Regular Paper

Anti-allergic properties of a matured fruit extract of the date palm tree (*Phoenix dactylifera* L.) in mite-sensitized mice

Food Science

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ナツメヤシ (*Phoenix dactylifera* L.) 果実 (デーツ) 抽出物のダニ感作マウスにおける抗アレルギー作用

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要約 ナツメヤシ (*Phoenix dactylifera* L.) 果実 (デーツ) の熱水抽出物の経口摂取が, ダニ感作マウスのアレルギー反応に及ぼす影響を調べた。デーツ抽出物添加飼料で飼育したマウスのくしゃみと鼻の引っ掻き回数は, 抽出物無添加 (コントロール) 飼料で飼育した場合と比較して有意に減少した。また, デーツ抽出物添加飼料で飼育したマウスの血清総およびダニ特異 IgE レベル, 並びに脾臓の IL-4$^+$CD4$^+$ 細胞数, IgE$^+$B220$^+$ 細胞数および FcεRIα$^+$CD117$^+$ 細胞数は, コントロール飼料で飼育した場合と比較して有意に減少した。マウス脾臓細胞培養系において, クロロゲン酸, ペラルゴニンおよびフェルラ酸は IgE$^+$B220$^+$ 細胞数を, クロロゲン酸とペラルゴニンは FcεRIα$^+$CD117$^+$ 細胞数を有意に減少させた。これらの結果は, デーツ抽出物中に含有される 3 種類のポリフェノールが, IgE 産生形質細胞数と高親和性 IgE 受容体発現肥満細胞数の減少を通して, ダニにより誘導されるマウスのアレルギー症状を軽減することを示唆している。

Summary

The effects of oral ingestion of a hot water extract of matured fruit of the date palm tree (*Phoenix dactylifera* L.) on allergic responses were investigated in mite-sensitized mice. Sneezing and nose rubbing events in mice given a date extract-added diet were significantly lower than in those given an extract-free (control) diet. The serum total and mite antigen-specific immunoglobulin (Ig) E levels, and the number of spleen interleukin-$4^{+}CD4^{+}$, $IgE^{+}B220^{+}$ and $Fc\varepsilon RI\alpha^{+}CD117^{+}$ cells was significantly lower in mice given the date extract-added diet than in those given the control diet. Chlorogenic acid, pelargonin and ferulic acid significantly reduced the number of $IgE^{+}B220^{+}$ cells, while chlorogenic acid and pelargonin significantly decreased the number of $Fc\varepsilon RI\alpha^{+}CD117^{+}$ cells in mouse spleen cell cultures. These results suggest that some polyphenols in the date may reduce mite-induced allergic symptoms in mice via a decrease in the number of IgE-producing plasma cells and high-affinity IgE receptor-expressing mast cells.

Key words: anti-allergic property, date polyphenol, Th1/Th2 balance, IgE-producing plasma cell, mast cell

Introduction

Type I allergy is caused by an inappropriate reaction against food and environmental allergens. The prevalence and severity of allergic diseases such as atopic dermatitis, allergic asthma and allergic rhinitis have increased dramatically during the past decade around the world, especially in developed countries. The allergies characterized by these symptoms are generally caused by degranulation that occurs following antigen cross-linking of immunoglobulin (Ig) E molecules on mast cells (*1*). Thus, IgE is essential for type I allergic reactions. It is established that interleukin (IL)-4, IL-5, and IL-13, which are produced by type 2 helper T (Th2) cells, stimulate IgE production (*1*). In particular, IL-4 is known to play a crucial role in IgE synthesis (*2*). In contrast, type 1 helper T (Th1) cells mainly secrete interferon (IFN)-γ, which inhibits IL-4 production by the Th2 cells (*3*, *4*). As a result, a strict balance between Th1 and Th2 activity is essential for the control of IgE production.

It has been demonstrated in recent years that some fruit extracts reduce allergic symptoms. Kim *et al*. reported that a water-soluble extract prepared from *Actinidia arguta* improved asthmatic symptoms via the down-regulation of IgE in mouse (*5*). Lee *et al*. found that resveratrol, which has been identified in various fruit and vegetable extracts, inhibited Th2-type cytokines such as IL-4 and IL-5, and suppressed airway hyperresponsiveness, eosinophilia, and mucus hypersecretion in the asthmatic mouse model (*6*).

More recently, our group observed that the ratio of Th1/Th2 cells in the spleen and

Peyer's patch was higher in C3H/HeN mice given a diet containing a hot water extract of the matured fruit of the date palm tree (*Phoenix dactylifera* L.) than in mice given an extract-free diet (*7*), due to an increase in the number of spleen and Peyer's patch Th1 cells and a tendency for the number of Th2 cells to decrease. These results suggested that a date extract might have a suppressive effect on allergic immune responses via the adjustment of Th1/Th2 balance.

This study therefore evaluated the effect of a date extract on the allergic responses induced by a mite (*Dermatophagoides farinae, Df*) allergen in BALB/c mice, and determined whether date polyphenols affect allergy-associated effector cells in C3H/HeN mouse spleen cell cultures.

Materials and Methods

Materials. Phycoerythrin (PE)-labeled anti-mouse IL-4 monoclonal antibody (mAb, clone 11B11), PE-labeled anti-mouse IFN-γ mAb (clone XMG1.2), PE-labeled anti-mouse CD80 mAb (clone 16-10A1), biotin-labeled anti-mouse CD4 mAb (clone RM4-5), biotin-labeled anti-mouse CD45R/B220 (B220) mAb (clone RA3-6B2), biotin-labeled anti-mouse CD117 mAb (clone 2B8), biotin-labeled anti-mouse CD11b mAb (clone M1/70) and phycoerythrin/cyanine 5 (PE/Cy5)-labeled streptavidin were purchased from BioLegend (San Diego, CA, USA). Anti-mouse FcεRIα (clone G-14) and PE-labeled anti-goat IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Brefeldin A (BFA), ionomycin, streptomycin and phorbol

12-myristate 13-acetate (PMA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Df-extract, a mite allergen, was obtained from LSL (Tokyo, Japan). IntraPrep was purchased from Beckman Coulter (Marseille, France). Defined fetal bovine serum (FBS) was obtained from HyClone Laboratories (Logan, UT, USA). Penicillin was purchased from MP Biomedicals (Costa Mesa, CA, USA). Roswell Park Memorial Institute (RPMI)-1640 was purchased from Nissui Pharmaceutical (Tokyo, Japan). Horseradish peroxidase (HRP)-labeled anti-mouse IgE was obtained from Bethyl Laboratories (Montgomery, TX, USA). 3,3′,5,5′-Tetramethyl benzidine (TMB) was purchased from KPL (Gaithersburg, MD, USA). Bovine serum albumin (BSA) and aluminum hydroxide gel were obtained from Sigma-Aldrich (St. Louis, MO, USA). Protocatechuic acid, chlorogenic acid, caffeic acid, pelargonin, and ferulic acid were obtained from Funakoshi Co., Ltd. (Tokyo, Japan) and syringic acid was purchased from Wako Pure Chemical Industries. All chemicals used in this study were of the highest analytical grade commercially available.

Preparation of a date extract. Dried date fruit that was harvested in the United Arab Emirates (UAE) was obtained from Marubeni Corporation (Tokyo, Japan). The fruit (1,000 g), including peel and pulp but not seeds, was cut into pieces of approximate 5 × 5 mm, and boiled in 9,000 ml of hot distilled water for 2 h under reflux. The supernatant was collected by centrifugation (5,000 × *g*, 30 min) and ultrafiltrated using a Stirred Ultrafiltration Unit (Model UHP-150K; Advantec Toyo Kaisha, Ltd., Tokyo, Japan) with a Molecular/Por Ultrafiltration Membrane MWCO 500 (Spectrum Laboratories,

Inc., Rancho Dominguez, CA, USA) to eliminate fructose and sucrose in the extract. The residue on the membrane was then dissolved in 500 ml of distilled water, freeze-dried and used as the date extract. The weight of date extract obtained from 1,000 g fruit was 70 g. The total content of polyphenols in the extract, determined according to the Folin-Ciocalteu method used by Al-Farsi *et al*. (*8*), was 32.07 mg of ferulic acid equivalents (FAE)/g. Hence, it was calculated that 1 g of the date fruit contained 2.24 mg of FAE of polyphenols. Al-Farsi *et al*. reported that the content of polyphenols in the date fruit was 2.17 to 3.43 mg of FAE/g (*8*). The polyphenol content of date fruit used in this study was within the amount of their report. On the other hand, we estimated that the content of protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, pelargonin and ferulic acid identified in the previous study (*7*) was 10.78, 0.88, 0.74, 7.55, 3.64 and 3.15 mg/g date extract, respectively, by ultra performance liquid chromatography analysis.

Feeding procedure. Four-week-old male BALB/c mice were obtained from Japan SLC (Shizuoka, Japan). The mice were assigned to different test regimens as shown in Fig. 1. The mice were given a commercial mouse powder feed (MF, Oriental Yeast Co., Tokyo, Japan) for 1 week, and were then given the defined protein-free purified diet P5765 (Purina Mills, St. Louis, MO, USA) supplemented with 25% ovalbumin (control diet) or a mixture of 24% ovalbumin and 1% date extract (date-diet) between 5 and 11 weeks of age (n = 5). The mice were intraperitoneally injected with 200 μl saline solution containing 100 μg Df-extract and 2 mg aluminum hydroxide gel at 5, 6 and 7

Fig. 1

weeks of age. The mice were intranasally challenged by instillation with 20 μl distilled water containing 20 μg Df-extract per week between 8 and 11 weeks of age. The number of sneezing and nose rubbing events was then counted for 30 min, starting 1 min after the intranasal instillation. Food and water were supplied *ad libitum* throughout the course of the experiment. The mice were housed at a temperature of 23 ± 2 °C under a standard 12-h light-dark cycle. Blood, spleen and Peyer's patch samples were collected immediately following a lethal dose of ether at 11 weeks of age. Serum was obtained by centrifugation at 450 × *g* for 60 min at 4 °C and was stored at -30 °C until use. All animal experimentations undertaken during this study were 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.

Spleen and Peyer's patch cell suspensions and cell cultures. Spleen and Peyer's patch cell suspensions were prepared as described previously (*9*). One thousand microliters of the cell suspensions were plated into the wells of a 24-well flat bottom plate (Sarstedt, Inc., Newton, NC, USA). Individual standard polyphenols were dissolved in dimethyl sulfoxide (DMSO) and added into the medium at a final concentration of 4 nmol/ml. The final concentration of DMSO was 0.01%, which has been determined to be noncytotoxic. The cells were cultured at 37 °C in a humidified 5% CO_2 incubator for 48 h for cell function analysis.

Cell function analysis. Cell surface markers and intracellular cytokines were labeled

according to a previously described procedure (*10*). The cell number was determined using a Guava personal cell functional analyzer (Guava PCA, Guava Technologies, Hayward, CA, USA).

Antibody analysis. Serum total and Df-specific IgE levels were measured by an enzyme-linked immunosorbent assay (ELISA) according to a previously described procedure (*9*).

Statistical analysis. Data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using a statistical software package (ystat2004.xls, Igakutosho Shuppan, Tokyo, Japan). Repeated measures analysis of variance (ANOVA) was used to determine if there were significant differences in the numbers of sneezing and nose rubbing events among group over time. When significant differences over time were detected, two-way ANOVA (diet and week as independent variables) was used to analyze the numbers of sneezing and nose rubbing events between the mice given the date-diet and the mice given the control diet at the same weeks of age. Dunnett's multiple comparison tests for one-way ANOVA were used to analyze the numbers of immunocompetent cells cultured with polyphenols. All other analyses were assessed using Student's *t*-test. All analyses were performed using the two-sided, and differences were considered significant when *P*-values were less than 0.05.

Results

Effects of a date-diet on allergic symptoms in mice

Five-week-old BALB/c mice were given a date-diet or a control diet for 6 weeks. No significant difference in body weight was observed between the mice given these diets (data not shown).

Fig. 2 represents the number of sneezes (A) and nose rubbings (B) of the mice. The number of sneezes was significantly lower in mice between 9 and 11 weeks of age that were given the date-diet than in mice given the control diet. The number of nose rubbings was significantly lower in mice between 8 and 11 weeks of age that were given the date-diet than in mice given the control diet. Fig. 2

Effects of a date-diet on total and Df-specific IgE levels in mouse serum

Fig. 3 shows the serum level of total (A) and Df-specific (B) IgE. The total IgE level was significantly lower in mice given the date-diet than in mice given the control diet. In addition, the Df-specific IgE level was significantly lower in mice given the date-diet than in mice given the control diet. Fig. 3

Effects of a date-diet on the number of immunocompetent cells in the spleen and Peyer's patch of mice

Fig. 4 represents the number of spleen IFN-γ^+CD4$^+$ (A) and IL-4$^+$CD4$^+$ (B) cells in 11-week-old mice. The number of IFN-γ^+CD4$^+$ cells was significantly higher, although the number of IL-4$^+$CD4$^+$ cells was significantly lower, in mice given the date-diet than in mice given the control diet. The ratio of IFN-γ^+CD4$^+$ cells/IL-4$^+$CD4$^+$ cells in the mice given the date-diet was 1.97, while that in the mice given the control diet was 0.56. Fig. 4

Fig. 5 shows the number of spleen and Peyer's patch CD80$^+$CD11b$^+$ (A, D), IgE$^+$B220$^+$ Fig. 5

(B, E) and FcεRIα^{+}CD117^{+} (C, F) cells in 11-week-old mice. The number of IgE^{+}B220^{+} and FcεRIα^{+}CD117^{+} cells was significantly lower in mice given the date-diet than in mice given the control diet, although the number of CD11b^{+}CD80^{+} cells did not differ significantly between these two mouse groups.

Effects of various date polyphenols on the number of immunocompetent cells in mouse spleen cell cultures

Table 1

Table 1 shows the effect of six polyphenols previously identified in the date extract (*7*) on cell number of two different types of cultured immunocompetent cells from C3H/HeN mouse spleen. Chlorogenic acid, pelargonin and ferulic acid significantly decreased the number of IgE^{+}B220^{+} cells compared to polyphenol-free, protocatechuic acid, caffeic acid and syringic acid. Chlorogenic acid and pelargonin significantly reduced the number of FcεRIα^{+}CD117^{+} cells compared to polyphenol-free, protocatechuic acid, caffeic acid, syringic acid and ferulic acid.

Discussion

Date fruit, or the fruit of the date palm tree, has been harvested in North Africa for at least 3,500 years (*11*), and its cultivation has now spread to the Middle East, parts of Central and South America, and Southern Europe (*12*, *13*). Date fruit has been used in the Middle East as a folk medicine to prevent various infectious diseases, indicating its antimicrobial capacity (*14, 15*). We recently demonstrated that a date extract more strongly enhanced the cellular immune system than prune or fig fruit extracts (*7*). In

addition, the ratio of spleen and Peyer's patch IFN-γ^+CD4^+/IL-4^+CD4^+ (Th1/Th2) cells was higher in C3H/HeN mice that were given the date extract-added diet than in mice given the extract-free diet.

Zhou *et al*. reported that type I allergic reactions were induced by intranasal administration of Df after intraperitoneal injection of Df in BALB/c mice (*16*). They also observed that the amount of total serum IgE in the mice without intraperitoneal immunization was approximately 0.2 μg/ml, while that in mice with the immunization was approximately 4 μg/ml or more (*16*). Hence, in the present study, we treated BALB/c mice with Df according to the procedure described by Zhou *et al*. The serum total IgE amount in the BALB/c mice given the control diet was 9.75 ± 2.87 μg/ml (Fig. 3). These facts indicate that the mice could be induced allergic reactions appropriately.

The number of sneezing and nose rubbing events was significantly lower in mice given the date-diet between the ages of 9 to 11, and 8 to 11 weeks, respectively, than in mice given the control diet (Fig. 2). Furthermore, the serum level of Df-specific IgE in the mice given the date-diet was significantly lower than that in the mice given the control diet (Fig. 3). These results indicate that the feeding of mice with the date-diet decreases allergic symptoms such as sneezing and nose rubbing via the suppression of Df-specific IgE production.

It is well established that IL-4, IL-5 and IL-13 produced by Th2 cells stimulate IgE production (*1*). The number of spleen Th2 cells in mice given the date-diet was significantly lower than that in mice given the control diet (Fig. 4). This result indicates

that the decrease in the serum level of Df-specific IgE in the mice given the date-diet was due to a decrease in the number of Th2 cells.

It is known that IgE^+B220^+ cells are IgE-producing B cells (plasma cells) (*17*). The number of spleen and Peyer's patch IgE^+B220^+ cells in mice given the date-diet was significantly lower than that in mice given the control diet (Fig. 5B, E). These results indicate that the date extract may reduce the number of IgE-producing plasma cells in both the systemic and local immune systems.

The onset of type I allergic diseases is ultimately associated with the degranulation of mast cells. Expression of the FcεRI, a high-affinity IgE receptor (*1*), on mast cells is necessary for degranulation. Mast cells are normally defined on the basis of cell surface expression of both FcεRIα and CD117 (*18*). The number of spleen and Peyer's patch $Fc\varepsilon RI\alpha^+CD117^+$ cells in mice given the date-diet was significantly lower than that in mice given the control diet (Fig. 5C, F). These results indicate that the anti-allergic effect of the date extract is also partly due to a reduction in the number of high-affinity IgE receptor-expressing mast cells.

We previously identified six kinds of polyphenols; protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, pelargonin and ferulic acid in a date extract on UPLC (*7*). In that study, chlorogenic acid and caffeic acid increased the number of $IFN\text{-}\gamma^+CD4^+$ cells, chlorogenic acid, pelargonin and ferulic acid enhanced the number of $IFN\text{-}\gamma^+CD49b^+$ cells, while chlorogenic acid, caffeic acid and ferulic acid increased the number of $IL\text{-}12^+CD11b^+$ cells in C3H/HeN mouse Peyer's patch cell cultures. In the

present study, we found that chlorogenic acid, pelargonin and ferulic acid significantly decreased the number of $IgE^{+}B220^{+}$ cells, whereas chlorogenic acid and pelargonin significantly reduced the number of $Fc\varepsilon RI\alpha^{+}CD117^{+}$ cells (Table 1). These results suggest that chlorogenic acid, pelargonin and ferulic acid might be associable with the reduction of IgE-producing plasma cell and high-affinity IgE receptor-expressing mast cell numbers in the mice given the date-diet. On the other hand, we also estimated that β-D-glucan could be present in the date extract (*7*). It has been reported that a certain structure of β-D-glucan suppressed the allergic symptoms (*19*). It could be therefore likely that the anti-allergic effect of the date extract might be partly due to the action of β-D-glucan.

In conclusion, we note that date fruit appears to suppress acquired allergic responses via a reduction in the number of IgE-producing plasma cells and high-affinity IgE receptor-expressing mast cells. In order to use date fruit as an effective anti-allergic food material, a further investigation including the confirmation of the anti-allergic effect of β-D-glucan in the date extract is now in progress.

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Figure captions

Fig. 1. Experimental schedules. After pre-breeding for a week, BALB/c mice were given the control diet or date-diet between 5 and 11 weeks of age. The mice were intraperitoneally injected with 200 μl saline solution containing 100 μg Df-extract and 2 mg aluminum hydroxide gel at 5, 6 and 7 weeks of age, and intranasally challenged by instillation with 20 μl distilled water containing 20 μg Df-extract between 8 and 11 weeks of age. Blood, spleen and Peyer's patch samples were collected at 11 weeks of age.

Fig. 2. The number of sneezes (A) and nose rubbings (B) in BALB/c mice given the control diet (the blank circles) or date-diet (the filled circles). Sneezing and nose rubbing events were counted for 30 min, starting 1 min after the intranasal instillation with Df-extract. Repeated measures ANOVA examining the numbers of sneezing and nose rubbing events among group showed significant differences. Two-way ANOVA was used to analyze those between the mice given the date-diet and the mice given the control diet at the same weeks of age. Data are presented as the mean ± SD (n = 5). *P<0.05, **P<0.01.

Fig. 3. Serum level of total (A) and Df-specific (B) IgE in BALB/c mice given the control diet (the blank columns) or date-diet (the filled columns). Data are presented as the mean ± SD (n = 5). *P<0.05, ***P<0.005.

Fig. 4. The number of spleen IFN-γ^{+}CD4^{+} cells (A) and IL-4^{+}CD4^{+} cells (B) in BALB/c mice given the control diet (the blank columns) or date-diet (the filled columns). Data

are presented as the mean ± SD (n = 5). ***P<0.005.

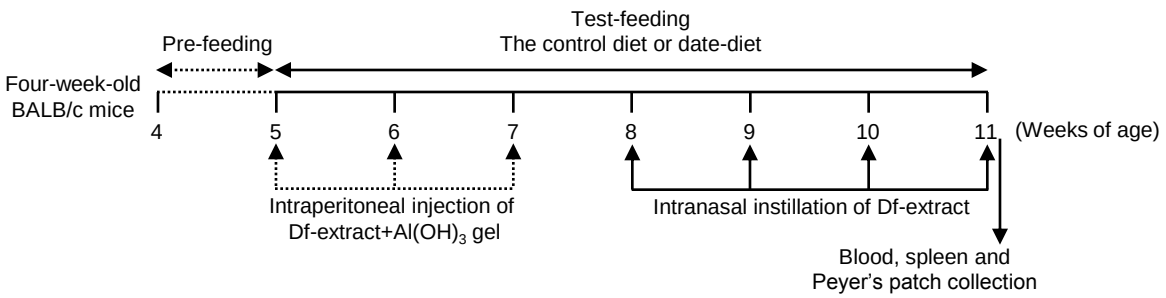
Fig. 5. The number of immunocompetent cells in the spleen and Peyer's patch of BALB/c mice given the control diet (the blank columns) or date-diet (the filled columns). Spleen $CD80^+CD11b^+$ cells (A); spleen IgE^+B220^+ cells (B); spleen $Fc\varepsilon RI\alpha^+CD117^+$ cells (C); Peyer's patch $CD80^+CD11b^+$ cells (D); Peyer's patch IgE^+B220^+ cells (E); Peyer's patch $Fc\varepsilon RI\alpha^+CD117^+$ cells (F). Data are presented as the mean ± SD (n = 5). *P<0.05, **P<0.01.

Table 1. Number of immunocompetent cells in spleen cells cultured with six polyphenols identified in the date extract.

Immunocompetent cell	Number of cells ($\times 10^4/10^6$ Spleen cells)						
	Polyphenol						
	Free	Protocatechuic acid	Chlorogenic acid	Caffeic acid	Syringic acid	Pelargonin	Ferulic acid
IgE^+B220^+	8.70 ± 0.73*a*	7.84 ± 0.63*a*	6.67 ± 0.55*b*	7.60 ± 0.57*a*	7.73 ± 0.72*a*	5.72 ± 0.43*c*	6.89 ± 0.31*b*
$Fc\varepsilon RI\alpha^+CD117^+$	1.51 ± 0.20*a*	1.18 ± 0.11*a*	0.81 ± 0.16*b*	1.23 ± 0.25*a*	1.47 ± 0.33*a*	0.77 ± 0.36*b*	1.29 ± 0.14*a*

Spleens were obtained from C3H/HeN mice at six weeks of age bred with a commercially available diet.
Data are represented as the mean ± SD (n = 5).
Items indicated with different letters (i.e. *a*, *b*, *c*) are significantly different (P<0.05).

Table 1. Karasawa & Otani.



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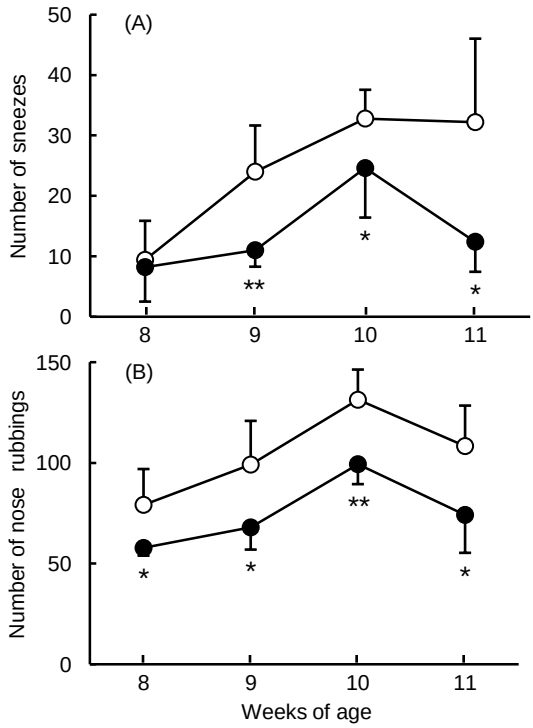

Fig. 2. Karasawa & Otani.

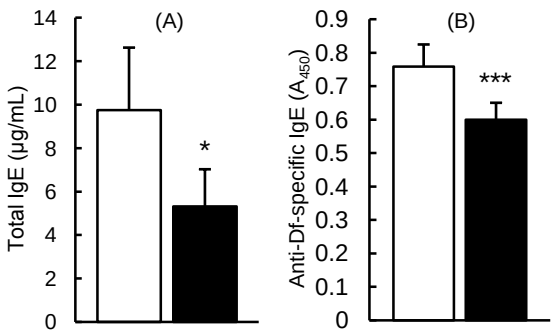

Fig. 3. Karasawa & Otani.

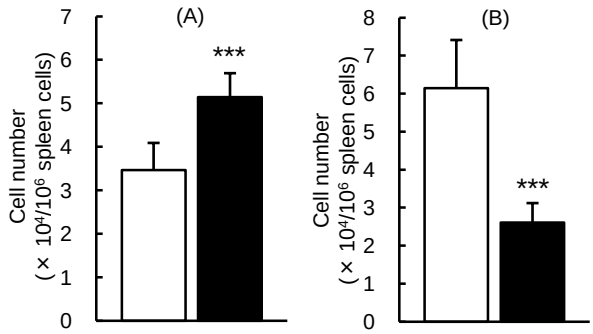

Fig. 4. Karasawa & Otani.

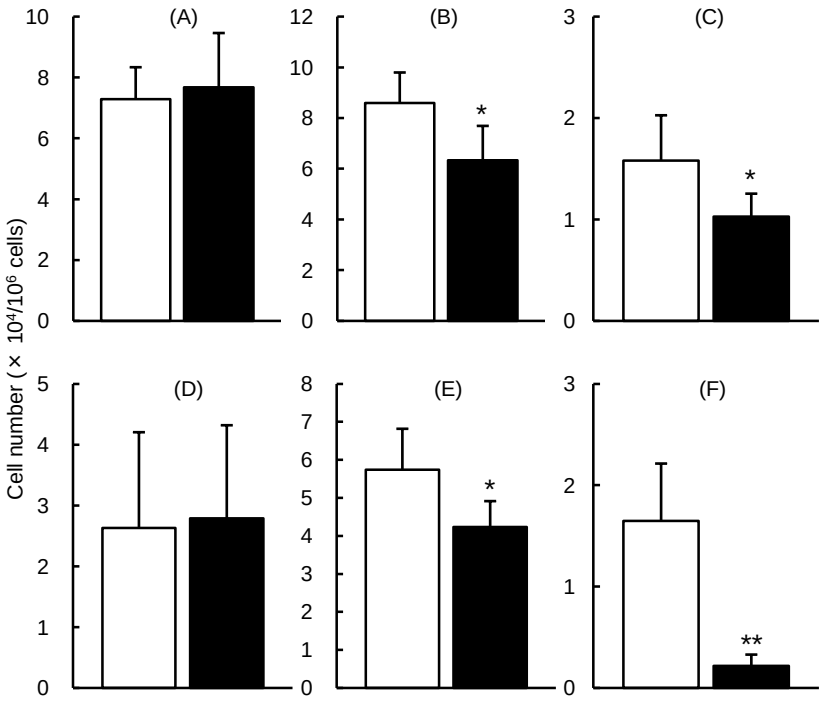

Fig. 5. Karasawa & Otani.