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LKM512 yogurt consumption improves the intestinal environment and induces the T-helper type 1 cytokine in adult patients with intractable atopic dermatitis

M. Matsumoto^{*†}, A. Aranami^{‡§}, A. Ishige[§], K. Watanabe[§], and Y. Benno[†]

^{*}Dairy Science and Technology Institute, Kyodo Milk Industry Co. Ltd., Hinode-cho, Tokyo, Japan, [†]Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama, Japan, [‡]Aranami Clinic, Shimada, Shizuoka, Japan, and [§]Department of Kampo Medicine, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan

Clinical and Experimental Allergy

Summary

Background In atopic dermatitis (AD) patients, the intestinal mucosal barrier function is weakened, permitting frequent invasion by antigens. Polyamines and short-chain fatty acids (SCFA) produced by intestinal bacteria are involved in the promotion of intestinal mucosal barrier functions.

Aim Our aim was to investigate the effect of pro-biotic yogurt containing *Bifidobacterium animalis* subsp. *lactis* LKM512 (LKM512 yogurt) on subjective symptoms, intestinal microbiota, intestinal bacterial metabolites (polyamines and SCFA), and T-helper type 1 (Th1)/Th2 balance in intractable AD patients.

Methods In a double-blind, placebo-controlled, crossover study, LKM512 yogurt was given for 4 weeks to 10 adult AD patients who were diagnosed with moderate AD (four males and six females; average age, 22.1 years). The subjective symptoms were recorded after each intervention. The dynamics of fecal microbiota were analysed by the terminal-restriction fragment length polymorphism method. The effects of LKM512 yogurt on fecal polyamines, SCFA, and serum cytokines were evaluated.

Results Scores of itch and burning tended to improve to a greater extent by LKM512 yogurt consumption than by placebo consumption. LKM512 yogurt ($P < 0.005$) and placebo consumption ($P < 0.05$) significantly increased the serum IFN- γ concentration by six- and threefold, respectively. Fecal microbiota was altered dynamically by LKM512 yogurt consumption, in particular, the bacterial species and phylotypes of *Bifidobacterium*, *Clostridium* cluster IV and subcluster XIVa were increased in number. In addition, fecal spermidine concentration was significantly ($P < 0.05$) increased, while fecal butyrate also tended to be increased by LKM512 yogurt consumption.

Conclusion We conclude that LKM512 yogurt consumption may be effective against intractable adult-type AD and this effect depends on the recovery of the intestinal mucosal barrier function and the induction of the Th1-type cytokine by polyamines and SCFA, particularly, butyrate, produced by the altered intestinal microbiota.

Keywords atopic dermatitis, intestinal microbiota, polyamine, pro-biotics, terminal-restriction fragment length polymorphism

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Correspondence:

Mitsuharu Matsumoto, Dairy Science and Technology Institute, Kyodo Milk Industry Co. Ltd., 20-1 Hirai, Hinode-cho, Tokyo 190-0182, Japan.
E-mail: m-matsumoto@meito.co.jp

Introduction

Since Björkstén et al. [1] first reported differences in the fecal microbiota between allergic and healthy infants, many researchers have demonstrated that the intestinal

microbiota plays an important role in the onset of the atopic diseases [2–5]. In particular, the intestinal microbiota of the newborns is considered to play an important role in the development of atopic diseases. Previous reports indicate that *Bifidobacterium* spp. and

Enterobacteriaceae in the intestines of newborns may be involved in atopic disease; the establishment of the intestinal microbiota, which predominantly consist of *Bifidobacterium* spp. during infancy, is effective in the prevention of atopic disease [1–5]. On the other hand, the number of patients with intractable adult-type atopic dermatitis (AD) has been found to be increasing in Japan [6], although it has been recognized that AD is contracted during infancy and resolves by adulthood. We presume that this phenomenon is associated with the change in the intestinal microbiota with westernization of the diet. In fact, by using a culture-dependent method [7] and terminal-restriction fragment length polymorphism (T-RFLP) analysis [8], we have demonstrated that the amount of Enterobacteriaceae and some uncultured bacteria in intractable adult-type AD patients is significantly higher than that in the healthy adults.

Many pro-biotic effects on the alleviation of allergic and atopic diseases have been reported [2, 9–13]. Some researchers show that these effects depend on the improvement of helper T cell balance [T-helper type 1 (Th1)/Th2 balance]; the consumption of pro-biotics was found to alter the balance of the immune condition from Th2-type in allergic and atopic patients to Th1-type [10, 11, 13]. However, this mechanism has not been elucidated completely. It is generally believed that the Th1/Th2 balance is improved on stimulation of the intestinal microbiota by consumption of pro-biotics. However, the dynamics of intestinal microbiota, including the uncultured bacteria that account for 60–80% of the total intestinal bacteria [14, 15], on consumption of pro-biotics has not been investigated thus far using the 16S rRNA gene sequence. In addition, using the cell culture systems, it has been demonstrated that pro-biotic and bifidobacterial cell components stimulate lymphocytes to alter the Th1/Th2 balance [16, 17]. However, the complexity of the intestinal microbiota has not been addressed in these experiments using single bacterial cultures. It is difficult to believe that pro-biotics, which control the host cytokine pattern, confer a special immunogenicity that influences the systemic immune system of the host, because most pro-biotic strains include *Bifidobacterium* spp. or *Lactobacillus* spp., which exhibit commensal colonization in the human intestine. We believe that there are other factors and mechanisms that induce the alleviation of the allergic and atopic diseases. In particular, in AD patients, the intestinal mucosal barrier function is weakened, permitting frequent invasion by antigens [18–21]. Therefore, in order to explain the mechanism of the alleviation of allergic and atopic diseases, we examined bacterial metabolites such as polyamines, putrescine (PUT), spermidine (SMD), and spermine (SPM) [22–25], and short-chain fatty acids (SCFA) [26–28], which are closely associated with the maturation and maintenance of the intestinal mucosal barrier function.

The properties of *Bifidobacterium animalis* subsp. *lactis* LKM512, including acid tolerance [29] and the ability to adhere to intestinal mucin [30], have been well studied. We confirmed that LKM512 yogurt has strong antimutagenicity and anti-acute inflammatory activities, and the health benefits conferred by the consumption of this yogurt depend on the increase in intestinal polyamine concentration produced by the altered intestinal microbiota [31–33]. In the present study, we investigated the effects of LKM512 yogurt consumption on intestinal microbiota, its metabolites (polyamines and SCFA), and Th1/Th2 balance in adult patients with intractable AD.

Materials and methods

Patients

Ten AD patients (four males and six females; average age, 22.1 years) who were diagnosed with moderate AD at Aranami Clinic (Shimada, Japan) using the criteria listed by the Keio University School of Medicine [34] were enrolled in this study. These patients had not been treated with steroid medicines in the past 1 year and had been undergoing long-term treatment with Kampo medicine (Table 1). At least during the study period, each patient took the same Kampo medicine. All the patients were asked to avoid fermented milk, lactic acid bacterial drinks, and fermented soybean (natto) during the experimental periods; otherwise, there were no specific dietary restrictions. In accordance with the Helsinki Declaration, the purpose and content of the examination were explained to the patients in advance, and the study was conducted with their agreement.

Schedule

The patients were randomly assigned in a double-blind manner to receive either placebo (4 weeks) followed by LKM512 yogurt (4 weeks), or LKM512 yogurt followed by placebo. Each intervention period was separated by a 4-week washout period. Feces and blood samples were collected from each subject at pre-consumption, 4 weeks after commencement of LKM512 yogurt/placebo consumption, and 4 weeks after commencement of placebo/LKM512 yogurt consumption.

Yogurt

LKM512 yogurt was fermented with *B. animalis* subsp. *lactis* LKM512, *Lactobacillus delbrueckii* subsp. *bulgaricus* LKM1759, and *Streptococcus thermophilus* LKM1742. The number of *B. animalis* subsp. *lactis* LKM512 and two lactic acid bacteria were 5.2×10^7 and 4.7×10^8 colony formation unit/g, respectively. The placebo consisted of yogurt fermented by *L. delbrueckii* subsp. *bulgaricus*

Table 1. Characteristics of intractable adult-type atopic dermatitis (AD) patients and Kampo medicines

Patient no.	Age	Sex	Age of onset	Kampo medicines	Dose (g/day)	Herbs in Kampo medicine*	Ingredients (pro-drugs) [†]	Active metabolites [‡]
1	19	F	19	Choreito	5	ND [§]		
				Hochuekito	5	<i>Ginseng Radix</i>	Ginsenoside	Protopanaxadiol
				Sanmotsuogonto	5	ND		
2	25	F	10	Unseiin	7.5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
						<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II
				Shiniseihaito	7.5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
3	18	M	18	Shouhusan	7.5	ND		
				Unseiin	7.5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
						<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II
4	20	F	20	Unseiin	7.5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
						<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II
				Keishibukuryogan	7.5	<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II
5	24	M	19	Choreito	5	ND		
				Hochuekito	5	<i>Ginseng Radix</i>	Ginsenoside	Protopanaxadiol
				Byakkokaninjinto	6	<i>Ginseng Radix</i>	Ginsenoside	Protopanaxadiol
6	18	F	4	Jumihaidokuto	7.5	ND		
				Tokiinshi	7.5	<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II
7	15	F	3	Jumihaidokuto	7.5	ND		
				Unseiin	7.5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
						<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II
8	15	M	10	Jumihaidokuto	5	ND		
				Choreito	5	ND		
				Ryutanshakanto	5	ND		
9	17	M	3	Byakkokaninjinto	6	<i>Ginseng Radix</i>	Ginsenoside	Protopanaxadiol
				Hochuekito	5	<i>Ginseng Radix</i>	Ginsenoside	Protopanaxadiol
				Unseiin	5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
						<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II
10	50	F	30	Orengedokuto	7.5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
				Unseiin	7.5	<i>Gardeniae Fructus</i>	Geniposide	Genipin
							Gardenoside	Gardenogenin
						<i>Paeoniae Radix</i>	Paoniflorin	Paconimetabolin I, II

*The listed herbs contain ingredients (pro-drugs) that are believed to be hydrolysed by β -glucosidase derived from intestinal bacteria. Other herbs that do not contain these ingredients (pro-drugs) have not been listed. *Bifidobacterium* spp. (*B. animalis* subsp. *lactis* LKM512) produce β -glucosidase.

[†]Listed here are the ingredients (pro-drugs) that are converted to the active form by β -glucosidase derived from intestinal bacteria.

[‡]Listed here are the active metabolites produced from pro-drugs by β -glucosidase.

[§]No data are available regarding the ingredients (pro-drugs) that are hydrolysed by β -glucosidase.

LKM1759, and *S. thermophilus* LKM1742. The amount of ingested LKM512 yogurt or placebo was 100 g/day/individual.

Questionnaire

The severity of atopic symptom, itch, burning, pain, irritation, and visual status experienced by the patients during the last 7 days of LKM512 yogurt/placebo consumption when compared with those during the pre-

consumption period were recorded through questionnaires. The scores were as follows: - 2 = deterioration; - 1 = a little deterioration; ± 0 = no change; + 1 = a little improvement; and + 2 = improvement.

Preparation of fecal extract

Feces were collected from each patient immediately after excretion and kept under anaerobic conditions using the AnaeroPack Anaero (Mitsubishi Gas Chemical, Tokyo,

Japan) until preservation at -80°C . Frozen feces were diluted 10-fold with 10 mM phosphate-buffered saline (PBS; pH 7.2) containing 10 mM sodium azide; a soluble substance was extracted twice by intense mixing for 1 min and was allowed to stand on ice for 15 min. After extraction, the precipitate was removed by centrifugation ($10\,000\text{ g}$ for 20 min at 4°C), and the supernatant was filtered ($0.45\text{ }\mu\text{m}$) and used as the fecal extract. This extract was stored at -20°C until analysis.

Determination of fecal polyamine concentration

Fecal polyamines, PUT, SMD, and SPM were determined from fecal extracts by reversed-phase high-performance liquid chromatography (HPLC) using 9-fluorenylmethyl chloroformate. The columns and analysis conditions have been described in our previous report [32].

Determination of fecal short-chain fatty acids concentration

Frozen feces were diluted 10-fold with 2% perchloric acid, and a soluble substance was extracted at 4°C for 18 h. After extraction, the precipitate was removed by centrifugation ($10\,000\text{ g}$ for 20 min at 4°C), and the supernatant was filtered ($0.45\text{ }\mu\text{m}$) and used. The columns and analysis conditions have been described in our previous report [33].

Bacterial DNA extraction from feces

The bacterial DNA was isolated from feces by the method described by Matsuki et al. [35]. The DNA obtained was suspended in $200\text{ }\mu\text{L}$ of distilled water and stored at -20°C until use.

Polymerase chain reaction amplification of 16S rRNA gene

A pair of universal primers – 529F (5'-ACGTGCCAG-CAGCCGCGG-3') [36] and 1492R (5'-GGTTACCTTGTTAC-GACTT-3') [37] – were used for amplification by polymerase chain reaction (PCR). The 529F primer was labelled at the 5' end with 6-carboxyfluorescein, which was synthesized by Applied Biosystems, Tokyo, Japan. The amplification reaction was performed as described in our previous report [38]. PCR products were purified by the polyethylene glycol precipitation method.

Comparison of fecal microbiota using terminal-restriction fragment length polymorphism analysis

T-RFLP analysis was performed as described by Sakamoto et al. [39], with some modifications. Briefly, the purified PCR product (100 ng) was digested with 4 units of *Hha*I, *Msp*I, *Hae*III, or *Alu*I (TaKaRa, Ohtsu, Japan) in a total

volume of $10\text{ }\mu\text{L}$ at 37°C for 3 h. The lengths of the terminal restriction fragments (T-RFs) were determined with the standard size markers GS 500 ROX and GS 1000 ROX (Applied Biosystems, Foster City, CA, USA) using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and GeneScanAnalysis Software (Applied Biosystems).

Assignment of terminal restriction fragments detected

The bacterial clusters, genus, or species composed in T-RFs were assigned using the phylogenetic assignment database for T-RFLP analysis of human colonic microbiota (PAD-HCM); this database includes the 16S rRNA gene sequences data of approximately 350 colonic bacterial species and phylotypes and enables the prediction of T-RFs at the species level, including those of uncultured bacteria [38]. In this study, we used the PAD-HCM for 529F primer (PAD-HCM-529F), which was modified by the above-mentioned PAD-HCM for the 27F primer. In this paper, the term 'phylotype' refers to a clone for which only the 16S rRNA gene sequence is known.

Measurement of the cytokine content in serum

Serum IL-4, IL-5, IL-10, IL-12, and IFN- γ were measured using the commercially available sandwich ELISA kits, the immunoassay kit for human IL-4, IL-5, IL-10, IL-12, and IFN- γ (BioSource International Inc., CA, USA).

Statistical analysis

Changes in each parameter caused by LKM512 yogurt consumption were analysed using the paired *t* test. Calculations were performed using the computer software STATISTICA (Design Technologies Inc., Tokyo, Japan).

Results

Subjective symptoms

The changes that were observed in the subjective symptoms are shown in Table 2. Some subjective symptoms tended to improved following LKM512 yogurt or placebo consumption. In particular, LKM512 yogurt consumption resulted in an improvement in 'itch' and 'burning' in four of 10 and three of eight patients, respectively. In contrast, placebo consumption resulted in an improvement in 'itch' and 'burning' in one of 10 patients and two of eight patients, respectively. Further, some of the symptoms during the period of placebo consumption tended to be lower than those during the period of LKM512 yogurt consumption, although all symptoms were slightly improved by placebo consumption.

Fecal polyamines concentration

The changes in fecal PUT and SMD concentrations are shown in Fig. 1a and b, respectively. PUT concentrations at pre-consumption, LKM512 yogurt consumption, and placebo consumption stages were $208.4 \pm 43.9 \mu\text{M}$, $400.0 \pm 128.5 \mu\text{M}$, and $390.8 \pm 106.8 \mu\text{M}$, respectively. SMD concentrations at pre-consumption, LKM512 yogurt consumption, and placebo consumption stages were $68.6 \pm 14.9 \mu\text{M}$, $160.2 \pm 22.1 \mu\text{M}$, and $139.7 \pm 38.7 \mu\text{M}$, respectively. The SMD concentration increased significantly after the consumption of LKM512 ($P < 0.005$) and placebo ($P < 0.05$). SPM concentrations were below the detection limit; therefore, we omitted SPM data from this study.

Fecal short-chain fatty acids concentration

Table 3 shows the changes in fecal SCFA concentration. The acetate, propionate, butyrate, and isobutyrate con-

Table 2. Change in subjective symptoms after the consumption of LKM512 yogurt and placebo

Symptoms	LKM512 yogurt		Placebo	
	Score* (mean)	The number of improved patients†	Score* (mean)	The number of improved patients†
Itch	0.5	4/10	0.1	1/10
Burning	0.3	3/8	0.2	2/8
Pain	0.3	2/6	0.3	3/6
Irritation	0.2	1/8	0.1	1/8
Visual status	0.4	3/10	0.3	3/10

*Patients were scored as follows: -2 = deterioration; -1 = a little deterioration; ± 0 = no change; $+1$ = a little improvement; and $+2$ = improvement.

†The number of improved patients was expressed as: the number of patients who registered 'a little improvement' and 'improvement' / the number of patients who exhibited the symptoms.

centrations tended to increase following LKM512 yogurt consumption when compared with the pre-consumption concentrations. In particular, the butyrate concentration tended to increase following LKM512 yogurt consumption in eight of 10 patients ($P = 0.089$).

Serum cytokine contents

The changes in serum cytokines are shown in Table 4. Serum IFN- γ was significantly increased by six- and threefold on consumption of LKM512 yogurt ($P < 0.005$) and placebo ($P < 0.05$), respectively. There was no change in the concentration of other cytokines on consumption of LKM512 yogurt and placebo. IL-10 level was below the detection limit.

Dynamics of fecal microbiota

Predicted bacterial species and phylotypes of T-RFs increased dramatically on consumption of LKM512 yogurt and placebo, as shown in Table 5. The bacterial species and phylotypes that were commonly increased in three or more patients after LKM512 yogurt consumption when compared with the pre-consumption were as follows: *Clostridium disporicum* and *Clostridium barati* in *Clostridium* cluster I, *Clostridium methylpentosum*, *Eubacterium desmolans*, *Eubacterium siraeum*, *Ruminococcus flavefaciens*, uncultured bacterium clone afhufec13, uncultured bacterium clone HuCA11, and uncultured bacterium clone p-5459-2Wb5 – all relatives of *Faecalibacterium prausnitzii*, and uncultured bacterium clone p-3567-9F3 (a relative of *Clostridium sporasphaeroides*) in *Clostridium* cluster IV, *Selenomonas ruminantium* in *Clostridium* cluster IX, *Clostridium sticklandii* in *Clostridium* cluster XI, *Clostridium coccoides*, *Clostridium methylpentosum*, *Eubacterium halii*, *Eubacterium*

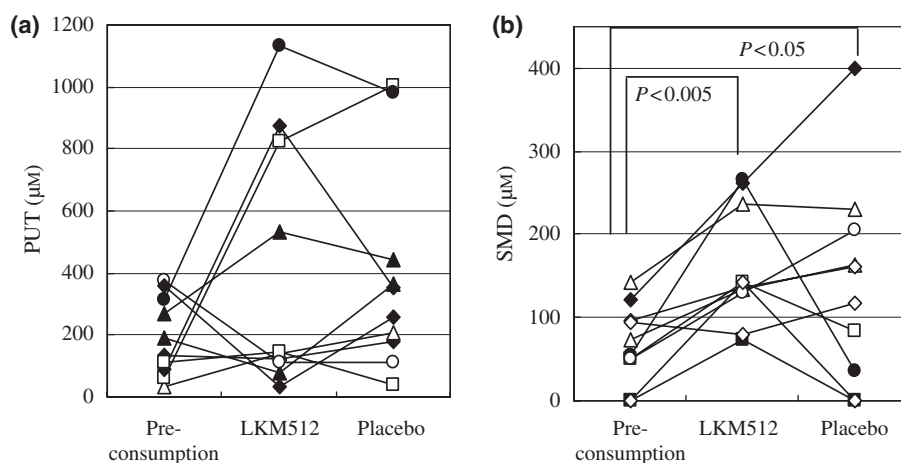


Fig. 1. Change in fecal polyamine concentration [(a) putrescine (PUT), (b) spermidine (SMD)] after the consumption of LKM512 yogurt and placebo. SMD concentration increased significantly after the consumption of LKM512 ($P < 0.005$) and placebo ($P < 0.05$). Spermine concentrations were below the detection limit.

Table 3. Change in fecal short-chain fatty acid concentration after the consumption of LKM512 yogurt and placebo

	Acetate	Propionate	Butyrate	Isobutyrate	Valerate	Isovalerate
Pre-consumption	106	28	19	12	0	5
LKM512 yogurt	122	36	36*	18	0	0
Placebo	74	17	31	12	0	7

*Butyrate concentration tended to increase following LKM512 yogurt consumption when compared with the pre-consumption concentration ($P = 0.0816$).

Table 4. Change in serum cytokines after the consumption of LKM512 yogurt and placebo

Cytokines (pg/mL)	Pre-consumption	LKM512	Placebo
IFN- γ	0.21 $\pm$ 0.15	1.26 $\pm$ 0.39**	0.59 $\pm$ 0.41*
IL-4	2.72 $\pm$ 1.17	2.79 $\pm$ 1.36	1.97 $\pm$ 0.53
IL-5	11.6 $\pm$ 4.6	12.7 $\pm$ 9.6	15.78 $\pm$ 3.66
IL-12	82.0 $\pm$ 30.6	70.1 $\pm$ 25.0	84.0 $\pm$ 21.1

**, *Serum IFN- γ was significantly increased after the consumption of LKM512 yogurt ($P < 0.005$) and placebo ($P < 0.05$), respectively.

ramulus, *Eubacterium ventriosum*, *Ruminococcus hanse-
nii*, *Ruminococcus obeum*, *Ruminococcus productus*,
Roseburia intestinalis, uncultured bacterium clone adhufec24 (a relative of *E. ventriosum*), uncultured bacterium clone adhufec172 (a relative of *R. obeum*), uncultured bacterium clone adhufec236 (a relative of *Clostridium nexile*), uncultured bacterium clone adhufec406 (no relative), uncultured bacterium clone HuCA2 and uncultured bacterium clone p-2000-s959-5 (both relatives of *R. obeum*), uncultured bacterium clone HuCB1 and butyrate-producing bacterium L1-93 (both relatives of *Ros. intestinalis*), and butyrate-producing bacterium T2-145 (a relative of *Butyrivibrio crossotus*) in *Clostridium* subcluster XIVa, *B. animalis* subsp. *lactis*, *Bifidobacterium adolescentis*, *Bifidobacterium catenulatum*, *Bifidobacterium gallicum*, and *Bifidobacterium pseudocatenulatum* in the genus of *Bifidobacterium*, *Lactobacillus gasseri*, *Enterococcus* group, and *Streptococcus* group. In contrast, the increase in the number of T-RFs was found to be greater after LKM512 yogurt consumption than after placebo consumption. The predicted bacterial species and phylotypes that were commonly increased in three or more patients after placebo consumption when compared with the pre-consumption were uncultured bacterium clone adhufec24 (a relative of *E. ventriosum*) and butyrate-producing bacterium T2-145 (a relative of *Bu. crossotus*) in *Clostridium* subcluster XIVa, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Klebsiella granulomatis* in *Gamma-proteobacteria*, *Enterococcus* group, *Lactobacillus* group, and *Streptococcus* group.

The predicted bacterial species and phylotypes of T-RFs that decreased dramatically on consumption of LKM512 yogurt and placebo are shown in Table 6. No T-RF was found to be commonly decreased in three or more patients

after LKM512 yogurt consumption when compared with the pre-consumption. On the other hand, some T-RFs that were commonly decreased in three or more patients after placebo consumption when compared with the pre-consumption were as follows: *E. desmolams*, *C. methylpentosum*, and uncultured bacterium clone p-3567-9F3 (a relative of *C. sporasphaeroides*) in *Clostridium* cluster IV, *Mitsuokella jalaludinii* in *Clostridium* cluster IX, butyrate-producing bacterium T2-145 (a relative of *Bu. crossotus*) and uncultured bacterium clone adhufec172 (a relative of *R. obeum*) in *Clostridium* subcluster XIVa, *Prevotella ruminicola* in the genus of *Bacteroides*, *B. adolescentis*, *B. pseudocatenulatum*, and *B. gallicum* in the genus of *Bifidobacterium*.

Discussion

The aim of the AD treatment that uses pro-biotics and Kampo medicine is to improve immune balance and intestinal barrier function without immunosuppression as observed in the case of steroidal medicines. These act on the immune balance and intestinal barrier function through the intestinal microbiota after oral consumption.

Kampo is a general term used for the unique system of traditional medicine of Chinese origin that has developed in Japan. Kampo is based on the therapeutic technique that has been developed by the accumulation, organization, and systemization of an extensive amount of clinical experience, and it is designed to be an individualized therapy. Kampo medicines are not indicated on the basis of the diseases. As each individual's physical response to the same disease varies, different Kampo medicines are often prescribed to patients with the same disease. The human body is organized as one whole system. Therefore, Kampo treatment is based on the concept that restoration of the body's balance heals the affected area. Thus, the same Kampo formula may be prescribed to patients with different diseases. This study should have ideally comprised AD patients who had been treated with identical Kampo medicines. However, this was impossible due to the above-mentioned reasons.

In the Kampo medicine system, glycoside is believed to act as a pro-drug that is metabolized to the active form by intestinal bacteria [40], and it appears that polysaccharides regulate the intestinal bacterial composition. For example, β -glucosidase derived from intestinal bacteria,

Table 5. Bacterial species and phylotypes increased dramatically after the consumption of LKM512 yogurt and placebo

LKM512 yogurt consumption			Placebo consumption		
Bacterial species or phylotypes	Group*	Volunteers†	Bacterial species or phylotypes	Group	Volunteers
<i>Clostridium disporicum</i>	I	1, 4, 6, 9, 10	<i>Clostridium disporicum</i>	I	8
<i>Clostridium barati</i>	I	1, 6, 9	<i>Clostridium barati</i>	I	8
<i>Anaerofilm pentosovorans</i>	IV	8, 10	<i>Clostridium cellulosi</i>	IV	8
<i>Anaerofilm agile</i>	IV	8, 10	<i>Eubacterium desmolans</i>	IV	5, 8
<i>Clostridium cellulosi</i>	IV	8	<i>Eubacterium siraeum</i>	IV	8
<i>Clostridium methylpentosum</i>	IV	4, 8, 9, 10	<i>Rumonococcus bromii</i>	IV	6
<i>Clostridium orbiscindens</i>	IV	8	<i>Rumonococcus callidus</i>	IV	8
<i>Clostridium viride</i>	IV	8	Uncultured bacterium clone adhufec21	IV	6
<i>Eubacterium desmolans</i>	IV	1, 4, 7, 8, 9	Uncultured bacterium clone HuCB2	IV	6
<i>Eubacterium siraeum</i>	IV	1, 6, 9			
<i>Faecalibacterium prausnitzii</i>	IV	8, 10			
<i>Rumonococcus albus</i>	IV	10			
<i>Rumonococcus callidus</i>	IV	6, 9			
<i>Ruminococcus flavefaciens</i>	IV	2, 8, 10			
Uncultured bacterium clone adhufec13	IV	6, 8, 10			
Uncultured bacterium clone adhufec365	IV	8, 10			
Uncultured bacterium clone adhufec78.25	IV	8			
Uncultured bacterium clone adhufec113	IV	8, 10			
Uncultured bacterium clone adhufec218	IV	8, 10			
Uncultured bacterium clone HuCA11	IV	1, 8, 10			
Uncultured bacterium clone HuCA24	IV	10			
Uncultured bacterium clone HuCA25	IV	8, 10			
Uncultured bacterium clone HuCB7	IV	8			
Uncultured bacterium p-3567-9F3	IV	2, 4, 8, 10			
Uncultured bacterium clone p-5459-2Wb5	IV	1, 8, 10			
Butyrate-producing bacterium A2-165	IV	8, 10			
Butyrate-producing bacterium A2-207	IV	10			
<i>Mitsuokella jalaludinii</i>	IX	8, 10	<i>Mitsuokella jalaludinii</i>	IX	8
<i>Selenomonas ruminantium</i>	IX	6, 8, 10	<i>Selenomonas ruminantium</i>	IX	8
Uncultured bacterium clone adhufec395	IX	10			
<i>Clostridium sticklandii</i>	XI	1, 9, 10	<i>Clostridium sticklandii</i>	XI	8
<i>Butyrivibrio fibrisolves</i>	XIVa	7, 9	<i>Butyrivibrio fibrisolves</i>	XIVa	7
<i>Clostridium celerecrescens</i>	XIVa	9	<i>Eubacterium ramulus</i>	XIVa	8
<i>Clostridium clostridioforme</i>	XIVa	6, 8	<i>Eubacterium ventriosum</i>	XIVa	8
<i>Clostridium coccoides</i>	XIVa	1, 4, 8, 9, 10	<i>Rumonococcus obeum</i>	XIVa	8
<i>Clostridium herbivorans</i>	XIVa	8, 10	<i>Ruminococcus productus</i>	XIVa	8
<i>Clostridium methylpentosum</i>	VIVa	2, 4, 8	<i>Rumonococcus torques</i>	XIVa	9
<i>Clostridium polysaccharolyticum</i>	XIVa	8, 10	Uncultured bacterium clone adhufec24	XIVa	1, 7, 8
<i>Eubacterium eligens</i>	XIVa	10	Uncultured bacterium clone adhufec52	XIVa	8
<i>Eubacterium halii</i>	XIVa	1, 4, 9, 10	Uncultured bacterium clone adhufec172	XIVa	1, 8
<i>Eubacterium ramulus</i>	XIVa	1, 4, 9, 10	Uncultured bacterium clone adhufec236	XIVa	8
<i>Eubacterium ventriosum</i>	XIVa	2, 8, 9, 10	Uncultured bacterium clone adhufec405	XIVa	7
<i>Lachnospira pectinoschiza</i>	XIVa	10	Uncultured bacterium clone adhufec406	XIVa	8
<i>Ruminococcus gnavus</i>	XIVa	1, 8	Uncultured bacterium clone p-3567-9F3	XIVa	7
<i>Rumonococcus hansenii</i>	XIVa	1, 2, 8	Uncultured bacterium clone p-2000-s959-5	XIVa	8
<i>Rumonococcus obeum</i>	XIVa	1, 9, 10	Butyrate-producing bacterium T2-145	XIVa	1, 7, 8
<i>Ruminococcus productus</i>	XIVa	1, 4, 9, 10			
<i>Roseburia intestinalis</i>	XIVa	1, 9, 10			
Uncultured bacterium clone adhufec24	XIVa	1, 2, 4, 8, 9, 10			
Uncultured bacterium clone adhufec52	XIVa	4, 10			
Uncultured bacterium clone adhufec172	XIVa	1, 4, 9, 10			
Uncultured bacterium clone adhufec236	XIVa	1, 4, 9, 10			
Uncultured bacterium clone adhufec250	XIVa	9, 10			
Uncultured bacterium clone adhufec405	XIVa	10			
Uncultured bacterium clone adhufec406	XIVa	1, 4, 9, 10			

Table 5. Continued

LKM512 yogurt consumption			Placebo consumption		
Bacterial species or phylotypes	Group*	Volunteers [†]	Bacterial species or phylotypes	Group	Volunteers
Uncultured bacterium clone adhufec425	XIVa	9			
Uncultured bacterium clone HuCA2	XIVa	1, 9, 10			
Uncultured bacterium clone HuCA8	XIVa	7			
Uncultured bacterium clone HuCA19	XIVa	9			
Uncultured bacterium clone HuCA20	XIVa	4			
Uncultured bacterium clone HuCA26	XIVa	1, 9			
Uncultured bacterium clone HuCA27	XIVa	9			
Uncultured bacterium clone HuCB1	XIVa	1, 7, 8, 10			
Uncultured bacterium clone HuCB12	XIVa	9			
Uncultured bacterium clone p-2000-s959-5	XIVa	1, 9, 10			
Butyrate-producing bacterium A2-194	XIVa	7			
Butyrate-producing bacterium L1-93	XIVa	1, 9, 10			
Butyrate-producing bacterium L1-92	XIVa	4			
Butyrate-producing bacterium L2-50	XIVa	4			
Butyrate-producing bacterium SSC/2	XIVa	4			
Butyrate-producing bacterium T2-145	XIVa	1, 2, 4, 8, 9, 10			
<i>Bacteroides merdae</i>	Bact.	6	Uncultured bacterium clone HuCA36	Bact.	8
<i>Prevotella ruminicola</i>	Bact.	7	Uncultured bacterium clone HuCB1	Bact.	8
			Uncultured bacterium clone HuCB23	Bact.	8
			<i>Bacteroides merdae</i>	Bact.	8
			<i>Bacteroides stercoris</i>	Bact.	8
			<i>Bacteroides splanchnicus</i>	Bact.	8
			<i>Bacteroides thetaiotaomicron</i>	Bact.	8
			<i>Bacteroides</i> sp. ASF519	Bact.	8
<i>Hafnia alvei</i>	Gamma-p	7	<i>Enterobacter cloacae</i>	Gamma-p	6, 7, 10
			<i>Escherichia coli</i>	Gamma-p	6
			<i>Hafnia alvei</i>	Gamma-p	2, 7
			<i>Klebsiella pneumoniae</i>	Gamma-p	6, 7, 10
			<i>Klebsiella granulomatis</i>	Gamma-p	6, 7, 10
			<i>Proteus mirabilis</i>	Gamma-p	2, 7
<i>Bifidobacterium adolescentis</i>	Bif.	1, 2, 3, 4, 6, 8, 9, 10	<i>Bifidobacterium adolescentis</i>	Bif.	8
<i>Bifidobacterium angulatum</i>	Bif.	2, 3	<i>Bifidobacterium angulatum</i>	Bif.	2
<i>Bifidobacterium catenulatum</i>	Bif.	2, 4, 5, 6	<i>Bifidobacterium catenulatum</i>	Bif.	2
<i>Bifidobacterium gallicum</i>	Bif.	2, 3, 4, 5, 6	<i>Bifidobacterium lactis</i>	Bif.	2
<i>Bifidobacterium lactis</i>	Bif.	1, 2, 3, 4, 5, 6, 8, 9	<i>Bifidobacterium pseudocatenulatum</i>	Bif.	2
<i>Bifidobacterium longum</i>	Bif.	2	<i>Bifidobacterium</i> sp. PL1	Bif.	8
<i>Bifidobacterium pseudocatenulatum</i>	Bif.	2, 3, 4, 5, 6			
<i>Bifidobacterium</i> sp. PL1	Bif.	1, 2, 3, 4, 5, 6, 8, 9			
<i>Enterococcus</i> group	Enteroc.	5, 6, 7	<i>Enterococcus</i> group	Enteroc.	3, 5, 6
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Enteroc.	1, 3, 5, 6, 7, 9			
<i>Lactobacillus gasseri</i>	Lact.	2, 4, 5, 6, 10	<i>Lactobacillus gasseri</i>	Lact.	8
<i>Lactobacillus salivarius</i> subsp. <i>salivarius</i>	Lact.	6, 7	<i>Lactobacillus salivarius</i> subsp. <i>salivarius</i>	Lact.	8
<i>Lactobacillus</i> group	Lact.	5, 6	<i>Lactobacillus</i> group		3, 5, 6
<i>Leuconostoc argentinum</i>	Leu.	7	<i>Leuconostoc argentinum</i>	Leu.	7
<i>Leuconostoc mesenteroides</i>	Leu.	7	<i>Leuconostoc mesenteroides</i>	Leu.	7
<i>Streptococcus bovis</i>	Streptoc.	5, 10	<i>Streptococcus bovis</i>	Streptoc.	8
<i>Streptococcus</i> group		5, 6, 7, 10	<i>Streptococcus</i> group	Streptoc.	3, 5, 6, 8
<i>Staphylococcus aureus</i>					

T-RFs commonly increased after LKM512 yogurt/placebo consumption are shown in bold.

*I, *Clostridium* cluster I; IV, *Clostridium* cluster IV; IX, *Clostridium* cluster IX; XI, *Clostridium* cluster XI; XIVa, *Clostridium* subcluster XIVa; Bact., *Bacteroides* group; Gamma-p, Gammaproteobacteria; Bif., *Bifidobacterium*; Enteroc., *Enterococcus*; Lact., *Lactobacillus*; Leu., *Leuconostoc*; and Streptoc., *Streptococcus*.

[†]Volunteer number that bacterial species or phylotypes were detected.

Table 6. Bacterial species and phylotypes decreased dramatically after the consumption of LKM512 yogurt and placebo

LKM512 yogurt consumption			Placebo consumption		
Bacterial species or phylotypes	Group*	Volunteers†	Bacterial species or phylotypes	Group	Volunteers
<i>Clostridium disporicum</i>	I	5	<i>Clostridium disporicum</i>	I	5, 7
			<i>Clostridium barati</i>	I	7
<i>Clostridium methylpentosum</i>	IV	1	<i>Clostridium cellulosi</i>	IV	8
Uncultured bacterium p-3567-9F3	IV	1	<i>Eubacterium desmolans</i>	IV	2, 8, 9
<i>Ruminococcus flavefaciens</i>	IV	1, 3	Uncultured bacterium clone p-3567-9F3	IV	2, 5, 7, 8
<i>Ruminococcus bromii</i>	IV	8	<i>Ruminococcus flavefaciens</i>	IV	3, 8
<i>Eubacterium desmolans</i>	IV	8	<i>Ruminococcus callidus</i>	IV	7
Uncultured bacterium clone HuCB2	IV	8	Uncultured bacterium clone p-5459-2Wb5	IV	8
			Butyrate-producing bacterium A2-165	IV	8
			<i>Faecalibacterium prausnitzii</i>	IV	8
			Uncultured bacterium clone adhufec24	IV	8
			Uncultured bacterium clone adhufec365	IV	8
			Uncultured bacterium clone adhufec113	IV	8
			Uncultured bacterium clone adhufec218	IV	8
			Uncultured bacterium clone adhufec13	IV	8, 10
			Uncultured bacterium clone HuCA11	IV	8
			Uncultured bacterium clone HuCA25	IV	8
			<i>Clostridium orbiscindens</i>	IV	4, 8
			Uncultured bacterium clone HuCB7	IV	4, 8
			<i>Clostridium methylpentosum</i>	IV	5, 7, 8
			<i>Eubacterium siraeum</i>	IV	7
			<i>Ruminococcus bromii</i>	IV	8
			Uncultured bacterium clone HuCB2	IV	8
			Uncultured bacterium clone A03	IV	8
			<i>Clostridium viride</i>	IV	8
<i>Veillonella ratti</i>	IX	7	<i>Veillonella ratti</i>	IX	1
<i>Veillonella atypica</i>	IX	7	<i>Veillonella atypica</i>	IX	1
<i>Veillonella parvula</i>	IX	7	<i>Veillonella parvula</i>	IX	1
<i>Veillonella dispar</i>	IX	7	<i>Veillonella dispar</i>	IX	1
<i>Megasphaera cerevisiae</i>	IX	7	<i>Megasphaera cerevisiae</i>	IX	1, 9
<i>Mitsuokella jalaludinii</i>	IX	3, 5	<i>Mitsuokella jalaludinii</i>	IX	3, 5, 8
<i>Phascolarctobacterium faecium</i>	IX	7	<i>Phascolarctobacterium faecium</i>	IX	7
			<i>Selenomonas ruminantium</i>	IX	8, 10
			<i>Clostridium halophilum</i>	XI	2
			<i>Clostridium sticklandii</i>	XI	7
Uncultured bacterium clone adhufec52	XIVa	1, 2	Uncultured bacterium clone adhufec52	XIVa	2, 5
<i>Butyrivibrio fibrisolvens</i>	XIVa	1	<i>Clostridium herbivorans</i>	XIVa	2
Uncultured bacterium clone adhufec250	XIVa	1	<i>Eubacterium ramulus</i>	XIVa	2
Uncultured bacterium clone adhufec405	XIVa	1, 6	<i>Eubacterium halii</i>	XIVa	2
Uncultured bacterium clone adhufec24	XIVa	1, 5	<i>Clostridium clostridioforme</i>	XIVa	2, 10
butyrate-producing bacterium T2-145	XIVa	1, 5	Uncultured bacterium clone HuCB1	XIVa	2, 8
<i>Eubacterium ventriosum</i>	XIVa	1	<i>Clostridium polysaccharolyticum</i>	XIVa	3, 8
<i>Clostridium herbivorans</i>	XIVa	2	Uncultured bacterium clone adhufec24	XIVa	5, 7
<i>Eubacterium ramulus</i>	XIVa	2	Butyrate-producing bacterium T2-145	XIVa	5, 7, 8
<i>Eubacterium halii</i>	XIVa	2	Butyrate-producing bacterium ART55/1	XIVa	8
<i>Clostridium polysaccharolyticum</i>	XIVa	3	Uncultured bacterium clone adhufec172	XIVa	5, 7, 8
Uncultured bacterium clone adhufec172	XIVa	5	<i>Ruminococcus productus</i>	XIVa	7
Uncultured bacterium clone HuCC15	XIVa	8	<i>Clostridium coccooides</i>	XIVa	7
Uncultured bacterium clone adhufec420	XIVa	8	Uncultured bacterium clone adhufec406	XIVa	7
butyrate-producing bacterium ART55/1	XIVa	8	Uncultured bacterium clone adhufec217	XIVa	10
<i>Clostridium xylanolyticum</i>	XIVa	8	Uncultured bacterium clone adhufec236	XIVa	7
Uncultured bacterium clone adhufec21	XIVa	8	Uncultured bacterium clone HuCA2	XIVa	7
			Uncultured bacterium clone p-2000-s959-5	XIVa	7
			<i>Ruminococcus obeum</i>	XIVa	7
			<i>Eubacterium ventriosum</i>	XIVa	7

Table 6. Continued

LKM512 yogurt consumption			Placebo consumption		
Bacterial species or phylotypes	Group*	Volunteers†	Bacterial species or phylotypes	Group	Volunteers
			Uncultured bacterium clone HuCC15	XIVa	8
			Uncultured bacterium clone adhufec420	XIVa	8
			<i>Clostridium xylanolyticum</i>	XIVa	8
			<i>Clostridium oroticum</i>	XIVa	8
			Uncultured bacterium clone adhufec21	XIVa	8
			Uncultured bacterium clone p-2330-s962	XVIII	3, 8
Uncultured bacterium clone HuCA36	Bact.	6	<i>Prevotella ruminicola</i>	Bact.	1, 6, 8
<i>Prevotella ruminicola</i>	Bact.	6	<i>Bacteroides merdae</i>	Bact.	6
<i>Enterobacter cloacae</i>	Gamma-p	2	<i>Hafnia alvei</i>	Gamma-p	9
<i>Klebsiella pneumoniae</i>	Gamma-p	2	<i>Proteus mirabilis</i>	Gamma-p	9
<i>Klebsiella granulomatis</i>	Gamma-p	2	<i>Enterobacter cloacae</i>	Gamma-p	2, 9
			<i>Klebsiella pneumoniae</i>	Gamma-p	2, 9
			<i>Klebsiella gnanulomatis</i>	Gamma-p	2, 9
<i>Lactobacillus gasseri</i>	Lact.	1	<i>Lactobacillus</i> group		3
			<i>Lactobacillus gasseri</i>	Lact.	4, 7
			<i>Lactobacillus salivarius</i> subsp. <i>salivarius</i>	Lact.	7
<i>Enterococcus</i> group	Enteroc.	10	<i>Enterococcus</i> group	Enteroc.	10
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Enteroc.	1	<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Enteroc.	2, 6
			<i>Bifidobacterium</i> sp. PL1	Bif.	2, 3, 8,
<i>Bifidobacterium pseudocatenulatum</i>	Bif.	10	<i>Bifidobacterium adolescentis</i>	Bif.	2, 7, 8
<i>Bifidobacterium gallicum</i>	Bif.	10	<i>Bifidobacterium pseudocatenulatum</i>	Bif.	3, 7, 9
<i>Bifidobacterium catenulatum</i>	Bif.	10	<i>Bifidobacterium gallicum</i>	Bif.	3, 7, 9
			<i>Bifidobacterium longum</i>	Bif.	3
			<i>Bifidobacterium catenulatum</i>	Bif.	3, 10
			<i>Streptococcus bovis</i>	Streptoc.	3, 4
			<i>Streptococcus</i> group	Streptoc.	10
<i>Staphylococcus aureus</i>	Staphy.	10	<i>Leuconostoc argentinum</i>	Leu.	3
			<i>Leuconostoc mesenteroides</i>	Leu.	3

T-RFs commonly decreased after LKM512 yogurt/placebo consumption are shown in bold.

*The abbreviation of the bacterial group is the same as in Table 5.

†Volunteer number that bacterial species or phylotypes were detected.

in particular *Bifidobacterium* spp., is known to hydrolyse the ingredients (pro-drugs) in Kampo-medicine herbs to active metabolites, and an increase in these bacterial levels was observed in all the patients after LKM512 yogurt consumption. We have included information about the herbs, their inactive ingredients (pro-drugs), and active metabolites in Table 1 [41]. This indicates that (1) all patients were administered Kampo medicines that can be utilized as pre-biotics by *Bifidobacterium* spp. and (2) active metabolites may be produced from Kampo medicines by altered intestinal microbiota, in particular *B. animalis* subsp. *lactis* LKM512 and *Bifidobacterium* spp.

Because all the patients enrolled in this study had been undergoing long-term Kampo treatment, all effects observed cannot be attributable to LKM512 yogurt consumption. However, at least during the study period, the patients were prescribed identical Kampo medicines. Furthermore, considering the fact that the fecal microbiota was dramatically altered after the 4-week consumption of LKM512 yogurt (Tables 5 and 6), we believe that

almost all the effects were probably attributable to this consumption.

AD patients have increased intestinal permeability. Therefore, a weakened intestinal barrier function permits the frequent invasion by antigens and induces intractable AD [18, 20, 21]. In fact, Rosenfeldt et al. [42] suggested that pro-biotic supplementation may stabilize the intestinal barrier function and decrease gastrointestinal symptoms in children with AD. Polyamines influence the maturation of post-natal intestinal tissue and, in particular, intestinal enzyme activity and mucosal barrier functions. Capano et al. [43] reported that the permeability of antigens tended to be decreased by SMD administration with gavage in neonatal rats. We believe that as the fecal SMD concentration was increased, the intestinal barrier function of AD patients was recovered by LKM512 yogurt consumption for protection against the invasion by antigens. To our knowledge, there is little information supporting the relationship between intestinal polyamines and allergies; however, Peulen et al. [44] reported that

children who had suffered from allergies when they were 5 years old consumed milk containing less SPM and SMD than children who were not allergic at the same age. In our previous study, we analysed the fecal microbiota of AD patients and healthy adults using T-RFLP. The fecal microbiota of volunteers was divided into two clusters: A and B; the number of AD patients was found to be higher in cluster B than in cluster A. In addition, the fecal SMD concentration in cluster B was statistically lower than that in cluster A [45]. Intestinal SCFA is also associated with intestinal barrier function. While polyamines contribute to the maturation of the intestinal mucosal layer, SCFA influences the maintenance of intestinal epithelial cells under the mucosal layer [27, 46]. In particular, butyrate has an important role in the metabolism and normal development of colonic epithelial cells [47, 48] and induces the inhibition/recovery of damages of the intestinal epithelial cell layer [26, 28]. In this study, fecal butyrate, propionate, and acetate tended to be increased by LKM512 yogurt consumption. Therefore, the increase in intestinal polyamines and SCFA due to LKM512 yogurt consumption promotes the recovery of the intestinal mucosal barrier function and inhibition of the invasion of antigens from the intestine.

Exogenous polyamines in meals are absorbed mainly in the upper parts of the gut [49, 50], and thus, most of the polyamines in the lower parts of the gut are likely to be synthesized by the intestinal bacteria [51, 52]. We presume that the predominant intestinal bacteria that proliferate in the intestine are associated with polyamine production, because polyamines serve as one of the cell growth factors. Therefore, there is a high possibility that the bacterial species increased by LKM512 yogurt consumption produce polyamines in the intestine. Firstly, *B. animalis* subsp. *lactis* probably produces polyamines in the intestine because this bacterium was detected from the feces obtained from nine of 10 patients, and the area of T-RF including this bacterium is increased dramatically. In our previous study, we provided this yogurt to elderly volunteers and confirmed the temporary colonization of this pro-biotic strain, which constitutes 10% of total bacterial count of the fecal microbiota using the cultivation method [53]. Secondly, other commensal bacteria dramatically increased after LKM512 yogurt consumption also appear to contribute to the intestinal polyamine production. Many bacterial species in *Clostridium* cluster IV, subcluster XIVa, and *Bifidobacterium* spp. in Table 5 appear to be involved in polyamine production. *R. obeum* and its relatives, *Ros. intestinalis*, and *E. ventriosum* in *Clostridium* subcluster XIVa and *Bifidobacterium* spp. were predicted to be polyamine-producing bacteria in our previous report [54], supporting the finding that the bacterial species increase after LKM512 yogurt consumption is associated with the intestinal polyamine production.

Most of SCFAs in the lower parts of the intestine are also likely to be synthesized by intestinal bacteria from meal ingredients, such as polysaccharides [46]. The increase in intestinal SCFA appears to be produced by bacterial species increased by LKM512 yogurt consumption. However, *B. animalis* subsp. *lactis* was probably not associated with the tendency towards increase of SCFA, because the genus of *Bifidobacterium* does not produce butyrate and propionate [55]. In contrast, *F. prausnitzii*, *Ros. intestinalis*, butyrate-producing bacterium L1-93, and butyrate-producing bacterium T2-145, which were isolated from human feces as butyrate-producing bacteria and their relatives (uncultured bacterial clones adhuhe13, HuCA11, HuCB1, and p-5459-2Wb5), were increased by LKM512 yogurt consumption, indicating that intestinal butyrate was probably produced by these bacteria. In our previous study, we confirmed that increased amounts of *F. prausnitzii* were detected from healthy adults than from adult AD patients using the 16S rRNA gene clone library study (M. Matsumoto et al., unpublished data). This finding and the obtained data suggest that *F. prausnitzii* contributes to the alleviation of AD through the production of butyrate. On the other hand, the influence of Kampo medicines that contain a considerable amount of polysaccharides is not ignored because colonic SCFA are produced by the intestinal bacteria from polysaccharides [46]. In fact, in our previous study, fecal SCFA was not increased by LKM512 yogurt consumption in healthy adults [33], suggesting that the increase in SCFA in this study depended on the synergic effects of LKM512 yogurt and Kampo medicines. Further studies are required to investigate the relationship between LKM512 yogurt and Kampo medicines.

Intestinal bacterial species that absorb/utilize polyamines and SCFA also inhabit the intestine. Therefore, it may be possible that the increase in polyamines and SCFA was induced by the decrease in the numbers of these bacteria. However, few bacterial species were found to be commonly decreased by LKM512 yogurt consumption; this possibility was denied in this case.

Many studies reported that the Th1/Th2 balance tended towards the Th1-type owing to the consumption of probiotics [9–11]. This benefit probably depends on two factors, namely, the pro-biotic bacterial cell components and metabolites produced by pro-biotics or the altered intestinal microbiota. The fact that the former stimulates lymphocytes has been demonstrated using cell culture systems [16, 17]. We agree that pro-biotics can directly stimulate the immature immune system during infancy. However, it is difficult to consider that pro-biotics can stimulate the mature immune system of adults who have developed immune tolerance and complex intestinal microbiota; it is natural that the improvement of Th1/Th2 balance in adult-type allergic patients due to pro-biotics depends on the metabolites produced by the improved

intestinal microbiota. In fact, we confirmed that the consumption of LKM512 yogurt produces anti-inflammatory metabolites in the intestine [56]. Seiler and Atanassov [57] reported that polyamines are involved in the differentiation and the proliferation of lymphocytes. We also confirmed that Th2-type cytokines (IL-4 and IL-10) secreted by human peripheral blood mononuclear cells stimulated with ConA were decreased in a dose-dependent manner by SPM (1–100 μ M) (M. Matsumoto et al., unpublished data). This suggests that the induction of the Th1-type cytokine due to LKM512 yogurt consumption is induced by intestinal polyamines produced by the altered intestinal microbiota.

From these findings, we conclude that LKM512 yogurt consumption may be effective against intractable adult-type AD, and this effect depends on the recovery of the intestinal mucosal barrier function and the induction of the Th1-type cytokine by polyamines and SCFA, particularly butyrate, produced by the altered intestinal microbiota.

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