Effects of Dietary Restriction on Age-Related Immune Dysfunction in the Senescence Accelerated Mouse (SAM)[1]

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ABSTRACT The effects of age and dietary restriction on immune response were investigated using an animal model of accelerated senescence (senescence accelerated mouse, SAM). The experimental groups consisted of control (ad libitum fed) and restricted groups (fed 60% of energy intake of the controls). Spleen weight and total number of splenic cells were significantly lower in the food-restricted group at 8 mo of age. Percentages of T (Thy-1.1$^+$) and B (surface Ig$^+$) cells in the splenic cells were not significantly different between the two groups. The number of direct hemolytic plaque-forming cells per 10^6 spleen cells 4 d following immunization with sheep red blood cells and dinitrophenyl-Ficoll was significantly greater in the 8-mo-old mice in the food-restricted group than in the control group. In the latter group, antibody responses progressively decreased with age. Mitogen responses to concanavalin A and lipopolysaccharide were maintained in the food-restricted group but were depressed in the control group at 8 mo. In addition, though autoantibody to single-stranded DNA increased in the control group with advancing age, there was a steady decrease in the food-restricted group until 8 mo. Serum immunoglobulin (IgA and IgM) concentrations were significantly lower in the food-restricted group than in controls at 8 mo of age. Therefore, our results suggest that when senescence accelerated mice are subjected to food restriction, there may be a modulatory effect on the immune dysfunction associated with advancing age. *J. Nutr.* 120:1393–1400,1990.

INDEXING KEY WORDS:

- *senescence accelerated mouse (SAM)*
- *dietary restriction* • *immunity* • *aging*

A murine model of accelerated senescence, the senescence accelerated mouse (SAM), consists of SAM-P strains that are markedly short-lived and SAM-R strains with normal characteristics of aging (1–6). The SAM-P strains grow normally, but then they show early signs of severe loss of physical activity and skin glossiness, coarse skin, hair loss, periophthalmic lesions and increased lordokyphosis. The life span of the SAM-P strains is about 26% shorter than that of the SAM-R strains. In view of evidence obtained from the SAM-P survivors, the growth rate and Gompertz function, it seems that manifestations of senescence do not occur in the developmental stage but occur in an accelerated manner after normal development. We considered that the aging pattern in SAM-P strains was an acceleration of senescence (1).

In previous work, it became evident that a 40% food restriction in the SAM-P/1 strain extended the mean and maximum survival time and reduced the grading score of senescence and the deposition of senile amyloid (7). Food restriction has extended mean and maximum longevity in rats and mice (8, 9), reduced the incidence and delayed the onset of various cancers and other late-life-related diseases (10–12). However, the mechanisms by which this restriction produces these effects are not well understood. In several studies, energy restriction was begun after weaning or in early adulthood, and a significant improvement in immunologic response and a decrease in autoantibody production were

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noted (13). The reduction in age-related phenomena may reflect the effect of food intake on the aging immune system. The objective of the present investigation was to use an animal model of accelerated senescence, SAM-P/1, to assess the effect of dietary restriction on changes in immune responses with aging. The in vivo immune functions were analyzed in terms of antibody responses with sheep red blood cell (SRBC)[3] (T-dependent antigen) and dinitrophenyl (DNP)-Ficoll (T-independent antigen), and the in vitro functions were assessed in terms of mitogen responses with concanavalin A (conA) and lipopolysaccharide (LPS). Levels of serum immunoglobulins and autoantibodies to single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) were also determined.

MATERIALS AND METHODS

Mice and diets. Six-week-old SAM-P/1 mice born and raised in our laboratory under conventional conditions were used. Both sexes were used for the longevity study, and female mice were used for the immunologic study. The mice were separated into two groups: a control group fed ad libitum and a group fed 60% of the energy in the diet of the controls. All mice were housed five per cage and were fed a purified diet (**Table 1**). The control, ad libitum–fed group was fed diet 1. Diet 2 was provided to the experimental group in amounts equal to 60% of the energy intake of the ad libitum group. Diet 2 was fed once a day at 1700 h. The composition of diet 2 was such that when it was fed at 60% of the energy intake of diet 1, it provided amounts of vitamins and minerals per animal that were equal to those supplied to the mice fed diet 1. Based on body weight gain during the preliminary period, a few mice considered to be dominant animals were excluded from the experimental group. All the mice had free access to tap water and were maintained in a temperature-controlled room (24 ± 2°C) with 12 h of light per day. Representative mice were killed at 6 wk and at 5, 8 and 11 mo of age for examination of immunologic function. However, the few mice fed ad libitum that were alive at age 11 mo were excluded from the study. All the mice were weighed once monthly beginning with the sixth postnatal week.

Hemolytic plaque-forming cell (PFC) response to SRBC and DNP-Ficoll. Sheep red blood cells were washed three times in medium (RPMI-1640; GIBCO, Grand Island, NY) before use as the T-dependent antigen. Ficoll 400 (Pharmacia, Uppsala, Sweden) coupled with 2,4-DNP to obtain DNP-Ficoll (14) was used as the T-independent antigen. To measure the primary response, mice were immunized intraperitoneally with 10^8 SRBC/20 g body weight or intravenously with 2.5 μg DNP-Ficoll dissolved in saline. Four days later, the mice were decapitated, and the spleens were removed. The spleen cells were assayed for PFC by the Cunningham and Szenberg (15) modification of the Jerne plaque technique. For determination of the anti-DNP antibody forming cells, hapten-coupled SRBC were prepared as indicator cells by the method of Rittenberg and Pratt (16).

TABLE 1

Diet composition[1]

Ingredient	Amount	
	Diet 1	Diet 2
	%	
Casein	25.0	25.0
DL-Methionine	0.3	0.3
Cornstarch	34.3	32.5
Sucrose	23.4	21.6
Cellulose powder	2.0	2.0
Soybean oil	9.0	9.0
Mineral mix[2]	5.0	8.0
Vitamin mix[3]	1.0	1.6

[1]Diet 1 was fed ad libitum. Diet 2 was fed to 40% diet-restricted mice (60% of control energy intake) and was enriched in mineral and vitamin mixtures relative to diet 1. The two diets were virtually isoenergetic (diet 1, 412 kcal/100 g; diet 2, 409 kcal/100 g).

[2]Mineral mix (Clea Japan, Tokyo, Japan) supplied the following (mg/100 g diet 1): $CaCO_3$, 1355.4; KH_2PO_4, 1730.0; $CaHPO_4 \cdot 2H_2O$, 1500.0; $MgSO_4 \cdot 7H_2O$, 800.0; NaCl, 600.0; $FeC_6H_5O_7 \cdot XH_2O$, 190.0; $5ZnO \cdot 2CO_2 \cdot 4H_2O$, 6.0; $CuSO_4 \cdot 5H_2O$, 1.26; $CoCl_2 \cdot 6H_2O$, 0.40; $Ca(IO_3)_2$, 1.54; $MnSO_4 \cdot 4H_2O$, 15.4.

[3]Vitamin mix (Clea Japan, Tokyo, Japan) supplied the following (mg/100 g diet 1 unless otherwise noted): retinyl acetate 1200 IU; cholecalciferol, 240 IU; thiamin, 1.5; riboflavin, 1.5; vitamin B-6, 1.0; vitamin B-12, 5.0; dl-α-tocopherol, 10.0; menadione, 0.20; biotin, 0.01; calcium pantothenate, 2.0; para-aminobenzoic acid, 10.0; niacin, 10.0; inositol, 15.0; folic acid, 0.20; choline chloride, 300.

Lymphocyte proliferation to mitogen. Mice were anesthetized with ether and killed by cardiac puncture. The spleens were weighed, and cells were counted and distributed into sterile tubes at a concentration of 5×10^6/mL of medium. Some of the cells were used to assay cell surface markers. The mitogens used were con A (Sigma Chemical, St Louis, MO) and LPS (Difco Laboratories, Detroit, MI). The response of the spleen cells to mitogens was determined by incubating 5×10^5 cells for 3 d at 37°C in an atmosphere of 5% CO_2 with 3.5 μg con A or 40 μg LPS, in volumes of 0.2 mL, in flat-bottomed wells of microtiter plates (triplicate samples). Each well was pulsed with 0.4 μCi [^{3}H]thymidine for the final 6 h before harvest. The radioactivity of ^{3}H-labeled samples

[3]Abbreviations: con A, concanavalin A; DNP, dinitrophenyl; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; LPS, lipopolysaccharide; PFC, plaque-forming cell; SRBC, sheep red blood cell; ssDNA, single-stranded DNA.

was determined in a toluene-based scintillation fluid. Counting efficiency was 30% for ^{3}H-labeled samples. The results are expressed as counts per minute per vial corrected for control counts. The control contained equivalent numbers of cells but no mitogens.

Cell surface markers. The percentage of lymphocytes bearing Thy-1.1 or surface immunoglobulin (sIg) marker in the spleen cell after labeling with monoclonal antibody conjugated with fluorescein isothiocyanate (FITC) was assayed by flow-cytometry. Spleen cells were incubated with FITC conjugated sheep anti-mouse Ig F(ab')2 (Silenus Laboratories, Victoria, Australia) or FITC conjugated Thy-1.1 (Meiji Institute of Health Science, Tokyo, Japan). The final concentration of the antibody was adjusted to 1/200. Cells were analyzed using flow-cytofluorometric procedures with a cell sorter (ABCAS-100 Showa Denko K.K., Tokyo, Japan) to obtain percentages of sIg^+ or $Thy\text{-}1.1^+$ cells. Nonlymphoid cells were gated on the basis of the forward and perpendicular light scatter signal.

Measurements of serum immunoglobulins and protein. Serum levels of IgG, IgM and IgA were determined using an enzyme-linked immunosorbent assay (ELISA). Assays were conducted according to a modification of the method described by Yarchoan et al. (17), using affinity purified goat anti-mouse γ, μ and α chain-specific antibodies conjugated with alkaline phosphatase (Zymed Laboratories, San Francisco, CA). Results are expressed in terms of milligrams per 100 mL. Thus, each value was calculated from a standard curve obtained by serial dilution of either purified IgG, IgM or IgA mouse myeloma protein, 1 mg/mL of UPC 10 (IgG_k), 1 mg/mL of MOPC 104E (IgMλ1) or 1 mg/mL of MOPC 315 (IgAλ2) (Litton Bionetics, Charleston, NC), respectively. Total serum proteins were measured by the method of Lowry et al. (18) with bovine serum albumin as a standard (18).

Measurement of anti-ssDNA and anti-dsDNA antibody levels. Heat-denatured calf thymus DNA (type V; Sigma Chemical, St Louis, MO) was used as the dsDNA. Single-stranded DNA was obtained by heating dsDNA at 100°C for 10 min and quickly chilling in an ice-water bath. Assays were performed according to a modification of the method of Zouali et al. (19), using ELISA plates coated with ssDNA and dsDNA. Serum samples were diluted to 1:60. Alkaline phosphatase–labeled goat anti-mouse IgG and IgM antibodies (Zymed Laboratories, San Francisco, CA) were used to monitor the binding. The levels of anti-ssDNA and dsDNA antibodies were expressed as the difference in absorbance at 415 and 450 nm after incubation with the substrate.

Statistical analysis. Analysis of variance and the Kaplan-Meier test (20) were used to compare the mean life spans and the 10th decile in animals of each group. For the other data, a two-way analysis of variance was used to compare results with the two types of energy intake across the various ages of the mice. Comparisons between results with the two types of energy intake at each age were made using Tukey's test (21).

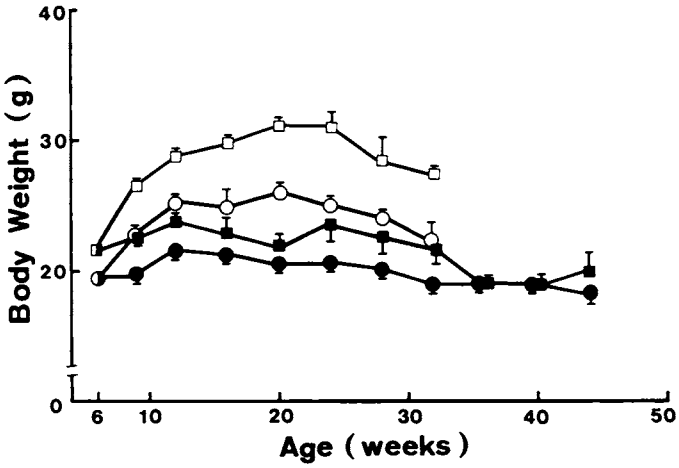


FIGURE 1 Average body weight in the ad libitum–fed group and in the group whose diet was restricted (60% of energy). Each point represents the mean ± SEM. Values are for males fed ad libitum (n = 39, □); males fed 60% of ad libitum intake (n = 34, ■); females fed ad libitum (n = 47, ○); and females fed 60% of ad libitum intake (n = 26, ●).

RESULTS

Growth curve and longevity. The mean body weights for each group are plotted in **Figure 1.** Mice fed the restricted diet showed little change in body weight throughout their life span, and they appeared vigorous and healthy until very late in life. The difference in weight between the group fed the restricted diet and the ad libitum–fed group was a 10 g in males and 6 g in females at age 20 wk. Survival curves for both sexes are shown in **Figure 2.** The mean life span of male mice in the restricted-diet group was 310 ± 17 d, that is 47% longer than that of the ad libitum–fed group ($p < 0.01$). The 10th decile of the restricted-diet group of males was 446 ± 11 d, or 34% longer than that of the ad libitum–fed group ($p < 0.01$). In the females fed the restricted diet, the mean life span was 331 ± 11 d, or 40% longer ($p < 0.01$) than that of the ad libitum–fed group, and the 10th decile was 408 ± 11 d, or 14% longer ($p < 0.01$).

Splenic weight and lymphocyte concentration. As shown in **Table 2,** at 5 mo of age, there was no significant difference in spleen weight between the groups. At 8 mo of age, spleen weight in the ad libitum–fed group was greater than it had been at 6 wk and was 3.4 times larger than that in the restricted-diet group, in which spleen weight had decreased to a minimum among the ages observed ($p < 0.01$). The effect of age on the spleen weight was not significant, but the interaction between age and energy intake was significant ($p < 0.01$) The effect of food restriction on spleen weight was signifi-

TABLE 2

Effect of age and nutrition on spleen weight, splenic cell numbers and the proportions of Thy-1.1⁺ and sIg⁺ cells in the ad libitum–fed and food-restricted mice[1]

Age and diet	n	Spleen weight		Splenic cell numbers	% of Splenic cells	
					Thy-1.1⁺	sIg⁺
		mg	*mg/g body wt*	$\times 10^8$	%	
6 Weeks	6	153 ± 15	6.4 ± 0.5	1.80 ± 0.07	20 ± 0.9	51 ± 5.0
5 Months						
Ad libitum	7	130 ± 10	4.2 ± 0.2	1.35 ± 0.13	20 ± 0.2	51 ± 1.5
60%	6	107 ± 9	4.8 ± 0.4	0.97 ± 0.10	21 ± 2.9	50 ± 2.3
8 Months						
Ad libitum	7	220 ± 17	8.5 ± 1.0	2.29 ± 0.23	21 ± 0.4	45 ± 4.4
60%	10	65 ± 9*	2.8 ± 0.3*	0.65 ± 0.08*	25 ± 3.5	47 ± 3.7
11 Months, 60%	8	96 ± 10	4.6 ± 0.4	1.04 ± 0.27	20 ± 0.3	46 ± 2.3

[1]Values are means ± SEM in each diet/age group. Two-way ANOVA of age and energy intake for spleen weights and the numbers of splenic cells showed a significant ($p < 0.01$) age × energy intake interaction, and the effect of energy intake was significant ($p < 0.01$). Restricted-diet group values that are significantly different from the corresponding ad libitum–fed group values are marked with an asterisk ($p < 0.01$, Tukey's test).

cant ($p < 0.01$). At 8 mo of age, the total number of splenic nucleated cells in the ad libitum–fed group was 3.5 times higher than that in the restricted-diet group ($p < 0.01$). Splenic cell numbers correlated with the spleen weights. The ratios of T (Thy-1.1⁺) and B (sIg⁺) cells among the splenic cells showed no differences between the groups.

Antibody responses to SRBC and DNP-Ficoll. As shown in **Figure 3**, at 5 mo of age, anti-SRBC and anti-DNP-Ficoll responses of spleen cells were not significantly different in the two groups of animals. At 8 mo of age, there was a progressive decrease in both antibody responses in the ad libitum–fed group. In the 8-mo-old mice fed the restricted diet, the anti-SRBC response was vigorous, and the level of anti-DNP-Ficoll response observed in the 5-mo-old food-restricted mice was maintained. Age did not influence the difference between the groups for both antibody responses, but the interaction between age and energy intake was significant ($p < 0.01$ for both responses). Food restriction had significant effects on the anti-SRBC ($p < 0.05$) and DNP-Ficoll ($p < 0.01$) responses. At 11 mo of age, though both antibody responses decreased in the group fed the restricted diet, the mean levels of both antibody responses were maintained at the level of 5-mo-old food-restricted mice.

Mitogen responses of lymphocyte. As shown in **Figure 4A**, the response to con A decreased with advancing age in the ad libitum–fed group. The group fed the restricted diet responded as did the control group at 5 mo of age. Con A response in the 8-mo-old mice fed the restricted diet was maintained at the level in the 5-mo-old mice. The effects of age and energy intake on the con A response were not significant, but their interaction was significant ($p < 0.05$) At 11 mo of age, the level of response to con A in the group fed the restricted diet decreased to nearly the level observed for 8-mo-old mice in the ad libitum–fed group. Response to the B cell mitogen, LPS, showed a striking decline with advancing age in the ad libitum–fed control group (Figure 4*B*). The level of LPS response in the restricted-diet group was maintained from 6 wk to 8 mo of age. The effect of age on the LPS response was not significant, but the interaction between age and energy intake was significant ($p < 0.01$), and the effect of food restriction on the LPS

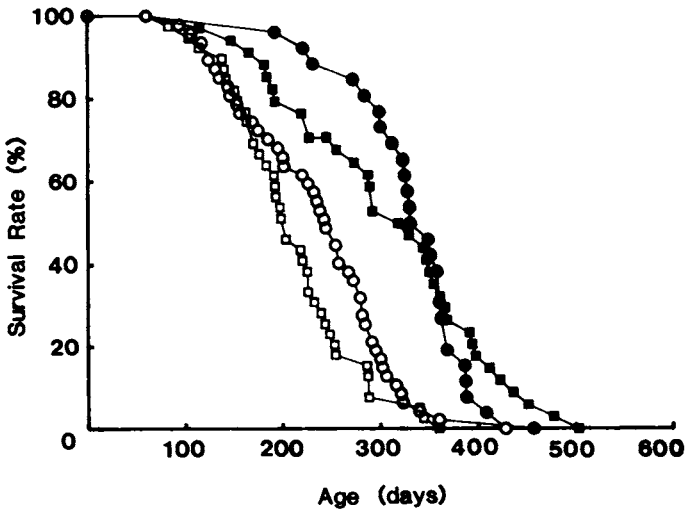


FIGURE 2 Survival curves of males fed ad libitum (□), males fed 60% of ad libitum intake (■), females fed ad libitum (○) and females fed 60% of ad libitum intake (●). See Figure 1 legend for the numbers of mice.

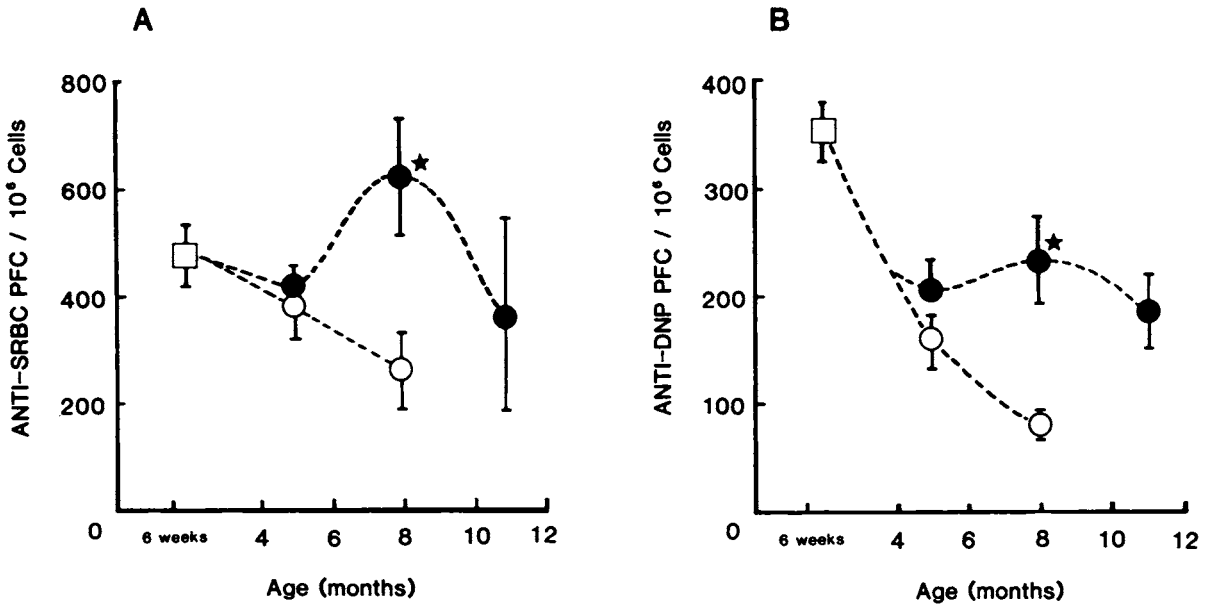


FIGURE 3 Influence of diet and age on number of direct plaque-forming cells (PFC) per million spleen cells after immunization with sheep red blood cells (SRBC) (*A*) or dinitrophenyl (DNP)-Ficoll (*B*) in mice fed ad libitum or fed 60% of ad libitum intake. Each data point represents the mean ± SEM for the 6-wk-old SAM-P/1 mice (□) before they were divided into two groups, the ad libitum–fed group (○) and the group fed 60% of ad libitum intake (●). Two-way ANOVA of age and energy intake showed a sigificant (A: $p < 0.05$; B: $p < 0.05$) age × energy intake interaction, and the effects of energy intake were significant (A: $p < 0.05$; B: $p < 0.01$). Restricted-diet group values that are significantly different (Tukey's test) from the corresponding ad libitum–fed group values are noted as follows: ★$p < 0.01$. The number of mice in each group was as follows. For panel A, there were seven ad libitum–fed mice at 6 wk and eight and seven at 5 and 8 mo of age; six, seven and six diet-restricted mice at 5, 8 and 11 mo of age, respectively. For panel B, there were five ad libitum–fed mice at 6 wk and six and eight and 5 and 8 mo of age, respectively; there were six diet-restricted mice each at 5, 8 and 11 mo of age.

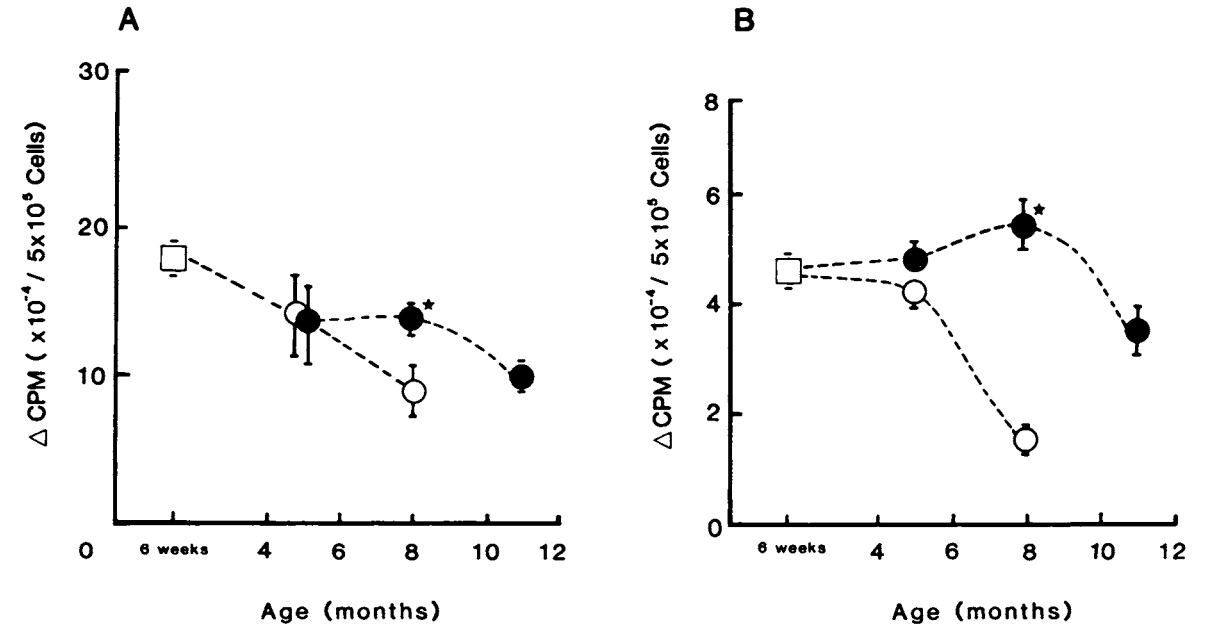


FIGURE 4 Influence of diet and age on the tritiated thymidine uptake of (*A*) concanavalin A and (*B*) lipopolysaccharide mitogen-stimulated splenic lymphocytes from mice of the ad libitum and energy-restricted diet groups. The values are counts per minute (cpm) of [^{3}H]thymidine uptake levels in mitogen-stimulated cultures minus cpm in unstimulated (media without mitogen) cultures (counting efficiency = 30%). Values are for 6-wk-old SAM-P/1 mice (□) and for the subsequent ad libitum-fed (○) and diet-restricted (●) groups. Two-way ANOVA of age and energy intake showed a significant (A: $p < 0.05$, B: $p < 0.01$) age × energy intake interaction, and the effect of energy intake for lipopolysaccharide response was significant ($p < 0.01$). Restricted-diet group values that are significantly different (Tukey's test) from the corresponding ad libitum–fed group values are indicated as follows: ★$p < 0.01$. For the ad libitum–fed mice, $n = 6$ at 6 wk and $n = 7$ both at 5 and 8 mo of age; for the diet-restricted mice, $n = 6$, 10 and 8 at 5, 8 and 11 mo of age, respectively.

TABLE 3

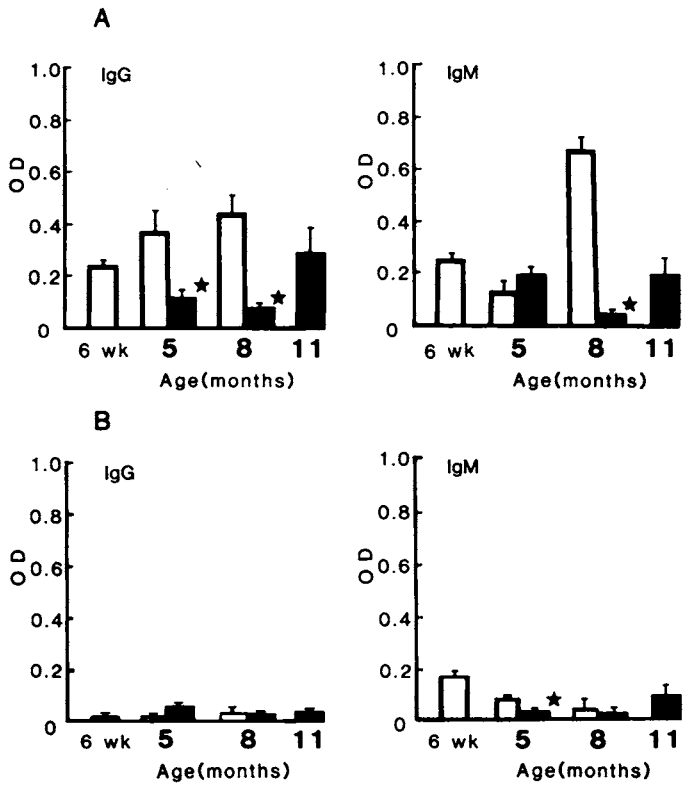
Effect of age and nutrition on total serum immunoglobulin and protein levels in the ad libitum–fed and food-restricted mice[1]

	Diet	6 Weeks	5 Months	8 Months	11 Months
			mg/100 mL		
Immunoglobulins					
IgG	Ad libitum	350 ± 65	711 ± 261	532 ± 84	n.d.
	60%		733 ± 124	134 ± 38	281 ± 76
IgA	Ad libitum	63 ± 7	109 ± 12	155 ± 18	n.d.
	60%		122 ± 3	88 ± 10*	134 ± 24
IgM	Ad libitum	92 ± 4	45 ± 4	54 ± 8	n.d.
	60%		33 ± 5	29 ± 5†	34 ± 10
			g/100 mL		
Total protein					
	Ad libitum	7.1 ± 0.32	7.0 ± 0.70	7.8 ± 0.80	n.d.
	60%		7.1 ± 0.14	7.5 ± 0.80	n.d.

[1]Values are means ± SEM in each diet/age group. See Table 1 for the number of mice in each group. Two-way ANOVA of age and energy intake for IgA (but not for IgG and IgM) showed a significant ($p < 0.01$) age × energy intake interaction. The effects of age for IgG and energy intake for IgM were significant ($p < 0.05$, $p < 0.01$). Restricted-diet group values that are significantly different (Tukey's test) from the corresponding ad libitum–fed group values are indicated as follows: *$p < 0.01$, †$p < 0.05$.

[2]Not determined.

response was evident ($p < 0.01$). At 11 mo of age, the response decreased in food-restricted animals; the level of response was nearly the same as that at 5 mo of age in the ad libitum–fed group.



Serum immunoglobulins and protein. As shown in Table 3, at 5 mo of age, there were no significant differences between the two groups in serum immunoglobulins levels. In the 8-mo-old mice fed the restricted diet, those levels tended to decrease. The effect of age was significant for serum IgG ($p < 0.05$) but not for serum IgA and IgM. The effects of energy intake on serum IgA and IgM, but not IgG, were significant ($p < 0.05$, $p < 0.01$). The interaction between age and energy intake was significant ($p < 0.01$) only for serum IgA. There was no significant difference between the groups with regard to the level of total serum protein. Serum immunoglobulin levels in the 11-mo-old group fed the restricted diet tended to be higher than levels at age 8 mo.

FIGURE 5 Influence of diet and age on the serum levels of (*A*) IgG and IgM anti-ssDNA and (*B*) IgG and IgM anti-dsDNA antibodies in mice fed ad libitum (open bar) or those fed 60% of ad libitum intake (closed bar). Sample sera were prepared from the mice killed for study of the lymphocyte proliferation in response to mitogens. The values are means ± SEM. The optical density (OD) is expressed as the difference in absorbance at 415 and 450 nm. Two-way ANOVA of age and energy intake for IgG and IgM anti-ssDNA and for IgM anti-dsDNA showed a significant (all $p < 0.01$) age × energy intake interaction, and the effects of energy intake for those antibodies were significant (IgG and IgM anti-ssDNA, $p < 0.01$; IgM anti-dsDNA, $p < 0.05$). Restricted-diet group values that are significantly different (Tukey's test) from the corresponding ad libitum–fed group values are indicated as follows: ★$p < 0.01$. For the ad libitum–fed mice, $n = 6$ and 6 wk and $n = 7$ at both 5 and 8 mo of age; for the restricted-diet mice, $n = 6$, 10 and 8 at 5, 8 and 11 mo of age, respectively.

Serum anti-ssDNA and anti-dsDNA antibody levels. As shown in **Figure 5A,** the mean levels of IgG and IgM anti-ssDNA antibodies in the ad libitum–fed group generally increased with age. By contrast, these antibody levels in the restricted-diet group decreased with age to 8 mo and then increased at 11 mo of age. Nonetheless, the effects of age on IgG and IgM anti-ssDNA were not significant. The effect of energy intake on those anti-ssDNA antibodies was significant (both $p < 0.01$), and the interactions between age and energy intake were significant for IgG and IgM anti-ssDNA (both $p < 0.01$).

In both groups, the mean levels of IgG and IgM anti-dsDNA antibodies were markedly low compared to the levels of anti-ssDNA antibodies (Fig. 5*B*). The effect of energy intake was significant for IgM anti-dsDNA ($p < 0.05$), and the interaction between age and energy intake was significant ($p < 0.05$) (Fig. 5*B*). The levels of IgA anti-ssDNA and anti-dsDNA antibodies were lower than those of IgG or IgM anti-ssDNA and anti-dsDNA antibodies, and there was no significant difference between the two groups (data not shown).

DISCUSSION

Restricting intake of energy retards aging, and among the putative mechanisms is a delay in immunologic aging (13). Two types of changes in immunologic aging occur in humans and mice: decreased capacity to respond to exogenous stimuli and increased autoimmunity with age (22). Mice fed energy-restricted diets after weaning or midadulthood exhibited vigorous immunologic responses to T and B cell mitogens, to injected SRBC and to cell-mediated lymphocytotoxicity; they also showed a decrease in autoantibodies (23–28).

Regarding changes in the immune system in SAM-P, we previously reported evidence of an age-associated early decline in immune activities of cultured spleen cells; also, the number of autoantibodies increased with advancing age, albeit at a rate less than that seen in autoimmune strains such as MRL1/1 and NZB (29, 30). In the present study, the in vivo humoral immune responses to both T-dependent and T-independent antigens (SRBC and DNP-Ficoll, respectively) and the in vitro polyclonal responses to T and B cell mitogens (con A and LPS, respectively) declined sharply with advancing age in the ad libitum–fed group but not in the group fed a restricted diet. The interaction between age and energy intake in both groups was statistically significant, but the main effects on those immune responses were related to energy deprivation rather than to advancing age. These findings suggest that the effect of dietary restriction on life span prolongation in SAM-P/1 may partly relate to vigorous immune systems. It was previously suggested that dietary restriction may cause a significant delay in maturation of the immune system and it was noted that diet-restricted animals remained vigorous for a longer time than did controls (23, 24). In light of our results, the greater activity of the immune system in the older mice fed a restricted diet relative to those fed ad libitum does not seem to be due to a delay in maturation but rather to maintenance of a more vigorous immune system. Evidence of immune activities in this strain between 6 wk and 5 mo of age has yet to be confirmed. The decrease in immune responses in the 11-mo-old group fed the restricted diet may be a result of the decline of immunologic function with aging. Though T cell activities (responses to SRBC and con A) decreased to near the levels seen in the 8-mo-old ad libitum–fed mice, B cell activities (responses to DNP-Ficoll and LPS) remained higher than those in the 8-mo-old ad libitum–fed group.

Data in the literature indicate that dietary restriction in both mice and rats leads to higher responses (compared to those in controls) in T cell activity, not in B cell activity, when dietary restriction is initiated just after weaning (13, 26, 27, 31). In the case of SAM-P/1, at age 8 mo, the T cell responses (to SRBC and con A) and B cell responses (to DNP-Ficoll and LPS) in the restricted diet were higher than those in the control group. In the 8-mo-old food-restricted mice, despite the decrease in number of nucleated cells in the spleen, the reactivity to exogenous stimuli increased relative to reactivity in the ad libitum–fed group. There were no significant changes with aging in the proportions of Thy-1.1$^+$ and sIg$^+$ cells between the groups, but qualitative changes in splenic immunocompetent cells might be caused by dietary restriction. A relative increase in T or B lymphocyte subpopulation(s) may account for the increase in splenic immune responses because dietary restriction may increase the proportion of a specific T cell subpopulation (26). Further, immune functions in food-restricted mice may be enhanced by mutual interaction among the splenic immunocompetent cells, including cells other than lymphocytes.

We also confirmed our previous results that autoantibody levels increased with advancing age in the SAM-P strain (30). We further noted that striking increases with advancing age were significantly suppressed in the group fed the restricted diet, for up to 8 mo of age (Table 3).

Thus, age-related immune dysfunctions in SAM-P/1 were alleviated by maintaining immune capability with dietary restriction. Those results suggest that dietary restriction contributes, at least in part, to improvement in the grading score of senescence (2, 7) and to prolongation of the mean life span and 10th decile of the senescence accelerated mice.

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LITERATURE CITED

1. Takeda, T., Hosokawa, M., Takeshita, S., Irino, M., Higuchi, K., Matsushita, T., Tomita, Y., Yasuhira, K., Hamamoto, H., Shimizu, K., Ishii, M. & Yamamuro, T. (1981) A new murine model of accelerated senescence. *Mech. Ageing Dev.* 17: 183–194.
2. Hosokawa, M., Kasai, R., Higuchi, K., Takeshita, S., Shimizu, K., Hamamoto, H., Honma, A., Irino, M., Toda, K., Matsumura, A., Matsushita, M. & Takeda, T. (1984) Grading score system: a method for evaluation of the degree of senescence in senescence accelerated mouse(SAM). *Mech.Ageing Dev.* 26: 91–102.
3. Naiki, H., Higuchi, K., Yonezu, T., Hosokawa, T. & Takeda, T. (1988) Metabolism of senile amyloid precursor and amyloidgenesis: age-related acceleration of apolipoprotein A-II clearance in the senescence accelerated mouse. *Am. J. Pathol.* 130: 579–588.
4. Matsushita, M., Tsuboyama, T., Kasai, R., Okumura, H., Yamamuro, T., Higuchi, K., Higuchi, K., Kohno, K., Yonezu, T., Utani, A., Umezawa, M & Takeda, T. (1986) Age-related changes in bone mass in the senescence accelerated mouse (SAM): SAM-R/3 and SAM-P/6 as new murine models for senile osteoporosis. *Am. J. Pathol.* 125: 276–283.
5. Miyamoto, M., Kiyota, Y., Yamazaki, N., Nagaoka, A., Matsuo, T., Nagawa, Y. & Takeda, T. (1986) Age-related changes in learning and memory in the senescence accelerated mouse (SAM). *Physiol. Behav.* 38: 399–406.
6. Yagi, H., Katoh, S., Akiguchi, I. & Takeda, T. (1988) Age-related deterioration of ability of acquisition in memory and learning in senescense accelerated mouse: SAM-P/8 as an animal model of disturbances in recent memory. *Brain Res.* 474:86–93.
7. Kohno, A., Yonezu, Y., Matsushita, M., Irino, M., Higuchi, K., Higuchi, K., Takeshita, D., Hosokawa, M. & Takeda, T. (1985) Chronic food restriction modulates the advance of senescence in the senescence accelerated mouse (SAM). *J. Nutr.* 115: 1259–1266.
8. McCay, C. M., Crowell, M. F. & Maynard, L. A. (1935) The effect of retarded growth upon the length of the lifespan and upon the ultimate body size. *J. Nutr.* 10: 63–79.
9. Ross, M. H. (1961) Length of life and nutrition in the rat. *J. Nutr.* 75: 197–210.
10. Ross, M. H. & Bras, G. (1965) Tumor incidence patterns and nutrition in the rat. *J. Nutr.* 87: 245–260.
11. Yu, B. P., Masoro, E. J., Murata, I., Bertrand, H. A. & Lynd, F. T. (1982) Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease. *J. Gerontol.* 37: 130–141.
12. Maeda, H., Gleiser, C. A., Masoro, E. J., Murata, I., McMahan, C. A. & Yu, B. P. (1985) Nutritional influences on aging of Fisher 344 rats. II. Pathology. *J. Gerontol.* 40: 671–688.
13. Weindruch, R. H., Kristie, J. A., Cheney, K. E. & Walford, R. L. (1979) Influence of controlled dietary restriction on immunologic function and aging. *Fed. Proc.* 38: 2007–2016.
14. Hosokawa, T. (1979) Studies on B-cell memory. II. *Immunology* 38: 291–299.
15. Cunningham, A. J. & Szenberg, A. (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology* 14: 599–600.
16. Rittenberg, M. B. & Pratt, K. L. (1969) Antitrinitrophenyl (TNP) plaque assay. *Proc. Soc. Exp. Biol. Med.* 132: 575–581.
17. Yarchoan, R., Tosato, G., Blaese, M., Simon, R. M. & Nelson, D. L. (1983) Limiting dilution analysis of Epstein-Barr virus-induced immunoglobulin production by human B cells. *J. Exp. Med.* 157: 1–14.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.
19. Zouali, M., Fine, J. M. & Eyquem, A. (1984) Anti-DNA autoantibody activity and idiotypic relationships of human monoclonal proteins. *Eur. J. Immunol.* 14: 1085–1089.
20. Kaplan, E. L. & Meier, P. (1958) Nonparametric estimation from incomplete observation. *J. Am. Stat. Assoc.* 53: 457–481.
21. Robert, G. D. S. & James, H. T. (1980) *Principles and Procedures of Statistics*, 2nd ed. pp. 112–115, McGraw-Hill, New York, NY.
22. Walford, R. L. (1974) Immunologic theory of aging: current status. *Fed. Proc.* 33: 2020–2027.
23. Walford, R. L., Liu, R. K., Gerbase-Delima, M., Mathies, M. & Smith, G. S. (1973/74) Long term dietary restriction and immune function in mice: response to sheep red blood cells and to mitogenic agents. *Mech. Ageing Dev.* 2: 447–454.
24. Gerbase-Delima, M., Liu, R. K., Cheney, K. E., Mickey, R. & Walford, R. L. (1975) Immune function and survival in a long-lived mouse strain subjected to undernutrition. *Gerontologia* 21: 184–202.
25. Weindruch, R., Gottesman, S. R. S. & Walford, R. L. (1982) Modification of age-related immune decline in mice dietarily restricted from or after midadulthood. *Proc. Natl. Acad. Sci. USA* 79: 898–902.
26. Weindruch, R. H., Kristie, J. A., Naeim, F., Mullen, B. G. & Walford, R. L. (1982) Influence of weaning-initiated dietary restriction on responses to T cell mitogens and on splenic T cell levels in a long-lived F_1-hybrid mouse strain. *Exp. Gerontol.* 17: 49–64.
27. Weindruch, R., Walford, R. L., Fligiel, S. & Guthrie, D. (1986) The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. *J. Nutr.* 116: 641–654.
28. Kubo, C., Johnson, B. C., Day, N. K. & Good, R. A. (1984) Calorie source, calorie restriction, immunity and aging of (NZB/NZW) F_1 mice. *J. Nutr.* 114: 1884–1899.
29. Hosokawa, T., Hosono, M., Higuchi, K., Aoki, A., Kawai, K. & Takeda, T. (1987) Immune responses in newly developed short-lived SAM mice.I. Age-associated early decline in immune activities of cultured spleen cells. *Immunology* 62: 419–423.
30. Yoshioka, H., Yoshida, H., Doi, T., Muso, E., Ohshio, G., Higuchi, K., Inada, M., Miyake, T., Kita, T., Hamashima, Y. & Takeda, T. (1989) Autoimmune abnormalities in a murine model of accelerated senescence. *Clin. Exp. Immunol.* 75: 129–135.
31. Good, R. A., West, A. & Fernandes, G. (1980) Nutritional modulation of immune responses. *Fed. Proc.* 39: 3098–3104.