Effects of Natural and Chemically Defined Nutrients on Candida albicans Water-soluble Fraction (CAWS) Vasculitis in Mice

Wataru Sato, Ken-ichi Ishibashi, Daisuke Yamanaka, Yoshiyuki Adachi and Naohito Ohno

School of Pharmacy, Tokyo University of Pharmacy and Life Sciences

ABSTRACT

Kawasaki disease (KD) is an inflammatory disease that was identified by Professor Tomisaku Kawasaki in 1961. Candida albicans-derived substances, such as C. albicans water-soluble fraction (CAWS), induce coronary arteritis similar to KD in mice. CAWS functions as a pathogen-associated molecular pattern (PAMP) by acting as a ligand for dectin-2. A gut-associated immunological system has developed specifically to segregate advantageous and detrimental stimuli, and the microbial flora has been found to markedly affect the development and severity of diseases. We herein investigated whether diet affects the onset and progression of CAWS vasculitis in mice. A standard diet, CE-2, and chemically defined diet, AIN93G, which is free of β-glucan, were used. Although all mice administered with CAWS died, the mean number of survival days was smaller in the AIN93G group because vasculitis was induced earlier than in the CE-2 group. Bacteroides, which are inflammatory flora, were enriched in the microbial flora of the AIN93G group. The results of the present study suggest that diet quality affects not only microbial flora changes, but also the progression of systemic disease.

Key words: Candida albicans, C. albicans water-soluble fraction (CAWS), DBA/2, Dectin-1, Dectin-2

Introduction

Kawasaki disease (KD) is an inflammatory disease that typically develops in children, particularly those younger than 4 years old, and was identified by Professor Tomisaku Kawasaki in 1961. Typical symptoms include fever; swollen lips, throat, and lymph nodes; and peeling of the skin on the hands and feet. KD is prevalent in Asian countries including Japan. Clinical studies recently demonstrated that the prevalence of KD was increased two-fold by an abnormality in the inositol-trisphosphate 3-kinase C (ITPKC) gene. We and others reported that Candida albicans-derived substances (CADS), such as C. albicans water-soluble fraction (CAWS), induced coronary arteritis similar to KD in mice. Our previous findings obtained using CAWS showed that the severity of vasculitis significantly depends on the strains of mice used, i.e., weak vasculitis developed in the CBA/j strain, whereas severe and lethal vasculitis occurred in the DBA/2 strain. CAWS is the polysaccharide fraction released into the culture supernatant of C. albicans NBRC 1385 cultured in C-limiting medium and is mainly composed of a mannoprotein β-glucan complex. Mannan in CAWS consists of the main chain of α-1,6-mannose with a side chain of α-1,2-mannose and α-1,3-mannose, while that of β-glucan has β-1,3- and β-1,6-glucose.
The gut is constantly exposed to various stimuli, such as diet, allergens, and pathogenic microorganisms from the environment; therefore, it has developed a gut-associated immunological system specifically to segregate advantageous and detrimental stimuli. The first lymphoid tissues to be affected by these stimuli in the gut are Peyer’s patches and microfold cells (M cells).

β-glucan is well known to have strong immunopotentiating action. Receptors for β-glucan, such as dectin-1, lactosylceramide, and complement receptor 3 (CR3), have recently been identified, and their molecular mechanisms are being examined in detail. Dectin-1 is expressed by antigen-presenting cells (APCs) such as macrophages (Mφ) or DCs, is activated by β-glucan, and promotes immunity. β-glucan is widely present in nature, such as in plant, bacterial, and fungal cell walls. In animal experiments, standard diets include yeast extract, which is rich in β-glucan. Since the gut mucosal membrane is known to express β-glucan receptors, β-glucan in the diet influences systemic immunity and inflammatory responses. Low-molecular-weight β-glucan has been shown to act as an antagonist of dectin-1 and improves inflammatory bowel disease (IBD).

The microbial flora in the human body is markedly influenced by the daily conditions of the host, such as energy production, metabolism, infectious diseases, and allergic diseases. The quality of the microbial flora has an impact on the maintenance of health and prevention of diseases. For example, the microbial flora is considered to balance the negative influences of diseases, such as IBD, irritable bowel syndrome (IBS), non-alcoholic steatohepatitis (NASH), colorectal cancer, diabetes mellitus, obesity, and autoimmune disease.

Pseudomembranous colitis (PMC), which is caused by the microbial flora, is treated by transplanting the microbial flora from healthy feces. These findings prompted us to speculate that changes in the microbial flora markedly influence the prevention and severity of diseases. Therefore, we investigated the effects of different diets on the onset and progression of CAWS vasculitis in mice.

Materials and Methods

Materials

Tween 20 was purchased from Wako Pure Chemical Co. (Osaka, Japan). Gentamicin sulfate and bovine serum albumin (BSA) were from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 medium and fetal bovine serum (FBS) were from Gibco Life Technologies Ltd. (Grand Island, NY, USA). YAC-1 was from RIKEN (Tsukuba, Japan). PKH-26 was from Sigma-Aldrich Co. (St. Louis, MO). TP3 was from Invitrogen Life Technologies Ltd. (Grand Island, NY, USA).

C-limiting medium

C-limiting medium, originally described by Shepherd et al., was used to grow C. albicans. C-limiting medium contained (per liter): 10 g sucrose, 2 g (NH₄)₂SO₄, 2 g KH₂PO₄, 0.05 g CaCl₂·2H₂O, 0.05 g MgSO₄·7H₂O, 1 mg ZnSO₄·7H₂O, 1 mg CuSO₄·5H₂O, 0.01 g FeSO₄·7H₂O, and 25 μg biotin at a final pH 5.2.

Preparation of CAWS

C. albicans strain NBRC1385 was purchased from the Biological Resource Center, NITE (NBRC), stored at 25°C on Sabouraud’s agar (Difco, New Jersey, USA), and passaged once every 3 months. CAWS was prepared from C. albicans strain NBRC1385 in accordance with conventional methods. The procedure used was as follows: 5 l of medium (C-limiting medium) was added to a glass incubator and cultured at 27°C for 2 days with air supplied at a rate of 5 l/min and rotation at 400 rpm. Following the culture, an equal volume of ethanol was added, and, after the mixture was allowed to stand overnight, the precipitate was collected. The precipitate was dissolved in 250 ml of distilled water, ethanol was added, and the mixture was left to stand overnight. The precipitate was collected and dried with acetone to obtain CAWS.

Animals

DBA/2 male mice between 4 and 12 weeks of age were purchased from Japan SLC (Shizuoka, Japan). Mice were maintained under specific pathogen-free (SPF) conditions at 23 ± 1°C with constant humidity of 55 ± 5% under a 12-h light/dark cycle, and had free access to food and tap water according to the Guidelines for Experimental Animal Care issued by the Prime Minister’s Office of Japan. The experimental protocol was approved by the Tokyo University Medical Mycology Journal Volume 58, Number 2, 2017
of Pharmacy and Life Sciences Laboratory Animal Committee (pp.15-42).

**Diet compositions**

A standard diet, CE-2, and chemically defined diet, AIN93G were used. CE-2 is a GLP-compliant, standard rodent diet consisting mainly of vegetable protein (soybean waste) with a proper balance of animal protein. AIN93G is standard purified diet for mice and rats, proposed by the American Institute of Nutrition (AIN) in 1993 for use in nutritional studies. The composition of the diet was determined by AIN based on the nutritional requirements of mice and rats issued by the National Academy of Sciences National Research Council (NAS-NRC). The mice were provided with CE-2 (CLEA Japan, Inc., Tokyo) or AIN93G (Oriental Yeast Co., LTD.). The composition of these diets is shown in the suppliers’ websites (http://www.clea-japan.com/Feed/ce2.html, http://www.oyc-bio.jp/files/download/lafeed_certified/ain_93g).

**Administration schedule for the induction of coronary arteritis**

After a 1-week acclimation period with each diet, CAWS was administered intraperitoneally (i.p.) for five consecutive days to each mouse. Animals were sacrificed, and their hearts were fixed with 10% neutral formalin and prepared in paraffin blocks. Tissues sections were stained with hematoxylin & eosin (HE).

**Analysis of serum biochemistry**

A serum biochemical analysis was performed by Oriental Yeast Co., Ltd. using DBA/2 mice after a 1-week acclimation period with each diet.

**Analysis of the microbial flora**

Feces were collected from DBA/2 mice following the consumption of the CE-2 and AIN93G diets for one week, and the microbial flora was analyzed using the T-RFLP method (TechnoSuruga Laboratory Co., Ltd., Shizuoka).

**Preparation of splenocytes**

Splenocytes were prepared as previously described. Briefly, the spleen was teased apart in RPMI 1640 medium. A single cell suspension was treated with AC-lysing buffer (8.3 g/l NH4Cl and 1.2 g/l Tris [2-Amino-2-hydroxymethyl-1,3-propanediol]) to lyse red blood cells. After centrifugation (1,250 rpm at 4°C for 5 min), splenocytes were maintained in RPMI 1640 medium supplemented with 50 μg/ml gentamicin sulfate and 10% FBS.

**Preparation of target cells (YAC-1)**

The NK cell-sensitive lymphoma cell line, YAC-1, was maintained in RPMI 1640 medium supplemented with 50 μg/ml gentamicin sulfate and 10% FBS, and cultured at 37°C in a humidified, 5% CO2, 95% air atmosphere. Harvested cells were labeled with the red membrane dye PKH-26 in accordance with the manufacturer’s instructions. Target cells were washed twice in RPMI 1640 without FBS and incubated with 1 ml of 4 μM PKH-26 dye in Duluent C buffer solution at RT for 5 min. Labeling was stopped by incubating with 1 ml FBS at RT for 1 min, after which cells were washed twice in RPMI 1640. Following the final wash, cells were resuspended in RPMI 1640.

**NK cytotoxicity assay**

Labeled target cells were incubated with the effector cells (splenocytes), in a 96-well U-bottom plate at a final volume of 200 μl. Effector-to-target cell (E:T) ratios of 100:1, 50:1, and 25:1 were used. The resulting cell suspensions were incubated at 37°C for 24 h in a humidified, 5% CO2, 95% air atmosphere. Spontaneous target cell death was assessed in PKH-26-labeled target cell cultures only.

Cells were analyzed using dual laser FACS Canto (BD Biosciences, San Diego, CA, USA). Forward and right-angle light scattering was used to include all cells but exclude debris. PKH-26-labeled cells were excited using an argon ion laser emitting at 488 nm and were detected in the PE channel. In order to identify dead cells, 5 μl of a 10-μM stock solution of TP3 was added. Samples were maintained at RT and analyzed between 10 and 40 min after the addition of TP3. TP3 was excited by a 633-nm red diode laser, and the emitted fluorescence was detected in the APC channel. Photomultiplier voltages were set to place the PKH-26-labeled cells in the first log decade of the APC channel and the fourth log decade of the PE channel. A gate was set to include all PKH-26-labeled cells, and APC fluorescence from these cells was displayed as a histogram. Acquisition was stopped after 3000 PKH-26-labeled cells had been acquired, and all scatter-gated events were saved. Files were analyzed using FlowJo (FlowJo, LLC. OR). Cytotoxicity was expressed as a percentage of cell death within the PKH-26+ target (dead labeled targets/dead labeled targets + live labeled targets) × 100. The percentage of target cell death was corrected for spontaneous background death by subtracting the percentage of dead cells in control samples (PKH-26-labeled...
targets only) from the percentage of dead cells within the test samples\textsuperscript{21}.

**Measurement of cytokines**

Cytokine levels in the cell culture supernatant and mouse serum were determined using commercial ELISA kits: Mouse TNF-\(\alpha\), IFN-\(\gamma\), and IL-10 (BioLegend, San Diego, CA).

**Limulus G test**

The activation of Factor G (limulus reactivity) by beta-1,3-glucans (\(\beta\)-glucan) was measured by a chromogenic method using a \(\beta\)-glucan-specific reagent (Fungitec G test MKII, NISSUI, Tokyo, Japan). Each fraction was dissolved in 0.5 N NaOH and diluted with 0.01 N NaOH. Dilutions were made with 0.01 N NaOH, and the sample solution was used directly for the limulus reaction without neutralization. Diluted NaOH was considered to be usable for the limulus reaction because of the high buffer action of the reagent. Reactions were performed in flat-bottomed 96-well Toxipet plate 96F (Seikagaku Co., Tokyo) as follows. Samples (50 \(\mu\)l) were placed in the wells, and Fungitec G test MKII reagent (50 \(\mu\)l) was added to each well. The plate was incubated at 37\(^\circ\)C and during incubation, the absorbance at 405 nm (reference 492 nm) was measured kinetically using a microplate reader (Well reader SK603, Seikagaku Co., Tokyo).

**Statistical analysis**

Results were expressed as means and their standard deviation (SD). The significance of differences between means was measured by the Student’s \(t\)-test. Survival rates were analyzed using the Kaplan-Meier method, and the significance of differences was analyzed using the Log-rank test.

**Results**

**Effects of diets on the CAWS vasculitis model**

The effects of the well-known and generally used standard diets, CE-2 and AIN93G, on vascular inflammation were investigated using the CAWS vasculitis model. As described in Materials and Methods, CE-2 is a GLP-compliant, standard rodent diet consisting mainly of vegetable protein (soybean waste) with a proper balance of animal protein, and AIN-93G is a standard purified and chemically defined diet proposed by the American Institute of Nutrition (AIN). Based on the suppliers’ documents shown on their websites, the composition of these diets, such as mineral and vitamin content, was different from each other. Since the \(\beta\)-glucan content of the diet was not indicated by the suppliers, we examined it by using the specific limulus factor G-test, and found that the CE-2 diet contained 2,070 \(\mu\)g/g of \(\beta\)-glucan, while the AIN93G diet contained 33.5 \(\mu\)g/g.

After 1 week of feeding with either diet, CAWS was administered i.p. for 5 consecutive days to induce CAWS vasculitis in DBA/2 mice. Survival, histology, body weight, and cytokine synthesis were examined for the whole experimental period.

The survival rate of mice is shown in Fig. 1-A. All CAWS-administered mice died, and the mean number of survival days was smaller in the AIN93G group. Fig. 1-B, C shows HE staining of the aortic root. In CAWS-administered mice, the severity of vasculitis in the aortic root and coronary vessels was similar in the CE-2 and AIN93G groups.

Various parameters were tested in order to accurately analyze the pathophysiology of CAWS vasculitis. As shown in Fig. 2, no significant differences were observed in the results obtained from the serum biochemical examination between the two groups. Fig. 3 and 4 show that body weight increased more in the AIN93G group than in the CE-2 group. The weights of major organs, such as the heart, liver, and kidney, were not significantly different. The lengths of the small intestine and colon were markedly shorter in the AIN93G group than in the CE-2 group, and, among CAWS-administered mice, these were also markedly shorter in the AIN93G group than in the CE-2 group. These results suggest that diet significantly influences physiology of the gut and also CAWS vasculitis in mice.

**Time course for the induction of CAWS vasculitis between CE-2 and AIN93G groups**

As shown in Fig. 1-A, differences were observed in the survival periods of the CE-2 and AIN93G groups. In an attempt to elucidate the underlying mechanisms for the differences, HE staining of the aorta was performed at different time periods, and the induction of inflammatory cells and thickness of vascular walls were compared. As shown in Fig. 5, vasculitis induced by CAWS (250 \(\mu\)g/mouse) was detected by day 7 in the CE-2 group, but by day 3 in the AIN93G group. Various kinds of inflammatory cells accumulated in the regions of the aorta and coronary vessels, as shown in Fig. 5. Similar to a previous study, one of the main leukocytes that accumulated was neutrophils\textsuperscript{22}.

Furthermore, penetration of inflammatory cells
Fig. 1. (A) Survival rates following i.p. PBS or CAWS (1 mg/mouse) -induced vasculitis in DBA/2 mice fed with CE-2 or AIN93G diet. Survival of CAWS vasculitis mice fed with (B) CE-2 or (C) AIN93G diet.

Fig. 2. Serum biochemical examination of DBA/2 mice fed with CE-2 or AIN93G diet. Significant differences: *p < 0.05, **p < 0.01.
into the vascular walls was observed from day 28 in the CE-2 group, but from day 14 in the AIN93G group. The severity of vasculitis was compared using the scoring method described by Takahashi et al.\(^{23,24}\). As shown in Fig. 6, the AIN93G group had significantly higher severity by day 14, and vasculitis deteriorated from an early stage. We attempted to induce vasculitis using a lower dose of CAWS (125 μg/mouse) and found that slight inflammation was induced on vascular walls in the AIN93G group, but not in the CE-2 group, as shown in Fig. 7. These results demonstrate that vasculitis developed earlier in the AIN93G group than in the CE-2 group.

**Effects of diets on cytokine production of splenocytes in CAWS-administered mice**

Nagi-Miura et al. previously demonstrated that the mouse strain, DBA/2, was highly sensitive to stimulation with CAWS, and this characteristic was considered useful for analyzing the molecu-
lar mechanism of CAWS vasculitis\(^6\). In addition, our group has demonstrated that the cytokine production of splenocytes and bone marrow cells stimulated with ligand of C-type lectin receptors, such as dectin-1 (β-glucan) and dectin-2 (CAWS), were significantly enhanced in the presence of GM-CSF due to the enhanced expression of variety of molecules including major histocompatibility complex (MHC), co-stimulatory molecules, as well as innate immune receptors\(^{25-28}\). Thus, we measured the CAWS-induced production of the cytokines, TNF-α, IFN-γ, and IL-10 in these mice in the presence or absence of GM-CSF. Fig. 8-10 show the production of TNF-α, IFN-γ, and IL-10 from splenocytes of PBS- and CAWS-administered mice, stimulated in vitro with either PBS, CAWS, or CAWS + rmGM-CSF. Splenocytes were collected at 1, 3, 7, 14, and 28 days of CAWS administration.

Comparing PBS- (Fig. 8A) and CAWS- (Fig. 8B) stimulated splenocytes, we found that CAWS induced 10 times higher concentration of TNF-α (Fig. 8A, B). In CAWS + GM-CSF- (Fig. 8C) stimulated splenocytes, TNF-α production was enhanced further. This is similar to the data previously shown by Harada et al\(^{15}\). Comparing data of PBS- and CAWS-administered mice, TNF-α production of CAWS-administered mice (Fig. 8D-F) was significantly higher compared with corresponding PBS-administered mice (Fig. 8A-C). Comparing effect of diets, CE-2 (black symbol) and AIN93G (white symbol), splenocytes of AIN93G...
Fig. 5 (a). Vasculitis induced by CAWS (250 μg/mouse) in DBA/2 mice fed with CE-2 (A-E) or AIN93G (F-J) diet.

Fig. 5 (b). Enlarged view of D and I (near the arrow). Large number of neutrophils accumulated both inside and outside of the aorta.
Fig. 6. Incidence of vasculitis induced by CAWS (250 μg/mouse) and severity scores of coronary arteritis in CE-2 and AIN93G groups. Severity score of each segment. Significant difference: ***p < 0.001.

Fig. 7. Vasculitis induced by CAWS (125 μg/mouse) on day 28 in DBA/2 mice fed with CE-2 or AIN93G diet.
group produced relatively higher concentration of TNF-α. These results suggest induction of enhanced inflammatory condition in mice by feeding with AIN93G.

For the production of IFN-γ, we found that: (1) i.p. CAWS administration enhanced IFN-γ production (Fig. 9A vs. D), (2) CAWS stimulated enhanced in vitro IFN-γ production (Fig. 9D vs. E), (3) GM-CSF further enhanced IFN-γ production (Fig. 9E vs. F), and (4) AIN93G diet enhanced IFN-γ production (Fig. 9, ■, ◆ vs. □, ◇).

Furthermore, comparing the kinetics of cytokine production by splenocytes, we found that IFN-γ production peaked at day 14 in addition to day 1 (Fig. 9). Comparing responses at day 28, TNF-α, IFN-γ, and IL-10 were all higher in the group fed with AIN93G. These results strongly suggest that inflammatory response continued for a longer period in the AIN93G group, and may be related to the severe vasculitis in the group.

**Effects on NK cytotoxicity by different diets**

A recent study reported that lung NK cells play a major counter-regulatory role in pulmonary vascular hyperpermeability after myocardial infarction through neutrophils. Therefore, we examined the effects of diet as well as the administration of CAWS on the NK cytotoxicity of splenocytes. As shown in Fig. 11, no significant differences were observed in NK cytotoxicity between the PBS-administered CE-2 and AIN93G groups. In addition, no significant differences were observed in NK cytotoxicity until day 14 in
both diet groups administered with CAWS, however, it decreased on day 28 in the AIN93G group. In addition, NK cytotoxicity decreased in i.p. CAWS-administered mice on days 1, 14, and 28. These results reveal that NK cytotoxicity was reduced by CAWS.

Effects of diet on the microbial flora

Dietary intake significantly influences various diseases, such as metabolic syndromes. Recent findings suggest that the intestinal microbial flora strongly influences gut inflammatory diseases, such as IBD. In some cases, dietary intake has been shown to modulate the intestinal microbial flora, resulting in a change to healthy conditions. To determine the influence of changes in microbial flora, we analyzed the microbial flora in the feces of DBA/2 mice fed with each diet for one week. As shown in Fig. 12, the relative abundance of Lactobacillales and Prevotella was lower, whereas that of Bacteroides was higher in the AIN93G group than in the CE-2 group. Bacteroides are inflammatory flora, and may thus play a role in the enhanced induction of CAWS vasculitis.

Discussion

CAWS functions as a pathogen-associated molecular pattern (PAMP) that induces vasculitis in mice, and dectin-2 is a major cell surface receptor for CAWS\(^8\). CAWS also activates the lectin pathway of complement activation by binding to mannose-binding lectin (MBL). Through

---

Fig. 9. IFN-γ production in splenocytes from DBA/2 mice fed the CE-2 or AIN93G diet. Cells were incubated for 48 h with PBS, CAWS (100 μg/ml) and CAWS + rmGM-CSF (5 ng/ml). (A-C) i.p. PBS or (D-F) i.p. CAWS (250 μg/mouse). The concentrations of IFN-γ were determined by ELISA. Significant differences: *p < 0.05, **p < 0.01, ***p < 0.001.
these molecular mechanisms, CAWS induces acute inflammatory reactions in mice. Various kinds of inflammatory cells accumulate in the regions of the aorta and coronary vessels. One of the main leukocytes that accumulate is neutrophils. The activation of neutrophils induces inflammatory molecules to degrade elastic fibers, and this is followed by damage to vascular walls. The relationship between diet and CAWS vasculitis has been attracting interest. In the present study, we demonstrated that the chemically defined diet, AIN93G, aggravated CAWS vasculitis more than the natural diet, CE-2. The main reason for this was the earlier onset of local inflammation around the aorta and coronary vessels.

The production of TNF-α and IFN-γ has been identified as a factor that leads to the deterioration of CAWS vasculitis. In the present study, among CAWS-administered mice, the production of TNF-α and IFN-γ was greater in the AIN93G group (Fig. 8-10). Thus, CAWS vasculitis appears to be aggravated by AIN93G. The activity of NK cells in the spleen was decreased by the administration of CAWS. One of the major functions of NK cells is to maintain homeostasis in self-defense mechanisms. A reduction in the activity of NK cells by CAWS may be directly or indirectly related to vasculitis. NK cell function was markedly decreased in the AIN93G group, which was consistent with the severity of CAWS vasculitis (Fig. 11).

![Graphs showing IL-10 production in splenocytes from DBA/2 mice fed the CE-2 or AIN93G diet.](image)
CE-2 is a commonly used animal diet that is composed of a protein source (soybean waste, whitefish meal, and yeast), fat source (cereal germ and soybean oil), fiber source (wheat bran, defatted rice bran, and alfalfa meal), carbohydrate source (wheat flour, corn, and Milo), vitamins, and minerals. As described in the results section, β-glucan concentration of CE-2 was significantly higher than that of AIN93G. The yeast cell wall ingredient of CE-2 is composed of β-
glucan and α-mannan. Cereal germ, rice bran, and alfalfa meal are also rich in β-glucan. Dietary β-glucan has been shown to stimulate β-glucan receptors, i.e. dectin-1, in the intestinal tract, and affects the immune system in the whole body. Stimulation of dectin-1 in the intestinal tract also influences the microbial flora, and in turn, affects the immune system. We have demonstrated both the modulation of the microbial flora and the aggravation of coronary vasculitis in DBA/2 mice. Our previous studies have shown that DBA/2 is the most sensitive strain for β-glucan due to the enhanced expression of dectin-1 and GM-CSF. From these findings, DBA/2 mice might be a key parameter for inducing diet-mediated immune modulation in mice.

Comparing the composition of CE-2 and AIN93G, we found that CE-2 also contained soybean waste and soybean oil, which are sources of phytoestrogens. Phytoestrogens are well known anti-inflammatory compounds. A previous study reported that CAWS vasculitis is aggravated by increases in linoleic acid due to elevations in arachidonate cascade-induced inflammatory molecules. In addition, heart diseases have been shown to deteriorate with decreases in retinol. Moreover, according to the information from suppliers, content of the crude fat is higher in AIN93G, and the higher fat ratio in the AIN93G diet may play a role in the aggravation of CAWS vasculitis.

Dietary intake has been shown to modulate the intestinal microbial flora, resulting in a change to healthy conditions. Significant imbalance of the intestinal microbial flora is a strong risk factor for the gut inflammatory diseases. In the present study, the relative ratios of Lactobacillales and Prevotella were lower, whereas that of Bacteroides was higher in the AIN93G group than in the CE-2 group. Bacteroides in the microbial flora has been shown to induce allergic diseases in childhood. An enhanced ratio of Bacteroides may be one of the factors that aggravate Bacteroides in the gastrointestinal immune system. These results strongly suggest that the quality of the diet affects not only the changes in microbial flora, but also the progression of diseases during the whole life period.

Self-declared COI content: none.

References

9) Hobson RP, Munro CA, Bates S, MacCallum DM, Cutler JE, Heinsbroek SE, Brown GD, Odds FC, Gow NA: Loss of cell wall mannosylphosphate in...


