



Immune Deviation and Alleviation of Allergic Reactions in Mice Subjected to Dietary Restriction

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We examined cytokine production and allergic reactions in mice fed *ad libitum* (AL) and subjected to dietary restriction (DR). DR retarded the increase in body weight, and peripheral blood T cells in the DR mice produced less IFN- γ and more IL-4 in response to immobilized anti-CD3 mAb. Systemic immunization and intranasal challenge with ovalbumin (OVA) induced accumulation of leukocytes into the lung, increase in IL-4 level in bronchoalveolar lavage fluid (BALF), and rise in serum IgE in the AL mice. In contrast, these allergic symptoms were alleviated in the DR mice. Furthermore, the relative proportion of IL-4-producing T cells responsive to OVA was less in the DR mice than the AL mice. DR tended to decrease the proportion and cytolytic activity of NK cells in the spleen, especially in younger mice. These results indicate that DR can prevent the expansion of allergen-specific IL-4-producing T cells followed by suppression of the allergic reaction, but might dampen NK cell activity.

Key words: dietary restriction; allergic reaction; IL-4; IFN- γ

There is accumulating evidence that restriction of food intake can delay the onset of various aging-related diseases and suppress their incidence. Restriction of food intake inhibited 3-methylcholanthrene-induced carcinogenesis in mice, delayed the onset of carcinogenesis in p53-deficient mice, and suppressed the polyp formation spontaneously occurring in the intestine of *Apc*^{Min} mice.¹⁻³⁾ Dietary restriction (DR) inhibited immune complex-based renal disease in BXSB mice and prevented occlusive coronary vascular disease in (NZW $\times$ BXSB)F₁ mice.^{4,5)} Moreover, DR attenuated severity of experimental autoimmune uveoretinitis in rats and alleviated the disease course of experimental autoimmune encephalomyelitis in both rats and mice.⁶⁻⁸⁾ These results indicate that DR restores disordered immune functions indispensable for defense against cancer cells and mitigate excessive immune responses to self-antigens.

Epidemiological study has shown that the serum IgE level is significantly lower in malnourished children (69.30 ng/ml) than in well-nourished ones (95.97 ng/ml).⁹⁾ A study in which rats were fed either a normal diet or a low-protein diet and were triggered to induce an allergic reaction in the lung, revealed that the low-protein diet dampened IgE production and anaphylactic

reaction.¹⁰⁾ When mice were fed diets containing protein at 5% or 20%, given OVA orally, and then immunized with OVA intraperitoneally, the low-protein diet down-regulated IL-4 production and the serum IgE level, and allowed the mice to achieve oral tolerance more efficiently.¹¹⁾ These findings indicate that DR is beneficial in the prevention of allergic reactions through alleviating the IgE response mediated by Th2 cytokines such as IL-4, but it has not been examined in detail how DR alleviates allergic responses.

In this study, the effects of DR on immune functions were analyzed in mice. We found that DR suppressed the allergic reaction induced by intranasal challenge with OVA and reduced the frequency of OVA-specific IL-4-producing T cells in the periphery, in association with decreases in NK cell activity in the spleen.

Materials and Methods

Mice. C57BL/6 mice, DBA/1 mice, and BALB/c mice (male, 6 weeks old or 17 weeks old) were purchased from CLEA Japan (Tokyo). The mice were given the AIN-76 diet (Oriental Yeast, Tokyo) *ad libitum* for 1 week before the experiment at the animal facility of the Yakult Central Institute for Microbiological Research. Thereafter, the mice were randomly divided into two or three groups. The mice in the first group were further fed the AIN-76 diet *ad libitum* (abbreviated AL), and the average weight of the diet eaten was measured. The mice in the other groups were given the AIN-76 diet at 80% and 60% of the average weight of the diet eaten by a mouse in the first group (20% and 40% dietary restriction, abbreviated 20% DR and 40% DR). They were maintained for 4 more weeks, and their immune functions were analyzed. Yakult Central Institute's Ethical Committee on Experimental Animals accepted the experimental protocol.

Induction of allergic response. An allergic response was induced in the 7-week-old and the 18-week-old mice with a slight modification of the method described elsewhere.¹²⁾ The mice were bred for 4 weeks under AL, 20% DR, or 40% DR (from day 0 to day 28). Ten μ g of OVA with alum was intraperitoneally injected on day 1, and 10 μ g of OVA alone was intraperitoneally injected on days 14 and 21. After OVA (100 μ g) was intranasally challenged twice (on days 26 and 27), the mice were sacrificed on day 28. To recover bronchoalveolar lavage fluid (BALF), the lungs were lavaged twice with 1 ml of pre-warmed PBS. Total leukocyte numbers were measured, and differential cell counts were carried out using cytocentrifuged preparations of BALF stained with Diff-Quick solution (Sysmex International Reagents, Kobe, Japan).

Cell preparation. Peripheral blood was collected from the heart under anesthesia, diluted with 1 ml of PBS containing heparin (5 U/ml, Ajinomoto Pharma, Tokyo), and applied to Lymphosepal II (Immunobiological Laboratories, Takasaki, Japan). After centrifugation,

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Abbreviations: OVA, ovalbumin; PBMC, peripheral blood mononuclear cell; BALF, bronchoalveolar lavage fluid

peripheral blood mononuclear cells (PBMCs) were recovered from the interface. The spleen-cell suspension was prepared by teasing over gauze, and mononuclear cells were recovered by centrifugation making use of Lymphosepal II.

Cell culture. Each well of a microtiter plate (TC Microwell 96F; NUNC, Roskilde, Denmark) was coated with anti-CD3 mAb (145-2C11) or hamster IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at 10 µg/ml. After extensive washing, PBMCs suspended in 10% FCS/5 × 10⁻⁵ M β-mercaptoethanol/RPMI1640 were added at a density of 2 × 10⁵ cells/well and cultured at 37 °C in 5% CO₂ for 1.5 or 7 d. To purify the T cells, the PBMCs were incubated with a cocktail of biotinylated anti-immunoglobulins (IgG, IgM, and IgA) Ab (Cappel Organon Teknika, West Chester, PA), biotinylated anti-CD11b mAb (BD Biosciences, San Jose, CA), and biotinylated anti-CD11c mAb (BD Biosciences), and then with Streptavidin MicroBeads (Miltenyi Biotec, Bergisch-Gladbach, Germany). After washing, the cells were passed through an LD column (Miltenyi Biotec), and the non-attached cells were collected (>95% CD3⁺ cells). To prepare antigen-presenting cells (APCs), spleen cells of the AL mice were incubated with biotinylated anti-CD3 mAb and then with Streptavidin MicroBeads. The cells were applied to an LD column, and the non-attached cells were recovered (<2% CD3⁺ cells). The APCs were irradiated at 3,000 Rad before culture. Purified T cells (2 × 10⁵ cells/well) were cultured in the presence of immobilized anti-CD3 mAb for 6 d using a microtiter plate (TC Microwell 96F) or were cultured with irradiated APCs (2 × 10⁵ cells/well) and OVA (0–100 µg/ml) for 6 d using a microtiter plate (TC Microwell 96U; NUNC). The supernatants were collected and stored at –30 °C until analysis.

Flow cytometry. Cells were stained with the following mAbs: fluorescent isothiocyanate-conjugated anti-CD3 mAb, phycoerythrin-conjugated anti-CD45RB (B220) mAb, phycoerythrin-conjugated anti-CD49b (DX5) mAb, biotinylated anti-CD8α (Ly-2) mAb, and phycoerythrin-conjugated anti-CD4 (L3T4) mAb (all from BD Biosciences). Biotinylated mAb was detected by incubating the cells with streptavidin-TC (Monosan, Uden, Netherlands). After washing, the cells were analyzed with a flow cytometer.

Limiting dilution analysis. T cells purified from the PBMCs were added at 5 × 10² cells, 1 × 10² cells, or 2 × 10 cells/well of a microtiter plate (TC Microwell 96U) and were cultured in the presence of irradiated APCs (2 × 10⁵ cells/well) and OVA (10 µg/ml) for 6 d. By measuring the proportion of wells where T cells capable of producing IL-4 were present, we calculated the frequency of T cells producing IL-4 in response to OVA.

Cytokine measurement. The IFN-γ and IL-4 concentrations were measured using mouse Cytoset (Invitrogen-BioSource Cytokines & Signaling, Camarillo, CA). Each well of a microtiter plate (Immuno-plate; NUNC) was coated with anti-mouse IFN-γ or anti-mouse IL-4 mAbs, and then blocked by adding 1% BSA in carbonate buffer. After washing, the samples were added and the plate was incubated for 1 h. After removal of unbound materials, biotinylated anti-IFN-γ or anti-IL-4 mAbs was added, followed by incubation for 1 h. Then, peroxidase-conjugated streptavidin was added and the plate was incubated for 45 min. Finally, TMB solution (Sigma-Aldrich, St. Louis, MO) was added, and 2.5 M H₂SO₄ was added 10 min later. The absorbance at 450 nm of each well was measured, and the concentration of cytokines was calculated. The lowest concentration of detectable IFN-γ and IL-4 was 7.8 pg/ml.

IgE measurement. The total IgE concentration was measured by sandwich ELISA. Each well of a microtiter plate (Immuno-plate) was coated with anti-mouse IgE mAb (BD Biosciences), followed by blocking with 1% BSA in carbonate buffer. After washing, the samples were added and the plate was incubated for 1.5 h. Then, biotinylated anti-mouse IgE mAb (AbD Serotec, Oxford, UK) was added, followed by incubation for 1.5 h. To assess OVA-specific IgE, biotinylated OVA solution (0.85 mg/ml) was added. After washing, peroxidase-conjugated streptavidin (Zymed, South San Francisco, CA) was added and the plate was incubated for 30 min. Finally, TMB solution was added, and 2.5 M H₂SO₄ was added 10 min later. Based on the absorbance at

450 nm, the IgE concentration was calculated. One unit of OVA-specific IgE was defined as the dilution exhibiting half the value of the absorbance given by the serum of the hyper-immunized mice.

Assay of the cytolytic activity of NK cells. YAC-1 cells (1 × 10⁶ cells) were labeled by incubation with 100 µCi of Na₂⁵¹CrO₄. Mononuclear cells prepared from the spleens were mixed with ⁵¹Cr-labeled YAC-1 cells at an effector-to-target ratio (E/T ratio) of 12.5–200 in a microtiter plate (TC Microwell 96U) and were incubated for 4 h. The supernatant was collected and the radioactivity released was measured. Cytolytic activity was calculated by the following formula: specific lysis (%) = (cpm of experimental release – cpm of spontaneous release)/(cpm of maximal release – cpm of spontaneous release) × 100. Maximal release was defined as the radioactivity released by standing ⁵¹Cr-labeled YAC-1 cells in 1% Triton X-100.

Statistical analysis. The significance of difference among the three groups was evaluated by ANOVA, and then the difference between two groups was assessed by Dunnett's test.

Results

Immune deviation in mice subjected to dietary restriction

The C57BL/6 mice, the DAB/1 mice, and the BALB/c mice (7 weeks old) were divided to two groups. The mice were given diet *ad libitum* or subjected to dietary restriction by 40%. The body weight of the AL group constantly increased during the study period. In contrast, the body weight of the DR group did not increase or declined after the beginning of DR (Fig. 1A). When the cell constitution of the PBMCs was compared in the AL and the DR mice, the number of cells recovered was lower in the mice subjected to 40% DR for 4 weeks than in the AL mice (C57BL/6 6.9 ± 2.2 × 10⁶ vs. 4.0 ± 1.3 × 10⁶ cells/mouse, *p* < 0.05; DBA/1 5.6 ± 0.6 × 10⁶ vs. 3.2 ± 0.9 × 10⁶ cells/mouse, *p* < 0.001; BALB/c 8.5 ± 1.4 × 10⁶ vs. 3.1 ± 0.5 × 10⁶ cells/mouse, *p* < 0.001). Flow cytometry analysis revealed that the relative proportion of B cells (B220⁺) decreased while that of T cells (CD3⁺) increased, while the ratio of the CD4⁺ and CD8⁺ subsets in the CD3⁺ population was not very different between the AL mice and the 40% DR mice (Fig. 1B). The effect of DR on body weight and PBMCs was greatest in the BALB/c mice among three inbred strains.

The 7-week-old and 18-week-old BALB/c mice were randomly divided to three groups. One group was given diet *ad libitum*, and other groups were subjected to DR at 20% or 40%. While the body weight of the AL group constantly increased during the study period, DR retarded the increase in body weight dependent on the degree of DR (Fig. 2A).

The production of cytokines by PBMCs stimulated with immobilized anti-CD3 mAb was measured in the AL and the DR mice. Compared with T cells from the AL mice 11 weeks old, the T cells from the 40% DR mice 11 weeks old produced less IFN-γ and higher levels of IL-4 (Fig. 2B). Although DR did decrease IFN-γ production and augmented IL-4 production by PBMCs in the mice 22 weeks old, the difference between the AL and the DR mice was not significant. While a tendency to produce higher levels of IL-4 in the DR mice was also observed in the C57BL/6 mice and the DBA/1 mice, the extent of the decline in IFN-γ production and the enhancement of IL-4 production due to DR was the most

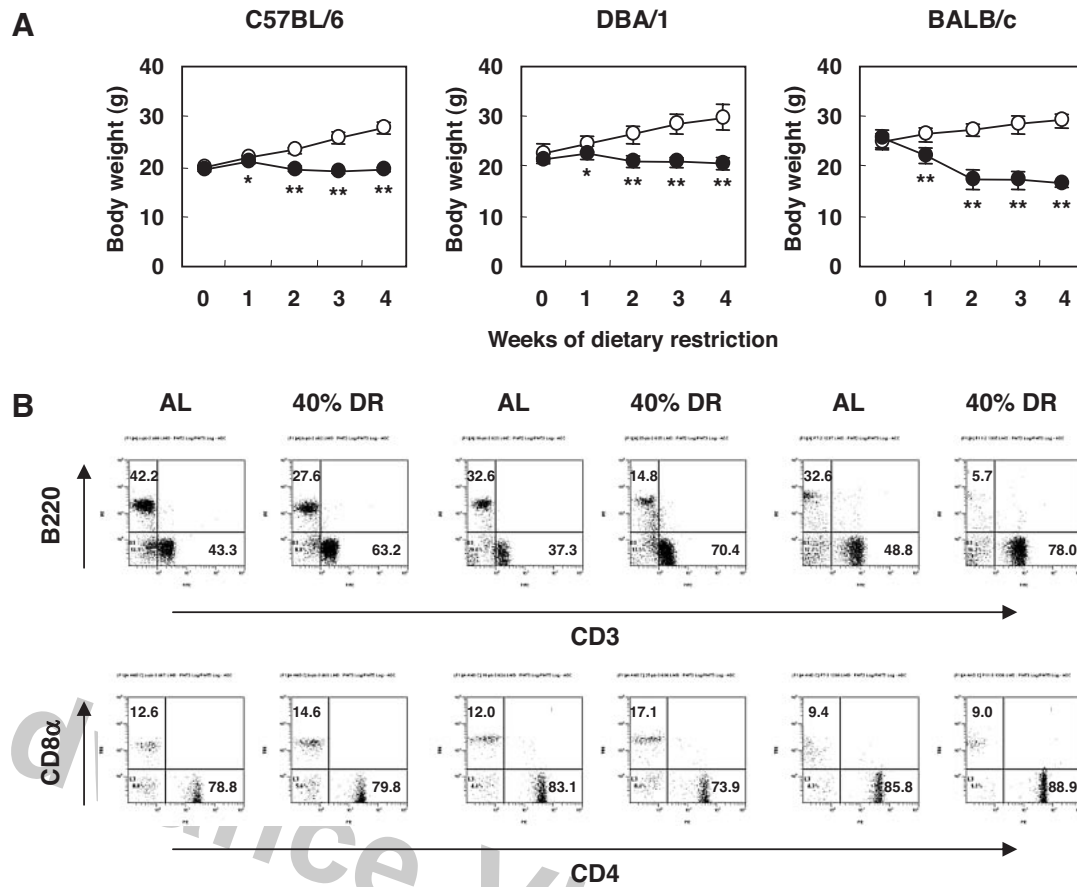


Fig. 1. Effects of Dietary Restriction (DR) on Body Weight and PBMCs in Three Inbred Mouse Strains.

A, Mice 7 weeks old were given the AIN-76 diet *ad libitum* (n = 6, ○) or subjected to 40% DR of the same diet (n = 6, ●) for 4 weeks, and body weight was measured. Data are shown as mean ± SD. Significant difference between the AL and the DR mice is shown as follows: * $p < 0.05$; ** $p < 0.01$. B, Flow cytometry analysis of PBMCs from mice given diet *ad libitum* (AL) or subjected to 40% DR. After gating out CD3⁺ cells, the proportion of CD4⁺ and CD8α⁺ cells was examined. Number in figures represents the relative proportion of each subset.

Table 1. Effect of Dietary Restriction on IFN-γ/IL-4-Production by PBMCs Stimulated with Immobilized Anti-CD3 mAb

Mouse	IFN-γ (ng/ml)		IL-4 (pg/ml)	
	AL	40% DR	AL	40% DR
C57BL/6 (n = 6)	24.7 ± 9.1	39.9 ± 2.1	10 ± 7	30 ± 13
DBA/1 (n = 6)	70.0 ± 8.3	54.6 ± 15.1	309 ± 27	369 ± 112
BALB/c (n = 5–6)	170.6 ± 13.8	11.8 ± 5.2**	154 ± 39	298 ± 84

The mice were bred under AL or 40% DR conditions for 4 weeks (from 7 weeks old to 11 weeks old), and their PBMCs were stimulated with immobilized anti-CD3 mAb for 1.5 d (for IL-4 production) or 7 d (for IFN-γ production).

** $p < 0.01$ as compared with the AL mice

drastic in the BALB/c mice among these three inbred strains (Table 1).

Alleviation of allergic reaction by dietary restriction

To determine the effects of DR on the occurrence of allergic reactions, we induced an allergic response in the BALB/c mice 11 weeks old and 22 weeks old that had been bred for 4 weeks under AL or DR conditions. When the AL mice were immunized and then intranasally challenged with OVA, leukocytes recovered in the BALF drastically increased. In contrast, in the 40% DR mice challenged with OVA in the same way, accumulation of leukocytes into the lung was hardly induced. The tendency to suppress accumulation of leukocytes was observed in mice bred even under the condition of 20% DR (Fig. 3A and B). The total IgE level in the serum induced by intranasal challenge with

OVA was significantly lower in the 40% DR mice than in the AL mice, although the OVA-specific IgE in serum was not decreased by DR (Fig. 3C and D). It should be noted that the IL-4 level was reduced in the BALF of the 40% DR mice as compared with the AL mice, while the influence of DR on IFN-γ level in the BALF was variable, dependent on age (Fig. 3E and F).

Reduction of antigen-specific T cells by dietary restriction

To determine how DR can suppress the allergic response, T cells were purified from the PBMCs of the AL and the DR mice and stimulated with immobilized anti-CD3 mAb. Forty % DR reduced the ability of T cells to produce IFN-γ in the younger mice (11 weeks old), but IFN-γ production was not different between the AL and the DR mice 22 weeks old. OVA-specific IFN-

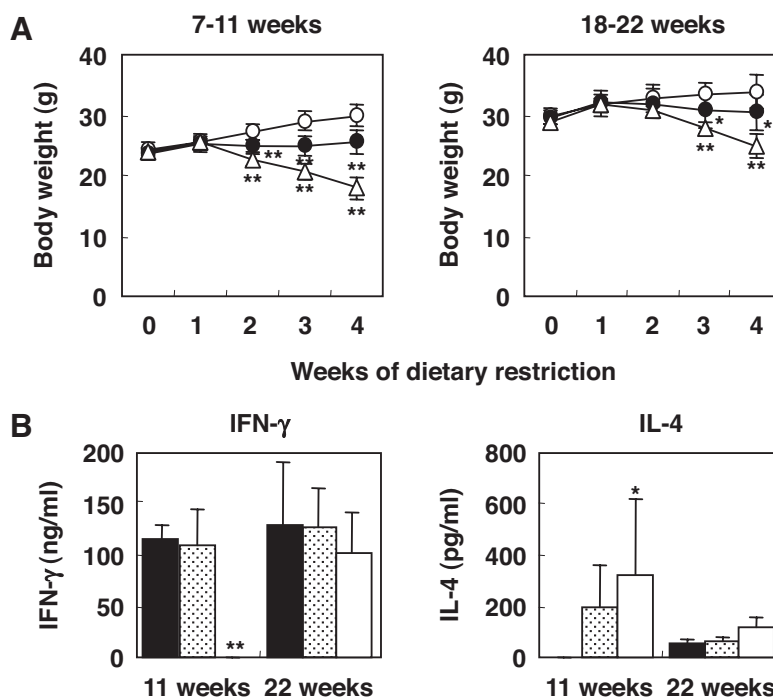


Fig. 2. Effects of Dietary Restriction (DR) on Body Weight and Cytokine Production by PBMCs.

A, BALB/c mice 7 weeks old or 18 weeks old were given the AIN-76 diet *ad libitum* (AL: $n = 10$, $\circ$), subjected to 20% DR ($n = 10$, $\bullet$), and 40% DR ($n = 10$, $\triangle$) with the same diet for 4 weeks, and body weight was measured. Data are presented as mean $\pm$ SD. Significant difference between the AL mice and the DR mice is shown as follows: * $p < 0.05$; ** $p < 0.01$. B, PBMCs from AL mice ($\blacksquare$), 20% DR mice ($\boxplus$), and 40% DR mice ($\square$) were cultured in a well coated with anti-CD3 mAb for 1.5 d (for IL-4 production) or 7 d (for IFN- γ production). Cells cultured in the well coated with unrelated hamster IgG produced neither IFN- γ nor IL-4 (data not shown). IFN- γ in the 40% DR mice 11 weeks old and IL-4 in the AL mice 11 weeks old was under the detectable level. Data obtained from 4 mice (11 weeks) and 3 mice (22 weeks) per group are presented as mean $\pm$ SE. Significant difference between the AL and the DR mice is shown as follows: * $p < 0.05$; ** $p < 0.01$.

γ -production was hardly detected under this immunization protocol irrespective of the age of the mice (Fig. 4A and B). The T cells from the DR mice exhibited an ability to produce higher quantities of IL-4 than the T cells from the AL mice when stimulated with immobilized anti-CD3 mAb. In contrast, OVA-induced IL-4 production was retarded in the T cells from the DR mice as compared with the AL mice (Fig. 4C and D).

These results suggest that DR down-regulates the ability of T cells to produce IL-4 in response to OVA or diminishes the clone size of OVA-specific IL-4-producing T cells. To distinguish these possibilities, we measured the relative proportion of T cells capable of producing IL-4 in response to OVA. Limiting dilution analysis revealed that the proportion of OVA-responsive IL-4-producing T cells among total T cells in the AL mice was 1/17 (11-week old mice) and 1/52 (22-week old mice), respectively. The relative proportion of OVA-responsive IL-4-producing T cells was lower in the DR mice, and the degree of decrease was dependent on the extent of DR (Table 2).

Influence of dietary restriction on NK cell activity

To determine the influence of DR on cellular immune functions, we compared the proportion and cytolytic activity of NK cells. The relative proportion of NK cells (DX5⁺CD3⁻) in the spleen was lower in the 40% DR mice than in the AL mice (Fig. 5A). In younger mice (11 weeks old), 40% DR almost deprived NK cells of cytolytic activity. In contrast, the cytolytic activity of the NK cells was not reduced so drastically in the 40% DR mice 22 weeks old (Fig. 5B).

Discussion

In this study, we examined the influence of DR on immune functions in mice. DR modified the relative ratio of B/T cells and induced immune deviation in terms of IFN- γ /IL-4 production by T cells in the peripheral blood. Peripheral blood T cells from the DR mice exhibited potent ability to produce higher quantities of IL-4 than the T cells from the AL mice. The extent of enhancement of IL-4 production by DR was the most drastic in the BALB/c mice among three inbred strains examined. DR affects various aspects of immune functions, including phagocyte function, antibody production, and cytokine production.¹³⁻¹⁵ Our results indicate that DR modulates IFN- γ /IL-4 production by peripheral blood T cells, resulting in a shift of Th1/Th2 balance toward Th2 dominance.

However, we found that DR alleviated the allergic responses induced by systemic immunization and subsequent intranasal challenge with OVA, in association with declines in the IL-4 concentration in the BALF and the serum IgE level. These results suggest that allergen-induced production of IL-4 and IgE declines in DR mice as compared with AL mice. The relative proportion of OVA-specific IL-4-producing T cells was much less in the DR mice than the AL mice. Therefore, it is thought that the expansion of OVA-specific IL-4-producing T cells is suppressed under DR, although we cannot rule out the possibility that the amount of IL-4 produced by one T cell was reduced in the DR mice as compared with the AL mice. Several types of lymphocytes, including TCR $\alpha\beta$ ⁺ T cells, TCR $\gamma\delta$ ⁺ T cells, and NKT cells, are

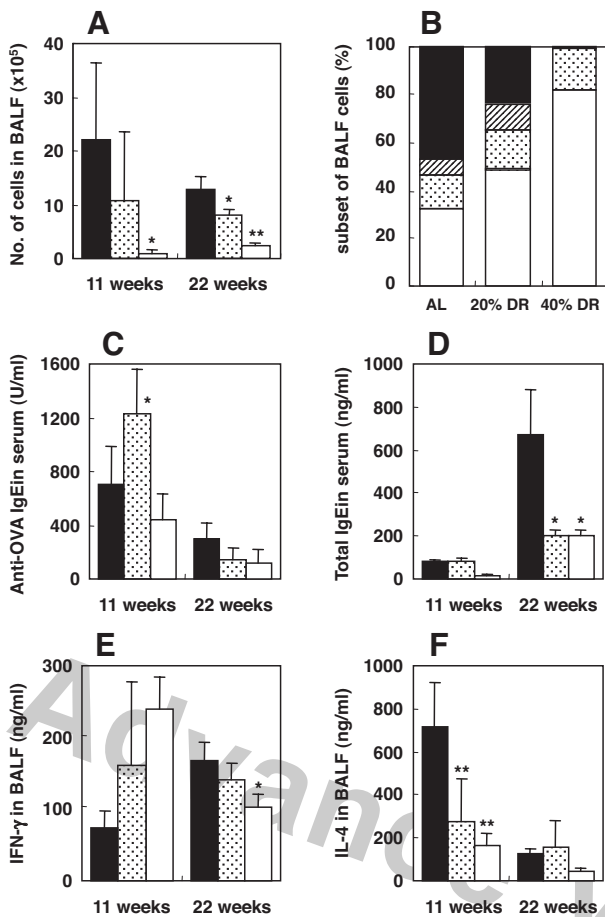


Fig. 3. Effects of Dietary Restriction (DR) on Allergic Responses Induced by Challenge with OVA.

A, Mice 7 weeks old or 18 weeks old were given the AIN-76 diet *ad libitum* ($n = 3-5$, ■), subjected to 20% DR ($n = 3-6$, ▨), and 40% DR ($n = 3$, □) for 4 weeks, and the total leukocyte number in the BALF was counted. B, Cytocentrifuged preparations of BALF obtained from mice 22 weeks old were stained with Diff-Quick solution and eosinophil (■), neutrophil (▨), lymphocyte (▨), and macrophage (□) were differentially counted. Relative proportions are shown. C, OVA-specific IgE level and (D) total IgE level in the serum and IFN- γ level (E) and IL-4 level (F) in the BALF from AL mice (■), 20% DR mice (▨), and 40% DR mice (□) were measured. Data were obtained from the following mice: 11 weeks old ($n = 3-6$ /group), 22 weeks old ($n = 3$ /group). * $p < 0.05$; ** $p < 0.01$ as compared with the AL mice.

Table 2. Effect of Dietary Restriction on Frequency of OVA-Specific IL-4-Producing T Cells

Age	Frequency of IL-4-producing T cells		
	AL	20% DR	40% DR
11 weeks	1/17	1/65	1/128
22 weeks	1/52	1/110	1/668

The mice were bred under AL, 20% DR, or 40% DR conditions for 4 weeks, and the relative ratio of IL-4-producing T cells in response to OVA was measured.

involved in the airway hypersensitivity induced by OVA, and all of them can produce IL-4.¹⁶⁾ Our results indicate that peripheral blood T cells were rendered less competent at producing IL-4 in response to OVA under DR. Judging from the fact that more than 95% of peripheral blood T cells are TCR $\alpha\beta$ ⁺ T cells, these results indicate that the main effect of DR is to restrain excessive expansion of IL-4-producing TCR $\alpha\beta$ ⁺ T cells

specific for OVA. From this viewpoint, it is of great interest that the maintenance and/or production of naïve T cells is improved and TCR diversity is consequently preserved under calorie restriction (CR) in nonhuman primates.¹⁷⁾ While it remains to be elucidated how DR modulates the excessive expansion of antigen-specific T cells, CR has been found to inhibit thymopoiesis and lessen the proliferative response of mature T cells.^{18,19)}

Our results confirm that DR is beneficial in the prevention of allergic responses, but DR may be harmful to defense against pathogenic infection. Peritoneal macrophages of mice bred under 40% CR have been found to express lower levels of mRNA of proinflammatory cytokines (IL-6, IL-12, TNF- α) and Toll-like receptors (TLR2, TLR4), and mice bred under 40% CR died earlier than AL mice after sepsis was induced by cecum ligation and puncture.²⁰⁾ The effect of CR on NK cells is controversial. Some reports indicate that CR reduces endogenous NK cell activity in the spleen of mice and impairs enhancement of NK cell activity in the lung after intranasal infection of the influenza virus,^{21,22)} but another study clarified that CR did not affect endogenous NK cell activity and enhanced IL-2-induced NK cell activity in rats.²³⁾ We have observed that the cytolytic activity of NK cells declined in younger mice (11 weeks old) but not in older mice (22 weeks old) even when they were similarly subjected to 40% DR. These results indicate that the influence of DR on NK cells varies depending on age-related changes in immune functions.

It should be noted that DR affected immune functions not only in the mice 11 weeks old but also in the mice 22 weeks old, but the degree of influence due to DR was more evident in the younger mice. One of the reasons the younger mice exhibited more drastic changes under DR may be that younger mice reserve less energy than older ones. During the course of influenza virus infection, mice subjected to energy restriction suffered from weight loss beyond the critical threshold and died, while the AL mice maintained the body weight above the critical level.²⁴⁾ Our study showed that weight loss due to DR was greater in mice 11 weeks old than mice 22 weeks old. Therefore, the energy necessary to control immune functions is insufficient in younger mice (11 weeks old) under DR, and they exhibit greater changes in cytokine production patterns than older mice (22 weeks old).

It has become a serious social problem that allergic patients are remarkably increasing in developed countries, and changes in the life style, including diet, are considered to be one of the causes of the increment of allergic patients in modern societies.²⁵⁾ Clinical studies have been performed to assess the beneficial effects of restriction of food intake on allergic symptoms. A pilot study showed that restriction of energy intake to 55% of nutritional requirements for 8 weeks significantly alleviated the symptoms of atopic dermatitis.²⁶⁾ Moreover, alternate-day restriction of food intake regimen (repetition of consumption of 320–380 calories one day and *ad libitum* intake the other) for 8 weeks improved quality of life in subjects with body-mass-index >30 and moderate asthma.²⁷⁾ These results encourage us to clarify the beneficial effects of restriction of food intake and to apply them to remedy dysregulated immune functions and to maintain health.

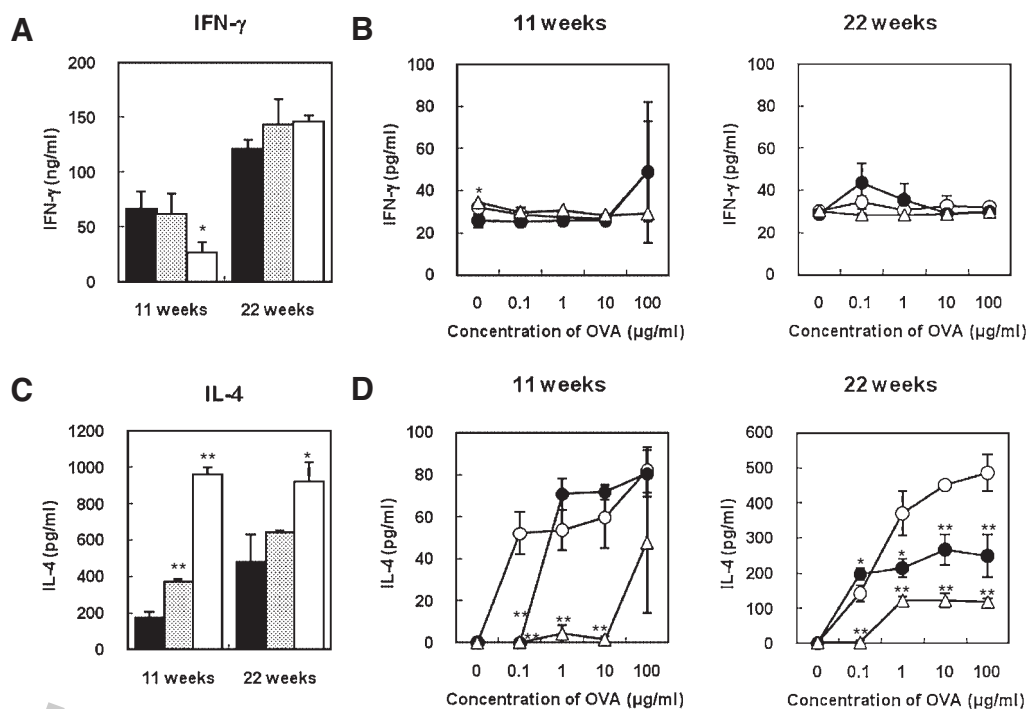


Fig. 4. Effects of Dietary Restriction (DR) on Antigen-Specific Cytokine Production by Peripheral Blood T Cells.

T cells purified from PBMCs were stimulated with immobilized anti-CD3 mAb (A, C) or cultured in the presence of APC and various concentration of OVA (0–100 μg/ml) (B, D) for 6 d. Culture supernatants were recovered, and the concentrations of IFN-γ and IL-4 were measured. Symbols: (A, C) ■, AL mice; ▨, 20% DR mice; □, 40% DR mice; (B, D) ○, AL mice; ●, 20% DR mice; △, 40% DR mice. OVA-triggered enhancement of IFN-γ production was minimal in this immunization protocol (B).

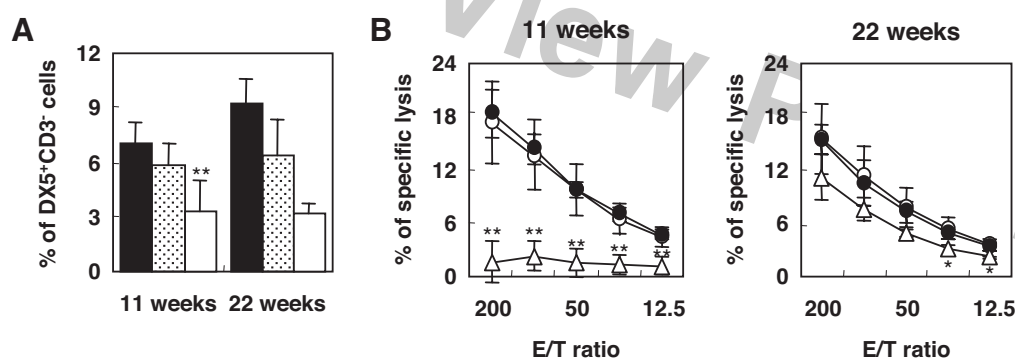


Fig. 5. Effects of Dietary Restriction (DR) on NK Cells.

Mice 7 weeks old or 18 weeks old were given the AIN-76 diet *ad libitum*, and subjected to 20% DR or 40% DR for 4 weeks. Mononuclear cells were prepared from the spleens, and (A) the relative proportion of NK cells (DX5⁺CD3[−]) and (B) cytolytic activity against YAC-1 cells were assayed. Column and symbols: (A) ■, AL mice; ▨, 20% DR mice; □, 40% DR mice; (B) ○, AL mice; ●, 20% DR mice; △, 40% DR mice. Data were obtained from the following mice: 11 weeks old (n = 4–10/group), 22 weeks old (n = 2–3/group). *p < 0.05; **p < 0.01 as compared with the AL mice.

Acknowledgments

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