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ARTICLE

The Antiallergic Effects and Acute Toxicity of *Lactobacillus crispatus* KT-11 Cultured in Food Grade Medium

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We synthesized a medium consisting of commercial food supplements (food grade medium) that could be used to cultivate *Lactobacillus crispatus* KT-11 (KT-11), and investigated the antiallergic effects and acute toxicity of KT-11 cultured in this medium. We found that the growth of KT-11 in the food grade medium was comparable to that in DeMan−Rogosa−Sharpe (MRS) medium. Sneezing event was reduced in ovalbumin (OVA)-sensitized BALB/c mice given a diet supplemented with KT-11 grown in the food grade medium (FG-KT-11 group) when compared to mice given a diet supplemented with KT-11 grown in MRS medium (MRS-KT-11 group). The number of $CD80^+CD11b^+$ Peyer's patch cells was significantly lower in the FG-KT-11 group than in the MRS-KT-11 group, while $IL\text{-}12^+CD11b^+$ Peyer's patch cells were higher in the FG-KT-11 group. Only minimal acute toxicity was observed in ICR mice given 1000 or 2000 mg of FG-KT-11/kg body weight. These results suggest that FG-KT-11 represents a safe antiallergic food material.

KEYWORDS: Antiallergic effects; food grade medium; food safety study; *Lactobacillus crispatus* KT-11; Peyer's patch

INTRODUCTION

Type I allergic diseases including atopic dermatitis, allergic asthma and allergic rhinitis are generally characterized by elevated serum immunoglobulin (Ig) E levels (*1*). Production of IgE is thought to be due to a skewed T helper type 1 (Th1)/T helper type 2 (Th2) cell balance (*2*).

In a previous paper, we demonstrated that *Lactobacillus crispatus* KT-11 (KT-11) cultured in DeMan−Rogosa−Sharpe (MRS) medium (*3*) reduced allergic symptoms via a decrease in antigen-specific IgE levels in NC/Nga mice, and that the reduction was due to adjustments in the Th1/Th2 balance via Toll-like receptor (TLR)2 and nucleotide-binding oligomerization domain (NOD) 1 and NOD2 (*4*). In a following paper, we revealed that KT-11 suppressed the acquired allergic response not only via the adjustment of Th1/Th2 balance but also through a decrease in spleen mast cell and antigen-presenting cell numbers in ovalbumin (OVA)-sensitized BALB/c mice (*5*). However, KT-11 cultured in MRS medium cannot be used for food materials, as components including polyoxyethylene sorbitan monooleate (Tween 80) and ammonium citrate dibasic are not permitted food supplements. Therefore, the development of a medium consisting of food supplements alone is required if KT-11 is to be used as an antiallergic food.

We have devised a novel medium (food grade medium) consisting of commercial food supplements such as Berlex 60, glucose and Sunsoft Q-17S. In this study, we report the growth properties of KT-11 cultured in the food grade medium, the antiallergic effects of KT-11 grown in the food grade medium (FG-KT-11) and the acute toxicity of FG-KT-11.

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MATERIALS AND METHODS

Materials. Phycoerythrin (PE)-labeled antimouse interleukin (IL)-12p40 monoclonal antibody (clone C15.6) was purchased from BioLegend (San Diego, CA). OVA and Tween 80 were purchased from Wako Pure Chemical Industries (Osaka, Japan). Aluminum hydroxide gel was obtained from Sigma-Aldrich (St. Louis, MO). All chemicals used in this study were of the highest analytical grade commercially available.

Food Grade Medium. Berlex 60 (Barley Fermentation Technologies, Oita, Japan), a commercial food medium for microorganisms, was used for the KT-11 cultures. Food grade medium was prepared from 13.33% Berlex 60 water solution containing 2% glucose and 0.1% Sunsoft Q-17S (Taiyo Kagaku, Tokyo, Japan), adjusted to pH 6.4 with caustic soda for food additives (Asahi Glass, Tokyo, Japan) and sterilized at 121 °C for 15 min.

Growth of KT-11. KT-11 was obtained as a stock culture from Kitii (Tokyo, Japan), inoculated into MRS medium, food grade medium or Sunsoft Q-17S-free food grade medium and grown at 37 °C. The degree of cloudiness was then read at 660 nm on a spectrophotometer (Ultrospec 3300 pro. Amersham Biosciences, Uppsala, Sweden), and shown as growth of microorganisms as reported previously (*6*).

Heat Treatment of KT-11. KT-11 was inoculated into MRS medium or food grade medium and cultured for 24 h at 37 °C. The cells were then collected by centrifugation, washed with sterile water, heat-treated at 65 °C for 30 min and lyophilized. KT-11 grown in MRS medium was termed MRS-KT-11, and KT-11 grown in food grade medium was termed FG-KT-11.

Feeding Procedure. BALB/c and ICR mice were obtained from Japan SLC (Shizuoka, Japan). The spleens of OVA-sensitized mice were used in the *in vitro* studies. Briefly, male BALB/c mice were injected intraperitoneally with 200 μL of saline containing 20 μg of OVA and 2 mg of

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aluminum hydroxide gel at 6 and 8 weeks of age. The spleen samples were then collected at 9 weeks of age.

OVA-sensitized BALB/c mice in the *in vivo* studies were fed according to our previous study (*5*). The mice were given either a commercial mouse powder feed (MF, Oriental Yeast, Tokyo, Japan: control diet), MF containing MRS-KT-11 (5×10^7 cfu/MF 1 g, MRS-KT-11-supplemented diet) or MF containing FG-KT-11 (5×10^7 cfu/MF 1 g, FG-KT-11-supplemented diet) between 6 and 13 weeks of age ($n = 6$). The total number of sneezing events was then counted for 10 min, 1 min after the intranasal instillation. Blood, spleen and Peyer's patch cells were collected immediately following a lethal dose of ether at 13 weeks of age.

ICR mice were used for the examination of acute toxicity. Briefly, 6-week-old male and female ICR mice were assigned to different test regimen groups, given MF for 1 week and then either 20 mL of saline/kg body weight containing 0, 1000, or 2000 mg of FG-KT-11/kg body weight once at 7 weeks of age ($n=5$). The mice were then observed for any abnormalities for 2 weeks after the administration of FG-KT-11. Blood, brain, heart, liver, kidney, lung, spleen, thymus, testes and ovary samples were collected immediately following a lethal dose of ether at 9 weeks of age.

Food and water was supplied *ad libitum* throughout the course of the experiment. The mice were housed at 23 ± 2 °C under a standard 12 h light−dark cycle. Serum was obtained by centrifugation at 450*g* for 60 min at 4 °C and stored at −30 °C until required. All animal experimentation undertaken during this study was conducted in accordance with the guidelines for the Regulation of Animal Experimentation at Shinshu University, and according to Law No. 105 and Notification No. 6 of the Japanese government.

Cell Suspensions and Cultures. Spleen and Peyer's patch suspensions were prepared as described previously (*5*).Then, 1000 μL of the spleen cell suspension was plated into the wells of a 24-well flat-bottomed plate (Sarstedt, Newton, NC). One hundred microliters of a lactic acid bacterium solution at a final whole cell concentration of 0 or 1×10^7 cells/mL in 0.01 M phosphate-buffered saline (PBS, pH 7.2) and 100 μL of an OVA solution at a final concentration of 100 μg/mL PBS were then added to each well. The cells were cultured at 37 °C in a humidified 5% CO_2 incubator for 48 h for cell functional analysis or 168 h for antibody analysis.

Cell Functional Analysis. The cell surface markers and intracellular cytokines were labeled according to our previous study (*5*). The cell number was determined using a Guava personal cell functional analyzer (Guava PCA: Guava Technologies, Hayward, CA).

Antibody Analysis. OVA-specific IgE levels were measured by an enzyme-linked immunosorbent assay (ELISA) (*5*). The antibody level was calculated using the following formula: antibody level = ELISA value (A450)×dilution-fold of the test sample.

Analysis of Serum Components. Total protein, albumin, creatinine and calcium levels in the serum were measured using a Hitachi 7180 automatic analyzer (Hitachi High-Technologies, Tokyo, Japan). The level of serum globulin was calculated by subtracting the level of serum albumin from the level of total serum protein.

Statistical Analysis. Data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using Dunnett's multiple comparison tests for one-way analysis of variance or Student's *t* tests of two independent samples. Differences were considered significant when *P* values were less than 0.05.

RESULTS AND DISCUSSION

Growth of KT-11. Figure 1 shows the growth of KT-11 in food grade medium and Sunsoft Q-17S-free food grade medium compared to that of the MRS medium. The growth pattern of KT-11 in the food grade medium was similar to that in the MRS medium. In contrast, the growth pattern of KT-11 in the Sunsoft Q-17S-free food grade medium was significantly lower than that in the other two media types examined. In general, oleic acid is considered to be an essential material for the growth of *Lactobacillus* strains (*7*). Sunsoft Q-17S, which is mainly composed of oleic acid and linoleic acid (*6*), was used in the food grade medium instead of the Tween 80 used in the MRS medium. These results indicate that Sunsoft Q-17S is a good source of oleic acid, and suggest that the food grade medium may be used for the cultivation of KT-11 as an antiallergic bacterium.

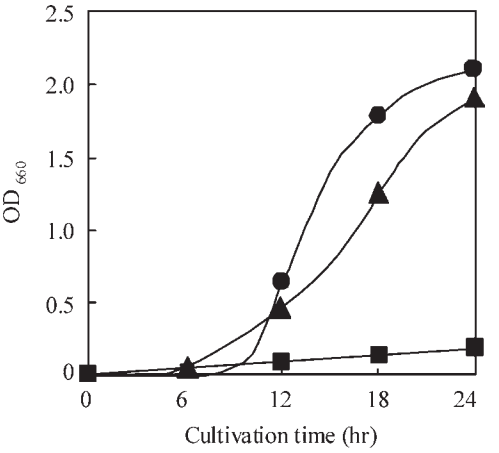

Figure 1. Growth of KT-11 at 37 °C. The growth of KT-11 was determined by calculating the absorbance at 660 nm. ●: MRS medium. ▲: Food grade medium. ■: Sunsoft Q-17S-free food grade medium.

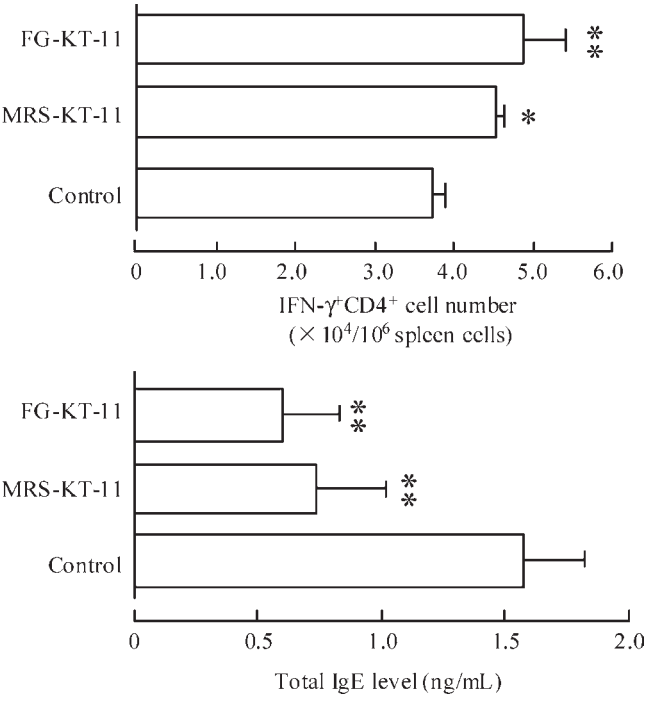

Figure 2. Number of IFN-γ^+CD4$^+$ cells and the level of IgE in OVA-sensitized BALB/c mouse spleen cell cultures in the presence of KT-11. Spleen cells from the mice were cultured with OVA (100 μg/mL) and lactic acid bacteria (0 or 1×10^7 cells/mL) for 48 h (IFN-γ^+CD4$^+$ cell number) or 168 h (IgE level). The number of IFN-γ^+CD4$^+$ cells was determined using Guava PCA. The IgE level was determined using ELISA. Data are presented as the mean ± SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$ (compared to control using the Dunnett's multiple comparison test).

The Effects of KT-11 Cultured in MRS Medium (MRS-KT-11) and KT-11 Cultured in the Food Grade Medium (FG-KT-11) on the Number of Interferon (IFN)-γ^+CD4$^+$ Cells and IgE Levels in OVA-Sensitized BALB/c Mouse Spleen Cultures. Figure 2 presents the number of IFN-γ^+CD4$^+$ cells and IgE levels in OVA-sensitized BALB/c mouse spleen cell cultures. The number of IFN-γ^+CD4$^+$ cells was significantly higher in the MRS-KT-11 and FG-KT-11 cultures than in the cell cultures without bacteria (control). In addition, the number of IFN-γ^+CD4$^+$ cells was higher in the cell

cultures with FG-KT-11 than in the cell cultures with MRS-KT-11. In contrast, the levels of IgE were lower in the cell cultures with MRS-KT-11 and FG-KT-11 than in the control cultures. It is generally thought that the Th1/Th2 balance of type I allergic disease patients inclines toward a Th2-dominant state, and it is well established that IL-4, IL-5 and IL-13 produced by Th2 cells stimulate IgE production (*8*). In particular, IL-4 is known to play a crucial role in IgE synthesis (*9*). Th1 cells predominantly secrete IFN-γ, which inhibits IL-4 production in Th2 cells (*10*). As already described in the introductory comments, we reported

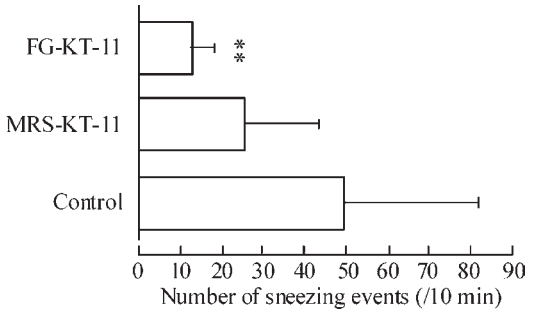

Figure 3. Number of sneezing events in OVA-sensitized BALB/c mice at 13 weeks of age. The sneezing events were counted during a 10 min period following 1 min of intranasal instillation with OVA. Data are presented as mean ± SD (n = 6). ** P < 0.01 (compared to control using the Dunnett's multiple comparison test).

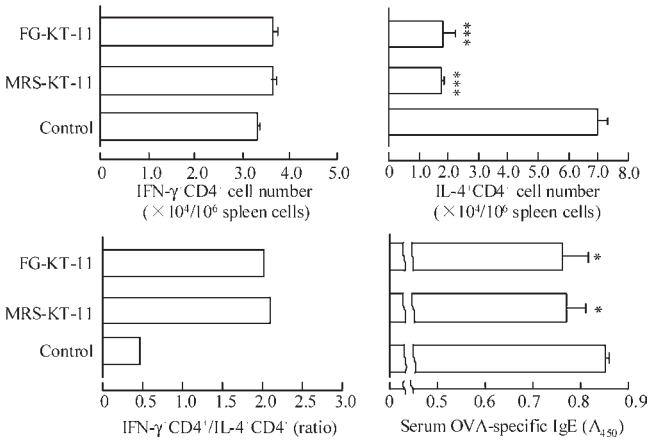

Figure 4. Number of spleen IFN-γ^+CD4$^+$ cells, number of spleen IL-4$^+$CD4$^+$ cells, IFN-γ^+CD4$^+$/IL-4$^+$CD4$^+$ cell ratio and level of serum OVA-specific IgE in OVA-sensitized BALB/c mice given the control, MRS-KT-11-supplemented and FG-KT-11-supplemented diets. The number of spleen IFN-γ^+CD4$^+$ and IL-4$^+$CD4$^+$ cells was determined using Guava PCA. The IFN-γ^+CD4$^+$/IL-4$^+$CD4$^+$ cell ratio was calculated from the mean IFN-γ^+CD4$^+$ cell number against the mean IL-4$^+$CD4$^+$ cell number. The level of serum OVA-specific IgE was determined using ELISA. Data are presented as the mean ± SD (n = 6). * P < 0.05, *** P < 0.001 (compared to control using Dunnett's multiple comparison test).

that KT-11 grown in MRS medium suppressed IgE production by shifting the Th1/Th2 balance from a Th2-dominant state toward a Th1-dominant state (*4*, *5*). Moreover, several studies have demonstrated that numerous lactic acid bacteria and their cellular components reduce allergic symptoms by shifting the Th1/Th2 balance from a Th2-dominant state to a Th1-dominant state (*11*, *12*). Thus, these results suggest that FG-KT-11 also suppresses IgE production by shifting the Th1/Th2 balance from a Th2-dominant state toward a Th1-dominant state.

The Effects of Oral Administration of MRS-KT-11 and FG-KT-11 on Allergic Symptoms and Immune Response in OVA-Sensitized BALB/c Mice. OVA-sensitized BALB/c mice were fed a control diet, MRS-KT-11-supplemented diet or FG-KT-11-supplemented diet between 6 and 13 weeks of age. Under these conditions, the average intake of food was approximately 3.46 g/day/mouse (control), 3.43 g/day/mouse (MRS-KT-11) and 3.54 g/day/mouse (FG-KT-11), and no significant differences in body weight among the mice given these diets were observed (data not shown). These results suggest that there is no difference in either the stress levels or nutritive value among the mice administered these diets.

Figure 3 shows the number of sneezing events in the mice fed the control diet, MRS-KT-11-supplemented diet or FG-KT-11-supplemented diet at 13 weeks of age. The number of sneezing events was significantly lower in the mice given the FG-KT-11-supplemented diet than in those administered the control diet. **Figure 4** presents the number of spleen IFN-γ^+CD4$^+$ cells and IL-4$^+$CD4$^+$ cells, the ratio of spleen IFN-γ^+CD4$^+$/IL-4$^+$CD4$^+$ cells and the level of serum OVA-specific IgE in mice at 13 weeks of age. The number of spleen IL-4$^+$CD4$^+$ cells and the level of serum OVA-specific IgE were significantly lower in the mice given the MRS-KT-11- and FG-KT-11-supplemented diets than in those fed the control diet. The ratio of spleen IFN-γ^+CD4$^+$/IL-4$^+$CD4$^+$ cells was remarkably higher in the mice given the MRS-KT-11- and FG-KT-11-supplemented diets than in those given the control diet. **Table 1** shows the number of spleen and Peyer's patch CD80$^+$CD11b$^+$, IL-12$^+$CD11b$^+$ and FcεRIα^+CD117$^+$ cells in the mice. The number of spleen CD80$^+$CD11b$^+$ and FcεRIα^+CD117$^+$ cells was found to be significantly lower in the mice given the MRS-KT-11- and FG-KT-11-supplemented diets than in those fed the control diet. The number of Peyer's patch CD80$^+$CD11b$^+$ cells was significantly lower in the mice given the FG-KT-11-supplemented diet than in those given the control diet. The number of Peyer's patch IL-12$^+$CD11b$^+$ cells tended to be higher in mice given the FG-KT-11-supplemented diet than in those given the control diet.

Saito et al. (*13*) reported that allergic rhinitis may be induced by the continuous nasal administration of OVA following an intraperitoneal injection of OVA in BALB/c mice. In this study, we induced an acquired allergic symptom via the continuous nasal administration of OVA in BALB/c mice. Oral administration of FG-KT-11 reduced the allergic symptoms in mice when compared with that of MRS-KT-11 (**Figure 3**). In a previous study, we observed a reduction in allergic symptoms in OVA-sensitized BALB/c mice given a diet supplemented with KT-11 grown in

Table 1. The Number of Spleen and Peyer's Patch Immunocompetent Cells in OVA-Sensitized BALB/c Mice[a]

	no. of cells (×10^4/10^6 cells/mL)					
	spleen			Peyer's patch		
immunocompetent cell	control	MRS-KT-11	FG-KT-11	control	MRS-KT-11	FG-KT-11
CD80$^+$CD11b$^+$	7.37 ± 0.80	3.24 ± 0.12***	2.81 ± 0.14***	2.83 ± 0.20	2.43 ± 0.31	0.79 ± 0.12***
IL-12$^+$CD11b$^+$	1.08 ± 0.21	1.03 ± 0.15	0.97 ± 0.10	0.51 ± 0.05	0.68 ± 0.19	1.39 ± 0.10
FcεRIα^+CD117$^+$	1.76 ± 0.13	0.82 ± 0.12***	0.92 ± 0.17***	1.17 ± 0.12	1.15 ± 0.05	1.07 ± 0.24

[a] Data are presented as the mean ± SD (n = 6). P < 0.001 (compared to control using Dunnett's multiple comparison test).

Table 2. The Organ Weight of ICR Mice Given FG-KT-11[a]

	organ weight (g)					
	male			female		
organ	0[b]	1000[b]	2000[b]	0[b]	1000[b]	2000[b]
brain	0.47 ± 0.04	0.48 ± 0.02	0.48 ± 0.02	0.48 ± 0.02	0.46 ± 0.03	0.46 ± 0.02
heart	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.15 ± 0.01
liver	2.84 ± 0.31	2.64 ± 0.25	2.63 ± 0.15	2.00 ± 0.16	1.95 ± 0.15	1.91 ± 0.14
kidney	0.80 ± 0.08	0.86 ± 0.11	0.74 ± 0.06	0.53 ± 0.09	0.48 ± 0.04	0.47 ± 0.03
lung	0.24 ± 0.02	0.27 ± 0.03	0.24 ± 0.02	0.24 ± 0.02	0.24 ± 0.04	0.23 ± 0.04
spleen	0.14 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.15 ± 0.02	0.14 ± 0.02	0.13 ± 0.02
thymus	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.09 ± 0.01	0.11 ± 0.02	0.09 ± 0.01
testicle	0.21 ± 0.01	0.22 ± 0.03	0.24 ± 0.04			
ovary				0.013 ± 0.003	0.011 ± 0.001	0.015 ± 0.003

[a] Data are presented as the mean ± SD (n = 5). [b] Dose of KT-11 (mg/kg body weight).

Table 3. The Serum Component of ICR Mice Given FG-KT-11[a]

	male		female	
serum component	0[b]	2000[b]	0[b]	2000[b]
total protein (mg/mL)	584.0 ± 11.4	592.0 ± 8.4	566.0 ± 16.7	560.0 ± 20.0
albumin (mg/mL)	336.0 ± 11.4	348.0 ± 4.5	362.0 ± 16.4	372.0 ± 11.0
globulin (mg/mL)	248.0 ± 4.5	244.0 ± 11.4	204.0 ± 15.2	188.0 ± 11.0
albumin/globulin (ratio)	1.4 ± 0.1	1.4 ± 0.1	1.8 ± 0.2	2.0 ± 0.1
creatinine (μg/mL)	10.2 ± 1.3	11.8 ± 1.8	10.6 ± 0.5	11.0 ± 0.7
calcium (μg/mL)	1252.0 ± 39.6	1260.0 ± 58.3	1178.0 ± 61.0	1180.0 ± 39.4

[a] Data are presented as the mean ± SD (n = 5). [b] Dose of KT-11 (mg/kg body weight).

MRS medium (*5*). Moreover, it was suggested that the antiallergic effect was due not only to an increase in the IFN-γ^+CD4$^+$/IL-4$^+$CD4$^+$ cell ratio but also to a decrease of spleen CD80$^+$CD11b$^+$ and FcεRIα^+CD117$^+$ cell numbers (*5*). CD80$^+$CD11b$^+$ and FcεRIα^+CD117$^+$ cells normally represent antigen-presenting cells and mast cells, respectively (*14*−*16*). Antigen-presenting cells activate helper T cells via their interaction with T cell receptors, major histocompatibility complex molecules and costimulatory molecules including CD80, and are involved in allergic immune responses (*14*, *15*). Mast cells cause type I allergic disease via degranulation following antigen cross-linking between IgE molecules bound to FcεRI, a high-affinity IgE receptor present on the cell (*9*). We observed that the ratio of spleen IFN-γ^+CD4$^+$/IL-4$^+$CD4$^+$ cells was remarkably higher in the mice given the FG-KT-11-supplemented diets than in those given the control diet, and the level of serum OVA-specific IgE was significantly lower in the mice given the FG-KT-11-supplemented diets than in those given the control diet (**Figure 4**). These results support that FG-KT-11 suppresses IgE production by shifting the Th1/Th2 balance from a Th2-dominant state toward a Th1-dominant state in OVA-sensitized BALB/c mouse spleen cell cultures. However, we did not observe any differences in the ratio of IFN-γ^+CD4$^+$/IL-4$^+$CD4$^+$ cells, the number of CD80$^+$CD11b$^+$ cells and the number of FcεRIα^+CD117$^+$ cells in the spleen between mice fed the FG-KT-11- and MRS-KT-11-supplemented diets (**Figure 4**, **Table 1**). These results suggest that FG-KT-11 also reduces the acquired allergic symptom via not only the adjustment of Th1/Th2 balance, but also via a decrease in mast cell and antigen-presenting cell numbers in the spleen of mice.

Ichikawa et al. (*17*) reported that IL-12 mRNA expression in Peyer's patch CD11b$^+$ cells and the level of blood IL-12 increased immediately following the oral administration of *Lactobacillus paracasei* KW3110 in mice. IL-12 induces IFN-γ production in Th1 cells, natural killer cells, dendritic cells and macrophages, and enhances Th1 immune response (*18*). Antigen-presenting cells migrate farther toward secondary lymphoid organs such as the spleen after activation by antigen stimulation in tissues (*19*). In this study, oral administration of FG-KT-11 induced a decrease in CD80$^+$CD11b$^+$ cell number and an increase in IL-12$^+$CD11b$^+$ cell number in the Peyer's patches (**Table 1**). These results suggest that FG-KT-11 reduces allergic symptoms more strongly than MRS-KT-11 by modulating the immune function of CD11b$^+$ cells in the Peyer's patch. However, we did not observe any differences in Peyer's patch CD80$^+$CD11b$^+$ and IL-12$^+$CD11b$^+$ cell numbers between mice administered the control diet and mice given the MRS-KT-11-supplemented diet (**Table 1**). The differences in medium composition for cultivation of KT-11 would influence the antiallergic effects.

Kimoto-Nira et al. (*20*) reported that the IL-12-inducing activity of lactic acid bacteria grown in M17 medium (*21*) supplemented with 0.5% glucose instead of lactose was higher than that in MRS medium. They also suggested that alterations in the cellular component or in the structure of the cell surface by different growth media affected the IL-12-inducing activity of the lactic acid bacteria. Therefore, the differences in antiallergic effects between MRS-KT-11 and FG-KT-11 may be attributed to their differing structural properties and cellular components.

Acute Toxicity of FG-KT-11. We did not observe any death and abnormal activities in both the male and female ICR mice administered 0, 1000, or 2000 mg of FG-KT-11/kg body weight throughout the observation period (data not shown). These results indicate that a LD_{50} of FG-KT-11 is more than 2000 mg/kg, suggesting that FG-KT-11 has little acute toxicity in mice.

Table 2 presents the weight of brain, heart, liver, kidney, lung, spleen, thymus, testes and ovary samples from mice given FG-KT-11. We did not observe any significant differences in organ weight among the mice given 0, 1000, and 2000 mg of FG-KT-11/kg body weight in either the male or female mice. **Table 3** shows the total protein, albumin, globulin, creatinine and calcium levels present in the serum, and the ratio of albumin/globulin. No significant differences in the serum components were observed between the mice fed 0 and 2000 mg of FG-KT-11/kg body weight in either the male or female mice.

Organ enlargement or atrophy is generally thought to be caused by a functional disorder in the organ (*22*), while fluctuation in total serum protein, albumin and globulin levels is mainly attributed to liver functional disorders (*23*). In addition, fluctuation in serum creatinine levels is thought to be due to kidney functional disorders (*24*, *25*), while the fluctuation of serum calcium levels is though to be due to kidney and parathyroid functional disorders (*26*, *27*). These findings support the hypothesis that only minimal toxic effects in the liver, kidney and parathyroid of mice administered FG-KT-11 were observed.

We also determined the required dosage of KT-11 based on the report by Hirose et al. (*28*). In a previous paper, we reported that the allergic symptoms of NC/Nga mice were reduced following oral administration of 1 mg KT-11 daily (*4*). The body weight of the mice fed this diet was maintained at approximately 20−30 g throughout the experimental period. Hence, it was concluded that the mouse ingests about 33.3−50.0 mg of KT-11/kg body weight daily. In the current study, minimal acute toxicity was observed in the ICR mice given 2000 mg of FG-KT-11/kg body weight. However, 2000 mg of KT-11/kg body weight is approximately 40−60 times higher than the dose that would be expected to induce antiallergic effects. Therefore, it is suggested that FG-KT-11 is safe to use as an antiallergic food material.

In conclusion, we successfully devised a food grade medium consisting of commercial food supplements including Berlex 60, glucose and Sunsoft Q-17S for cultivation of KT-11. We demonstrated that KT-11 cultured in our food grade medium significantly reduced allergic symptoms in OVA-sensitized BALB/c mice when compared with KT-11 cultured in MRS medium. We also found that KT-11 cultured in the food grade medium exhibits minimal acute toxicity in ICR mice. We propose that KT-11 cultured in the food grade medium may be used as a safe antiallergic food material.

ABBREVIATIONS USED

ELISA, enzyme-linked immunosorbent assay; Guava PCA, Guava personal cell functional analyzer; IFN, interferon; Ig, immunoglobulin; IL, interleukin; KT-11, *Lactobacillus crispatus* KT-11 strain; MRS, DeMan−Rogosa−Sharpe; NOD, nucleotide-binding oligomerization domain; OVA, ovalbumin; PE, phycoerythrin; SD, standard deviation; Th1, T helper type 1; Th2, T helper type 2; TLR, Toll-like receptors; Tween 80, polyoxyethylene sorbitan monooleate.

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