

Preliminary Human Study for Possible Alteration of Serum Immunoglobulin E Production in Perennial Allergic Rhinitis with Fermented Milk Prepared with *Lactobacillus gasseri* TMC0356

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Abstract: The fermented milk prepared with *Lactobacillus gasseri* TMC0356 was administered at 200 ml per day for 4 weeks to 15 subjects with high serum IgE levels and perennial allergic rhinitis. The serum total IgE concentration was significantly reduced after 28 days' exposure to the fermented milk ($P<0.05$) compared to that before the intervention. The serum IgE specific to Acari and those to Japanese cedar pollen also significantly declined ($P<0.05$). T helper 1 (Th1) cells in the composition of their peripheral blood mononuclear cells (PBMCs) significantly increased after 14 days ($P<0.01$) and after 28 days ($P<0.05$). These results suggest that the fermented milk prepared with *L. gasseri* TMC0356 may alter serum IgE concentration, at least partly by enhancement of Th1 immune responses of the subjects with high concentration of serum IgE. However, further studies are still necessary to know the underlying mechanisms by which the tested fermented milk could influence the host immunity.

Key words: Fermented milk *Lactobacillus gasseri* TMC0356, Serum IgE, Th1/Th2 balance, Allergic rhinitis

The prevalence of allergic disease characterized by enhanced immunoglobulin E (IgE) responses to common environmental antigens is increasing worldwide especially in Western industrialized countries (1, 19, 32). Accruing data on the cross-talk between the endogenous bacteria and the gut-associated lymphoid tissue (GALT) suggest that the intestinal microbiota may play a critical role in the homeostasis of the human immune function, and the intestinal microbiota can be considered as one of the prospective targets in the management of allergic disorders (2, 3, 10, 16). Special probiotics offer a safe and practical means of modulating the composition and activity of the human intestinal microflora (28, 29).

Probiotics have been defined as microbial cell preparations or components of microbial cells which have a beneficial effect on the health and well being of the host (5, 30). Most current probiotics are lactic acid bacteria

and bifidobacteria, which often possess adhesive properties and the ability to at least transiently colonize the human gastrointestinal tract (28, 29). The mechanisms underlying these documented clinical effects include both immunoregulatory and microbial activities exhibited by these bacteria while they persist in human intestinal epithelium (28).

In the present study, the fermented milk prepared with *Lactobacillus gasseri* TMC0356 was administered to 15 subjects with high serum IgE levels and allergic symptoms. The changes in the serum IgE of the subjects and the population of T helper 1 cell (Th1) and Th2 cells of their peripheral blood mononuclear cells (PBMCs) were evaluated after the intervention of the tested fermented milk.

In the preparation of the tested fermented milk, pasteurized milk base (8.0% SNF, 3.0% milk fat, 5.0% sucrose, 1% glucose and 1% yeast extract) was inoculated with *L. gasseri* TMC0356 and incubated at 37 C

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Abbreviations: CFU, colony-forming units; IFN, interferon; Ig, immunoglobulin; IL, interleukin; Th1, T helper 1 cell; Th2, T helper 2 cell.

for 18 hr. The pH, acidity and bacterial counts were 4.60, 0.8% and 4.3×10^8 CFU/ml.

The study protocol was in accordance with the Declaration of Helsinki, and it was approved by the Ethics Committee of Koyei, Inc. A total of 45 subjects were screened by medical examination of the nasal cavity and blood clinical chemistry at the Koyei Clinic (Osaka, Japan). After screening, 15 subjects (males=7, females=8; aged from 17 to 56 years old) with perennial allergic rhinitis and other allergic symptoms and a high concentration of non-specific IgE were selected for the present study. None of them were allergic to milk, and none had received specific immunotherapy. The subjects were orally administered with the tested fermented milk at 200 ml per day for 4 weeks. Blood samples collected were used to determine the concentration of total-IgE, anti-Japanese cedar IgE, anti-Acari IgE, anti-house dust IgE and anti-fungi IgE with Fluorescence Enzyme Immunoassay (FEIA) at Mitsubishi Kagaku Bio-Chemical Laboratories, Inc., Tokyo. The numbers of Th1 and Th2 cells (Th1/Th2 ratio) were evaluated by analyzing IFN- γ ×IL-4/CD4 of PBMCs isolated from blood samples at Osaka Kessei Research Laboratories, Inc., Osaka, Japan (14, 15). The results were expressed as mean values with standard error. Statistical analysis was conducted by Dunnett of two-way ANOVA using software of JMP4J (JMP Sales, N.C., U.S.A.).

The serum IgE concentration of the subjects 2 and 4 weeks after the oral administration of the tested fermented milk indicated changes. The serum total IgE concentrations in the subjects were significantly reduced after 28 days' exposure ($P<0.05$) (Fig. 1). The serum IgE specific to Acari and those to Japanese cedar pollen also significantly declined after 28 days ($P<0.05$) (Figs. 2 and 3). Furthermore, the tested fermented milk also decreased the serum IgE specific to house dust and fungi after 28 days but these changes were not significant statistically. After 28 days of exposure to the tested fermented milk, Th1 cells in PBMCs of the subjects significantly increased ($P<0.01$) (Fig. 4). Th-1 cells in the composition of PBMCs increased significantly from 7.84 ± 2.81 before administration of the test strain to 9.52 ± 4.06 after 14 days ($P<0.01$), and 11.0 ± 4.5 after 28 days ($P<0.05$) (Fig. 5). No clinical problems have been observed in the medical examination during the intervention of the tested fermented milk.

IgE is the largest immunoglobulin monomer with four CH domains and is present in the serum of healthy individuals at extremely low levels (1). However, the IgE level rises in response to parasitic infection and in individuals who are atopic or patients with immediate

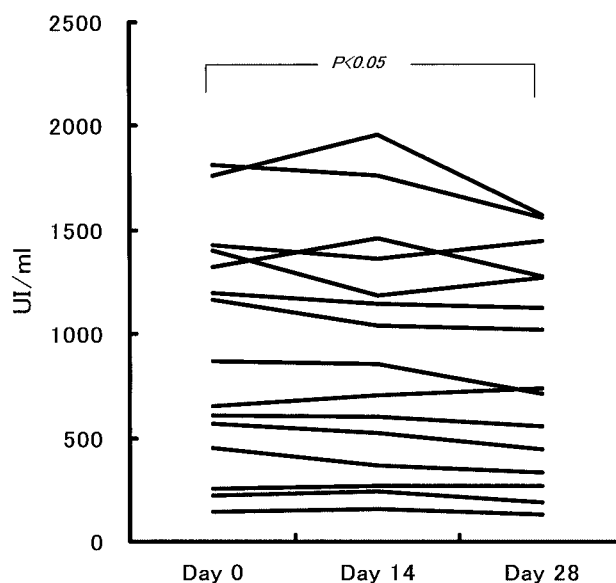


Fig. 1. Serum total IgE of the subjects before and after administration of the fermented milk prepared with *L. gasseri* TMC0356.

hypersensitivity in response to allergens. Enhanced IgE expression is probably a consequence of the Th2 type skewing immune responses (4, 15). Recently, several studies have suggested that lactic acid bacteria may act as prospective dietary immunoregulators. Some probiotic strains, such as *L. casei* Shirota and *L. plantarum* L-137, *L. paracasei* KW3110 appear to activate macrophages to secrete more pro-inflammatory cytokine IL-12 which can favor Th1 type immunity (6, 18, 24, 33). Furthermore, these probiotic strains have been found to down-regulate antigen-induced serum IgE production in mice (18, 21, 25). This would suggest the possibility of using probiotic bacteria or related food such as fermented milk in the management of allergic disorders by altering the over-expression of IgE implicated in the pathogenesis of allergic diseases. However, no direct evidence from human studies is available to support this hypothesis as the probiotic and the food supplemented with probiotics strain did not exhibit significant inhibitory effect to serum IgE concentration in the studies reported, although some subjective parameters improved following the treatment using some of probiotic strains and probiotic contained food (7, 13, 14).

L. gasseri TMC0356 was originally isolated from healthy adult intestine (original isolate No. 5B61) (11). In our previous studies, this bacterium characteristically adhered to intestinal epithelium and mucosal surface,

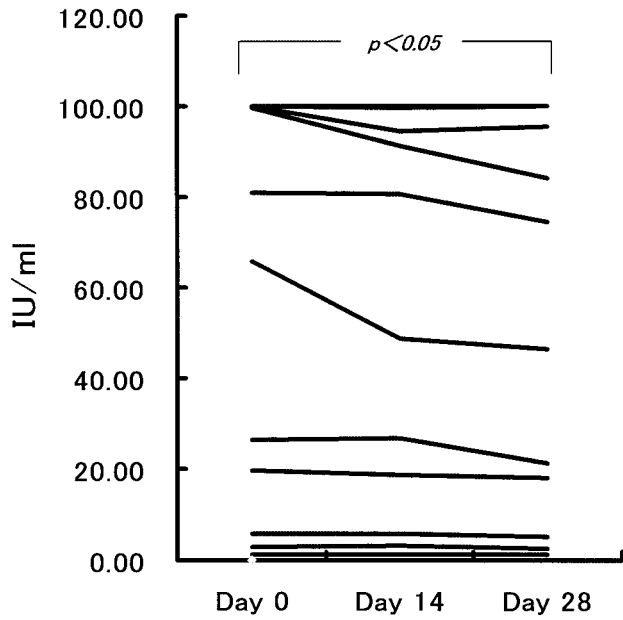


Fig. 2. Serum IgE specific to Acari of the subjects before and after administration of the fermented milk prepared with *L. gasseri* TMC0356.

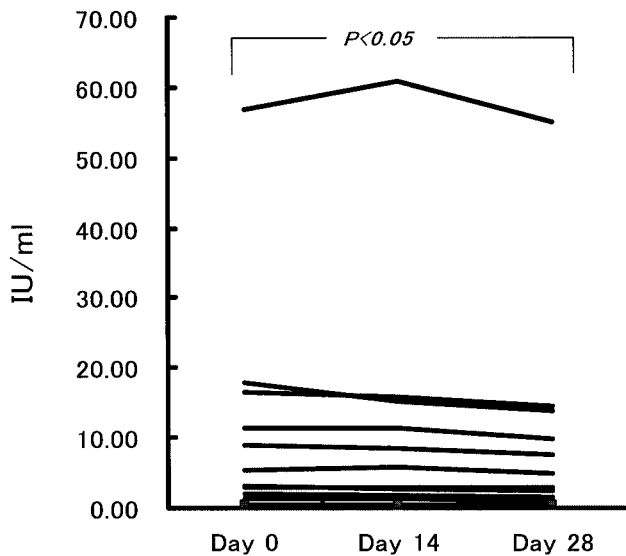


Fig. 3. Serum IgE specific to Japanese cedar of the subjects before and after administration of the fermented milk prepared with *L. gasseri* TMC0356.

and activated the macrophage to secrete more cytokine and show enhanced phagocytes compared to other lactobacilli (8, 22, 23, personal communication with Dr. Manabe). Furthermore, this bacterium effectively inhibited the antigen-augmented serum IgE in the tested BALB/c mice immunized intraperitoneally with the food antigen ovalbumin (OVA) (data not shown). In

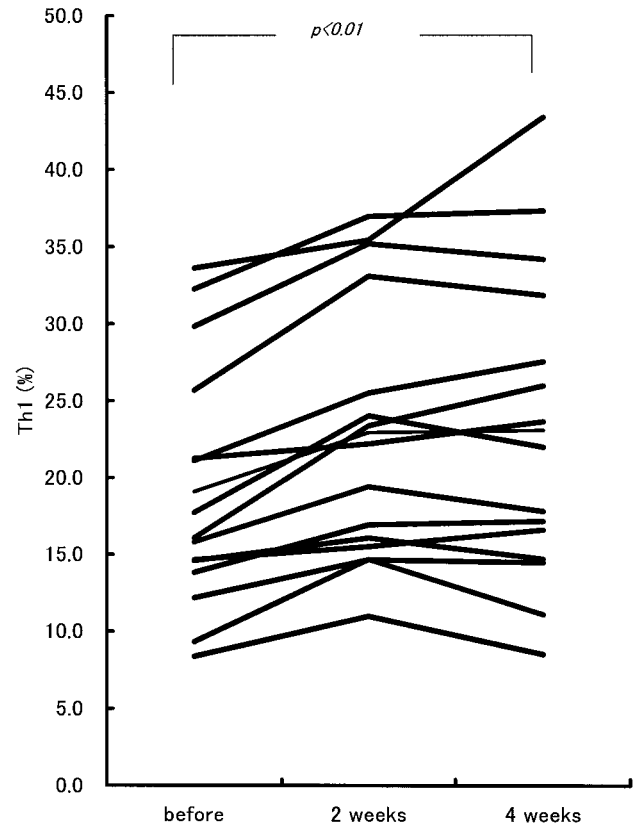


Fig. 4. Th1 cells in the PBMCs of the subjects before and after administration of the fermented milk prepared with *L. gasseri* TMC0356.

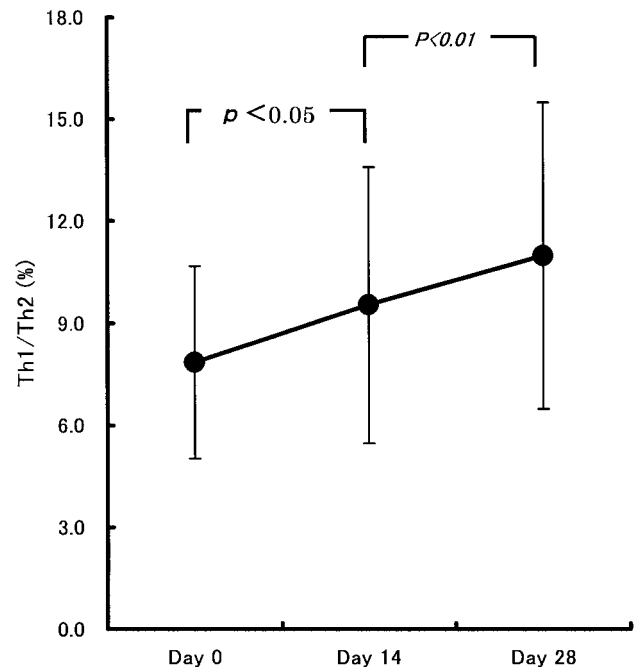


Fig. 5. Th1/Th2 in PBMCs of the subjects before and after administration of the fermented milk prepared with *L. gasseri* TMC0356.

light of these studies, we hypothesize that this bacterium supplemented food may influence the serum IgE of those with allergic rhinitis.

In the present study, the fermented milk prepared with *L. gasseri* TMC0356 significantly reduced the serum total and antigen-specific IgE concentration of the tested subjects after intervention. To our knowledge, this is the first evidence obtained from a human study indicating that probiotics supplemented fermented milk may down-regulate serum IgE of allergic disorders. However, the present study is still a preliminary human study and larger scale double-blind and placebo-controlled human studies should be conducted to confirm the prospective effects of the fermented milk prepared with *L. gasseri* TMC0356 to serum IgE and allergic symptoms.

In the present study, Th1 cells in the composition of PBMCs in the subjects were increased significantly after administration of *L. gasseri* TMC0356 prepared fermented milk. Consequently, the Th1/Th2 balance was enhanced to Th1 skewed direction in subjects receiving *L. gasseri* TMC0356. These results indicate that the alteration of serum IgE in the allergic rhinitis by *L. gasseri* TMC0356 may be, at least partly, related to the effects of this bacterium to enhance the Th1 immune responses by increasing Th1 cells in PBMCs. Unmethylated CpG motifs frequently found in the bacteria have recently been shown to stimulate the innate immune responses through interaction with the toll-like receptor on the surfaces of leukocytes, which may alleviate the IgE-mediated allergic disorders. Recently, the chromosomal DNA from *L. gasseri* TMC0356 was found to have strong immunostimulation in murine cells (12). These suggest that the enhancement of Th1 immunity in the subjects after exposure to *L. gasseri* TMC0356 may result from the activities of the chromosomal DNA of this bacterium, at least partly. Furthermore, other components originally present in the milk such as various milk proteins, peptides and the metabolites which are produced in the fermentation process can also work as potent boosts to the host human immunity (34, 35). Therefore, the alteration of serum IgE in allergic disorders by the tested fermented milk may result from the combination of the activities of bacteria and components and metabolites present in the fermented milk. Further studies should be done to evaluate and differentiate the role of each component of the fermented milk supplemented with *L. gasseri* TMC0356 in the alteration of immune responses, which can enhance knowledge about the underlying mechanisms by which probiotic affects the immunity of the host.

Lactobacillus GG is widely used in the dairy industry

as a probiotic bacterium with well documented health promoting benefits (31). Majamaa and Isolauri (20) demonstrated that the clinical symptoms of atopic eczema could be improved in nursing infants whose mothers were given *Lactobacillus* GG and in milk-hypersensitive infants who consumed a hydrolyzed whey formula supplemented with *Lactobacillus* GG. In a 4-year follow up clinical study, this probiotic bacterium effectively protected infants with predisposition for allergic diseases (16, 17). In these studies, 23% of the children who received probiotic perinatally for 6 months, compared with 46% receiving placebo, suffered from atopic dermatitis by the age of 2. At the age of 4, cumulative prevalence of atopic dermatitis was 26% in the probiotic group compared with 46% in the placebo group. An elevation of the anti-inflammatory cytokine, IL-10 and TGF- β were found as one of the important events relating such effects of *Lactobacillus* GG to atopic dermatitis and food allergy but no significant changes were found in serum IgE (16, 26, 27, 29). *Lactobacillus* GG also did not modify the antigens-augmented serum IgE in an animal study (9). In the present study, the fermented milk supplemented with *L. gasseri* TMC0356 altered the serum IgE concentration and enhanced Th1 immune response of the host, which are different from the immune responses of the host after exposure to *Lactobacillus* GG. These results suggest that the immunomodulatory effects of the probiotic contained fermented milk are individual and should be carefully evaluated.

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