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Effects of yogurt containing probiotics on respiratory virus infections: Influenza H1N1 and SARS-CoV-2

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ABSTRACT

Respiratory virus infections are an escalating issue and have become common worldwide. Influenza and COVID-19 are typical infectious respiratory diseases, and they sometimes lead to various complications. In a situation in which no established drug or treatment exists, consumption of proper food might be beneficial in maintaining health against external infections. We studied the potential effects of mixtures of probiotic strains on various viral infections. The purpose of this study was to assess the ability of yogurt containing probiotics to reduce the risk of respiratory viruses such as influenza H1N1 and SARS-CoV-2 infection. First, we performed in vitro tests using infected Madin-Darby canine kidney (MDCK) and Vero E6 cells, to evaluate the potential effects of yogurt containing high-dose probiotics against influenza H1N1 and SARS-CoV-2 infection. The yogurt significantly reduced plaque formation in the virus-infected cells. We also performed in vivo tests using influenza H1N1-infected C57BL/6 mice and SARS-CoV-2-infected Syrian golden hamsters, to evaluate the potential effects of yogurt. Yogurt was administered orally once daily during the experimental period. Yogurt was also administered orally as pretreatment once daily for 3 wk before viral infection. Regarding influenza H1N1, it was found that yogurt caused an increase in the survival rate, body weight, and IFN- γ , IgG1, and IL-10 levels against viral infection and a decrease in the inflammatory cytokines TNF- α and IL-6. Although the SARS-CoV-2 copy number was not significantly reduced in the lungs of yogurt-treated SARS-CoV-2-infected hamsters, the body weights and histopathological findings of the lungs were improved in the yogurt-treated group. In conclusion, we suggest that consumption of yogurt containing probiotics can lead to beneficial effects to prevent respiratory viral infections.

Key words: probiotics, respiratory virus infection, H1N1, SARS-CoV-2, prevention

INTRODUCTION

In recent years, coronavirus infection (CoV) has emerged as a replacement for influenza, which is a classic respiratory viral infection, having been identified as a large problem and a risk to human health worldwide (Avolio et al., 2022). The risk of respiratory viral infection is because of its rapid and various routes of transmission, such as via droplets and air in a closed environment (von Linstow et al., 2008; Tagliamonte et al., 2021).

It is known that the main causes of epidemic respiratory diseases in mammals, including humans and other species, are influenza viruses (Maragkoudakis et al., 2010; DeGrandi-Hoffman and Chen, 2015). Influenza viruses are transmitted almost every winter, and influenza virus infections pose social and economic risks in many countries. Severe influenza infection increases the levels of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), which are inflammatory cytokines. Increases in the levels of these cytokines have often been observed during the lethal course of infection (Van Reeth et al., 1998; Julkunen et al., 2001; Kaiser et al., 2001),

Moreover, influenza virus infection is dangerous to high-risk groups, including pregnant women, patients with diabetes, infants, and the elderly (Boyd et al., 2006; Lynch and Walsh, 2007). Such high-risk groups do not have sufficient immune response against viral infections and frequently show severe and lethal conditions. Thus, proper maintenance of the immune system, which forms a barrier to foreign antigens, is essential to prevent the severe and lethal conditions of influenza (Nakayama et al., 2014).

Coronaviruses, which belong to the *Orthocoronavirinae* family, infect mammalian species, including humans and other species (Islam et al., 2021). Various CoV have threatened human society over the past 20 years. In particular, severe acute respiratory syndrome

Received April 15, 2022.

Accepted September 25, 2022.

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CoV (SARS-CoV), Middle East respiratory syndrome CoV (MERS-CoV), and SARS-CoV-2, which causes coronavirus disease 2019 (COVID-19) have adversely affected global health in 2002, 2012, and 2019 (Ning et al., 2021). The mild symptoms of SARS-CoV-2 infection are similar to those of influenza and common cold-like illness, such as cough, fever, and fatigue, whereas the severe symptoms of SARS-CoV-2 include severe dyspnea, multifocal pneumonia, acute respiratory distress syndrome, and multi-organ dysfunction syndrome (Elhamzaoui et al., 2021). Immune-naïve populations across the world are the cause of the efficient SARS-CoV-2 transmission, and the virus had infected more than 536 million people and killed nearly 6.3 million people by June 2022 (WHO, 2022).

For influenza and CoV infections, prevention through vaccination is very important; however, if the inoculated virus vaccine does not match the vaccine-produced antibodies and the current pandemic virus strains, spread of the virus cannot be prevented. Therefore, it is important to increase immunity, maintain personal hygiene, and eat nutritious food in daily life.

Probiotics from dairy products are consumed worldwide every day. Probiotics include many lactic acid bacteria, a group of bacteria belonging to many genera, including *Lactobacillus* and *Bifidobacterium*. They are also reported to have beneficial effects in gut disorders, diabetes, and cancer, and to improve defenses against infectious diseases through immune regulation (MacDonald and Bell, 2010; Hill et al., 2017). Our test product is a yogurt (Bulgaria, provided by Namyang Dairy Products Co. Ltd.) containing high-dose probiotics, such as *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacterium animalis* ssp. *lactis* Bb-12, *Limosilactobacillus fermentum* PL9988 (bulgaria Kor91), *Lactiplantibacillus plantarum* SN35N, *L. acidophilus* NCFM, and *B. lactis* Bi-07, which are known to have beneficial effects in humans.

The purpose of this study was to determine the overall effect of high-dose probiotic yogurt (Bulgaria) treatment on viral respiratory infections such as influenza and SARS-CoV-2. To achieve this purpose, we evaluated the potential effects of yogurt on influenza H1N1 and SARS-CoV-2 infection in vitro. We performed an in vivo experiment using virus-infected animal models to evaluate the positive effects of yogurt on influenza H1N1 and SARS-CoV-2.

MATERIALS AND METHODS

Test Product

The test product is a yogurt (Bulgaria, Namyang Dairy Products Co. Ltd.) containing high-dose probiot-

ics, such as *S. thermophilus*, *L. acidophilus*, *Bifidobacterium animalis* ssp. *lactis* Bb-12, *Limosilactobacillus fermentum* PL9988 (bulgaria Kor91), *Lactiplantibacillus plantarum* SN35N, *L. acidophilus* NCFM, and *B. lactis* Bi-07, at 2.1×10^9 cfu/mL. The test product was purchased from a dairy retail store in Iksan, Korea, and used within 1 week of manufacture. The test product was stored at 4°C during experimental period.

Cell Culture and In Vitro Experiment

Madin-Darby canine kidney (MDCK) cells were acquired from the Korean Cell Line Bank for influenza in vitro experiments. Cells were maintained in minimal essential medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Incubation conditions were 37°C in 5% CO₂.

Vero E6 cells were acquired from the American Type Culture Collection for SARS-CoV-2 in vitro experiments. Cells were maintained in minimal essential medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Incubation conditions were 37°C in 5% CO₂.

Human influenza virus type A/PR/8/1934 (H1N1) and SARS-CoV-2 (L clade) were provided by the Centers for Disease Control and Prevention of Korea. To evaluate the inhibitory effect of yogurt, a plaque assay was performed.

Briefly, yogurt was mixed with each virus stock (4×10^6 pfu/mL) at a ratio of 1:9 (vol/vol) for 2 h at $25 \pm 1^\circ\text{C}$. Confluent monolayers of each cell in 6- or 12-well plates were treated with a mixture (0.5 mL) containing yogurt and virus serially diluted in cell culture medium to 10^{-6} at 37°C in 5% CO₂ for 2 h. The mixture in each well was removed, and overlay medium (1 mL) was subsequently added to each well. The plates were incubated in an inverted position for 72 h to observe whether plaque was formed, and 1 mL of 4% neutral buffered formalin (NBF) was added and left for at least 30 min to fix the infected cells. The overlay medium and 4% NBF were removed, and 500 µL of 0.05% crystal violet solution was added to stain viable cells. Excess crystal violet was removed, and the plaques formed were counted to calculate the number of plaques per milliliter. The reduction rate was calculated by the following formula:

$$\text{Reduction rate (\%)} = \frac{(\text{Titer of virus control} - \text{Titer of yogurt})}{\text{Titer of virus control}} \times 100.$$

Influenza In Vivo Experiment

A total of 75 7-wk-old specific-pathogen-free mice (C57BL/6) were used in this study. The mice were housed in an environmentally controlled room. Temperature was $23 \pm 3^\circ\text{C}$, relative humidity was $55 \pm 15\%$, ventilation frequency was 10 to 20 times per hour, lighting time was 12 h/d (0800 to 2000 h), and illuminance was 150 to 300 lx. Food and water were freely available. This animal study was approved by the Institutional Animal Care and Use Committee of Knotus (Incheon, Korea), certificate number IACUC 21-KE-380.

All the experimental mice were treated with the indicated substance, such as distilled water (**DW**; 225 μL /mouse) and yogurt (225 μL /mouse), once a day from 3 wk before viral infection and then for 2 more weeks.

Additionally, the noninfected group (**G1**) was given only DW during the experimental period. The virus-infected group (**G2**) was given only DW during the experimental period, and the treated group (**G3**) was treated with yogurt during the experimental period.

In all, 30 mice were used for the virus challenge test, and 45 mice were used for the immune response test.

Fifty microliters of prepared viral solution H1N1 influenza A virus (A/Puerto Rico/08/1934, H1N1) was administered to the left nasal cavity of the mice. The clinical score and BW were observed once a day during the experimental period. All mice were individually weighed using an electronic scale once before administration. The mice were euthanized, and the survival curve was obtained.

The clinical score was determined based on the clinical signs according to the behavior of the mouse after influenza virus infection, using the following scoring system: (1) slight ruffling of fur, (2) ruffled fur and reduced mobility, (3) ruffled fur, reduced mobility, and rapid breathing, (4) ruffled fur, reduced mobility, huddled appearance, rapid or labored breathing indicative of pneumonia (animals displaying evidence of pneumonia or having lost $>20\%$ of their original BW were euthanized), and (5) death.

Necropsy sampling day was determined using the virus challenge test. Five mice per group after 3 d post-infection (**DPI**) and 5 DPI were euthanized. The mice were anesthetized with a 3:1 solution of Zoletil 50 (Virbac Laboratories) and 2% Rompun (Bayer) through intraperitoneal injection at 1 mL/kg of BW, as previously described (Rahman et al., 2017).

After anesthesia, the thorax was exposed so that tracheal bronchus was revealed, and the right bronchus was ligated with a 3-0 silk suture. A catheter-linked syringe was mounted on the left bronchus, and we collected bronchoalveolar lavage fluid (**BALF**) by injection

of 1 mL of PBS into the connected syringe. The BALF was centrifuged at $1,000 \times g$ for 10 min at 4°C , and the supernatant was moved to a cryogenic freezer and stored at -70°C .

The entire lung was collected and sufficiently fixed in 10% NBF. After blood sampling from the inferior vena cava using a syringe, some blood was added to an EDTA-3K tube and stored for further analysis. The rest of the blood was added to an evacuated tube containing clot activators and stored at room temperature for 15 to 20 min for blood clotting.

We performed TNF- α , IL-6, IL-10, IFN- γ , and IgG1 ELISA analyses to confirm the presence of inflammatory cytokines, using a commercially available ELISA kit (Thermo Fisher). The TNF- α , IL-6, and IL-10 ELISA analyses were performed using serum samples which were isolated by centrifugation for 10 min at $1,000 \times g$ at 4°C .

Serum samples for ELISA of interferon-gamma (IFN- γ) and IgG1 were collected at the indicated time point, such as 3 wk before viral infection and day of virus infection.

The BALF was used for differential cell counting, including neutrophils and monocytes, using flow cytometry. Briefly, cells were collected from BALF for flow cytometry and incubated with the following antibodies: CD45 (30-F11), CD11b (M1/70), Ly6c (HK1.4), and Ly6G (1A8-Ly6g), all of which were purchased from Thermo Fisher. Flow cytometry was performed using ThermoCyte3000 (Agilent), and data were analyzed using NovoExpress 1.4.1 (Agilent). The cell populations in BALF were identified using the following surface markers: neutrophils ($\text{CD45}^+\text{CD11b}^+\text{Ly6C}^{\text{int}}\text{Ly6G}^+$) and inflammatory monocytes ($\text{CD45}^+\text{CD11b}^+\text{Ly6C}^{\text{hi}}\text{Ly6G}^-$).

SARS-CoV-2 In Vivo Experiment

A total of 18 4-wk-old specific-pathogen-free Syrian golden hamsters were used in this study. The hamsters were housed in an environmentally controlled room, temperature was $23 \pm 3^\circ\text{C}$, relative humidity was $55 \pm 15\%$, ventilation frequency was 10 to 20 times per hour, lighting time was 12 h/d (0800 to 2000 h), and illuminance was 150 to 300 lx. Food and water were available freely.

This animal study was approved by the Institutional Animal Care and Use Committee of Knotus (Incheon, Korea), certificate number IACUC 21-KE-391.

All experimental hamsters were administered the indicated substances, such as DW (900 μL /hamster) and yogurt (900 μL /hamster), once a day from 3 wk before viral infection and then for 1 more week. The virus-infected group (**G1**) was administered only DW during the experimental period, whereas the treated

group (G2) was administered yogurt during the experimental period. A total of 200 μ L of prepared viral solution SARS-CoV-2 (10^3 TCID₅₀/mL, NCCP43326, S clade) was administered to the left nasal cavity of the hamsters.

The BW were measured once a day during the experimental period. All the hamsters were individually weighed using an electronic scale once before oral administration of yogurt.

Three animals' lungs in each group were collected after euthanasia at 3, 5, and 7 DPI and used for virological and histopathological analyses. The lungs for virological analysis were stored in a cryogenic freezer at approximately -70°C before real-time reverse-transcription PCR. The lungs for histopathological analysis were sufficiently fixed in 10% NBF.

SARS-CoV-2 RNA Quantification

RNA was extracted from the tissues using the Qiagen RNeasy Mini Kit (cat. no. 74106, Qiagen) according to the manufacturer's instructions. vRNA was extracted from nasal washes using the Qiagen QIAamp Viral RNA Mini Kit (cat. no. 52904, Qiagen) and quantified using Qiagen Quanti-Fast RT probe master mix.

We used the following primer and probe sets: E_Sarbeco_F1: ACAGGTACGTTAATAGTTAATAGCGT; E_Sarbeco_R2: ATATTGCAGCAGTACGCACACA; E_Sarbeco_P1: [FAM]ACACTAGCCATCCTTACTGCGCTTCG[BHQ1]; nCoV_IP2-12669Fw: ATGAGCTTAGTCCTGTTG; nCoV_IP2-12759Rv: CTCCCTTTGTTGTGTTGT; nCoV_IP2-12696bProbe(+): [HEX]AGATGTCTTGTGCTGCCGTA[BHQ1]. These are specific primer/probe sets for the SARS-CoV-2 *E* and *RdRP* genes (Corman et al., 2020).

The reactions were performed on a CFX96 Touch real-time RT-PCR (Bio-Rad). Cycle threshold values were compared with a standard of diluted vRNA from a stock of SARS-CoV-2. The PCR results were expressed in copy numbers per total RNA nanogram equivalents.

Histopathological Analysis

The collected tissues were immersed in formalin for at least 7 d. Formalin-fixed tissues were stained with hematoxylin and eosin after pre-processing as follows: embedding in paraffin, sectioning, mounting on slides.

The scores of hematoxylin and eosin-stained slides were determined based on the percentage of infiltration of inflammatory cells using the following scoring system: 0 = no pathological change, 1 = affected area $\leq 10\%$, 2 = affected area $< 50\%$ and $> 10\%$, and 3 = affected area $\geq 50\%$ (Imai et al., 2020).

Statistical Analysis

All data were expressed as mean \pm standard deviation. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software). The unpaired *t*-test was used to perform comparisons between 2 groups. All statistical tests were 2-sided, and significance was defined as $P < 0.05$.

RESULTS

In Vitro Results Revealed the Potential Effects of Yogurt Against Respiratory Virus Infection

As shown in Figure 1, plaque formation of influenza H1N1 and SARS-CoV-2 was inhibited by yogurt treatment. Influenza H1N1 in the yogurt-treated group showed no plaque formation compared with the untreated influenza H1N1 group. The SARS-CoV-2 yogurt-treated group showed some plaque formation at 10^{-3} dilution, determined as 2×10^5 PFU/mL. The results of the untreated SARS-CoV-2 group were counted as 9×10^5 PFU/mL. This result suggests a significant reduction in viral infection compared with the untreated SARS-CoV-2 group, and the reduction rate was 77.78%.

Yogurt Improved the Condition of Influenza H1N1-Infected Mice

We performed in vivo experiments using mice infected with influenza H1N1, which confirmed the positive results in the previous in vitro experiment. Survival rates were confirmed up to 14 d in the group fed with yogurt (G3) and the influenza H1N1-infected group (G2).

As shown in Figure 2A, the first death occurred on 7 DPI in G2 and on 8 DPI in G3. In the case of G2, 50% of the infected mice died on 9 DPI, whereas only one mouse died in G3 until 14 DPI.

The results of BW (Figure 2B) and clinical scores (Figure 2C) revealed tendencies similar to the survival rate. On 1 DPI, the BW of G2 and G3 were significantly lower than that of G1 (uninfected control; $P < 0.01$ and $P < 0.05$, respectively). On 2 DPI, the BW of G3 was significantly lower than that of G1 ($P < 0.01$). From 3 DPI to 11 DPI, the BW of G2 was significantly lower than that of G1 ($P < 0.001$ and $P < 0.05$, respectively). From 13 DPI to 14 DPI, the BW of G2 was significantly lower than that of G1 ($P < 0.05$). On 14 DPI, that BW of G3 was significantly higher than that of G2 ($P < 0.05$). The clinical score of G3 was lower than that of G2 and remained consistently low until 14 DPI.

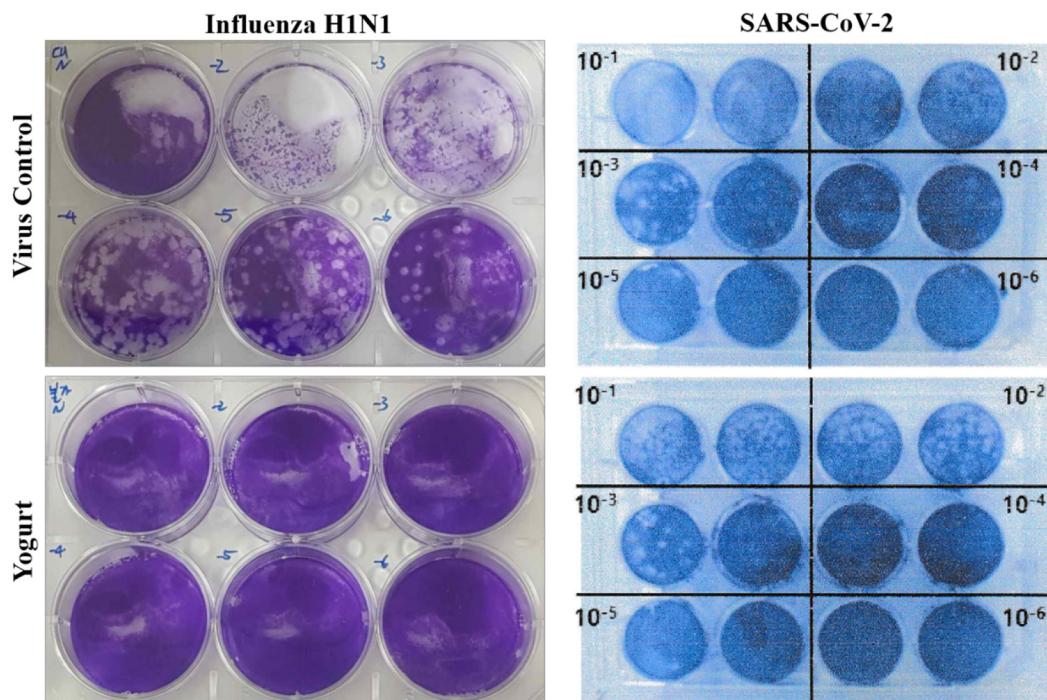


Figure 1. Plaque formation with and without yogurt in influenza H1N1 and SARS-CoV-2. Plaque assay for influenza H1N1 in 6-well plates and SARS-CoV-2 in 12-well plates. Yogurt was treated at the indicated concentrations from 10^{-1} to 10^{-6} in each plate. The clear zone indicates plaque induced by virus infection. Upper left, virus control group under influenza H1N1 infection in 6-well plate; lower left, yogurt-treated group under influenza H1N1 infection in 6-well plate; upper right, virus control group under SARS-CoV-2 infection in 12-well plate; lower right, yogurt-treated group under SARS-CoV-2 infection in 12-well plate.

Based on these results, yogurt improved the condition of influenza H1N1-infected mice.

In Vivo Results Revealed the Immune Response of Yogurt Against Influenza H1N1

To evaluate the possible relationship of immune response with yogurt, we performed IFN- γ ELISA and IgG1 ELISA using serum samples. Serum samples were obtained at different time points: before oral administration of the yogurt, before viral infection, and 3 wk after oral administration of the yogurt (Figure 2D and 2E).

The degree of change was quantified by subtracting the results obtained before oral administration of yogurt from the results obtained before viral infection 3 wk after oral yogurt administration. The degrees of change in IFN- γ and IgG1 levels in G3 were significantly higher than in G2 ($P < 0.05$) from -21 DPI to 0 DPI. Accordingly, we determined the populations of neutrophils and monocytes in BALF (Figure 3A and 3B) to evaluate inflammatory myeloid cell infiltration in BALF after virus infection. On 3 DPI, the neutrophil levels (%) of G2 and G3 were significantly higher than that of G1 ($P < 0.001$ and $P < 0.05$, respectively), and the mono-

cyte levels (%) of G2 and G3 were significantly higher than that of G1 ($P < 0.01$ and $P < 0.05$, respectively). On 5 DPI, the neutrophil and monocyte levels (n, %) of G2 and G3 were significantly higher than those of G1 ($P < 0.001$ and $P < 0.01$, respectively).

As shown in Figure 3C, we performed ELISA of serum and BALF at the indicated time points, such as 3 DPI and 5 DPI. Unfortunately, the ELISA results showed no difference among the groups. Therefore, only a few meaningful results are described. On 3 DPI, the IL-6 level in BALF in G2 was significantly lower than in G1 ($P < 0.05$). On 5 DPI, the IL-6 level in the blood in G3 was significantly lower than in G2 ($P < 0.01$), the TNF- α level in BALF in G3 was significantly higher than in G1 ($P < 0.001$ and $P < 0.05$, respectively), the IL-10 level in BALF in G2 was significantly lower than in G1 ($P < 0.05$), and the IL-10 level in BALF in G3 was significantly higher than in G2 ($P < 0.01$). Some of these results might imply that the yogurt treatment induced an immune response.

The histopathological results support the beneficial effect of yogurt against influenza H1N1 infection (Figure 4). On 3 DPI, the G2 score was significantly higher than that of G1 ($P < 0.01$), whereas the G3 score was significantly lower than that of G2 ($P < 0.05$). On 5

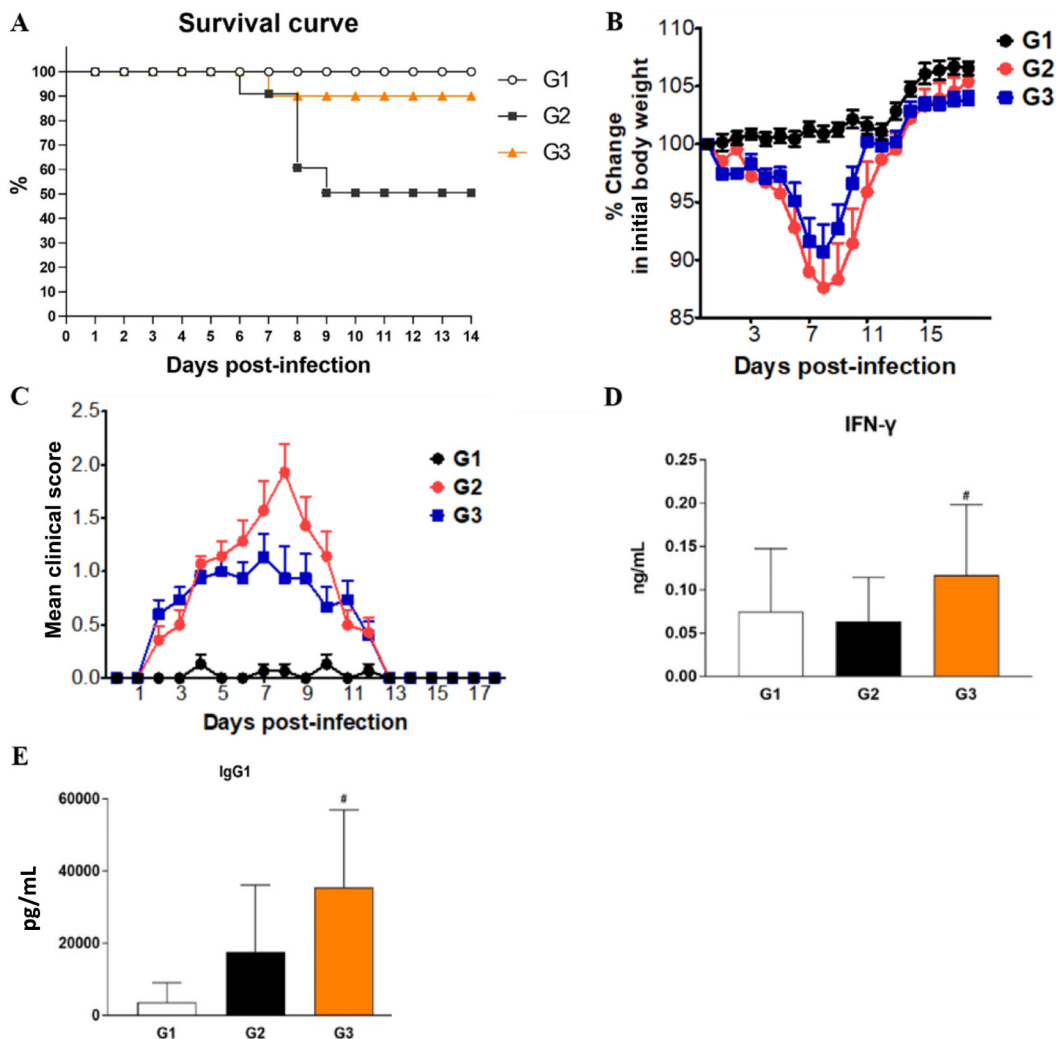


Figure 2. Beneficial effects of yogurt treatment in influenza H1N1-infected mice. (A) Survival curve, (B) BW change, and (C) clinical score of yogurt-treated influenza H1N1-infected mice over 14 d. (D) IFN- γ and (E) IgG1 values in the blood were obtained by subtracting value before yogurt treatment in each group from value after yogurt treatment for 3 wk in each group. Data are expressed as mean \pm SD. Unpaired *t*-tests were used to perform comparisons between 2 groups. All statistical tests were 2-sided, and significance was defined as $P < 0.05$. # indicates significant difference at $P < 0.05$ compared with group G2. G1 = noninfected group; G2 = influenza H1N1-infected group; G3 = yogurt-treated influenza H1N1-infected group.

DPI, the G2 and G3 scores were significantly higher than that of G1 ($P < 0.001$), whereas the G3 score was significantly lower than that of G2 ($P < 0.05$).

Yogurt Improved the Condition of SARS-CoV-2-Infected Hamsters

To examine the effect of yogurt on SARS-CoV-2 infection, the BW of the hamsters and viral gene expression were measured. Histopathological analysis was then performed.

As shown in Figure 5A, the BW of hamsters in G2, the yogurt-treated group, were significantly higher than those of hamsters in G1, the virus-infected group,

from 2 DPI to the end of the experiment ($P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively). This implies an improved body condition against SARS-CoV-2 infection in yogurt-treated hamsters. However, the difference between the virus-infected group and the yogurt-treated group in viral gene quantification was not confirmed (Figure 5B). In the results of the real-time RT-PCR, FAM expression (*E* gene) in G2 were lower than in G1, although the difference was not statistically significant over the entire experimental period. At 3 DPI, HEX expression (*RdRP* gene) in G2 was higher than in G1 and recovered to the levels of G1 at 5 and 7 DPI. Histopathological findings of the lungs scored the percentage of inflammatory cell

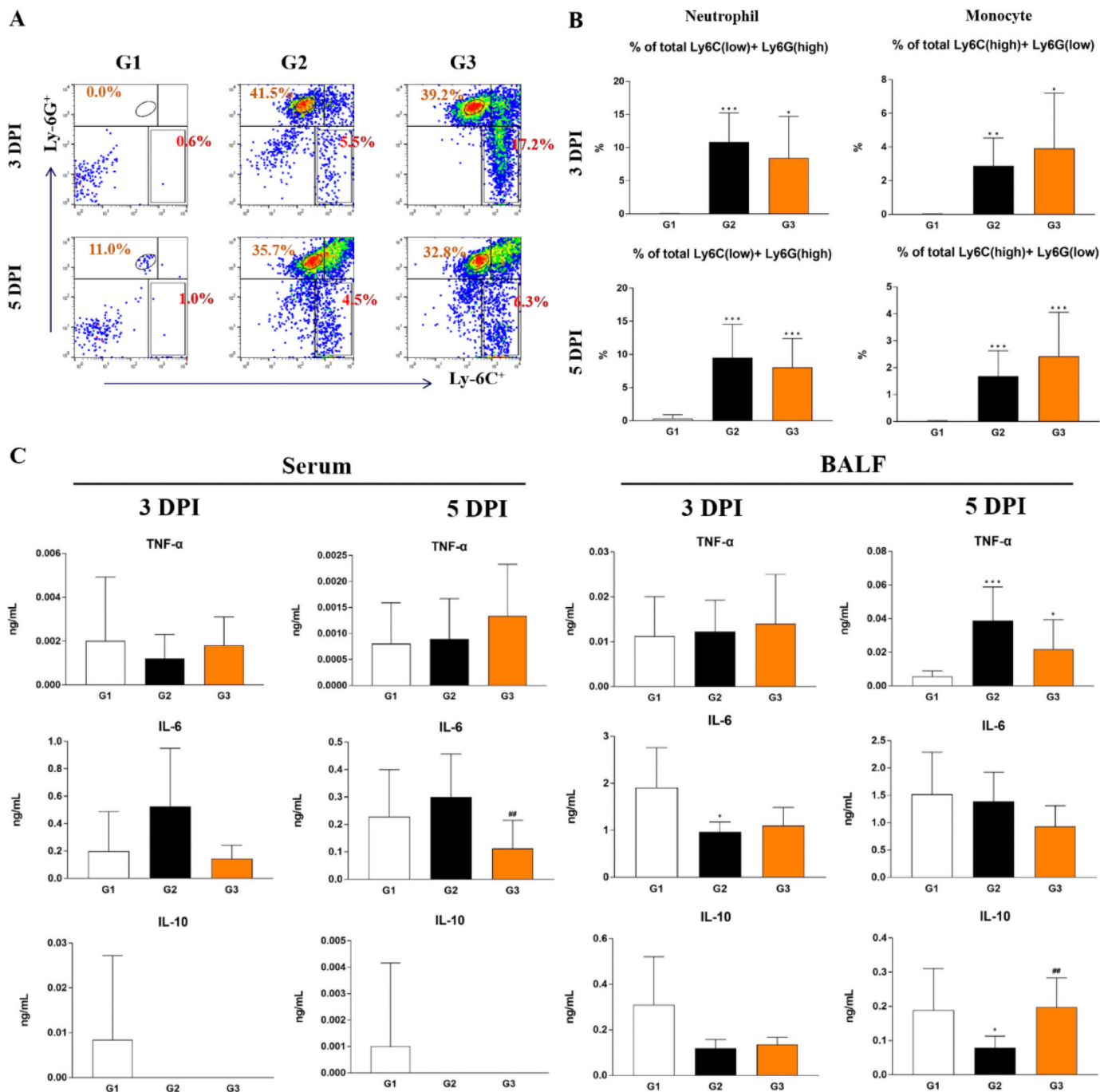


Figure 3. Immune responses in yogurt-treated influenza H1N1-infected mice. (A) Representative dot graphs showing the population of Ly-6G or Ly-6C-stained cells, analyzed using flow cytometry analysis. (B) Neutrophil and monocyte populations of bronchoalveolar lavage fluid (BALF) in influenza H1N1-infected mice at 3 and 5 d post-infection (DPI). (C) TNF- α , IL-6, and IL-10 expressions in both serum and BALF at 3 and 5 DPI in influenza H1N1-infected mice. Data are expressed as mean \pm SD. Unpaired *t*-tests were used to perform comparisons between 2 groups. All statistical tests were 2-sided, and significance was defined as $P < 0.05$. ***, * indicate significant difference at $P < 0.001$ and $P < 0.05$, respectively, compared with group G1; ## indicates significant difference at $P < 0.01$ compared with group G2. G1 = noninfected group; G2 = influenza H1N1-infected group; G3 = yogurt-treated influenza H1N1-infected group.

infiltration using the following scoring system: 0 = no pathological change; 1 = affected area $\leq 10\%$; 2 = affected area $< 50\%$, $> 10\%$; and 3 = affected area

$\geq 50\%$. The histopathological examination showed that the scoring of inflammation in lung tissue was not related between G1 and G2, although the score of

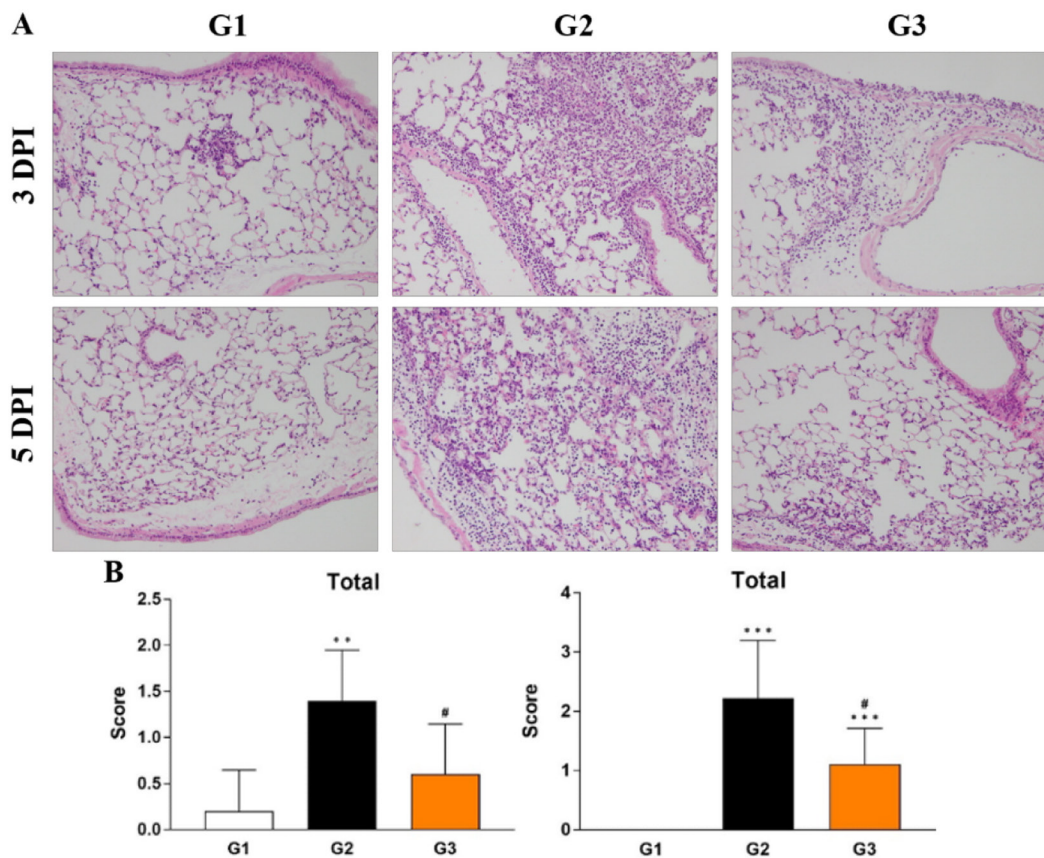


Figure 4. Histopathological findings of lungs from yogurt-treated influenza H1N1-infected mice. (A) Histopathological analysis of the lung was performed using hematoxylin and eosin staining in yogurt-treated influenza H1N1-infected mice. (B) Scores of hematoxylin and eosin-stained slides were determined based on the percentage of inflammatory cell infiltration using the following scoring system: 0 = no pathological change; 1 = affected area $\leq 10\%$; 2 = affected area $< 50\%$, $> 10\%$; and 3 = affected area $\geq 50\%$. Data are expressed as mean \pm SD. Unpaired *t*-tests were used to perform comparisons between 2 groups. All statistical tests were 2-sided, and significance was defined as $P < 0.05$. ***, ** indicate significant difference at $P < 0.001$ and $P < 0.01$, respectively, compared with group G1; # indicates significant difference at $P < 0.05$ compared with group G2. G1 = noninfected group; G2 = influenza H1N1-infected group; G3 = yogurt-treated influenza H1N1-infected group; DPI = d post-infection.

G2 was lower than that of G1 at 3 and 5 DPI (Figure 6A and 6B).

In this study, we performed oral administrations of yogurt to hamsters infected with SARS-CoV-2. The BW of group G2 was significantly higher than that of G1 from the beginning of the experiment, and we confirmed that histological inflammation in G2 was slightly better than G1, although we could not confirm direct effects against SARS-CoV-2 by yogurt according to the results of real-time RT-PCR.

Therefore, although oral administration of yogurt to hamsters infected with SARS-CoV-2 did not directly inhibit the virus, it showed limited improvement effects.

DISCUSSION

This study showed that consumption of yogurt containing probiotics could lead to beneficial effects to

prevent and treat influenza. However, yogurt showed a limited improvement effect for SARS-CoV-2.

Probiotic supplementation has many benefits that improve the ability to respond to external substances and immune responses biochemically, reduce susceptibility to virus infection, and improve the effectiveness of vaccine administration (Bae et al., 2018). Several studies have strongly suggested that probiotics ameliorate respiratory virus infection. It has been reported that dietary consumption of yogurt decreased the duration and severity of symptoms in the elderly (de Vrese et al., 2006). Consumption of dairy products containing the probiotic *Lactobacillus* reduced the symptomatic period induced by respiratory virus infection in the elderly (Guillemard et al., 2010). Based on this research on probiotics, 2 randomized control trials in elderly people vaccinated against influenza reported that daily consumption of dairy drinks containing probiotics improved antibody responses to

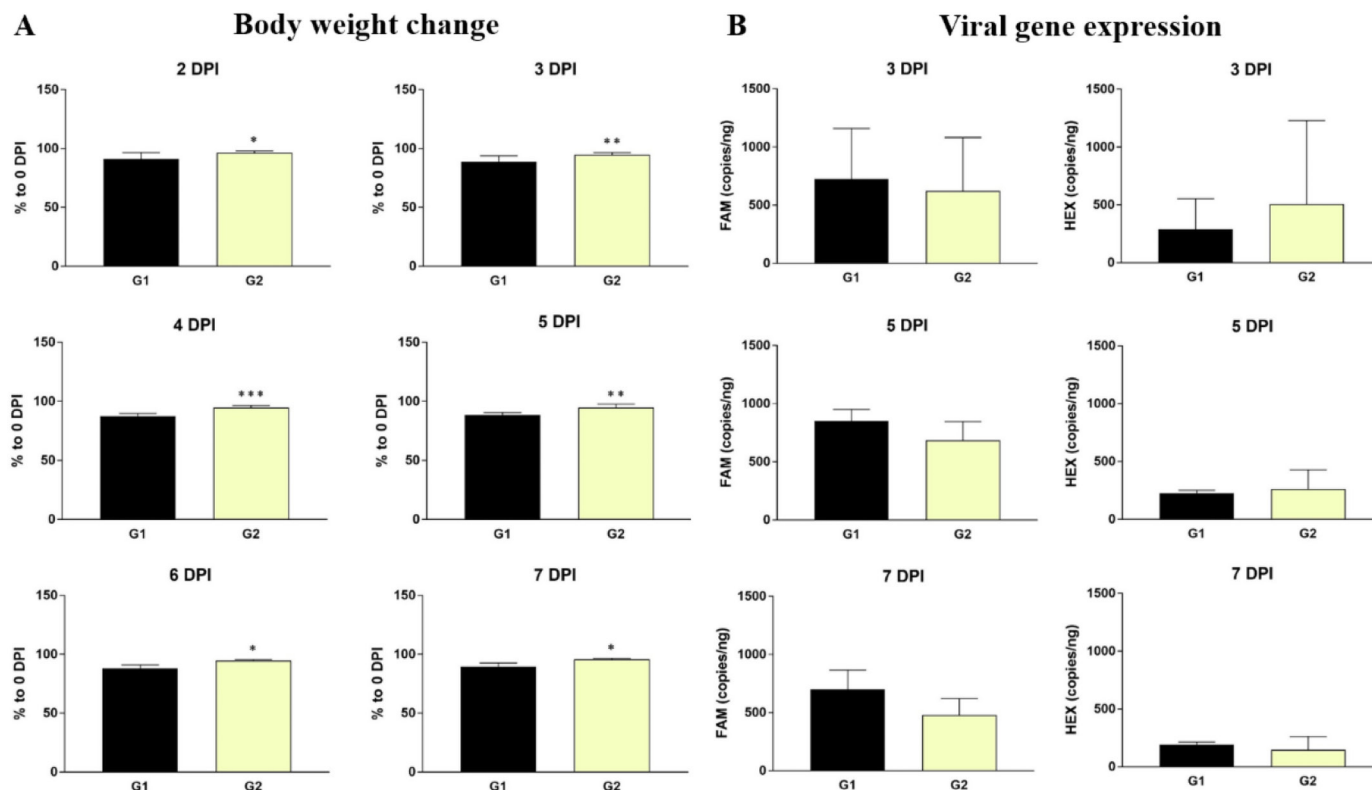


Figure 5. Beneficial effects of yogurt in SARS-CoV-2-infected hamsters. (A) Change in BW of yogurt-treated SARS-CoV-2-infected hamsters over 7 d. (B) Viral gene quantification of SARS-CoV-2: *E* (FAM expression) and *RdRP* (HEX expression) were quantified using real-time reverse-transcription PCR in the lungs from hamsters euthanized at 3 d post-infection (DPI), 5 DPI, and 7 DPI. Data are expressed as mean \pm SD. Unpaired *t*-tests were used to perform comparisons between 2 groups. All statistical tests were 2-sided, and significance was defined as $P < 0.05$. ***, **, and * indicate significant difference at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively, compared with group G1. G1 = SARS-CoV-2-infected group; G2 = yogurt-treated SARS-CoV-2-infected group.

influenza, although a human trial has not explicitly shown that lactic acid bacteria enhance the immune response (Boge et al., 2009).

Probiotics enhance the immune response, and these effects could be one of the potential mechanisms that can explain the above results in pre-clinical tests. Previous studies have suggested that specific antiviral mechanisms are involved in the immune response (Patra et al., 2021; Singh and Rao, 2021). First, the suggested initial mechanisms of probiotics against viral infection include direct probiotic cell interactions with the targeted viruses, production of antiviral metabolites, and modulation of the immune system (Tiwari et al., 2020). The levels of IL-1 β , IL-6, and TNF- α were reduced by suppressing NF- κ B signaling by probiotics. Indeed, our results showed a decrease in TNF- α concentration at 5 DPI in the yogurt-treated group compared with the infected group (Figure 3C). The anti-inflammatory cytokines such as IL-10 enhanced by probiotics have been shown to reduce cytokine storm and inflammation for reduction of hyaluronan synthesis, which could improve acute respiratory disorder syndrome (Patra et

al., 2021). Similarly, the levels of IL-10 were increased in the BALF of the yogurt-treated group at 5 DPI in this study (Figure 3C).

Our test product is a yogurt containing high-dose probiotics such as *S. thermophilus*, *L. acidophilus*, *Bifidobacterium animalis* ssp. *lactis* Bb-12, *Limosilactobacillus fermentum* PL9988 (*bulgaris* Kor91), *Lactiplantibacillus plantarum* SN35N, *L. acidophilus* NCFM, and *B. lactis* Bi-07, which have well-known beneficial effects in humans. A previous study on this yogurt demonstrated a beneficial effect on dental health, related to decreased numbers of oral bacteria and inhibition of the formation of biofilms induced by oral bacteria (Shin et al., 2009). Although there are differences between viruses and bacteria in terms of biology and pathology, we investigated whether consumption of this yogurt inhibited respiratory virus infection based on other studies on probiotics related to viral infection.

To investigate whether yogurt affects both influenza H1N1 and SARS-CoV-2, we first confirmed the results of the in vitro studies on influenza H1N1 and SARS-CoV-2. As shown in Figure 1, plaque formation

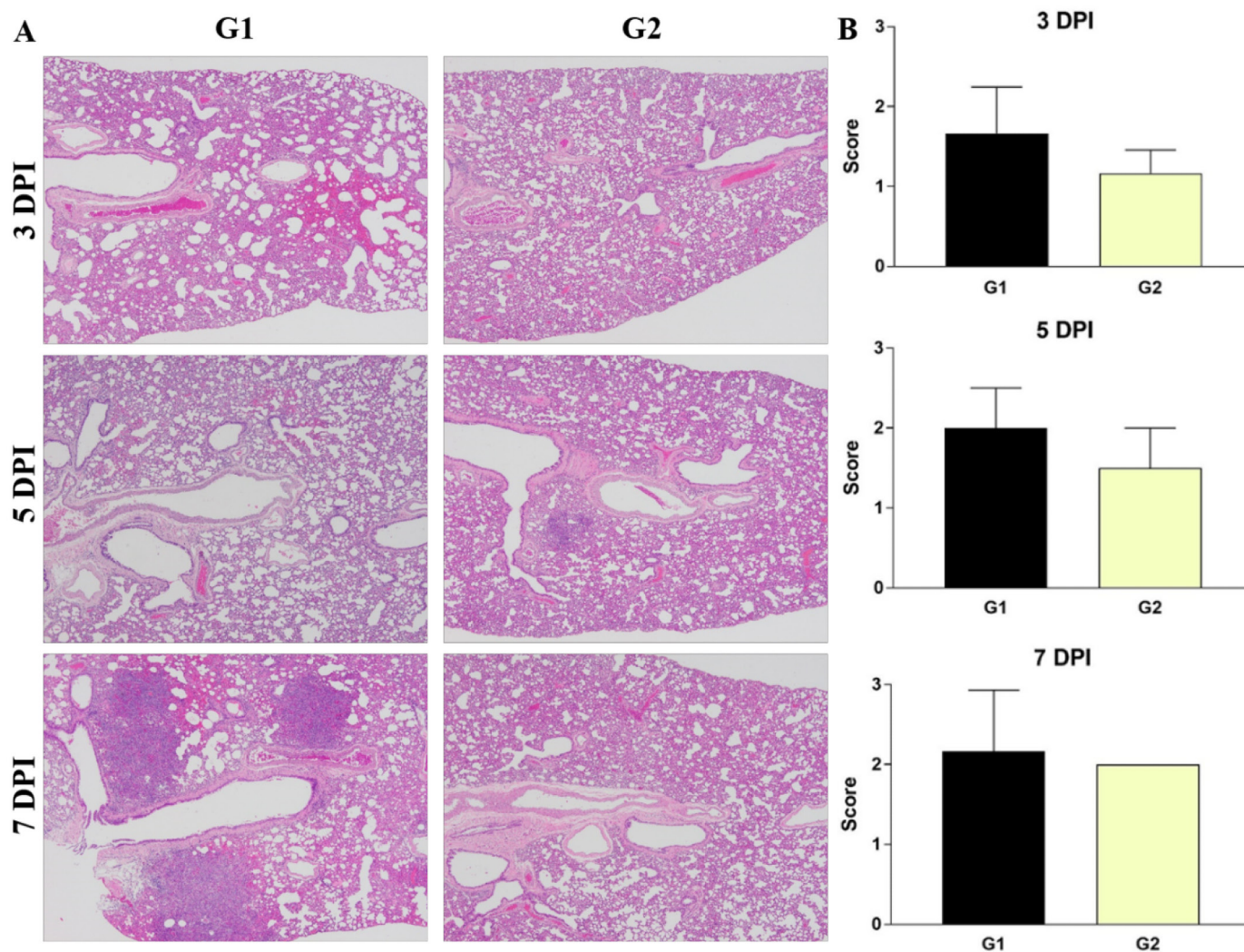


Figure 6. Histopathological findings of the lungs of yogurt-treated SARS-CoV-2-infected hamsters. (A) Histopathological analyses of the lungs were conducted using hematoxylin and eosin staining in yogurt-treated SARS-CoV-2-infected hamsters. (B) Scores of hematoxylin and eosin-stained slides were determined based on percentage of inflammatory cell infiltration using the following scoring system: 0 = no pathological change; 1 = affected area $\leq 10\%$; 2 = affected area $< 50\%$, $> 10\%$; and 3 = affected area $\geq 50\%$. Data are expressed as mean \pm SD. Unpaired *t*-tests used to perform comparisons between 2 groups. All statistical tests were 2-sided, and significance was defined as $P < 0.05$. G1 = SARS-CoV-2-infected group; G2 = yogurt-treated SARS-CoV-2-infected group; DPI = days post-infection.

of influenza H1N1 and SARS-CoV-2 was inhibited by yogurt treatment. Influenza H1N1 in the yogurt-treated group showed no plaque formation compared with the untreated influenza H1N1 group. This result could refer to the reduction of virus titer with probiotics in virus-cultured cells found by previous studies of influenza H1N1 (Bae et al., 2018; Rather et al., 2022). SARS-CoV-2 in the yogurt-treated group showed some plaque formation at 10^{-3} dilution. Previous studies on SARS-CoV-2 with probiotic treatment in virus-cultured cells have showed reduction of virus titer similar to our result (Wang et al., 2020; Islam et al., 2021; Rather et al., 2021; Salaris et al., 2021).

Accordingly, we performed in vivo studies with influenza H1N1 and SARS-CoV-2. Yogurt was administered to the mice 3 wk before the day of infection. Mice were infected with influenza H1N1, and yogurt administration was maintained until the end of the experimental period.

As shown in Figure 2, the yogurt-treated group showed a better survival rate than the virus-infected group, and showed rapid recovery of BW. Clinical scores were lower in the yogurt-treated group than in the virus-infected group. To confirm whether the immune response was induced by yogurt, serum analysis was performed. The IFN- γ and IgG1 levels were increased

3 wk after administration of yogurt. IFN- γ plays an important role as a cytokine involved in viral infection and induction of the Th1 response, and could play a role in preventing viral infection by increasing immune response ability (Julkunen et al., 2001; Mahooti et al., 2019). IgG1 is expected to help increase the resistance to viral infection by supporting the activation of complement and NK cell activation (Mahooti et al., 2019). Therefore, we analyzed neutrophils and monocytes in BALF to confirm the effects of influenza virus infection (Figure 3A and 3B). Unexpectedly, the tendencies of neutrophils and monocytes were not differentiated between the virus-infected group and the yogurt-treated group, whereas the number of monocytes was slightly increased in the yogurt-treated group compared with the virus-infected group. The CD11b⁺Ly-6C⁺ monocyte is an essential cell in opposition to viral infection, and they are differentiated into inflammatory macrophages, which play a role in eliminating viruses, and, in the middle-to-late stages of infection, anti-inflammatory macrophages, which are important immune cells involved in the repair of damaged tissues (Chen et al., 2017; Coates et al., 2018).

Additionally, we performed *in vivo* studies to analyze serum and BALF TNF- α , IL-6, and IL-10 levels. In serum at 5 DPI, the level of IL-6, which is a pro-inflammatory cytokine induced by virus infection (Julkunen et al., 2001; Kaiser et al., 2001), was significantly reduced in the yogurt-treated group compared with the virus-infected group. In BALF at 5 DPI, the level of IL-10, which is an anti-inflammatory cytokine (Kaiser et al., 2001), was significantly increased in the yogurt-treated group compared with the virus-infected group (Figure 3C). These results indicate that yogurt ameliorated inflammation induced by the influenza virus in mice at 5 DPI. It has been reported previously that administration of probiotics is effective for preventing influenza in mice at 5 DPI (Nakayama et al., 2014). The histopathological results support the beneficial effects of yogurt against the influenza H1N1 virus via reduction of inflammatory cell infiltration in lung tissues (Figure 4). These results suggest that the consumption of yogurt has an anti-influenza effect on C57BL/6 mice infected with influenza H1N1.

In this study, SARS-CoV-2-infected hamsters orally administered yogurt were examined. As expected, the BW of yogurt-treated hamsters infected with SARS-CoV-2 were significantly increased compared with the virus-infected group at every time point (Figure 5A). We confirmed viral gene expression and calculated virus copy number in lung tissues in each experimental group. Yogurt generally reduced the levels of the *E* gene at each time point, including at 3, 5, and 7 DPI, although no significant difference was found between

the virus-infected group and the yogurt-treated group. RNA-dependent RNA polymerase (RdRp) is responsible for the viral genome replication and transcription processes, playing a pivotal physiological role in viral invasion and replication of SARS-CoV2 (Ning et al., 2021). Unexpectedly, the levels of the *RdRP* gene were not reduced during the experimental period (Figure 5B). The histopathological analysis showed generally reduced inflammatory scores in the yogurt-treated group compared with the virus-infected group, although no significant relationship was detected between the virus-infected group and the yogurt-treated group (Figure 6A and 6B). These results suggest that yogurt has no direct anti-SARS-CoV-2 effect on hamsters. However, improved BW recovery and reduced infiltration of inflammatory cells in the lung tissue with administration of yogurt can be considered to be of some help in SARS-CoV-2 infection.

Recently, respiratory virus infection, especially with SARS-CoV-2, is becoming a large problem in human health. Although vaccination is the most important form of prevention, the pandemic changes faster than our response. Therefore, it is important to increase immunity, maintain personal hygiene, and eat nutritious food in daily life.

Despite the lack of significant effects of the yogurt tested here on SARS-CoV-2 infection of hamsters, our results suggest that consumption of yogurt containing probiotics could lead to beneficial effects to improve the response to respiratory virus infection. We suggest that yogurt can be used as an integrative therapeutic method with vaccines against external infectious factors, including viruses, contagious pathogens, and their variants, which can lead to epidemics.

ACKNOWLEDGMENTS

The influenza H1N1 *in vitro* study was supported in part by the Korea Research Institute of Bio-Medical Science (Daejeon, Korea). The SARS-CoV-2 *in vitro* study was supported in part by the Laboratory of Veterinary Public Health, College of Veterinary Medicine, Chungnam National University, Daejeon, Korea. The authors have not stated any conflicts of interest.

REFERENCES

- Avolio, M., S. Venturini, R. de Rosa, M. Crapis, and G. Basaglia. 2022. Epidemiology of respiratory virus before and during COVID-19 pandemic. *Infez. Med.* 30:104–108. <https://doi.org/10.53854/liim-3001-12>.
- Bae, J. Y., J. I. Kim, S. Park, K. Yoo, I. H. Kim, W. Joo, B. H. Ryu, M. S. Park, I. Lee, and M. S. Park. 2018. Effects of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* probiotics on human seasonal and avian influenza viruses. *J. Microbiol. Biotechnol.* 28:893–901. <https://doi.org/10.4014/jmb.1804.04001>.

- Boge, T., M. Remigy, S. Vaudaine, J. Tanguy, R. Bourdet-Sicard, and S. van der Werf. 2009. A probiotic fermented dairy drink improves antibody response to influenza vaccination in the elderly in two randomised controlled trials. *Vaccine* 27:5677–5684. <https://doi.org/10.1016/j.vaccine.2009.06.094>.
- Boyd, M., K. Clezy, R. Lindley, and R. Pearce. 2006. Pandemic influenza: Clinical issues. *Med. J. Aust.* 185(Suppl. 10):S44–S47. <https://doi.org/10.5694/j.1326-5377.2006.tb00706.x>.
- Chen, M. F., K. F. Weng, S. Y. Huang, Y. C. Liu, S. N. Tseng, D. M. Ojcius, and S. R. Shih. 2017. Pretreatment with a heat-killed probiotic modulates monocyte chemoattractant protein-1 and reduces the pathogenicity of influenza and enterovirus 71 infections. *Mucosal Immunol.* 10:215–227. <https://doi.org/10.1038/mi.2016.31>.
- Coates, B. M., K. L. Staricha, C. M. Koch, Y. Cheng, D. K. Shumaker, G. R. S. Budinger, H. Perlman, A. V. Misharin, and K. M. Ridge. 2018. Inflammatory monocytes drive influenza A virus-mediated lung injury in juvenile mice. *J. Immunol.* 200:2391–2404. <https://doi.org/10.4049/jimmunol.1701543>.
- Corman, V. M., O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D. K. Chu, T. Bleicker, S. Brunink, J. Schneider, M. L. Schmidt, D. G. Mulders, B. L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J. L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M. P. Koopmans, and C. Drosten. 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill.* 25:2000045. <https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045>.
- de Vrese, M., P. Winkler, P. Rautenberg, T. Harder, C. Noah, C. Laue, S. Ott, J. Hampe, S. Schreiber, K. Heller, and J. Schrezenmeir. 2006. Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. *Vaccine* 24:6670–6674. <https://doi.org/10.1016/j.vaccine.2006.05.048>.
- DeGrandi-Hoffman, G., and Y. Chen. 2015. Nutrition, immunity and viral infections in honey bees. *Curr. Opin. Insect Sci.* 10:170–176. <https://doi.org/10.1016/j.cois.2015.05.007>.
- Elhamzaoui, H., H. Rebahi, and A. Hachimi. 2021. Coronavirus disease 2019 (COVID-19) pathogenesis: A concise narrative review. *Pan Afr. Med. J.* 39:8. <https://doi.org/10.11604/pamj.2021.39.8.23546>.
- Guillemard, E., F. Tondou, F. Lacoïn, and J. Schrezenmeir. 2010. Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial. *Br. J. Nutr.* 103:58–68. <https://doi.org/10.1017/S0007114509991395>.
- Hill, D., I. Sugrue, E. Arendt, C. Hill, C. Stanton, and R. P. Ross. 2017. Recent advances in microbial fermentation for dairy and health. *F1000Res.* 6:751. <https://doi.org/10.12688/f1000research.10896.1>.
- Imai, M., K. Iwatsuki-Horimoto, M. Hatta, S. Loeber, P. J. Halfmann, N. Nakajima, T. Watanabe, M. Ujje, K. Takahashi, M. Ito, S. Yamada, S. Fan, S. Chiba, M. Kuroda, L. Guan, K. Takada, T. Armbrust, A. Balogh, Y. Furusawa, M. Okuda, H. Ueki, A. Yasuhara, Y. Sakai-Tagawa, T. J. S. Lopes, M. Kiso, S. Yamayoshi, N. Kinoshita, N. Ohmagari, S. I. Hattori, M. Takeda, H. Mitsuya, F. Krammer, T. Suzuki, and Y. Kawaoka. 2020. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc. Natl. Acad. Sci. USA* 117:16587–16595. <https://doi.org/10.1073/pnas.2009799117>.
- Islam, M. A., L. Albarracin, M. Tomokiyo, J. C. Valdez, J. Sacur, M. G. Vizoso-Pinto, B. G. N. Andrade, R. R. C. Cuadrat, H. Kitazawa, and J. Villena. 2021. Immunobiotic lactobacilli improve resistance of respiratory epithelial cells to SARS-CoV-2 infection. *Pathogens* 10:1197. <https://doi.org/10.3390/pathogens10091197>.
- Julkunen, I., T. Sareneva, J. Pirhonen, T. Ronni, K. Melen, and S. Matikainen. 2001. Molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression. *Cytokine Growth Factor Rev.* 12:171–180. [https://doi.org/10.1016/S1359-6101\(00\)00026-5](https://doi.org/10.1016/S1359-6101(00)00026-5).
- Kaiser, L., R. S. Fritz, S. E. Straus, L. Gubareva, and F. G. Hayden. 2001. Symptom pathogenesis during acute influenza: Interleukin-6 and other cytokine responses. *J. Med. Virol.* 64:262–268. <https://doi.org/10.1002/jmv.1045>.
- Lynch, J. P. III, and E. E. Walsh. 2007. Influenza: Evolving strategies in treatment and prevention. *Semin. Respir. Crit. Care Med.* 28:144–158. <https://doi.org/10.1055/s-2007-976487>.
- MacDonald, T. T., and I. Bell. 2010. Probiotics and the immune response to vaccines. *Proc. Nutr. Soc.* 69:442–446. <https://doi.org/10.1017/S0029665110001758>.
- Mahooti, M., E. Abdolalipour, A. Salehzadeh, S. R. Mohebbi, A. Gorji, and A. Ghaemi. 2019. Immunomodulatory and prophylactic effects of *Bifidobacterium bifidum* probiotic strain on influenza infection in mice. *World J. Microbiol. Biotechnol.* 35:91. <https://doi.org/10.1007/s11274-019-2667-0>.
- Maragkoudakis, P. A., W. Chingwaru, L. Gradisnik, E. Tsakalidou, and A. Cencic. 2010. Lactic acid bacteria efficiently protect human and animal intestinal epithelial and immune cells from enteric virus infection. *Int. J. Food Microbiol.* 141:S91–S97. <https://doi.org/10.1016/j.ijfoodmicro.2009.12.024>.
- Nakayama, Y., T. Moriya, F. Sakai, N. Ikeda, T. Shiozaki, T. Hosoya, H. Nakagawa, and T. Miyazaki. 2014. Oral administration of *Lactobacillus gasseri* SBT2055 is effective for preventing influenza in mice. *Sci. Rep.* 4:4638. <https://doi.org/10.1038/srep04638>.
- Ning, S., B. Yu, Y. Wang, and F. Wang. 2021. SARS-CoV-2: Origin, evolution, and targeting inhibition. *Front. Cell. Infect. Microbiol.* 11:676451. <https://doi.org/10.3389/fcimb.2021.676451>.
- Patra, S., S. Saxena, N. Sahu, B. Pradhan, and A. Roychowdhury. 2021. Systematic network and meta-analysis on the antiviral mechanisms of probiotics: A preventive and treatment strategy to mitigate SARS-CoV-2 infection. *Probiotics Antimicrob. Proteins* 13:1138–1156. <https://doi.org/10.1007/s12602-021-09748-w>.
- Rahman, M. M., H. S. Kwon, M. J. Kim, H. K. Go, M. H. Oak, and D. H. Kim. 2017. Melatonin supplementation plus exercise behavior ameliorate insulin resistance, hypertension and fatigue in a rat model of type 2 diabetes mellitus. *Biomed. Pharmacother.* 92:606–614. <https://doi.org/10.1016/j.biopha.2017.05.035>.
- Rather, I. A., S. B. Choi, M. R. Kamli, K. R. Hakeem, J. S. M. Sabir, Y. H. Park, and Y. Y. Hor. 2021. Potential adjuvant therapeutic effect of *Lactobacillus plantarum* probio-88 postbiotics against SARS-CoV-2. *Vaccines (Basel)* 9:1067. <https://doi.org/10.3390/vaccines9101067>.
- Rather, I. A., M. R. Kamli, J. S. M. Sabir, and B. A. Paray. 2022. Potential antiviral activity of *Lactiplantibacillus plantarum* KAU007 against influenza virus H1N1. *Vaccines (Basel)* 10:456. <https://doi.org/10.3390/vaccines10030456>.
- Salaris, C., M. Scarpa, M. Elli, A. Bertolini, S. Guglielmetti, F. Pregliasco, P. Brun, and I. Castagliuolo. 2021. *Lactocaseibacillus paracasei* DG enhances the lactoferrin anti-SARS-CoV-2 response in Caco-2 cells. *Gut Microbes* 13:1961970. <https://doi.org/10.1080/19490976.2021.1961970>.
- Shin, H. S., S. M. Kim, N. K. Choi, K. H. Yang, and M. S. Kang. 2009. The effect of fermented milk on viable cell count and biofilm formation of streptococcus mutans. *J. Korean Academy of Pediatric Dentistry* 36:358–366.
- Singh, K., and A. Rao. 2021. Probiotics: A potential immunomodulator in COVID-19 infection management. *Nutr. Res.* 87:1–12. <https://doi.org/10.1016/j.nutres.2020.12.014>.
- Tagliamonte, M. S., C. Mavian, K. Zainabadi, M. N. Cash, J. A. Lednicky, B. R. Magalis, A. Riva, M. M. Deschamps, B. Liautaud, V. Rouzier, D. W. Fitzgerald, J. W. Pape, J. G. Morris, and M. Salemi. 2021. Rapid emergence and spread of SARS-CoV-2 gamma (P.1) variant in Haiti. *Clin. Infect. Dis.* 74:2057–2060. <https://doi.org/10.1093/cid/ciab736>.
- Tiwari, S. K., L. M. T. Dicks, I. V. Popov, A. Karaseva, A. M. Ermakov, A. Suvorov, J. R. Tagg, R. Weeks, and M. L. Chikindas. 2020. Probiotics at war against viruses: What is missing from the picture? *Front. Microbiol.* 11:1877. <https://doi.org/10.3389/fmicb.2020.01877>.
- Van Reeth, K., H. Nauwynck, and M. Pensaert. 1998. Bronchoalveolar interferon- α , tumor necrosis factor- α , interleukin-1, and inflammation during acute influenza in pigs: A possible model for humans? *J. Infect. Dis.* 177:1076–1079. <https://doi.org/10.1086/517398>.

- von Linstow, M. L., M. Høgh, S. A. Nordbo, J. Eugen-Olsen, A. Koch, and B. Høgh. 2008. A community study of clinical traits and risk factors for human metapneumovirus and respiratory syncytial virus infection during the first year of life. *Eur. J. Pediatr.* 167:1125–1133. <https://doi.org/10.1007/s00431-007-0643-4>.
- Wang, M., T. Fu, J. Hao, L. Li, M. Tian, N. Jin, L. Ren, and C. Li. 2020. A recombinant *Lactobacillus plantarum* strain expressing the spike protein of SARS-CoV-2. *Int. J. Biol. Macromol.* 160:736–740. <https://doi.org/10.1016/j.ijbiomac.2020.05.239>.
- WHO. 2022. Weekly Epidemiological and Operational Updates. Accessed Jun. 22, 2022. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>.

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