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Tyndallized *Lactobacillus plantarum* HY7712 Restores Whole-Body γ-Irradiation-Impaired Th Cell Differentiation in Mice

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In the present study, we investigated the effect of tyndallized HY7712 (tHY7712) on the expression of Th cell specific transcription factors and cytokines in whole-body γ-irradiated mice. Oral administration of tHY7712 strongly recovered the γ-irradiation-suppressed expression of helper T (Th) cell- and regulatory T cell-related transcription factors and cytokines, such as T-bet, Foxp3, IFN-γ, TNF-α, and IL-10, and suppressed Th2 cell-associated transcription factor and cytokine GATA3 and IL-5, respectively. Furthermore, compared with the control, tHY7712 treatment also restored γ-irradiation-impaired natural killer and cytotoxic T cell activities against YAC-1 tumor cells to 97.8% and 98.6%, respectively.

Keywords: *Lactobacillus plantarum* HY7712, γ-irradiation, NK cell activity

T lymphocytes derived from hematopoietic progenitors of the bone marrow undergo differentiation in the thymus to cytotoxic (Tc), helper (Th), regulatory (Treg), memory, natural killer (NK), and gamma delta T cells [1, 2]. These differentiation processes are affected by various endogenous and exogenous factors, such as hormones, infection, and radiation [3, 4]. For instance, exposure to γ-irradiation impairs the innate and adaptive immune systems by modulating NK and T cell cytotoxicity and Th cell differentiation [5, 6]. Exposure to γ-irradiation in rodents induces the differentiation of Th cells into Th2 cells rather than Th1 cells [5] and reduces the interferon (IFN)-γ and interleukin (IL)-12 levels in blood, which further stimulate the differentiation of T cells into Th1 cells [8]. Therefore, whole-body γ-irradiation causes Th1/Th2 imbalance, as is observed in aged mice [5, 7, 8].

Lactobacilli, which are the representative Generally Recognized as Safe microorganims, exhibit various biological activities, including prevention of infection and activation of the host's immune system [9, 10]. Their biological effects could be changeable by tyndallization (heat inactivation) [11]. Lactobacilli stimulate the expression of IL-12 and IFN-γ in monocytes and induce the differentiation of Th cells into Th1 subsets [12, 13]. We also found that live *Lactobacillus plantarum* HY7712, which was isolated from kimchi (a traditional Korean fermented vegetable) [14], ameliorated cyclophosphamide-suppressed NK and T cell activities and γ-irradiation-suppressed NK cell activity in mice [14, 15]. However, studies on the effect of live or inactivated lactobacilli on T cell differentiation in γ-irradiation-immunosuppressed mice are limited.

Therefore, in the present study, we examined the effect of tyndallized HY7712 (tHY7712) on adaptive immunity, particularly Th cell differentiation, in mice with whole-body γ-irradiation-induced immunosuppression.

Male C57BL/6 mice (20–23 g, 6 weeks old) were supplied by Samtako Co., Ltd. (Korea). All mice were maintained under controlled conditions of light (12/12 h light/dark cycle), temperature (25 ± 2°C), and humidity (50 ± 10%). Mice were permitted ad libitum access to diet and water. The animal experiment was approved by the Committee for the Care and Use of Laboratory Animals and performed

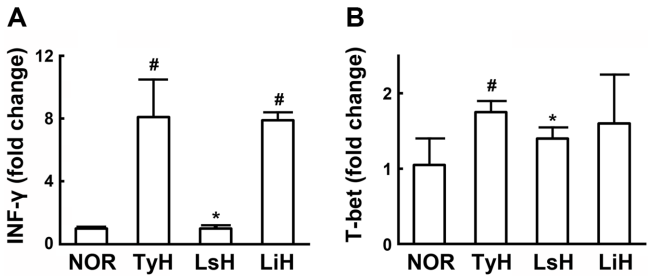

Fig. 1. Effect of *Lactobacillus plantarum* HY7712 on the differentiation of splenic T cells into Th1 cells.

(**A**) Effect on IFN-γ expression. (**B**) Effect on T-bet expression. HY7712 (TyH, tyndallized HY7712 (1×10^5 CFU/ml); LsH, lysozyme-treated HY7712 (1×10^5 CFU); LiH, live HY7712 (1×10^5 CFU/ml)) was treated to splenocytes (5×10^5 cells/ml). Lysozyme (1 mg/ml) with HY7712 (1×10^6 cells/ml) was treated for 4 h. Data indicate the mean ± SD (4–5). #Significantly different vs. normal control group ($p < 0.05$). *Significantly different vs. TyH-treated group ($p < 0.05$).

in accordance with the Kyung Hee University Guideline for Laboratory Animals Care and Usage (IRB No. KHPASP(SE)-15-072).

Exposure to γ-irradiation in mice was performed according to the modified method of Lee *et al.* [14]. Mice were divided into three groups; normal control group treated with vehicle (1% dextrose) alone, γ-irradiation-alone-treated group with tHY7712 (1×10^9 CFU/mouse/day) administration, and γ-irradiated group without tHY7712. Each group consisted of eight mice. A γ-ray generator (IBL 147 C; CIS Bio-International, France) was used for generating irradiation (^{137}Cs, 0.8 Gy/min). After 2 h of γ-irradiation (3 Gy) the probiotic or the vehicle was orally administered for 5 days from 2 h after treatment with γ-irradiation (3 Gy). Mice with or without γ-irradiation were sacrificed 3 days after the final administration of test agents. Blood samples were collected from the inferior vena cava of the mice. Expression

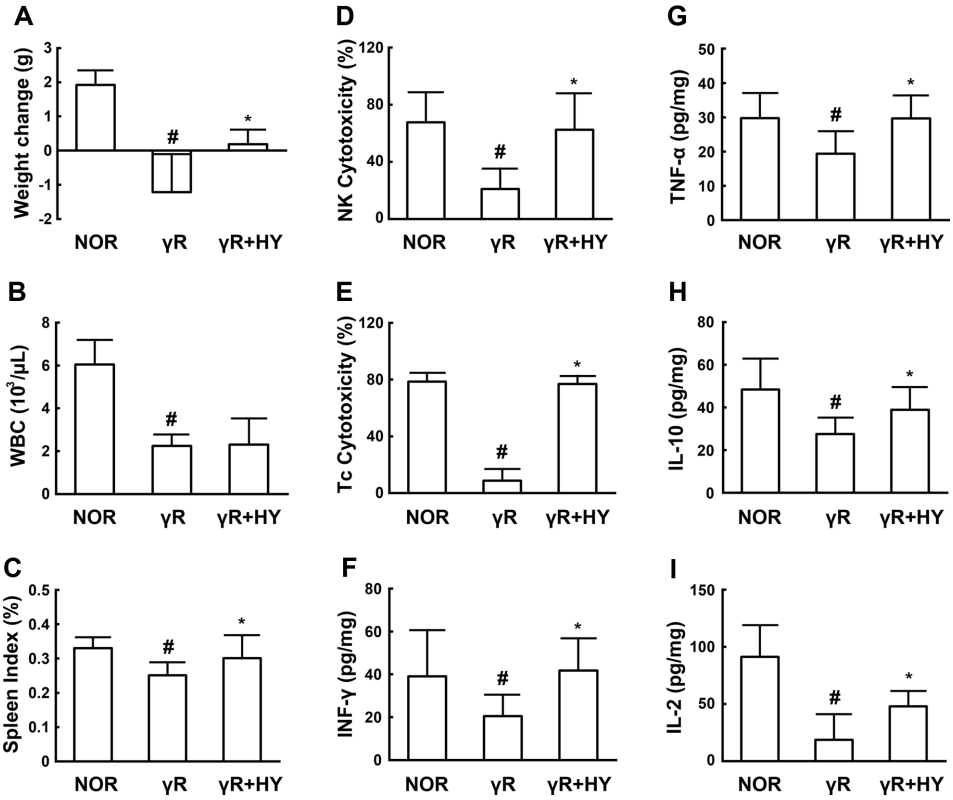

Fig. 2. Effect of *L. plantarum* HY7712 on the body weight (**A**), number of white blood cells (**B**), spleen index (**C**; spleen weight/body weight), cytotoxicities of NK (**D**) and Tc cells (**E**) against YAC-1 cells, and levels of blood cytokines IFN-γ (**F**), TNF-α (**G**), IL-10 (**H**), and IL-2 (**I**) in mice with γ-irradiated immunosuppression (γR).

L. plantarum HY7712 (HY; 1×10^9 CFU/day) suspended in 1% dextrose was orally administered once a day for 5 weeks. Normal control group (NOR) was treated with vehicle alone. Cytokines were assayed using commercial ELISA kits. Cytotoxicities of NK and Tc cells against YAC-1 cells at the ratio of the effector:target (= 50:1) was measured. Data indicate the mean ± SD ($n = 8$). #Significantly different vs. normal control group ($p < 0.05$). *Significantly different vs. γ-irradiation-treated group ($p < 0.05$).

levels of cytokines and transcription factors were analzyed by enzyme-linked immunosorbent assay (ELISA) and quantitative real time polymerase chain reaction (qPCR), respectively. NK and cytotoxic T cell activities were measured by the method of Jang *et al.* [15].

Data were analyzed by one-way analysis of variance followed by the Newman-Keuls test for multiple comparisons, and the results are represented as the mean ± standard deviation (SD).

To investigate the effect of tHY7712 on modulation of Th cell differentiation, splenocytes were isolated from mice and cultured with tHY7712 or live HY7712 for 5 days. Live HY7712 was prepared according to the method of Lee *et al.* [14] and tHY7712 by heat treatment for 1 h at 95°C on three consecutive days. The expression levels of Th1 transcription factor T-bet and its cytokine (IFN-γ) were analyzed by qPCR (Fig. 1). Treatment with tHY7712 and live HY7712 significantly induced T-bet and IFN-γ expression. However, treatment with lysozyme-treated tHY7712 abolished the increase in the expression of T-bet and IFN-γ. Next, we investigated the effect of tHY7712 in mice with γ-irradiation-induced immunosuppression. Exposure to γ-irradiation significantly reduced the body weight, number of white blood cells, and spleen index but did not affect the number of red blood cells (Fig. 2). Results of ELISA showed that γ-irradiation reduced the levels of IFN-γ, TNF-α, IL-2, and IL-10 in blood. However, treatment with tHY7712 significantly increased blood IFN-γ, TNF-α, IL-2, and IL-10 levels. Furthermore, analysis with qPCR showed that exposure to γ-irradiation decreased the expression of Th and Treg cell-related transcription factors and cytokines such as T-bet, Foxp3, RORγt, IFN-γ, TNF-α, IL-10, and IL17, but increased the expression of the Th2 cell-associated transcription factor and cytokine (GATA3 and IL-5, respectively) in the spleen (Fig. 3). However, treatment with tHY7712 increased γ-irradiation-suppressed expression of T-bet, Foxp3, TNF-α, IL-10, and IFN-γ, and decreased GATA3 and IL-5 expression. In contrast, expression of IL-17 and RORγt was not affected by tHY7712 treatment.

In agreement with previous reports [15], this study also

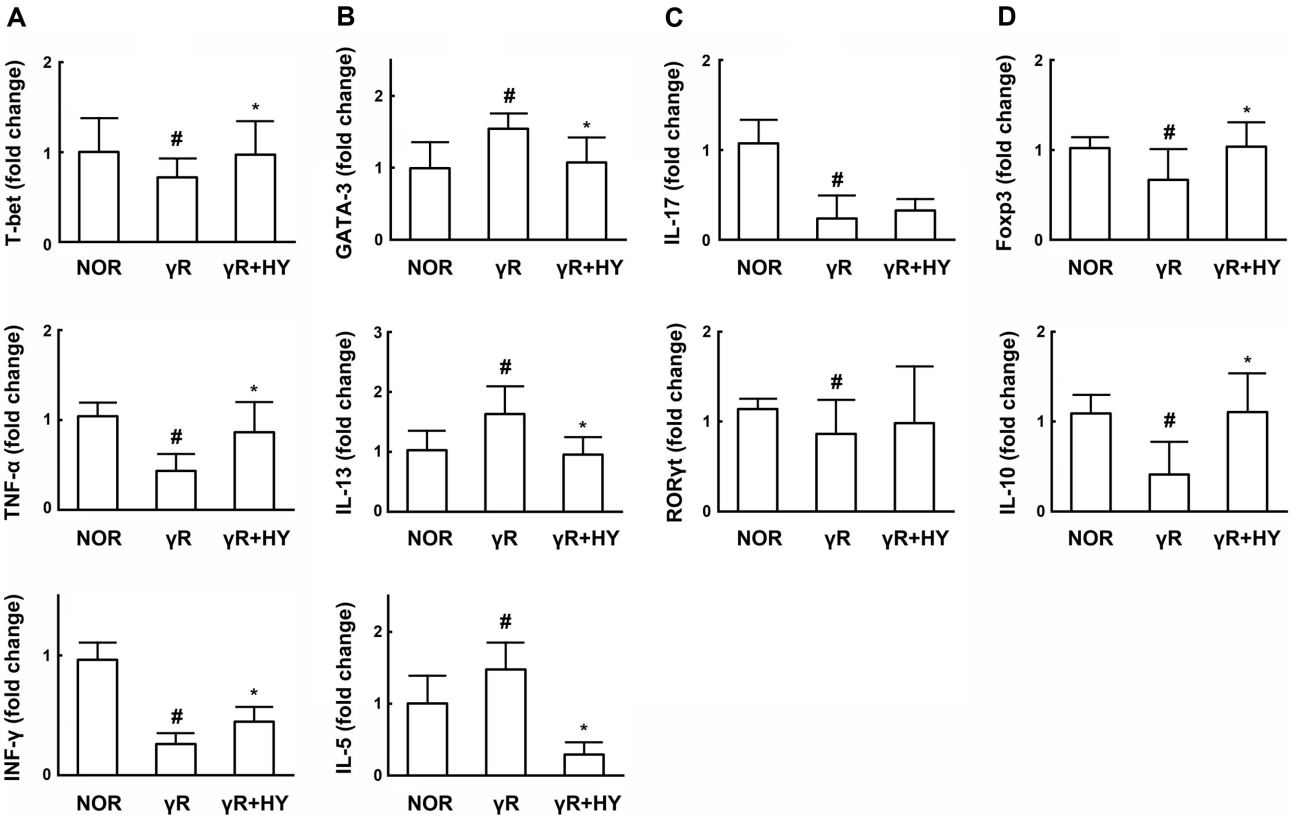

Fig. 3. Effect of *L. plantarum* HY7712 on Th cell differentiation in mice with or without γ-irradiated immunosuppression.
(**A**) Effect on T-bet, TNF-α, and IFN-γ expression. (**B**) Effect on GATA3, IL-13, and IL-5 expression. (**C**) Effect on RORγt and IL-17 expression. (**D**) Effect on Foxp3 and IL-10 expression. Transcription factors and cytokines were assessed by qPCR. Data indicate the mean ± SD (n = 8). #Significantly different vs. normal control group ($p < 0.05$). *Significantly different vs. γ-irradiation-treated group ($p < 0.05$).

confirmed that exposure to γ-irradiation reduced the cytotoxic effects of NK and Tc cells against YAC-1 tumor cells. However, oral administration of tHY7712 increased the cytotoxic effects of NK and Tc cells to 97.8% and 98.6%, respectively, in γ-irradiated mice compared with the control mice.

Whole-body exposure to γ-irradiation impairs the innate and adaptive immunity and thus facilitates increase in the occurrence of infection, tumors, and hypersensitivity [5, 16]. For example, exposure to γ-irradiation suppresses the differentiation of Th1 and Treg cells and increases the differentiation of Th2, resulting in Th1/Th2/Treg immune imbalance [16]. Lactobacilli alleviate γ-irradiation-induced immune diseases. In a previous study, we found that pretreatment with live HY7712 prevented the impairment of NK cell cytotoxicity against YAC-1 tumor cells and the downregulation of IFN-γ induced by γ-irradiation or cyclophosphamide [14, 15]. In the present study, we observed that the cytotoxic activities of NK and Tc cells in the tHY7712-treated groups were comparable to those of the live HY7712-treated groups. These results suggest that the immunopotentiating effect of HY7712 with respect to cytotoxic activity of NK cells against tumor cells was not affected by tyndallization. Furthermore, we found that treatment with tHY7712 recovered the γ-irradiation-impaired expression of Th1 and Treg cell-associated cytokines, such as TNF-α, IFN-γ, and IL-10, and suppressed the γ-irradiation-induced IL-5 and IL-13 expression.

These findings suggest that tHY7712 can restore the impaired cytotoxic activity of NK and T cells and differentiation of Th1 and Treg cells caused by exposure to γ-irradiation.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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