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The effects of dietary restriction on immune function and development of autoimmune disease in BXSB mice

(calorie restriction/nutrition/T cells/short-lived mice)

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ABSTRACT **Chronic energy intake restriction (CEIR) prolonged the median life span and inhibited autoimmunity and development of autoimmune disease in BXSB mice, as has been established for mice of several other autoimmune-prone, short-lived strains. Whether imposed just after weaning or delayed until manifestations of disease had appeared, CEIR inhibited or reversed development of autoimmunity and immune complex-based renal disease in male BXSB mice. CEIR also prevented the formation of anti-DNA antibodies and prevented the increase in circulating immune complex levels that is typically observed in male mice of this strain. Moreover, CEIR inhibited development of splenomegaly and prevented the normal age-associated decline of a number of immunological functions, including interleukin 2 production, cell-mediated cytotoxic responses, and mixed lymphocyte reactivity. The observed improvement in cell-mediated immune responses was attributed largely to the capacity of CEIR to inhibit development of the splenomegaly that occurs concomitant with expansion of a non-T, non-B lymphoid cell population. These findings emphasize that CEIR, even when imposed relatively late in life in BXSB mice, can influence expression of autoimmunities and autoimmune diseases of different genetic origins and presumed pathogenetic bases.**

In previous studies we determined that, in mice of several genetically short-lived, autoimmune-prone strains of mice, autoimmunity and autoimmune disease are prevented or delayed and that the median and maximal life span are extended by a chronic energy intake restriction (CEIR) of 40%. In such mice, even when CEIR was delayed until manifestations of renal disease had appeared, calorie restriction nonetheless significantly inhibited the progression of immunologically based renal disease and thus extended survival (1, 2). In NZB/NZWF_1 (B/W) and MRL/lpr mice, the extension of life span achieved by CEIR maintained certain immunological responses that normally decline relatively early in life in these short-lived mice (1–3). The diets that most dramatically extended life span were those relatively low in fat and relatively high in carbohydrate (4, 5).

BXSB mice, a more recently developed autoimmune strain, exhibit a human lupus-like disease associated with B-cell hyperplasia in peripheral lymphoid organs (6). Unlike other experimental models of autoimmunity or the human disorder systemic lupus erythematosus, wherein disease is more frequent and its course is more accelerated in females, in the BXSB mouse strain the males manifest accelerated autoimmune phenomena through the influence of a Y chromosome-linked enhancing factor (7, 8). BXSB autoimmune-prone mice are also small compared to other autoimmune-prone mice (i.e., B/W, MRL/lpr, and NZB). BXSB male mice develop an inflammatory vascular disease of the heart and autoimmune or immune complex-based renal disease that is somewhat different in distribution and manifestation than the diseases characteristic of mice of other autoimmune-prone strains (9).

In the present experiments we studied the influence of CEIR imposed early (just after weaning) or later (at 4 months of age, following the onset of renal disease) in life on immunological functions and manifestations of autoimmune disease in BXSB mice to determine whether CEIR inhibits development of disease in male mice and/or prevents or delays development of autoimmunity and increases life span in a short-lived strain of mice in which the individuals are not prone to obesity, are small in size, and have unique pathological features.

MATERIALS AND METHODS

Mice. Inbred 6-week-old male and female BXSB mice (The Jackson Laboratory) were housed individually and fed as specified. Animal rooms were operated on 12-hr light/dark cycles at constant temperature and humidity. Each group consisted of 20 mice. Long-lived C57BL/6 mice studied for comparison were housed in groups and fed a standard lab chow diet ad libitum.

Diets. Diets used in this study were as described (4, 5). Full-fed mice consumed a ration herein designated diet A, a fixed amount of food equal to 12 kcal per day per mouse. CEIR mice were fed 60% of the calorie intake provided by diet A (7.2 kcal per day per mouse) but received protein, vitamin, and salt mixtures in increased proportion so that consumption of these nutrients was equal to that of mice on the higher calorie intake. In diet B, CEIR was initiated at 6 weeks of age (early). In diet C full-feeding continued until initiation of CEIR at 4 months (late); the latter age is the usual time of onset of manifestations of autoimmunity (i.e., proteinuria). Representative mice were killed at 3 and 5 months of age for studies of immunological function and also to provide for serological evaluation to be used as correlates of disease development.

Cell Preparation for Immunologic Assay. Mice were anesthetized, bled by cutting the femoral arteries, and sacrificed by cervical dislocation. Spleens were collected aseptically. Single-cell suspensions of spleen were prepared by gently squeezing the tissue between two glass slides in Hanks' balanced salt solution (GIBCO). The cell suspensions were passed through a layer of gauze to remove residual large fragments. The cells were washed three times with Hanks' balanced salt solution before use.

Culture Medium. RPMI 1640 medium (GIBCO) was made 1 μM in sodium pyruvate and 5 mM in Hepes. It contained

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Abbreviations: CEIR, chronic energy intake restriction; CIC, circulating immune complexes; dsDNA, double-stranded DNA; IL-2, interleukin 2; MLR, mixed lymphocyte reaction; sIg, surface immunoglobulin.

penicillin at 100 units/ml, streptomycin at 100 g/ml, 50 μmol of 2-mercaptoethanol, and 10% fetal calf serum. Normal C57BL/6 mouse serum (1%) was used instead of fetal calf serum for assay of responses to mitogen stimulation or mixed lymphocyte reaction (MLR).

Immunofluorescence. Cells bearing surface immunoglobulin (sIg) or Thy 1.2 antigen were detected with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (Southern Biotechnology Associates, Birmingham, AL) or fluorescein-labeled anti-Thy 1.2 monoclonal antibodies (Becton Dickinson).

MLR. MLR was carried out in 200 μl of RPMI 1640 medium with cultures containing 4×10^5 responding cells and the same number of allogeneic stimulator cells that had been subjected to 2000 rad (1 rad = 0.01 Gy) of γ-irradiation (Cesium-137). These cultures were set up in flat-bottom microtiter plates and incubated for 64 hr at 37°C in 5% CO_2; then 0.4 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine were added for an additional 8-hr incubation. Samples were collected onto glass fiber filters by using a mini-MASH harvestor (Microbiological Associates), then placed into vials containing Scintilene (Fisher), and assayed for radioactivity with a liquid scintillation spectrometer.

Induction of Cytotoxic Cells. *In vitro* allogeneic immunization was performed in 24-well flat-bottom plates (Limbro). Duplicate cultures from each mouse were set up with 500 μl of suspension containing 1×10^7 responding spleen cells per ml and 250 μl containing 1×10^7 CBA/H spleen stimulator cells (in RPMI 1640 complete medium) that had been irradiated with 2000 rad; the cultures were incubated for 4 days at 37°C in an atmosphere containing 5% CO_2. After incubation, the cultures were collected, and the cytotoxic activity of the viable cell population was determined. CBA/H spleen cells that had been stimulated with Con A for 48 hr were used as target cells. Cytotoxic activity was tested by a microcytotoxic assay as described (3).

Interleukin 2 (IL-2) Production. Spleen cells (5×10^6) were suspended in 1 ml of RPMI 1640 complete medium that contained Con A at 2 μg/ml. The cells were cultured in 24-well tissue culture plates (Limbro) for 24 hr at 37°C in chambers containing 5% CO_2. Cells were removed from the culture supernatants by centrifugation at 1500 × *g* for 10 min. Cell-free supernatants were stored at −20°C until the IL-2 assay was carried out.

IL-2 Assay. The IL-2 activity of supernatants was determined by quantifying the influence of the supernatants on growth of cells of an IL-2-dependent T cell line, HT-2 (10). The HT-2 cells, a BALB/c (H-2^d), sheep red blood cell-specific IL-2-dependent helper T-cell line (11), were cultured in flat-bottom microtiter plates (5×10^3 per well) with the IL-2-containing sample that had been serially diluted from 1:2 to 1:64 in a total of 200 μl of RPMI 1640 medium. After 20 hr of culture at 37°C, the wells were pulsed for 4 hr with 0.4 μCi of [^{3}H]thymidine. After harvest of cultures, the radioactivity was determined in a liquid scintillation counter. The concentration of IL-2 was determined by probit analysis. One unit of IL-2 activity was defined as the amount of IL-2-containing crude culture supernate that produced 50% of the maximal proliferative response generated by the reference IL-2 preparation (standard rat recombinant IL-2; Collaborative Research).

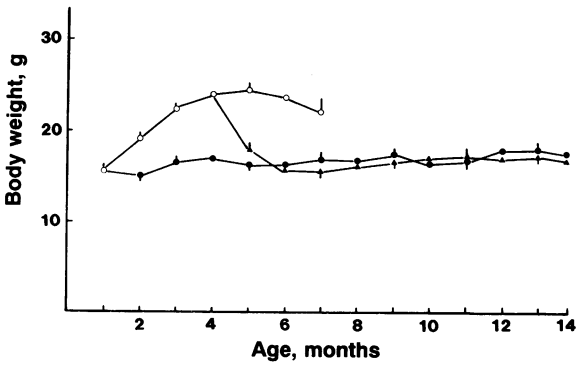

FIG. 1. Weight of male BXSB mice fed diet A (○), diet B (●), or switched from full-feeding to calorie restriction at 4 months of age (▲) (diet C).

Assay of Circulating Immune Complexes (CIC). The Raji cell RIA as adapted for mice was used to measure serum levels of CIC (12), with results expressed as μg equivalents of aggregated murine IgG per ml of serum.

Assay of Anti-Double-Stranded (ds) DNA. Serum antibodies specific for dsDNA were determined by using a solid-phase ELISA (13). The serum was diluted 1:200 for assay. The concentration of anti-dsDNA was determined by reading the OD at 410 nm.

Proteinuria. Proteinuria was assayed with tetrabromphenol paper (Combistix; Ames, Elkhart, IN) on a fresh urine sample. The test is graded 1–4$^+$ as follows: 1$^+$, <30 mg/100 ml; 2$^+$, 30–100 mg/100 ml; 3$^+$, 100–300 mg/100 ml; 4$^+$, >300 mg/ml. In this experiment, high-grade proteinuria was designated as 2$^+$ or greater.

Statistics. Statistical analyses were performed by using Student's *t* test; *P* values <0.05 were considered significant.

RESULTS

Growth of Mice. Growth curves for mice fed the different diets are shown in Fig. 1. Full-fed BXSB mice gained weight gradually; they reached a median weight of 25 g by 5 months

Table 1. Influence of CEIR on body and organ weights of BXSB mice

Age, months	Strain	Sex	Diet	Weight				
				Body, g	Spleen, mg	Thymus, mg	Liver, mg	Kidney, mg
3	BXSB	M	A	23.9 ± 0.7	47 ± 14	39 ± 5	1242 ± 89	161 ± 12
			B	17.6 ± 0.9	59 ± 59	30 ± 4	1199 ± 11	137 ± 5
		F	A	23.4 ± 0.6	93 ± 2	45 ± 3	1256 ± 16	123 ± 6
			B	16.9 ± 0.4	43 ± 4	33 ± 8	998 ± 60	127 ± 5
	C57BL/6		Chow	28.8 ± 1.0	85 ± 12	39 ± 3	1744 ± 75	163 ± 3
5	BXSB	M	A	25.9 ± 0.9	775 ± 82*	16 ± 1	1868 ± 117	206 ± 30
			C	21.2 ± 1.5	243 ± 9	22 ± 2	1510 ± 147	146 ± 13
			B	19.8 ± 0.4	110 ± 19	31 ± 1	1227 ± 123	184 ± 29
		F	A	27.8 ± 1.0	97 ± 25	28 ± 2	1116 ± 47	118 ± 6
			B	19.2 ± 0.3	60 ± 8	27 ± 3	1077 ± 106	119 ± 3
	C57BL/6		Chow	33.6 ± 0.1	80 ± 1	23 ± 2	1583 ± 46	194 ± 3

Each group consisted of three mice. Values represent the mean ± SEM. Diet A, 12 kcal per day per mouse; diet B, 7.2 kcal per day per mouse; diet C, 4 months of diet A then diet B. M, male; F, female.
*, $P < 0.01$ compared with diet B or C.

Table 2. Influence of CEIR on spleen cell populations in BXSB mice

Age, months	Strain	Sex	Diet	Cell number $\times 10^{-7}$	sIg^+ cells, %	Thy 1.2^+ cells, %
3	BXSB	M	A	23.5 ± 2.3	46.3 ± 4.7	11.0 ± 1.7*
			B	16.1 ± 5.5	45.6 ± 5.2	17.0 ± 4.5
		F	A	15.0 ± 0.6	50.0 ± 2.0	19.3 ± 0.6*
			B	6.8 ± 0.2	40.3 ± 3.2	30.0 ± 1.5
5	BXSB	M	A	58.1 ± 6.9	32.8 ± 1.8	8.5 ± 1.0†
			C	24.8 ± 13.0	31.2 ± 7.4	12.6 ± 6.0
			B	16.4 ± 4.0	43.6 ± 1.8	11.8 ± 2.0
		F	A	12.9 ± 3.1	41.1 ± 2.5	13.9 ± 4.6
			B	10.5 ± 0.5	42.6 ± 3.4	12.2 ± 1.1
	C57BL/B		Chow	10.8 ± 1.1	36.8 ± 1.7	19.7 ± 0.6

Each group consisted of three mice. Values represent the means ± SEM.
*, $P < 0.05$ compared with diet B; †, $P < 0.05$ versus diet B or C.

of age, after which time the mice lost weight as their renal disease progressed. In contrast, mice placed on CEIR from time of weaning showed little change in weight throughout the experiment. Mice subjected to CEIR later (at 4 months) lost weight rapidly over a course of 6 weeks and then maintained a nearly constant weight thereafter.

Organ Weights. Spleen, thymus, liver, and kidney weights are presented in Table 1. At age 3 months, spleen weights were significantly higher in the male BXSB mice compared with females. Actual spleen weight as well as spleen weight as percent of body weight were significantly increased at 5 months of age in the male mice fed diet A. On the other hand, male BXSB mice fed diet B or C did not exhibit increased spleen weight; their spleen weight as a percentage of body weight was similar to that observed in the females of this strain.

Cell Numbers and Percentages of sIg^+ or Thy 1.2^+ Cells in Spleen. Table 2 gives the absolute numbers of spleen cells as well as the percentages of sIg^+ and Thy 1.2^+ cell populations in this lymphoid organ. Male BXSB mice fed diet A exhibited splenomegaly and had the highest numbers of spleen cells at 3 and 5 months of age. These numbers were significantly decreased in BXSB male mice fed the calorie-restricted diets—even the diet C mice who were not restricted until 4 months of age ($P < 0.05$). CEIR also reduced spleen cell numbers in BXSB female mice.

The relative proportion of Thy 1.2^+ cells present in spleen taken from BXSB mice was lower in the mice fed diet A than in mice fed diet B at 3 months of age. By 5 months of age this change was striking and was also reflected in normal T-cell numbers in the mice on diet C. At 5 months of age as well, the proportions of sIg^+ cells in the spleen of BXSB male mice fed diet A was lower than in male mice on diet B. By contrast, calorie intake did not appear to influence sIg^+ or Thy 2.1^+ cells in the female BXSB mice studied.

The most dramatic change noted was in the total number of non-T and non-B (Thy 1^-, sIg^-) cells present in mice fed the different diets. BXSB males fed diet A developed far more of these "null-type" lymphocytes than did male mice fed diet B or C.

MLR. Responses of spleen cells taken from the BXSB mice to CBA/H spleen cells are shown in Table 3. At 3 months of age, no significant difference in response was observed as a function of diet. By 5 months, however, only very low responses could be detected among BXSB male mice fed diet A. CEIR, whether early or late, prevented this decline in responsiveness. Impaired MLR activity was not observed in BXSB female mice.

Cytotoxic Responses to *in Vitro* Immunization with Alloantigen. Table 4 includes cytotoxic responses of BXSB spleen cells to *in vitro* immunization with CBA/H allogeneic spleen cells. As observed for MLR responses, no differences were noted as a function of diet at 3 months of age, but by 5 months of age spleen cells of BXSB males fed diet A generated only low responses. By contrast, BXSB males fed diet C maintained vigorous cytotoxic responses. Among female mice, diet did not influence cytotoxic responsiveness.

IL-2 Production. Spleen cells taken from BXSB mice produced less IL-2 than did spleen cells of mice of the autoimmune-resistant C57BL/6 strain. Male BXSB mice showed impaired IL-2 production compared with BXSB females ($P < 0.05$). BXSB males fed diet A showed markedly decreased IL-2 production at 5 months of age. It was noteworthy, however, that even late imposition of CEIR (diet C) could reverse that decline. Among females, full-feeding (diet

Table 3. Influence of CEIR on MLR responses of splenocytes from BXSB mice

Age, months	Strain	Sex	Diet	Stimulator cells: None	Stimulator cells: CBA/H
3	BXSB	M	A	97 ± 17	2717 ± 806
			B	279 ± 76	3778 ± 1356
		F	A	558 ± 207	4774 ± 520
			B	547 ± 119	8527 ± 2680
	C57BL/6		Chow	281 ± 24	3731 ± 1035
5	BXSB	M	A	753 ± 219	709 ± 249*
			C	1026 ± 236	6580 ± 4129
			B	1211 ± 285	4988 ± 1836
		F	A	698 ± 164	5011 ± 2380
			B	543 ± 95	6042 ± 1571
	C57BL/6		Chow	575 ± 67	5102 ± 158

Three individual spleens were tested in each group using triplicate samples. Cell suspensions were incubated for 64 hr at 37°C, and then [^{3}H]thymidine was added for an additional 8 hr. Results are shown as the means ± SEM.
*, $P < 0.01$ compared with diet B or C.

Table 4. Effects of CEIR on *in vitro* cytotoxic T cells by BXSB spleen cells

Strain	Sex	Diet	% cytotoxicity: 3 months	% cytotoxicity: 5 months
BXSB	M	A	34.2 ± 5.5	12.5 ± 5.5*
		C	—	62.7 ± 16.4
		B	35.0 ± 7.7	59.6 ± 12.5
	F	A	43.2 ± 3.1	69.7 ± 2.2
		B	49.9 ± 2.1	79.0 ± 3.1
C57BL/6		Chow	42.4 ± 2.7	84.3 ± 0.1

Each group consisted of three mice. Values represent the means ± SEM. Spleen cells were immunized with γ-irradiated CBA/H spleen cells for 4 days. CBA/H spleen cells that had been stimulated with Con A for 48 hr were used as target cells. The effector-to-target cell ratio was 100:1.
*, $P < 0.01$ compared with diet B or C.

Table 5. Influence of CEIR on serum anti-dsDNA antibody and CIC formation in BXSB mice

Strain	Sex	Diet	Anti-dsDNA, OD		CIC, μg/ml	
			3 months	5 months	3 months	5 months
BXSB	M	A	0.11 ± 0.01	0.18 ± 0.11*	344 ± 15	4933 ± 1533†
		C	—	0.09 ± 0.03	—	315 ± 177
		B	0.09 ± 0.02	0.07 ± 0.02	336 ± 140	324 ± 16
	F	A	0.05 ± 0.01	0.07 ± 0.03	<4	284 ± 135
		B	0.05 ± 0.01	0.03 ± 0.01	<4	<4
C57BL/6		Chow	0.07 ± 0.02	0.02 ± 0.01	<4	<4

Each group consisted of three mice. Anti-dsDNA was determined by an ELISA. CIC levels are expressed as μg equivalents of aggregated murine IgG per ml of murine serum. Values represent the means ± SEM.
*, $P < 0.05$ versus diet B or C; †, $P < 0.01$ compared with diet B or C.

A) resulted in impaired IL-2 production of spleen cells by 5 months of age.

Levels of Anti-dsDNA and CIC. As shown in Table 5, male BXSB mice fed diet B or C had lower levels of anti-dsDNA and lower levels of CIC than did mice fed diet A.

Proteinuria. Male BXSB mice fed diet A began to develop severe proteinuria at 5 months of age; by 6 months, 90% had manifest proteinuria (Fig. 2). However, the mice fed diet B or C had a lower incidence of high-grade proteinuria, even at 15 months of age.

Longevity. The results reported for proteinuria correlated well with the survival curves for each dietary group (Fig. 3). Male BXSB mice fed diet A began to die at ≈5 months of age, and by 9 months of age all had died. Male BXSB mice placed on CEIR early (diet B) or late (diet C) survived significantly longer: 70% of mice of both these groups survived to 17 months of age, the endpoint of the study. Thus, CEIR more than doubled the life span of the normally short-lived males of this strain, and this occurred even when such restriction was delayed until early signs of renal disease had been noted.

DISCUSSION

In the present investigations we demonstrated that in male BXSB mice CEIR inhibits development of autoimmunities, slows or even reverses the progression of immune complex-based renal disease, and decreases the level of CIC present in these autoimmune-prone subjects. CEIR also appeared to maintain the vigor of immunological responses that otherwise wane rapidly as autoimmune disease progresses in the males of this strain. These responses include proliferation of spleen cells in response to stimulation with allogeneic spleen cells, *in vitro* cytotoxic T lymphocytes, and decreased production of IL-2. Furthermore, as we have previously shown for mice of the B/W and MRL/lpr strains, CEIR could be delayed until after the onset of disease yet still result in a greatly increased median life span, decreased expression of autoimmune phenomena, decreased immune complex formation, and inhibition of progression of renal disease. These influences of CEIR were much more striking in the male BXSB mice, which in this strain develop autoimmune disease featured by inflammatory cardiovascular disease and immune complex-based renal disease. Even in the female BXSB mice, however, significant influences on these same immunologic parameters could be attributed to CEIR.

These findings seem particularly provoking from several points of view. Unlike other autoimmune-prone strains of mice that we have studied (NZB, B/W, and MRL/lpr), BXSB mice appear to be genetically small mice. When fed ad libitum, BXSB mice do not readily exhibit obesity and consume much smaller amounts of food than do mice of the other autoimmune-prone strains. Indeed, the daily caloric intake of full-fed BXSB mice (diet A) was only slightly greater than the caloric intake of the larger autoimmune-prone mice fed an experimental ration restricted in calories by 40%. In mice of the larger autoimmune-prone strains, CEIR of 40% increased life and health span. But when fed a caloric intake similar to the restricted diet for the larger autoimmune-prone strains, male BXSB mice exhibit rapidly developing autoimmunities and renal disease. Nonetheless, in male BXSB mice, CEIR consisting of 40% calorie restriction greatly prolonged median life span and inhibited development of immunologically based diseases.

Among the most dramatic influences of CEIR in BXSB male mice has been in reduction of spleen size. Correction of some of the apparent abnormalities of cellular immunity in these mice may be attributed to the inhibition of splenomegaly that otherwise occurs in BXSB mice as a result of the expansion of a population of immature, non-T, non-B lymphocytes at this site. The renal disease that develops in BXSB male mice is pathologically quite different from that that develops in B/W or MRL/lpr mice. In the former, IgG and complement are deposited largely in a mesangial distribution, whereas in B/W and MRL/lpr mice, the renal lesions more typically are associated with IgG depositions in a capillary

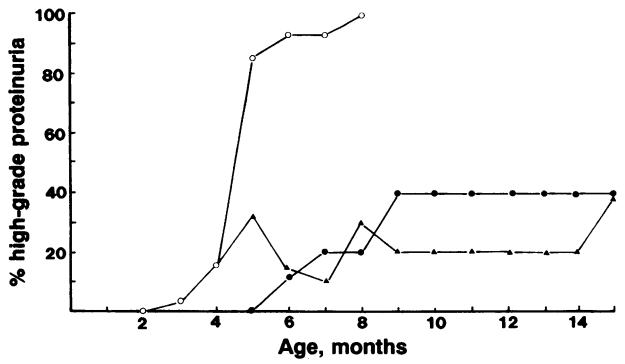

FIG. 2. CEIR inhibited cumulative progression to high-grade proteinuria (>100 mg/ml) in BXSB male mice up to 11 months of age. ○, Diet A; ●, diet B; ▲, diet C.

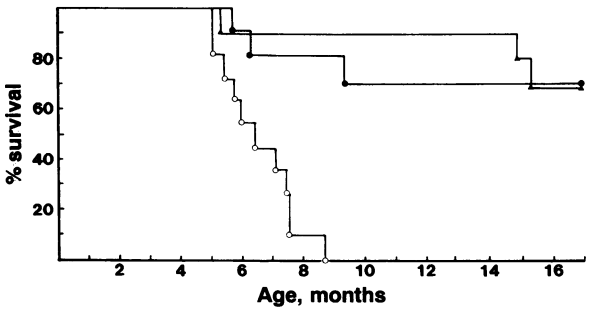

FIG. 3. Survival of male BXSB mice fed diet A (○), diet B (●), or switched from full-feeding to calorie restriction (▲) (diet C).

distribution (14). CEIR appears to have similar protective effects on these quite different renal pathologies.

BXSB male mice are also known to have a striking propensity to develop cardiac inflammatory vascular lesions, a manifestation of autoimmune disease that is not frequent in the other autoimmune-prone mice. Nonetheless, CEIR prevents development of disease and prolongs the spans of life and good health as dramatically in BXSB male mice as in mice of the other autoimmune-prone strains in which autoimmunity is manifest through different pathologies. These findings indicate that calorie restriction, or undernutrition without malnutrition, must exert its influence through the most fundamental and profound mechanisms, mechanisms that are only just beginning to be understood (15–17). The challenge now is to elucidate the mechanisms that underlie both the development of autoimmune disease in these genetically and pathologically different subjects and the capacity of CEIR to regulate this pathophysiology.

1. Fernandes, G., Friend, P., Yunis, E. J. & Good, R. A. (1977) *Proc. Natl. Acad. Sci. USA* **75,** 1500–1504.
2. Fernandes, G., Yunis, E. J. & Good, R. A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1279–1283.
3. Kubo, C., Day, N. K. & Good, R. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5831–5835.
4. Kubo, C., Johnson, B. C., Day, N. K. & Good, R. A. (1984) *J. Nutr.* **114,** 1884–1898.
5. Kubo, C., Johnson, B. C., Gajjar, A. & Good, R. A. (1987) *J. Nutr.* **117,** 1129–1135.
6. Murphy, E. D. & Roth, J. B. (1979) *Arthritis Rheum.* **22,** 1188–1194.
7. Andrews, B. S., Eisenberg, R. A., Theofilopoulos, A. N., Izui, S., Wilson, C. B., McConahey, P. J., Murphy, E. D., Roths, J. B. & Dixon, F. J. (1978) *J. Exp. Med.* **148,** 1198–1215.
8. Eisenberg, R. A., Izui, S., McConahey, P. J., Hang, L., Peters, C. J., Theofilopoulos, A. N. & Dixon, F. J. (1980) *J. Immunol.* **125,** 1032–1036.
9. Theofilopoulos, A. N., McConahey, P. J., Izui, S., Eisenberg, R. A., Pereira, A. B. & Creighton, W. D. (1980) *Clin. Immunol. Immunopathol.* **15,** 258–278.
10. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
11. Watson, J. (1979) *J. Exp. Med.* **150,** 1510–1519.
12. Safai-Kutti, S., Fernandes, G., Wang, Y., Safai, B., Good, R. A. & Day, N. K. (1980) *Clin. Immunol. Immunopathol.* **15,** 293–300.
13. Rubin, R. L., Joslin, F. G. & Tan, E. M. (1983) *J. Immunol. Methods* **63,** 359–366.
14. Ikehara, S., Yasumizu, R., Inaba, M., Izui, S., Hayakawa, K., Sekita, K., Toki, J., Sugiura, K., Iwai, H., Nakamura, T., Muso, E., Hamashima, Y. & Good, R. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3306–3310.
15. Walford, R. L. & Crew, M. (1989) *Growth Dev. Aging* **53,** 139–140.
16. Masoro, E. J., Katz, M. S. & McMahan, C. A. (1989) *J. Gerontol.* **44,** B20–B22.
17. Lipman, J. M., Turturro, A. & Hart, R. W. (1989) *Mech. Ageing Dev.* **48,** 135–144.