Original Papers

Preparation of goat milk anti-*Saccharomyces cerevisiae* immunoglobulin G (IgG)-rich fraction and immunological functions of mice orally administered a mixture of the antigen and its specific IgG-rich fraction

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Abstract

In the current study, we investigated the effects of *Saccharomyces* (*S.*) *cerevisiae* and its specific immunoglobulin G-rich fraction (anti-yeast IgG) prepared from goat's milk on the mouse immune system. Real-time polymerase chain reaction (PCR) analysis of mRNA extracted from C3H/HeN mouse Peyer's patch cells revealed that the expression of *Rnf128* on regulatory T cells was higher in the cells cultured with anti-yeast IgG alone or with a mixture of *S. cerevisiae* and anti-yeast IgG than in the cells cultured with *S. cerevisiae* alone. In contrast, the expression of *Stat6* related to polarize type 2 helper T (Th2) cells was lower in the cells cultured with the mixture than *S. cerevisiae* alone, although that was higher than IgG alone. Hence, 5-week-old C3H/HeN mice were orally administered with either saline solution (control) group or a mixture of *S. cerevisiae* and anti-yeast IgG in saline (test) group once a day for 5 weeks. We found total serum IgG levels to be significantly lower in mice administered the test solution than those that were given the control solution. Microarray analysis of mRNA extracted from the mouse Peyer's patch cells revealed that the expression profile of genes related to proliferation and differentiation of B cells, T cell activation and differentiation of Th2 cells was lower in the test mice than in the control group. In contrast, the genes related to regulatory T cells were more highly expressed in mice administered with the test solution. Moreover, oral administration of the test solution was found to reduce allergic symptoms in NC/Nga mice induced with a mite antigen. The number of spleen interleukin-4$^+$CD4$^+$ cells was reduced in test mice when compared to the control group. These findings indicate that oral administration of the mixture of *S. cerevisiae* and its specific goat milk IgG-rich fraction may suppress the development of type I allergic symptoms in mice.

Key words: anti-yeast IgG, oral administration, C3H/HeN and NC/Nga mice, microarray analysis, anti-allergic effect.

1. Introduction

Milk is the primary food source for mammalian neonates and contains high levels of physiologically active and nutritional components. The best characterized physiologically active components present in milk are the anti-infectious proteins including immunoglobulin (Ig) G, secretory IgA, lac-

toferrin and lysozyme. These components are thought to support the immature immune system of the neonate.

It has been reported that some cow milk proteins and their digestion products influence the development of the immune system. Wong and Watson[1] reported that oral ingestion of cow's milk whey proteins enhances the effects of secondary humoral antibody response in mice. Otani et al.[2,3] found that dietary casein phosphopeptides (CPP) enhance intestinal IgA response to peritoneally and orally administered antigens in both piglets and mice, while Kitamura et al.[4] discovered that oral

administration of CPP by pregnant sows results in higher observed levels of colostral IgA and IgG than in sows who were not administered CPP during pregnancy. Moreover, Yoshioka et al.[5] demonstrated that colostrum produced during the first 4 days of post-parturition directly stimulates the intestinal intraepithelial lymphocytes to polarize type 1 helper T (Th1) cells.

More recently, Ohnuki et al.[6,7] identified that oral administration of cow's milk IgG suppresses immunoglobulin production through the binding of Escherichia (E.) coli and its specific IgG on antigen-presenting cells via Fcγ receptor IIb (FcγIIbR) in mice. Sueda and Otani[8] demonstrated via microarray analysis of mRNA extracted from mouse Peyer's patch cells that the expression profile of genes related to Ig production and the development of immune diseases is reduced in mice administered a mixture of E. coli and its specific cow's milk IgG in their diet when compared to those not fed this mixture. In contrast, gene expression of marker proteins on Th1, Th3 and negative regulatory T cells was found to increase significantly. These findings suggest that the oral administration of a mixture of edible microorganisms such as yeasts for bread-making (Saccharomyces (S.) cerevisiae) or beer-making (S. carlsbergensis) and their specific milk IgG fraction suppresses acquired humoral immune responses and reduces development of type I allergic symptoms.

In the current study, we prepared an anti-S. cerevisiae IgG–rich fraction (anti-yeast IgG) from milk produced by Shiba goats that had been immunized with a commercial yeast (S. cerevisiae) powder for bread-making, and investigated the effect of S. cerevisiae and anti-yeast IgG on immunological functions of 2 strains of mice.

2.　Materials and Methods

2.1　Materials

A mite allergen extracted from Dermatophagoides farinae (Df-extract) was obtained from LSL (Tokyo, Japan). Complete Freund's adjuvant was purchased from Nacalai Tesque (Kyoto, Japan). Bovine serum albumin (BSA, fraction V) and tetramethylbenzene (TMB) were purchased from Sigma Chemical (St. Louis, MO). Horserad-ish peroxidase-conjugated rabbit anti-goat IgG (H +L) was obtained from Ana Spec, Inc. (San Jose, CA). Defined fetal bovine serum (FBS) was obtained from HyClone Laboratories (Road Logan, UT). RPMI–1640 medium was purchased from Nissui Pharmaceutical (Tokyo, Japan). Biolegend (San Diego, CA) supplied phycoerythrin (PE)–labeled anti-mouse interleukin (IL)–4 monoclonal antibodies (mAb, clone 11B11), PE–labeled anti-mouse interferon (IFN)–γ mAb (clone XMG1.2), biotin-labeled anti-mouse CD4 mAb (clone RM4–5) and phycoerythrin/cyanine 5 (PE/Cy5)–labeled streptavidin. Brefeldin A (BFA), ionomycin and phorbol 12–myristate 13–acetate (PMA) were obtained from Wako Pure Chemical Industries (Osaka, Japan). IntraPrep Permeabilization Reagent was bought from Beckman Coulter Inc. (Tokyo, Japan). Penicillin G potassium and streptomycin sulfate were obtained from Meiji Seika (Tokyo, Japan). Other chemicals were of the highest analytical grade commercially available.

2.2　Yeast preparation

A commercial yeast (S. cerevisiae) powder for bread-making was obtained from Hoshino Natural Leaven (Tokyo, Japan). S. cerevisiae JCM7255 was obtained from the Japan Collection of Microorganisms (JCM, Saitama, Japan). The yeast was inoculated in YM broth containing 1% glucose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract (pH 6.2), cultivated for 72 h at 25℃, collected by centrifugation, washed 3 times with sterile saline and lyophilized.

2.3　Preparation of anti-yeast IgG

Two Shiba goats (Capra hircus) were immunized with an intradermal injection of 10 mg commercial yeast powder suspended in an equal mixture of sterile saline solution and complete Freund's adjuvant. The immunization was carried out a total of 10 times at 7–day intervals approximately 3 months prior to delivery. Milk was then collected every day from delivery to 60 days postpartum. The collected milk was centrifuged at 1,200 × g for 30 min at 4℃ to obtain skim milk. The skim milk was adjusted to pH 4.6 with 1 M HCl. The whey was collected by centrifugation at 1,200 × g for 20 min at 4℃, and 40%–saturated with am-
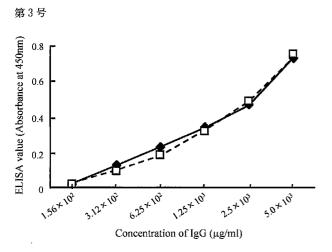
Fig. 1 Antigenic reactivity of *S. cerevisiae* with colostral IgG prepared from pooled milk produced during the first 7 days of post-parturition (□), or mature milk IgG prepared from pooled milk produced between 8 and 63 days post-parturition (◆).

monium sulfate. The precipitate was collected by centrifugation, dissolved in a small amount of distilled water, dialyzed against tap water and lyophilized. Both the lyophilized powders prepared from pooled milk produced during the first 7 days of post-parturition (colostral IgG-rich fraction) and prepared from pooled milk produced between 8-63 days post-parturition (mature milk IgG-rich fraction) were determined approximately 0.89 mg IgG per mg by a single radial immunodiffusion analysis. As the reactivity of *S. cerevisiae* with the colostral IgG-rich fraction was almost same to that of the mature milk IgG-rich fraction (Fig. 1), the colostral IgG-rich fraction and the mature milk IgG-rich fraction were pooled and used as anti-yeast IgG in the experiments outlined in this study.

2.4 Mice

Pathogen-free 4-week-old male or 6-week-old male C3H/HeN and 4-week-old male NC/Nga mice were obtained from Japan SLC (Shizuoka, Japan) and Charles River Japan (Kanagawa, Japan), respectively. All animal experiments were conducted in accordance with the Guidelines for Regulation of Animal Experimentation at Shinshu University, and Law No.105 and Notification No.6 of Japan.

2.5 Preparation of spleen and Peyer's patch cell suspensions, and cell cultures

The mice were sacrificed by vertebral disloca-

tion and their spleens were removed aseptically. The spleen and Peyer's patch cell suspensins were prepared according to the procedure described previously[7]. The resulting cell suspension was washed three times in RPMI-1640 medium containing 5% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin and adjusted to 1×10^6 viable cells /ml. 1,000 μl of the Peyer's patch cell suspensions prepared from 6-week-old male C3H/HeN mice was then plated into the wells of a 24 well flat bottom plate (Sarstedt, Inc, Newton, NC) and 100 μl of *S. cerevisiae* or its specific goat milk IgG solution. The final concentration of *S. cerevisiae* and anti-yeast IgG in the RPMI-1640 medium was 0 or 100 μg/ml. The cells were cultured at 37°C in a humidified 5% CO_2 incubator for 6 h for analysis of mRNA expressions.

2.6 Feeding procedure

The mice were assigned to the test regimes for either 1 (C3H/HeN) or 2 weeks (NC/Nga). They were then further divided into 2 groups (6 mice/group for C3H/HeN; 5 mice/group for NC/Nga), in which they were orally administered 0.5 ml of sterile saline solution (control) or 0.5 ml of sterile saline solution containing a mixture of 0.2 mg *S. cerevisiae* and 0.2 mg milk anti-yeast IgG (test) once a day. C3H/HeN mice were given the solution for 5 weeks (5-10 weeks of age), while NC/Nga mice were given the solution for 15 weeks (6-21 weeks of age). Mite allergy in NC/Nga mice was induced according to the description of Sasakawa et al.[9]. Briefly, the mice were injected intradermally into the ventral side of their right ear and their dorsum with 5 μg Df-extract dissolved in 10 μl sterile saline weekly from 7 to 21 weeks of age. The thickness of the ear injected with Df-extract was measured weekly prior to the first injection and 24 h after each intradermal injection. Mice were supplied with commercial mouse pellets (MF, Oriental Yeast Company, Tokyo, Japan) from stainless-steel feeders and water *ad libitum* through out of the course of the experiment. The mice were housed at 22±2°C under a 12-h light-dark cycle. Blood, intestine, Peyer's patch and spleen specimens were collected immediately following a lethal dose of ether at 10 (C3H/HeN) or 21 weeks of age (NC/Nga). Serum was obtained

by centrifugation at 1,000×g for 15 min at 4℃ and stored at −30℃ until use. Intestinal extract, Peyer's patch and spleen cell suspensions were prepared as described below, and stored at −30℃ until required.

2.7 Allergic score

Allergic symptoms in the ear of NC/Nga mice injected with Df-extract were scored in accordance to the description of Matsuda et al.[10]. The degree of erythema, hemorrhage, edema, scab/excoriation and scaling/dryness was scored from 0 points (none) to 2 points (severe). The ear was observed weekly and at random by 2 scorers, and each mean score was calculated. The sum of these scores was defined as the allergic score.

2.8 Preparation of intestinal extract

A 1 g sample of intestinal tract tissue (duodenum to rectum) including contents was ground using a pestle for 20 min at 2±1℃ with sea sand (1 g) in 2.5 ml of 0.01 M sodium phosphate buffer (PBS, pH 7.2) containing 0.15 M sodium chloride. The ground material was then centrifuged at 1,200 ×g for 30 min at 4℃ and the supernatant collected.

2.9 Analysis of antibodies

Total mouse serum and intestinal IgG levels were measured using a mouse ELISA quantitation kit, respectively, in accordance with the manufacturer's protocol. *S. cervisiae* (yeast)-specific IgG levels in goat milk IgG-rich fractions were measured using ELISA as follows. The wells of microplates (Nunc, Roskilde, Denmark) were coated with 100 μl *S. cervisiae* JCM7255 (100 μg/ ml) in 0.1 M carbonate buffer (pH 9.6) overnight at 4℃ and washed with PBS containing 0.05% Tween 20 (PBS-T). The plate was then post-coated with 300 μl of 0.1 Mcarbonate buffer (pH 9.6) containing 0.4% bovine serum albumin for 2 h at 25℃ and washed with PBS-T. One hundred microliters of goat milk or IgG sample was then added to each well and the plate incubated for 2 h at 25℃. After washing the plate as above, 100 μl of diluted horseradish peroxidase-conjugated anti-goat IgG was added to each well. The plate was then incubated for 1 h at 25℃ and washed in PBS-T after which 100 μl of 3, 3′, 5, 5′-tetramethyl ben-

zidine was added to the wells for 30 min at 25℃, and the reaction stopped by adding 100 μl 4 N H_2SO_4. Yeast-specific IgG levels were then read at 450 nm on Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA). Results are presented as ELISA values at A450 nm.

2.10 Preparation of total RNA and real-time polymerase chain reaction (PCR)

Analysis of mRNA expression was measured using real-time PCR. Total RNA from Peyer's patch cells was extracted using TRIzol Reagent (Invitrogen, Carsbad, CA) in accordance to the manufacturer's recommendations. The reaction was carried out by adding 1 mM of each dNTP, 2.5 units/μl M-MLV reverse transcriptase and 10 pmol/μl oligo d $(T)_{18}$ primer to 0.5 μg of total RNA, before incubating at 42℃ for 50 min. Q-PCR was conducted using a Thermal Cycler Dice Real Time TP800 system (Takara Bio, Tokyo, Japan) using 2xSYBR premix Ex Taq mixture according to the manufacturer's instructions. The primer sequences for amplifying *Rnf128*[11], *Stat6*[12] and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)[13] were as follows: *Rnf128* (forward): 5′-GCGCAG-TCAGCAAATGAA-3′, *Rnf128* (reverse): 5′-TGTCAACATGGGGAACAACA-3′, *Stat6* (forward): 5′-CTCTGTGGGGCCTAATTTCCA-3′, *Stat6* (reverse): 5′-CATCTGAACCGACCAGGAACT-3′, *GAPDH* (forward): 5′-CCACAGTCCATGCCATCACTGCC-3′, *GAPDH* (reverse): 5′-GGTCCACCACCCTGTTGCTGTAG-3. The PCR reaction involved 40 cycles of 95℃ for 5 s and 60℃ for 30 s. The relative amount of each mRNA was normalized using *GAPDH* expression as an internal control. An expression index was calculated from the normalized relative amount in the absence of *S. cerevisiae* and anti-yeast IgG to the normalized relative amount in the presence of *S. cerevisiae*, anti-yeast IgG, or a mixture *S. cerevisiae* and anti-yeast IgG. This analysis was carried out at least in triplicate and representative results are presented.

2.11 Cell function analysis

Spleen cells were cultured at 37℃ in RPMI-1640 medium supplemented with 10% FBS, 100 U /ml penicillin, 100 μg/ml streptomycin, 20 μg/ml BFA, 2 μg/ml ionomycin and 20 ng/ml PMA for 4

h. The cell surface markers for CD4 were then labeled using biotin-conjugated anti-mouse mAb for 15 min at 4℃, followed by incubation with PE/Cy5 -labeled streptavidin for 15 min at 4℃. Intracellular cytokines were measured following permeabilization and labeling with a PE–labeled anti-mouse cytokine mAb specific for IL–4 or IFN–γ. In order to achieve this, cells were fixed with IntraPrep reagent 1 for 15 min, washed and permeabilized with IntraPrep regent 2. The cells were then incubated with appropriate antibodies and intracellular cytokine levels determined using a Guava personal cell function analyzer (Guava PCA: Guava Technologies, Hayward, CA).

2.12 Microarray analysis

The genome-wide gene expression profile of Peyer's patch cells was examined using the Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA), that contains 45101 probe sets for approximately 34,000 mouse genes. Total RNA was extracted from Peyer's patch cells using TRIzol reagent and GeneChip analysis performed according to the GeneChip Eukaryotic Target Preparation & Hybridization Manual (Affymetrix). Data analysis was performed with GeneChip Operating software 1.4 (Affymetrix). Expression data was selected when there was more than a 1.5–fold difference or less than a 0.6–fold difference between the control and the test groups.
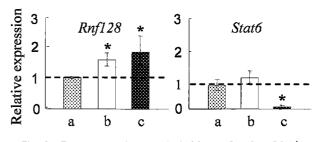
2.13 Statistical analysis

Data is expressed as mean ± standard deviation. Statistical differences were tested using the Student's t-test, and a p value < 0.05 was considered significant.
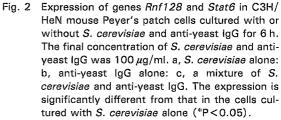
3. Results and Discussion

3.1 Effect of yeast, anti-yeast IgG, and a mixture of yeast and anti-yeast IgG on expression of

Rnf128 and *Stat6* in C3H/HeN mouse Peyer's path cell cultures

Figure 2 shows the expression of *Rnf128* and *Stat6* mRNA on C3H/HeN mouse Peyer's patch cells cultured in the presence of *S. cervisiae*, anti-yeast IgG, and a mixture of *S. cervisiae* and anti-

Fig. 2 Expression of genes *Rnf128* and *Stat6* in C3H/ HeN mouse Peyer's patch cells cultured with or without *S. cerevisiae* and anti-yeast IgG for 6 h. The final concentration of *S. cerevisiae* and anti-yeast IgG was 100 μg/ml. a, *S. cerevisiae* alone: b, anti-yeast IgG alone: c, a mixture of *S. cerevisiae* and anti-yeast IgG. The expression is significantly different from that in the cells cultured with *S. cerevisiae* alone (*P<0.05).

yeast IgG. The expression of *Rnf128* expressed on regulatory T cells[11] was significantly higher in the cells cultured with anti-yeast IgG alone (1.6–fold) or with a mixture of *S. cerevisiae* and anti-yeast IgG (1.8–fold) than in the cells cultured with *S. cerevisiae* alone. In contrast, the expression of *Stat6* related to porlize Th2 cells[12] was significantly lower in the cells cultured with the mixture (0.25–fold) than in the cells cultured with *S. cerevisiae* alone, although that was slightly higher in the cells cultured with IgG alone (1.25–fold). These results suggest that the mixture of *S. cerevisiae* and anti-yeast IgG suppresses antibody responses. We have reported that antigen-free milk IgG stimulates the production of immunoglobulins by binding to Fcγ receptor type I, whereas antigen-bound milk IgG suppresses it by binding to Fcγ receptor type IIb[7]. Thus, the suppression of antibody responses may be due to the complex of *S. cerevisiae* and its specific IgG in anti-yeast IgG, suggesting that oral administration of the mixture of *S. cerevisiae* and anti-yeast IgG may possess anti-allergic effect in mice.

3.2 Immunological properties of C3H/HeN mice orally administered with a mixture of yeast and anti-yeast IgG

Mice orally administered with the test solution demonstrated similar gains in body weight as those administered control solution (data not shown). This result suggests that there was little difference in both the nutritional value and the stress levels between mice given the control solution and those
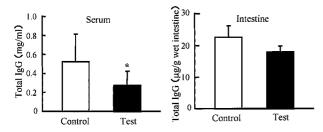
Fig. 3 Serum and intestinal total IgG levels in C3H/HeN mice administered with the control or test solution for 5 weeks. The data is presented as mean ±standard deviation (n=6). Serum values are significantly different from that of mice given the control solution (*P<0.05).

Fig. 4 Gene expression related to immune system in the Peyer's patch cells of C3H/HeN mice administered with the control or test solution.

administered the test solution. As mice housed under stressful conditions generally gain less weight than their control counterparts, the differences between the effects of the control and test solutions in the current study was considered to reflect the physiological function of the test solution.

Figure 3 presents the serum and intestinal IgG levels in C3H/HeN mice given control or test solution for 5 weeks. Serum IgG levels were found to be significantly reduced in mice administered the test solution (0.28±0.15 mg/ml) when compared to mice given the control solution (0.52±0.29 mg/ml; $P < 0.05$). The intestinal IgG levels were also noticeably reduced in mice administered test solution (test, 17.9±2.0 μg/g wet intestine; control, 22.7±3.5 μg/g wet intestine). Similarly, the intestinal IgA levels also tended to decrease in mice administered test solution, although serum IgE levels were little difference because of small amounts. These results suggest that oral ingestion of the mixture of *S. cerevisiae* and anti-yeast IgG suppresses immunoglobulin production.

Figure 4 shows that there was a less than 0.6-fold decrease in the levels of transcription factor genes associated with immune response in the Peyer's patch cells of test mice compared with control mice. Transcriptomes representing *Fos, Jun, Lck, Nfatc1, Btg3, Nf-kb2, Itk, Ikkb* and *Stat6* were decreased by less than 0.6-fold in mice administered test solution compared with those given the control solution. In contrast, transcriptomes representing *FcyrIIb, Btla, Muc13, Il-18, Ccl25, Leap2, Rnf128, Muc3, Ccl21* and *Defcr5* increased by greater than 1.5-fold in test mice. The proteins produced by transcription of *Fos, Jun, Lck, Nfatc1,*
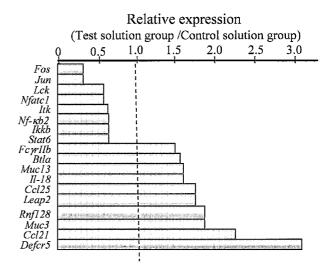
Btg3, Nf-kb2, Itk, Ikkb and *Stat6* are Fos, Jun, leukocyte-specific protein tyrosine kinase (Lck), nuclear factor of activated cells (NFAT) c1, nuclear factor kappa B (NF-κB) 2, IL-2-inducible T cell kinase (Itk), 3-phosphoinositide-dependent protein kinase-1-mediated IkappaB kinase beta (IkkB) and signal transducer and activator of transcription (Stat) 6, respectively. Similarly, the proteins produced by transcription of *FcyrIIb, Btla, Muc13, Il-18, Cc25, Leap2, Rnf128, Muc3, Ccl21* and *Defcr5* are Fcγ receptor IIb (FcγrIIb, CD32), Btla, mucin 13, IL-18, chemokine (C-C motif) ligand 25 (CCL25), liver-expressed antimicrobial peptide 2 (Leap2), ring finger protein 128 (GRAIL), mucin 3, chemokine (C-C motif) ligand 21 (CCL21) and defensin related cryptdin 5, respectively.

The activation of NF-κB by T cell receptor (TCR) signaling is crucial for T cell activation during the adaptive immune response, while IkkB is essential for rapid NF-κB activation by proinflammatory signaling cascades[14]. Lck is a member of the Src family of protein tyrosine kinases and is essential for T cell activation triggered by receptor ligation[15]. The expression of CD80 is essential for humoral immune responses and is suppressed by the blockage of FcγIIb [16]. IL-18 plays an important role in the induction and/or maintenance of regulatory T cells[17]. Given these previous reported findings, the results arising from our study sug-

gest that oral ingestion of a mixture of *S. cerevisiae* and anti-yeast IgG suppress adaptive immune responses. The pro-inflammatory transcriptional element activator protein-1 (AP-1) is an important contributor to expression of the asthma-relevant Th2 cytokines IL-4, IL-5 and IL-13. AP-1 comprises variable heterodimers of Jun and Fos family members[18], and is required for FcεR-mediated degranulation in mast cells. Hence, deficiency of c-Fos, a component of the AP-1 pathway, markedly inhibits FcεR-induced degranulation[19]. The NFAT family of transcription factors is important for the rapid induction of IL-4 gene expression in effecter T cells[20]. Stat 6 regulates Th2 cell activity by controlling the expression and responsiveness to IL-4, the regulation of which plays a key role in numerous allergic maladies[21]. Itk is known to be produced by T cells and mast cells, and a reduction of Itk production reduces IgE -induced histamine release[22]. GRAIL expression is increased in naturally occurring CD4+CD25+ T regulatory cells and is linked to their functional regulatory activity[11]. These findings therefore suggest that oral ingestion of a mixture of *S. cerevisiae* and anti-yeast IgG also reduce type I allergic reactions.

On the other hand, CCL21 and CCL25 are small cytokines belonging to the CC-chemokine family. CCL21 stimulates the phagocytic activity of dendritic cells[23], while CCL25 plays essential roles in intestinal homing of IgA antibody-secreting cells, primarily by mediating their extravasation into the intenstinal lamina propria[24]. Defensins and LEAP -2 are antimicrobial peptides with broad-spectrum activities[25,26]. Defensins play an important role in innate immunity and are known to contribute to the regulation of host adaptive immunity[27]. Leap2 is produced in tissues including the intestinal tissues and is known to function as the body's "natural antibiotic"[26]. Mucin 3 belongs to a family of heavily glycosylated proteins that protect epithelial membranes[28], while mucin 13 is a transmembrane mucin expressed at the epithelial surface of the gastrointestinal tract[28]. These mucins are known to play an important role in intestinal barrier formation.[27] These findings suggest that oral ingestion of a mixture of *S. cerevisiae* and anti-yeast IgG enhances mucosal immunity, in particular, in-

nate intestinal immunity. Thus, the microarray data arising from the current study indicates that the oral ingestion of a mixture of *S. cerevisiae* and anti-yeast IgG may suppress adaptive immune responses, reduce type I allergic reactions and enhance innate mucosal immunity in mice.

3.3 Allergic symptoms of NC/Nga mice orally administered a mixture of *S. cerevisiae* and anti-yeast IgG

Figure 5 presents the ear thickness (left panel) between 4 and 21 weeks of age and the allergic score at 21 weeks of age (right panel) in NC/Nga mice orally administered with control or test solutions. Ear thickness was noticeably reduced in mice administered the test solution when compared to mice given the control solution. The allergic score at 21 weeks of age was significantly ($P < 0.05$) lower in the test mice (1.7 ± 0.6) than the control group (3.4 ± 0.9).

Figure 6 shows the number of IFN-γ+CD4+ and IL-4+CD4+ cells in the spleen of 21-week-old mice. We observed a significantly smaller number of IL-4+CD4+ cells in mice administered test solution ($0.58 \pm 0.35 \pm 10^4/10^6$ spleen cells) when compared to the control group ($1.29 \pm 0.19 \times 10^4/10^6$ spleen cells, $P < 0.05$). In contrast, the number of IFN-γ+CD4+ cells to be appeared similar in the mice given the control ($2.59 \pm 0.68 \times 10^4/10^6$ spleen cells) and test solutions ($2.38 \pm 0.30 \times 10^4/$
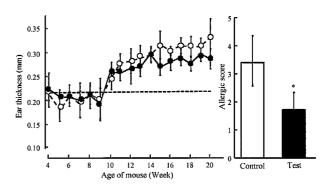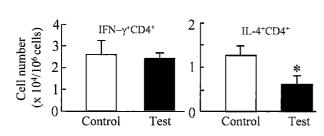

Fig. 5 Ear thickness (left panel) and allergic scores (right panel) of NC/Nga mice administered with the control (○) or test solution (●). Data is presented as mean ± standard deviation (n = 5). The allergic score value is significantly reduced in the mice given the test solution (*P < 0.05). Hashed line represents the ear thickness of mouse before administration of the control and test solutions.

Fig. 6　Helper T cell subsets in the spleens of NC/Nga mice administered the control or test solution. Results are presented as mean±standard deviation from experiments performed in triplicate. The cell number is significantly reduced from that of mice given the control solution (*P<0.05).

10^6 spleen cells). NC/Nga mice are widely accepted as a model of human atopic dermatitis. These mice develop atopic dermatitis-like skin lesions under conventional conditions, the symptoms of which are increased following intradermal injection of mite antigens. Sasakawa et al.[9] reported that regional inflammation in the ears of mite-injected NC/Nga mice was the result of antigen cross-linking between IgE molecules and FcεRI on mast cells. IL-4, IL-5 and IL-13 produced by Th2 cells are known to stimulate IgE production[29]. Thus, it is suggested that a reduction in allergic symptoms following oral ingestion of a mixture of S. cerevisiae and anti-yeast IgG may lead in part to the suppression of Th2 immune response.

In conclusion, the present findings suggest that the oral ingestion of the mixture of S. cerevisiae and its specific goat milk IgG-rich fraction suppresses type I allergic reactions via the suppression of genes relating to T cell activation during the adaptive immune response. Furthermore, it appears that this process also suppresses development of type I allergic symptoms via the stimulation of genes associated with T regulatory cells and via the suppression of Th2 immune response, although it remains to be investigated whether oral ingestion of S. cerevisiae alone improves atopic dermatitis in NC/Nga mice. In addition, it is suggested that the oral ingestion of the mixture may stimulate the innate immune system via the expression of proteins relating to the intestinal innate immune systems in mice.

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Saccharomyces cerevisiae に対するヤギ乳 IgG 画分の調製と
その酵母と IgG 画分を経口摂取したマウスの免疫機能

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　本研究では, *Saccharomyces* (*S.*) *cerevisiae* (酵母) とヤギ乳から調製した酵母に対する IgG を豊富に含む画分 (抗-酵母 IgG) のマウスの免疫系に及ぼす作用を検討した。まず, C3H/HeN 系マウスのパイエル板細胞に, 酵母単独, 抗-酵母 IgG 単独または酵母と抗-酵母 IgG の混合物を添加して培養したところ, 酵母単独の場合と比べて, 抗-酵母 IgG 単独および酵母と抗-酵母 IgG 混合物を添加した場合は制御性 T 細胞に発現する *Rnf128* レベルが有意に増加し, 2 型ヘルパー T (Th2) 細胞優位に導く *Stat6* レベルが酵母と抗-酵母 IgG 混合物添加では顕著に低下し, 抗-酵母 IgG 単独では僅かに増加することがリアルタイム PCR 法により確認された。そこで, 同系統のマウスに酵母と抗-酵母 IgG 混合物を経口投与し, 血清 IgG レベルを調べたところ, 非投与群と比べて顕著に低いことが示された。また, それら両群のマウスパイエル板細胞の遺伝子の網羅的解析を行ったところ, 非投与群と比べて混合物投与群では B 細胞の増殖や分化および T 細胞の活性化や Th2 細胞優位に導く遺伝子の発現が低下し, 制御性 T 細胞に発現する *Rnf128* の発現が上昇していた。さらに, 酵母と抗-酵母 IgG 混合物を I 型アレルギーモデルマウスに経口投与すると, 非投与群と比べてアレルギー症状が軽減される傾向にあり, IL-4$^+$CD4$^+$ (Th2) 細胞数が有意に減少した。これらの結果は, *S. cerevisiae* とそれに対するヤギ乳 IgG の混合物の経口摂取はマウスにおいて I 型アレルギーを抑えることを示している。