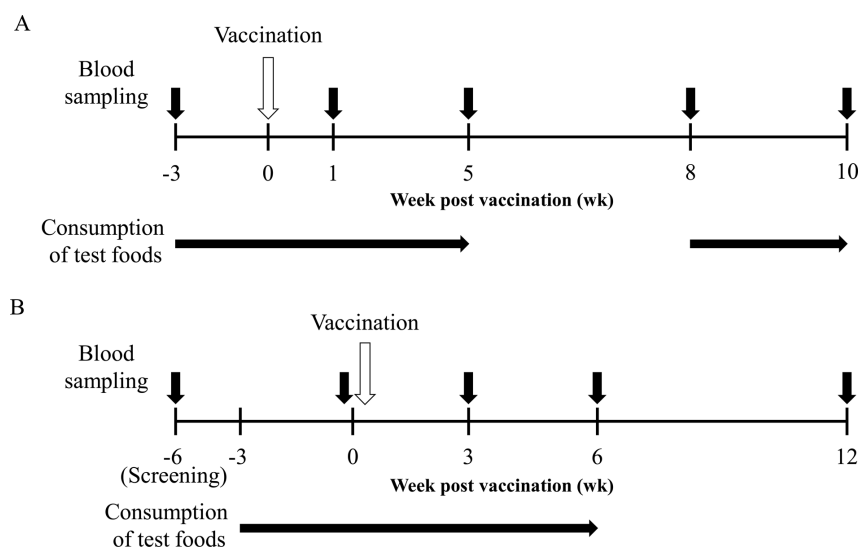

Fig. 1. Study designs of the first (A) and second (B) trials.

Ethical statement

The first trial conformed to the Declaration of Helsinki guidelines. This trial was approved by the ethics committees of Juntendo University (approval No. 26-85) and Meiji Co., Ltd. (approval No. 01) and registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) under registration number UMIN000040232.

Second trial

Subjects

Healthy individuals residing in the Kanto region of Japan were recruited. Subjects who met the following criteria were included: 20–59 years of age, BMI of 17.0–30.0 kg/m^2, able to receive the influenza vaccine according to the test schedule, and provided informed consent. Subjects who met at least one of the following criteria were excluded: history of allergy to vaccines; vaccinated against or infected with influenza in the previous 3 years; had a food and/or drug allergy; had lactose intolerance; had pollinosis, immunodeficiency, a malignant tumor, diabetes, hyperlipidemia, or a chronic disease; had a habit of consuming yogurt fermented with OLL1073R-1 at least once a week in the previous 3 months; had participated in another clinical trial in the previous 1 month; or planned to be pregnant or breastfeeding during the study period.

Study design and vaccine

The second trial was a randomized, double-blind, placebo-controlled trial conducted during the 2013/2014 influenza season. Blood samples were collected at −6 wk from subjects who provided informed consent, and antibody titers against influenza were measured using a hemagglutination-inhibition (HI) test. Subjects with low antibody titers (HI titer <40) were randomly assigned to one of two groups, which were matched for age, gender, and HI titer. The subjects consumed 112 mL of the test food or placebo once a day from −3 to 6 wk (Fig. 1B). They were subcutaneously vaccinated at 0 wk, and blood samples were collected at 0, 3, 6, and 12 wk. The vaccine used in this trial was the influenza HA vaccine (Daiichi Sankyo Biotech Co., Ltd., Kitamoto, Saitama, Japan), which contained A/California/7/2009pdm9 (X179-A), A/Texas/50/2012 (X-223), and B/Massachusetts/2/2012 (BX-51B).

The subjects were instructed to consult a doctor if they experienced any symptoms of respiratory disease, such as cough, sneezing, runny nose, stuffy nose, sore throat, throat swelling, or fever, and to document the duration of the symptoms in their health diary.

Ethical statement

The second trial conformed to the Declaration of Helsinki guidelines. This trial was approved by the ethics committees of Yaesu Sakuradori Clinic (approval No. 50010-0922) and Meiji Co., Ltd. (approval No. 17) and registered in the UMIN-CTR under registration number UMIN000040233.

Test food and placebo

The test food was yogurt fermented with OLL1073R-1 and *S. thermophilus* OLS3059. These strains were originally isolated from traditional Bulgarian yogurt [18]. The ingredients of the yogurt were milk, skimmed milk, high-fructose corn syrup, sugar, and food additives, such as pectin. A volume of 112 mL of the yogurt had a nutritional value of 76 kcal and contained 13.9 g of carbohydrates, 0.67 g of fat, and 3.6 g of protein. The test food contained 3.3 mg or more of exopolysaccharide (EPS). The placebo was acidified milk, which was prepared by adding lactic acid to the same acidity as that of the test food. Other ingredients of the placebo were skimmed milk, butter, high-fructose corn syrup, sugar, and food additives, such as pectin. A volume of 112 mL of the placebo had a nutritional value of 77.5 kcal and contained 13.1 g of carbohydrates, 0.67 g of fat, and 5.31 g of protein. Either the test food or the placebo was delivered to each subject every week and stored in a refrigerator until consumption.

Assessment of blood samples

Serum antibody titers were measured using the HI test. SRL, Inc. and LSI Medience Corporation performed the HI testing in the first and second trial, respectively. Geometric mean titer (GMT), seroprotection, seroconversion rate, and mean geometric increase were calculated from the HI titer and evaluated according to EMA criteria for the assessment of vaccines [17]. The EMA states that at least one of the assessments should meet the following requirements: rate of seroconversions or significant increase in anti-hemagglutinin antibody titer >40%; mean geometric increase >2.5; or proportion of subjects achieving an HI titer ≥40 of >70%.

Statistical analyses

Individual HI titers at each time point were log-transformed. The statistical analyses were performed using the BellCurve for Excel software (version 2.11; Social Survey Research Information Co., Ltd., Tokyo, Japan). Comparisons of the GMTs between groups were performed using the Tukey–Kramer test. Comparisons of the characteristics of the subjects were performed using an unpaired *t*-test, Welch's *t*-test, or Fisher's exact test. Comparisons of seroprotection, seroconversion, and cumulative days of respiratory disease symptoms between the groups were performed using Fisher's exact test. The mean geometric increases were compared between the two groups using the Mann–Whitney U test. In all of the analyses, p values <0.05 indicated statistical significance.

RESULTS

First trial

Recruitment

In the first trial, 49 healthy male university students who were 18–25 years of age provided informed consent and were randomly divided into two groups: the yogurt group (n=25) and the placebo group (n=24). During the course of the trial, one subject in the placebo group refused vaccination and dropped out of the study. Three subjects in the yogurt group and one in the placebo group were excluded from the analysis because of noncompliance with the food intake level, as they had low ingestion rates (<80%). One subject in the yogurt group and three in the placebo group were excluded from the analysis because of missing blood samples. There were no significant differences in the ages, heights, weights, or BMIs between the groups (Supplementary Table 1).

None of the participants were infected with influenza during the study period.

Antibody titers and evaluation according to the EMA criteria

At −3 wk, the GMTs of H1N1 and H3N2 in both groups were higher than 40, which was regarded as indicating that they were seroprotected, even though no vaccinations had been administered (Fig. 2). There were significant differences in the GMTs of the H1N1 and B viruses between the groups at −3 wk. Vaccination significantly increased the GMTs of all viruses in the yogurt group and those of the H3N2 and B viruses in the placebo group. In contrast, it had no impact on the GMT of H1N1 in the placebo group, which was due to the high titer before vaccination. The GMTs of the H3N2 and B viruses after vaccination were significantly higher in the yogurt group than in the placebo group.

Seroprotection, seroconversion rate, and mean geometric increase were calculated when the GMT was at its peak: at 8 wk for H1N1 and H3N2 and at 5 wk for B virus. The seroprotection against H1N1 and H3N2 met the EMA criteria (>70%) in both groups, whereas only the yogurt group met the criteria for the B

virus (Table 1). In terms of the seroconversion rate, H1N1 did not meet the criteria in either group, whereas the B virus met the criteria (>40%) in both groups; H3N2 met the criteria only in the yogurt group. The seroconversion rate of H3N2 was high in the yogurt group. In terms of the mean geometric increase, H1N1 did not meet the criteria in either group, whereas the B virus met the criteria (>2.5) in both groups; H3N2 met the criteria only in the yogurt group. The mean geometric increases in H1N1 and H3N2 were significantly higher in the yogurt group than in the placebo group.

Adverse events

No adverse events were reported during the first trial.

Second trial

The first trial demonstrated some effects of yogurt consumption on antibody production. However, the GMTs of H1N1 and H3N2 were so high at baseline that the subjects were already seroprotected before vaccination, which made it difficult to evaluate the effects properly. To confirm the effects, a second
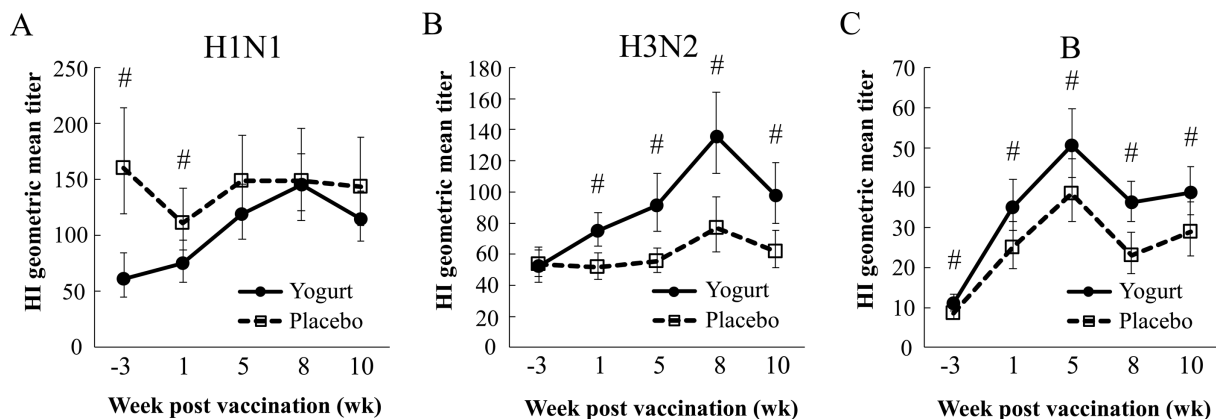

Fig. 2. Changes in geometric mean titers over time in the first trial.
Hemagglutination-inhibition (HI) titers against the influenza H1N1 (A), H3N2 (B), and B (C) viruses are presented as the geometric mean ± geometric standard error of the mean. #$p<0.05$ for comparisons between the two groups by the Tukey–Kramer test.

Table 1. Serological assessment according to the European Medicines Agency (EMA) criteria in the first trial

	Yogurt group	Placebo group	p-value
Seroprotection (%)			
H1N1	100.0*	89.5*	0.219[a]
H3N2	91.7*	90.0*	1.000[a]
B	75.0*	60.0	0.314[a]
Seroconversion rate (%)			
H1N1	20.8	10.0	0.345[a]
H3N2	41.7*	15.0	0.089[a]
B	62.5*	60.0*	1.000[a]
Mean geometric increase (ratio)			
H1N1	2.38	1.00	0.010[b]
H3N2	2.52*	1.46	0.032[b]
B	4.36*	4.59*	0.978[b]

The seroprotection, seroconversion rate, and mean geometric increase were calculated from the hemagglutination-inhibition titer at 8 wk for the H1N1 and H3N2 viruses and at 5 wk for the B virus. The seroconversion rate and mean geometric increase were calculated in comparison with those at −3 wk. *Meets the EMA criteria for vaccine assessment. [a]Fisher's exact test. [b]Mann–Whitney U test.

trial was conducted with healthy adults having low HI titers. The sample size for the second trial was calculated on the basis of the results of the first trial. In the first trial, the initial antibody titer against H3N2 in 10 subjects in the yogurt group and 11 subjects in the placebo group was 40 or less. Among these subjects, the averages of the log-transformed GMT values in the yogurt and placebo groups were 6.302 and 5.316, respectively, and the standard deviations in the yogurt and placebo groups were 1.630 and 1.183, respectively. From these values, the effect size was calculated as 0.70. Assuming an alpha error of 5% and a beta error of 20%, the number of subjects needed for each group in the second trial was calculated to be 32.

Recruitment

In the second trial, 120 healthy adults who were 20–59 years of age provided informed consent and were screened, and 64 subjects with low antibody titers against the H1N1, H3N2, and B viruses were randomly divided into two groups of 32 subjects each (Fig. 3). During the course of the trial, one subject in the yogurt group missed vaccination due to a cold and dropped out of the study, and one subject in the placebo group discontinued participation after vaccination and dropped out of the study. One subject in each group missed blood sampling at 12 wk within the prescribed period. One subject in the yogurt group was infected with influenza A virus between 6 and 12 wk. These subjects were excluded from the analysis. In terms of the characteristics of the subjects, there were no significant differences in the ages, gender, heights, and weights between the groups (Supplementary Table 2). The BMI of the placebo group was significantly higher than that of the yogurt group, but this had little impact on the trial because the BMI of each group was within the normal range.

Antibody titers and evaluation according to the EMA criteria

There were no significant differences in the GMTs between the groups at 0 wk. The GMT of H1N1 was significantly higher in the yogurt group at 6 wk, and the GMT of the B virus was significantly higher in the yogurt group from 3 to 12 wk (Fig. 4C). No significant difference was observed in the GMTs of H3N2

between the groups. Serological assessments according to the EMA criteria revealed that H1N1 and H3N2 met the criteria for seroprotection, seroconversion rate, and mean geometric increase in both groups (Table 2). In terms of the B virus, the placebo group failed to meet the criteria for any of the three assessments, whereas the yogurt group met the criteria for seroconversion and mean geometric increase. The mean geometric increase of the B virus tended to be high in the yogurt group.
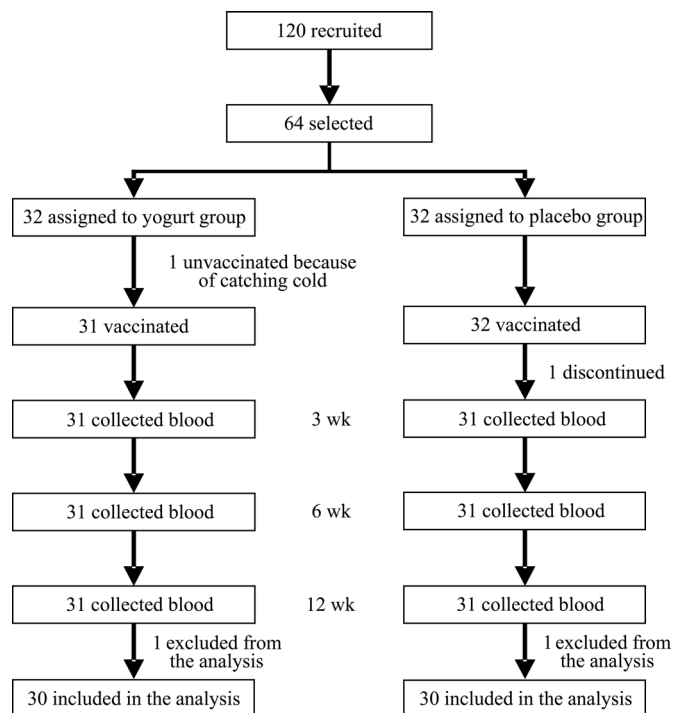

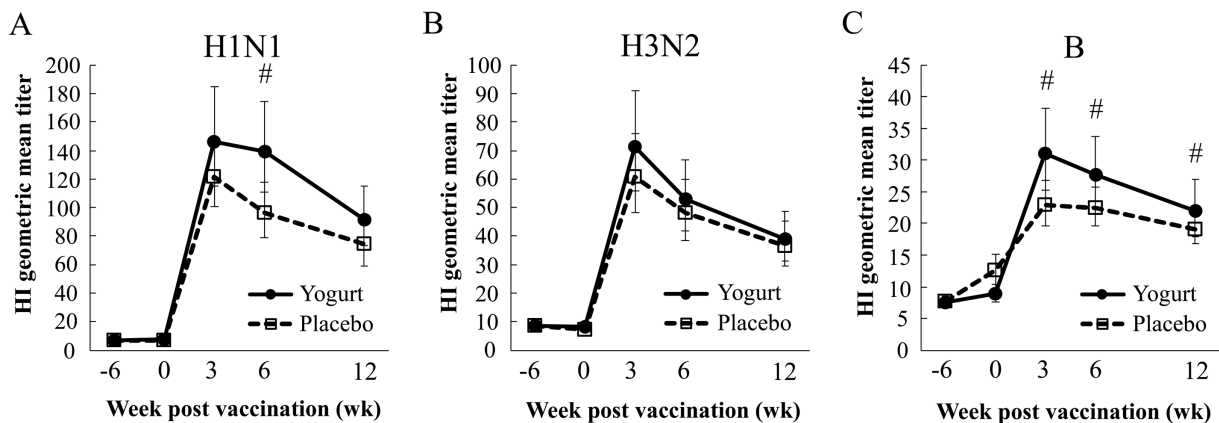**Fig. 3.** Consolidated Standards of Reporting Trials flow diagram for the second trial.

Fig. 4. Changes in the geometric mean titers in the second trial.

Hemagglutination-inhibition (HI) titers against the influenza H1N1 (A), H3N2 (B), and B (C) viruses are presented as the geometric mean ± geometric standard error of the mean. #p<0.05 for comparisons between the two groups by the Tukey–Kramer test.

Cumulative days of symptoms of respiratory diseases

To evaluate whether yogurt consumption improved vaccine effectiveness, the number of influenza incidents was measured. However, no subjects in either group were infected with influenza during the intake period; one subject in the yogurt group was infected with influenza A after that period. Therefore, there were no significant differences in the incidence rates of influenza between the groups. Instead, the cumulative days of symptoms among all subjects between 3 and 12 wk were calculated to evaluate whether the combination of vaccine and yogurt intake reduced ill health, such as throat complaints, upper respiratory inflammation, and cold. There were no significant differences in the numbers of subjects analyzed (Table 3). The cumulative days of symptoms were 22 and 45 in the yogurt and placebo groups, respectively, and the difference between the groups was significant.

Adverse events

During the second trial, 26 adverse events were reported: common cold in 16 subjects; gastroenteritis in 3 subjects; influenza, diarrhea, hemorrhoids, eczema, and migraine in 1 subject each; and uterine fibroids and ovarian cyst resection in 1 subject. None of these adverse events were related to the test food or placebo.

DISCUSSION

This study showed that the daily consumption of yogurt fermented with OLL1073R-1 augmented serum antibody titers against a seasonal influenza vaccine. In the first trial, the GMTs of the H3N2 and B viruses were significantly higher in the yogurt group than in the placebo group. No effect of yogurt intake was observed on the GMT of H1N1 due to a higher initial GMT in the placebo group. Assessment according to the EMA criteria revealed that the seroprotection of the B virus, seroconversion rate of H3N2, and mean geometric increase of H3N2 met the EMA criteria only in the yogurt group. This indicates that yogurt consumption enhances antibody production, which leads to improved vaccine immunogenicity. In addition, it was estimated that an HI titer of 40 was an approximately 50% protective dose against influenza infection [19] and that increase of the HI titer up to 100 substantially enhanced the clinical protection against influenza [20]. Therefore, a further increase in GMT in the yogurt group compared with the placebo group, which was observed in the present study, indicates that yogurt consumption might enhance protection against influenza infection.

On the other hand, the initial GMTs of H1N1 and H3N2 were higher than 40, which is regarded as indicating seroprotection by the EMA criteria, and this made it impossible to evaluate H1N1. These high baseline values might be due to a history of vaccination and seasonal influenza infection in the previous season. It has been reported that antibodies persist for several seasons [21].

Table 2. Serological assessment according to the European Medicines Agency (EMA) criteria in the second trial

	Yogurt group	Placebo group	p-value
Seroprotection (%)			
H1N1	90.0*	90.0*	1.000[a]
H3N2	76.7*	73.3*	1.000[a]
B	50.0	46.7	1.000[a]
Seroconversion rate (%)			
H1N1	86.7*	90.0*	1.000[a]
H3N2	73.3*	70.0*	1.000[a]
B	46.7*	33.3	0.430[a]
Mean geometric increase (ratio)			
H1N1	19.70*	17.50*	0.558[b]
H3N2	8.77*	8.38*	0.904[b]
B	3.48*	1.82	0.095[b]

The seroprotection, seroconversion rate, and mean geometric increase were calculated from the hemagglutination-inhibition titer at 3 wk. The seroconversion rate and mean geometric increase were calculated in comparison with those at 0 wk. *Meets the EMA criteria for vaccine assessment. [a]Fisher's exact test. [b]Mann–Whitney U test.

Table 3. Cumulative days of respiratory disease symptoms between 3 and 12 wk

	Yogurt group	Placebo group	p-value
Number of subjects			
Total	30	30	
Without symptoms	25	24	1.000[a]
With symptoms	5	6	
Cumulative number of days			
Total	1,890	1,890	
Without symptoms	1,868	1,845	0.006[a]
With symptoms	22	45	

[a]Fisher's exact test.

In terms of H1N1, vaccines available during the 2010/2011 and 2011/2012 seasons in Japan contained A/California/7/2009 pdm9 (X179-A), which is the same strain contained in the vaccine used in the 2012/2013 season. In terms of H3N2, the vaccines available during the 2010/2011 and 2011/2012 seasons contained A/Victoria/210/2009 (X-187), whereas the vaccine used in the trial during the 2012/2013 season contained A/Victoria/361/2011 (IVR-165). The similarity between these antigens was estimated to be ≤91.6% [22]. Despite the relatively low similarity of the antigens, one possible reason for the high initial GMT of H3N2 is that antibodies produced in the previous season might have cross-reacted with the HA used in the HI test in the trial.

To precisely evaluate the effects of yogurt intake, a second trial was conducted among subjects with low antibody titers against influenza. In this trial, the GMTs of the H1N1 and B viruses were significantly higher in the yogurt group than in the placebo group, indicating that the consumption of yogurt fermented with OLL1073R-1 enhanced the production of antibodies against the vaccine. However, yogurt had no significant effect on the GMT of the H3N2 virus. The subjects of this trial were healthy adults who were <60 years of age and had low baseline serum antibody titers against influenza. Since subjects like these are responsive to vaccines, the enhancing effect of yogurt might be unclear. The GMT of H3N2 in the yogurt group was not significant but was higher than that in the placebo group. Although the sample size of the second trial was calculated according to the first trial, the differences and distributions were different between the two trials, possibly because the characteristics of the subjects were completely different in terms of age, gender, and initial antibody titer. Therefore, the sample size may have been insufficient to evaluate the effect of yogurt on the GMT of H3N2. Since elderly individuals are known to have reduced antibody production [6, 7], trials with healthy elderly subjects would show the effect of yogurt more clearly.

To evaluate vaccine effectiveness, the number of influenza incidents was first evaluated. However, the number of incidents was too low to evaluate vaccine effectiveness. Instead, the cumulative days of symptoms of respiratory diseases between 3 and 12 wk were calculated. The yogurt group showed a significantly lower number of days than the placebo group, indicating that the combination of vaccine and yogurt intake might enhance protection against respiratory diseases. However, since the symptoms of influenza and the common cold are similar, this result might be due to enhancing protection against the common cold by yogurt consumption. It has been reported that consumption of yogurt reduces the risk of catching the common cold in healthy adults [15]. That study also showed that the quality-of-life score for the eye/nose/throat system was significantly improved in its yogurt group, which is consistent with the results of the present study.

To our knowledge, this is the first report to demonstrate that the consumption of traditional Bulgarian yogurt, which contains only *L. bulgaricus* and *S. thermophilus*, improves vaccine immunogenicity by augmenting serum antibody production. Probiotic bacteria such as *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*, *Limosilactobacillus fermentum*, and *Bifidobacterium animalis* have been reported to increase serum antibody production [23–26]. Unlike these probiotic bacteria, the consumption of yogurt fermented with OLL1073R-1 has also been reported to enhance the flow rate of IgA in the saliva of elderly subjects residing in a nursing home [27]. In addition, consumption of the yogurt augmented influenza virus-bound IgA levels in the saliva of elderly subjects in a randomized controlled trial [28]. Although the mechanism of induction of influenza virus-bound IgA by yogurt has not been determined, it is possible that consumption of yogurt induces salivary IgA in response to seasonal influenza vaccination, which is usually performed on most residents in nursing homes. IgA is predominant on the mucosal surface and prevents pathogens from penetrating the human body. IgA can also cross-react with various antigens. Therefore, inducing IgA by vaccine is a promising method of preventing seasonal influenza, which varies annually [29]. Since the seasonal influenza vaccine in use induces mainly serum IgG and not mucosal IgA, consumption of yogurt might act as a mucosal adjuvant to induce IgA.

The precise mechanism by which yogurt fermented with OLL1073R-1 increases antibody production remains unclear. The possible active ingredient in yogurt is the EPS secreted by OLL1073R-1 [30]. EPS produced by OLL1073R-1 contains acidic EPS with a phosphorylated residue, which has been reported to act as a B-cell mitogen [31]. EPS has also been reported to have immunomodulatory and anti-influenza virus effects [16]. Splenocytes from mice orally administered EPS secreted more interferon-γ under CD3-antibody stimulation than those from mice administered water, which indicates that EPS enhanced T-cell activity [18]. Since T cells help B cells differentiate into antibody-secreting cells, EPS might enhance B-cell differentiation, leading to the secretion of antibodies with a higher affinity. Thus, EPS may enhance antibody production by both direct and indirect activation of B cells as a B-cell mitogen and T-cell activator, respectively. How yogurt consumption enhances the immune response to antibody production remains unclear. The site of stimulation by yogurt is the intestine, which is distant from the site of inoculation of the vaccine antigen. Future studies investigating immune responses in the gastrointestinal tract and draining lymph nodes at the site of vaccination are needed to determine how OLL1073R-1 enhances antibody production. This might lead to the use of EPS as an oral adjuvant, a combination of which with a subcutaneous vaccine increases systemic IgG and possibly induces mucosal IgA.

In conclusion, the consumption of yogurt fermented with OLL1073R-1 augmented serum antibody titers against seasonal influenza vaccines. Thus, daily yogurt consumption has the potential to be a mucosal adjuvant that improves vaccine immunogenicity and possibly induces mucosal IgA, leading to the improvement of public health.

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