Undernutrition without Malnutrition Restricts the Numbers and Proportions of Ly-1 B Lymphocytes in Autoimmune (MRL/l and BXSB) Mice (42981)

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Abstract. **MRL/Mp-*lpr/lpr* (MRL/l) and BXSB mice represent inbred mouse strains in which lymphoproliferative disease and autoimmune disease that includes lethal renal disease routinely occurs by 6 months of age. Chronic energy intake restriction increases longevity and health span of MRL/l and BXSB mice as it does in mice of other short-lived as well as long-lived strains. Chronic energy intake restriction forestalls development of the lymphoproliferative process, prevents development of renal lesions, decreases levels of circulating immune complexes, and permits maintenance of vigorous immunologic function with age. We have reported that in autoimmune-prone mice, a population of Ly-1 B lymphocytes that is associated with autoimmune disease and is greatly expanded among cells of the spleen, peritoneal exudate, and peripheral blood can be reduced in proportion as a consequence of undernutrition without malnutrition. Herein, we demonstrate that in MRL/l and BXSB mice, chronic energy intake restriction imposed at weaning inhibited accumulation of Ly-1 B lymphocytes throughout the lymphoid system, i.e., among cells of the spleen, thymus, mesenteric lymph nodes, bone marrow, peritoneal exudate, and peripheral blood when these tissues or fluids were studied at age 3 or 5 months. These results extend our previous finding that autoimmune-prone mice possess unusually large numbers of Ly-1 B cells in their lymphoid tissues which can be reduced in frequency as a function of diet toward the levels present in long-lived autoimmune-resistant mice.** [P.S.E.B.M. 1990, Vol 193]

Over the past two decades we have investigated the influence of diet on longevity and health in genetically short-lived, autoimmune-prone mice (1–6). We have shown that diets low in both total energy and fat can extend maximum and median longevity, delay expression of autoimmune phenomena and development of autoimmune disease, and prevent the waning of immunologic vigor that normally occurs with aging. In each of the major autoimmune-prone strains of mice we have studied, reduction of energy intake dramatically prolongs life and inhibits the development of diseases associated with aging, i.e., autoimmunities, neoplasia, hyalinizing renal disease, vascular disease, and cardiac disease (1–6). We observed that dietary restriction could be initiated in mid-life or even later and still significantly inhibit pathology of aging and autoimmune disease in these genetically short-lived mice (7). These findings were supported by other investigator's reports that in long-lived strains dietary restriction, even when delayed until mid-life or later in life, extends longevity and preserves immunologic function (8).

Several of the autoimmune-prone strains of mice we have studied are known to possess unusual numbers of Ly-1 B lymphocytes in their peritoneal and lymphoid tissues (9–13). In studying human B cell leukemias and lymphomas, we (14, 15) and others (16) noted that a proportion of such patients had certain B cells that possessed Leu-1 antigens at their surface as well as the IgM and IgD surface markers characteristic of B lymphocytes. The biochemistry of these surface antigens showed certain similarities to those exhibited by human T cells (14–16). Certain mouse T cell malignancies have also been found to bear antigens characteristics of both T and B lymphocytes (15, 16). The Ly-1 B lymphocytes were found to be present in the lymphoid tissues of normal mice and to be increased in NZB mice, pointing

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to a role for these cells in the development of autoimmunity in the latter strain (9–12). Hayakawa *et al.* (10–12) have conducted studies which confirmed the observations of Manohar *et al.* (9) that linked the Ly-1$^+$ B cells to autoimmunity in (NZB × NZW)F_1 (B/W) inbred mice. Their findings showed that these cells were greatly increased in number and were abnormally distributed in mice of autoimmune-prone strains (12). Hayakawa *et al.* (10–12) also found that a population of Ly-1 B lymphocytes was greatly increased among the peritoneal exudate cells (PEC) and among splenic lymphocytes of mice of the NZB and B/W strains. More recently, Raveche *et al.* (17) reported that hyperdiploid Ly-1 B cells of clonal origin become a dominant subpopulation in the spleens of older NZB mice, and Stall *et al.* (18) observed that hyperplastic or neoplastic monoclonal Ly-1 B subpopulations were present in the lymphoid tissues of older normal mice as well as in autoimmune-prone mice.

In previous investigations with B/W mice and NZB mice (19 and unpublished data), we found that this interesting population of Ly-1$^+$ B cells is greatly decreased among spleen, PEC, and blood mononuclears as a consequence of chronic energy intake restriction (CEIR) (19). We extended our investigations of the role of diet on Ly-1 B with investigations of two relatively new murine models of autoimmune disease, MRL/l and BXSB mice, two strains that are prone to lymphoproliferative disorders and renal disease. We report herein a dramatic influence of CEIR on Ly-1 B cells in the spleen, thymus, mesenteric lymph node, bone marrow, PEC, and blood mononuclear cells of genetically short-lived, autoimmune-prone MRL/l mice.

Materials and Methods

Animals. Inbred 6-week-old female MRL/l mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in a nonbarrier environment in the Animal Research Center at the University of South Florida-St. Petersburg. The mice were housed individually and fed as specified. Animal rooms were operated on a 12-hr light and 12-hr dark cycle at 20°C. Long-lived, autoimmune-resistant C57BL/6 (B6) mice used for comparison were housed in groups and fed a commercial laboratory diet *ad libitum*. The number of mice studied in each experiment and sacrificed at either 12 or 20 weeks of age is recorded in Table I.

Diets. Diets were prepared weekly and stored at 4°C. The composition of the high-carbohydrate, low-fat Diets A and B fed to the autoimmune-prone mice and the ratios of diet and energy intake for each diet has been described previously (9). The full-fed mice (Diet A) were begun on *ad libitum* feedings at weaning. They were fed this diet for 1 to 2 weeks to establish an average daily intake. The animals placed on the restricted diet (Diet B) were then fed 60.2 g of this diet per 100 g of Diet A consumed by the *ad libitum*-fed mice. Diet B contained increased amounts of vitamins, minerals, and essential fatty acids so that it provided 100% of the amounts of those ingredients delivered by Diet A. Protein intake expressed per animal per day was decreased 40% for the mice fed Diet B. However, protein intake expressed as percentage of dietary energy was 30.18% for both diets. The level of protein intake in relation to metabolic mass (wt/kg$^{0.75}$) was essentially the same for both *ad libitum*-fed and CEIR mice due to the smaller size of the latter.

Table I. Composition of Diet

Constituent	Diet A		Diet B	
	g	kcal	g	kcal
Casein	29.4	117.6	17.64	70.56
Methionine	0.6	2.4	0.36	1.44
Sucrose	47.25	189.0	26.51	106.05
Glycerol	16.0	64.0	8.98	35.91
Safflower oil	2.0	18.0	2.0	18.0
AIN vitamin mix 76 (20)	1.0	3.95	1.0	3.95
AIN mineral mix 76 (20)	3.5	1.652	3.5	1.652
Inositol	0.05	0.2	0.05	0.2
Choline bitartrate	0.2	0.8	0.2	0.8
Total	100.00	397.6	60.24	238.56
Ratio of g fed to mice	100.00	—	60.24	—
kcal as percentage of Diet A	—	100	—	60
Protein energy as percentage of energy of diet	—	30.18	—	30.18

The CEIR mice were fed twice weekly, and their food intake was adjusted weekly to represent 60% of the previous week's consumption of the mice on Diet A. CEIR mice were fed Diet B daily during the week prior to sacrifice to eliminate influences attributable to food restriction terminally.

Cell Preparations for Fluorescence-Activated Cell Sorter (FACS) Analysis. Mice were sacrificed by cervical dislocation, spleens were collected aseptically, and single-cell suspensions were prepared by gently squeezing each tissue into phosphate-buffered saline solution (0.1 *M* phosphate, 0.9% NaCl without calcium or magnesium [PBS]; Gibco Laboratories, Chagrin Falls, OH). Cell suspensions were passed through a layer of gauze with PBS before use. Blood mononuclear cells were taken from cervical veins, and lymphocytes were collected after Mono-poly Resolving Medium Ficoll-Hypaque isolation (density 114; Flow Laboratories, McLean, VA). PEC were obtained by washing the peritoneal cavity with 5 ml of PBS.

Cell Enumerations. All cell enumerations were performed using a hemocytometer. Viability was determined by trypan blue dye exclusion.

Two-Color Immunofluorescence Staining. One million fully dispersed cells (from which erythrocytes

had been removed by lysis with 0.165 *M* ammonium chloride) in 50 μl of PBS supplemented with 3% heat-inactivated fetal calf serum and 10 mM Hepes buffer solution/500 ml of PBS (hereafter termed diluent) were placed into 12- × 75-mm polystyrene tubes (Falcon Plastics, Oxnard, CA). Five microliters of fluorescein isothiocyanate-conjugated monoclonal anti-mouse IgM (Ig heavy chain, clone 331.12; Becton Dickinson, Mountainview, CA) and 5 μl of biotin-conjugated monoclonal anti-Ly-1 clone 53.7 (Becton Dickinson) made up to 50 μl with diluent were added to each tube. The tubes were mixed gently and stored on ice for 30 min, after which 1 ml of diluent was added to each and the samples were centrifuged at 800*g* for 5 min. Supernatant was discarded, and 20 μl of Streptavidin-phycoerythrin (Streptavidin PE; Becton Dickinson) to make 100 μl of red staining solution were added to the pellet in each tube. After another 30 min on ice, the cells were washed with diluent and stored at 4°C in the dark until analyzed.

Two-Color Immunofluorescence Sorting by FACS. Cells were analyzed for two-color fluorescence using an Epics C flow cytometer (Epics Division, Coulter Corporation, Hialeah, FL). Experimental cells which were stained with fluorescein reagents exhibited a green fluorescence. Background values were obtained by carrying out the same procedure but replacing both fluorescein and phycoerythrin with diluent. Cell populations were at least 90–95% viable at the time of analysis, as determined by low-angle light scatter of 0.4% trypan blue-stained cells. Single-color FACS histograms were compared with double-stained FACS histograms. For two-color analysis, the argon laser emitted light at 514 nm through an LP 520 filter. A dichroic mirror (LP 580) was used to split the light emitted from the laser. Fluorescein light emissions were detected by photomultiplier tube #1 equipped with an SP 560 filter; phycoerythrin light emissions were detected using a photomultiplier tube #2 with an LP 580 filter.

In each experiment, data were recorded for 5000 cells. The green and red fluorescence intensities of lymphocytes were collected from an area delineated by a bit map. In this system, Ly-1 antigen responds with a red fluorescence and IgM responds with a green fluorescence. Cells carrying both markers show an orange fluorescence.

To establish absolute counts of Ly-1 B cells, the proportion of Ly-1 B cells was established per 5000 cells. The number of cells per cubic mm of tissue sample was counted by hemocytometer, and the absolute number of cells per tissue was determined by multiplying total tissue weight in mg by the number of cells/ml. The absolute number of Ly-1 B cells was then calculated by multiplying this total by the percentage of Ly-1 and dividing by 100.

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

Results

Body and Organ Weights. The mean weights of mice of both dietary groups at 12 and 20 weeks are listed in Table II. *Ad libitum*-fed mice of the two strains, with a mean energy intake of approximately 16 kcal/day, gained weight rapidly and reached peak weights at approximately 18 weeks of age and then began to lose weight after the onset of autoimmunities and renal disease. Throughout the experiment CEIR mice showed little change in weight; upon imposition of dietary restriction these mice lost a small amount of weight, quickly regained it, and then maintained a relatively constant weight for the duration of the study. The mean weight of the CEIR mice was approximately 60% that of the *ad libitum*-fed mice in the MRL/l strain and 70% in the BXSB mice. Thymus, spleen, mesenteric lymph nodes (MLN), and kidney weights are recorded in Table II. For further comparison, peripheral lymph node weights are given in Table III.

Spleen, thymus, MLN, and peripheral nodes were regularly enlarged relative to total body weight in the MRL/l mice. These findings reflect the lymphoproliferative disease which is characteristic of MRL/l mice

Table II. Effect of CEIR on Body and Organ Weight in MRL/l and BXSB Mice[a]

Strain	Age (weeks)	Group	Body weight (g)	Thymus (mg)	Spleen (mg)	MLN (mg)	Kidney (mg)
MRL/l	12	A(13)	38.9 ± 1.9	128 ± 40[b]	335 ± 80[c]	618 ± 165[c]	291 ± 20[d]
		B(11)	21.8 ± 2.2	59 ± 13	76 ± 34	92 ± 27	230 ± 21
	20	A(16)	41.8 ± 7.1	92 ± 26[d]	694 ± 146[c]	2146 ± 269[c]	376 ± 64[b]
		B(16)	28.7 ± 2.7	70 ± 28	151 ± 18	365 ± 163	266 ± 23
BXSB	12	A(13)	23.0 ± 1.2	39 ± 4	218 ± 101	71 ± 22	184 ± 19
		B(13)	15.6 ± 0.9	25 ± 6	71 ± 21	22 ± 6	142 ± 10
	20	A(16)	25.5 ± 1.5	30 ± 6	514 ± 256	109 ± 48	205 ± 16
		B(16)	18.0 ± 0.5	27 ± 7	66 ± 23	32 ± 15	156 ± 8

[a] Values are mean ± SD; number of mice per group is in parentheses.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] Value of difference not significant.

Table III. Effect of CEIR on Lymph Node Weights in MRL/l and BXSB Mice[a]

Strain	Age (weeks)	Group	Cervical	Axillary/brachial	Thoracic	Renal/lumbar	Inguinal
MRL/l[b]	12	A(13)	401 ± 347	232 ± 176	49 ± 28	222 ± 194	202 ± 105
		B(11)	7 ± 11	21 ± 12	13 ± 17	29 ± 20	26 ± 23
	20	A(16)	1360 ± 713	1401 ± 589	333 ± 209	1379 ± 537	556 ± 498
		B(16)	61 ± 28	124 ± 128	56 ± 43	120 ± 39	28 ± 31
BXSB[c]	12	A(13)	131 ± 97	134 ± 97	14 ± 9	50 ± 33	70 ± 35
		B(13)	6 ± 9	24 ± 21	2 ± 4	9 ± 13	5 ± 6
	20	A(16)	510 ± 292	535 ± 410	58 ± 49	357 ± 256	197 ± 110
		B(16)	3 ± 4	13 ± 13	2 ± 3	4 ± 4	2 ± 4

[a] Values are mean ± SD; number of mice per group is in parentheses.
[b] $P < 0.001$ for all node sites sampled in MRL/l mice.
[c] Lymph nodes could not be located or dissected in over 50% of the CEIR BXSB mice.

and which, under normal conditions of growth, is manifest by the time the mice reach 12 weeks of age. By 20 weeks, striking differences between the weights of the lymphoid tissues of the *ad libitum*-fed and CEIR MRL/l mice were apparent. In the BXSB male mice, spleen, MLN, and peripheral nodes also regularly were enlarged. Kidney weights in the *ad libitum*-fed mice increased substantially as the mice became ill with glomerulonephritis.

Spleen Cell Analysis by Flow Cytometry (FACS). Figure 1 shows the absolute numbers of double-marked Ly-1 B cells in spleens taken from MRL/l mice or BXSB mice fed either Diet A or Diet B. The absolute number of Ly-1 B cells present in spleens from CEIR mice was significantly lower ($P < 0.001$) than that found in spleens taken from *ad libitum*-fed mice at either 12 or 20 weeks of age. Moreover, these values were similar to those observed in spleens taken from normal B6 mice, especially at 12 weeks of age.

MLN Analysis by Flow Cytometry (FACS). Figure 2 compares the absolute numbers of Ly-1 B lymphocytes in MLN of MRL/l mice and BXSB fed the experimental diets and, for comparison, the absolute number of these cells in the MLN from the *ad libitum*-fed MRL/l mice was greatly increased ($P < 0.001$) compared with the number of such cells in age-matched CEIR mice at 12 or 20 weeks of age. The absolute number of Ly-1 B cells present in the *ad libitum*-fed BXSB mice was not nearly as great as that observed in the MRL/l mice, reflecting the fact that the genetically based lymphoproliferative disorder is more acute in the latter strain. However, the *ad libitum*-fed BXSB mice still exhibited an increased level of Ly-1 B proliferation ($P < 0.001$) compared with the number of such cells observed in the CEIR mice, which possessed numbers of Ly-1 B similar to those observed in normal B6 mice fed a standard commercial diet.

Thymus Cell Analysis by Flow Cytometry (FACS). As shown in Table IV, we also observed substantially fewer Ly-1 B cells present in thymuses of 12-week-old CEIR mice compared with those taken from *ad libitum*-fed mice; these differences were especially

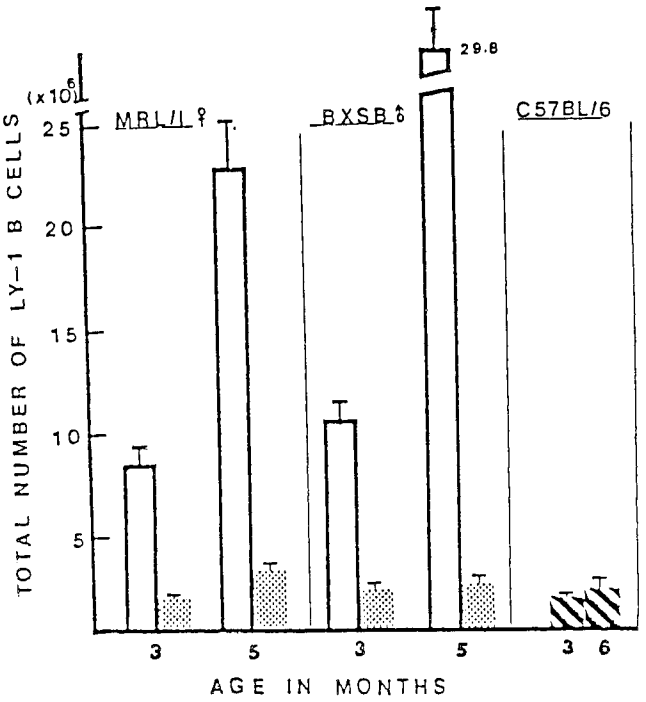

Figure 1. FACS analysis of numbers of Ly-1 B lymphocytes among spleen cells taken from MRL/l and BXSB mice at age 3 months or 5 months fed either the *ad libitum* (open bar) or CEIR (stippled bar) ration. Data for long-lived B6 mice fed a nonpurified diet are included for comparison.

striking at 20 weeks in the BXSB mice ($P < 0.001$). In the MRL/l mice, the percentage of thymic Ly-1 B cells was reduced 1.3-fold in the CEIR mice at 12 weeks of age, but by 20 weeks of age this difference was almost 2-fold in BXSB mice on the restricted diet, and at 20 weeks this difference was 4.1-fold. No Ly-1 B lymphocytes could be detected in thymus samples of B6 mice sacrificed at 12 weeks of age.

Bone Marrow Cell Analysis by Flow Cytometry (FACS). Table IV also presents FACS analysis data detailing the proportions of Ly-1 B lymphocytes among bone marrow cells taken from MRL/l and BXSB mice. The growth of Ly-1 B populations was also decreased as a function of CEIR, so that at 12 and 20 weeks of

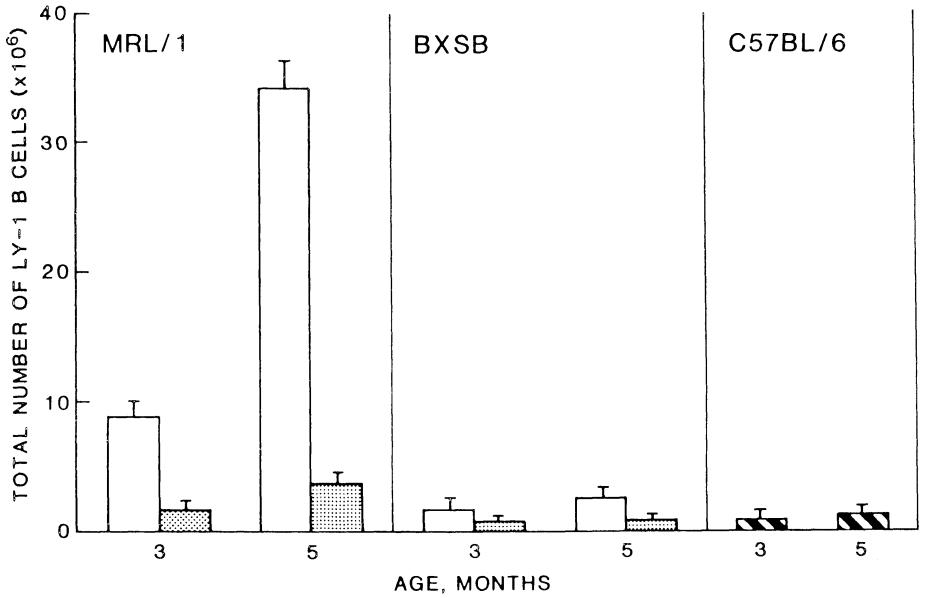

Figure 2. FACS analysis of numbers of Ly-1 B lymphocytes present among spleen cells taken from MRL/l and BXSB mice at age 3 months or 5 months fed either the *ad libitum* (open bar) or CEIR (stippled bar) ration. Data for long-lived B6 mice fed a nonpurified diet are shown for comparison.

Table IV. Effect of CEIR on Proportion and Number of Ly-1 B Lymphocytes in Thymus and Bone Marrow of MRL/l and BXSB Mice[a]

Strain	Age (weeks)	Group	% Ly-1 B cells in thymus	% Ly-1 B cells in bone marrow
MRL/l	12	A(13)	1.7 ± 1.0[b]	1.4 ± 0.5[b]
		B(11)	1.0 ± 0.4	1.1 ± 0.4
	20	A(16)	2.1 ± 1.4[c]	1.1 ± 0.3[c]
		B(16)	1.1 ± 0.2	0.7 ± 0.3
BXSB	12	A(13)	3.0 ± 1.8[b]	2.6 ± 1.8[b]
		B(13)	1.9 ± 0.2	1.0 ± 0.8
	20	A(16)	7.0 ± 4.5[d]	2.6 ± 1.8[b]
		B(16)	1.7 ± 0.8	1.3 ± 1.0

[a] Values are mean ± SD; number of mice per group is in parentheses.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.001$.

Table V. Effect of CEIR on Proportions of Ly-1 B Lymphocytes among PEC or Blood Mononuclear Cells in MRL/l and BXSB Mice[a]

Strain	Age (weeks)	Group	% Ly-1 B cells in PEC	% Ly-1 B cells in BMC
MRL/l	12	A(6)	13.7 ± 5.4[b]	7.9 ± 9.4[c]
		B(6)	1.0 ± 0.2	1.1 ± 0.5
	20	A(8)	0.9 ± 0.2[d]	25.0 ± 24.9[e]
		B(8)	0.7 ± 0.2	1.5 ± 1.6
BXSB	12	A(17)	22.4 ± 10.9[b]	23.2 ± 17.0[e]
		B(10)	7.3 ± 7.8	0.9 ± 0.3
	20	A(13)	16.3 ± 9.8[b]	11.2 ± 3.4[c]
		B(10)	2.2 ± 2.9	1.0 ± 0.2

[a] Values are mean ± SD; number of mice per group is in parentheses.
[b] $P < 0.001$.
[c] $P < 0.05$.
[d] Value of difference not significant.
[e] $P < 0.01$.

age the proportions of Ly-1$^+$ B lymphocytes were reduced 1.3-fold and 1.6-fold, respectively, in the MRL/l mice.

In BXSB mice, Ly-1 B populations in bone marrow were also influenced by diet and were decreased 2.1-fold at 12 weeks of age and 2.0-fold at 20 weeks of age.

PEC Analysis by Flow Cytometry (FACS). Table V shows the proportions of Ly-1 B cells present among PEC in *ad libitum*-fed or CEIR mice of the two experimental strains. In the CEIR mice, the proportion of Ly-1 B lymphocytes in the peritoneum was dramatically lower than that in age-matched *ad libitum*-fed mice. Indeed, the proportions of Ly-1 B cells present among PEC were highest in the younger mice, reflecting the characteristic pattern of distribution of the Ly-1 B population in lymphoid tissues, arising first in the peritoneum and subsequently in spleen, lymph nodes, and then bone marrow. At age 20 weeks, a significant difference in Ly-1 B populations in the PEC was not observed. These results may be due to the difficult techniques necessary to collect cells of the peritoneal exudate.

Peripheral Blood Mononuclear Cell Analysis by Flow Cytometry (FACS). Table V also shows the proportions of Ly-1 B cells among blood mononuclear cells. As determined by FACS analysis, a lower distribution of Ly-1 B cells was found among blood mononuclear cells of CEIR mice than of *ad libitum*-fed mice of both strains. In BXSB mice, the proportions of Ly-1 B cells present among blood mononuclear cells was greater in the *ad libitum*-fed mice at 12 weeks than at

20 weeks; this may reflect the fact that leukocyte counts were stable or increased with age in these animals.

Discussion

Mice of the MRL/l and BXSB strains represent two relatively recently developed models of autoimmune disease. MRL/l mice develop an extraordinary lymphoproliferative disease, autoimmunity, and rapidly progressing renal disease. Renal disease may be manifest as early as 2–3 months of age, and a flagrant lymphoproliferative disorder usually appears between 3 and 4 months (21, 22). Median survival time of these mice is approximately 6 months for males and 5 months for females, and almost all animals of this strain die by 6–8 months of age. BXSB mice exhibit a human lupus-like disease associated with B cell hyperplasia in peripheral lymphoid organs and renal disease (23–26). These mice are smaller in size compared with the MRL/l mice and mice of other autoimmune-prone strains. Unlike other experimental mouse strains in which the autoimmune disease is more frequent and more accelerated in females, in the BXSB strain it is the male mouse that manifests rapidly progressing autoimmune phenomena through the influence of a Y chromosome-linked accelerating factor (26, 27).

In previous studies with mice of these two strains, we found that imposition of CEIR using a ration relatively low in fat and relatively high in carbohydrate and reduced 40% in total caloric intake prolonged life-span, forestalled development of renal disease, inhibited development of lymphoproliferative disease, prevented early involution of immunologic function, prevented the growth of thymic lymphoid lesions, and reduced cell accumulation in the spleen and lymph nodes in MRL/l mice (4, 5). CEIR also maintained lymphoproliferative responses to phytohemagglutin, pokeweed mitogen, and allogeneic cells and corrected deficient interleukin 2 production by cells of the spleen and lymph nodes (4, 5 and unpublished data) in mice of these autoimmune-prone strains.

The mechanisms by which undernutrition without malnutrition prolongs life and maintains immunologic vigor in short-lived mice remain unclear. We have found that CEIR not only reduces the number of proliferating cells in the lymphoid tissues of autoimmune-prone mice (28) but also curbs the rapid expansion of the Ly-1 B lymphocyte subpopulation in such mice.

Ly-1 B cells, a subset of B lymphocytes that is apparently distinct and separate from other B lymphocytes present in the hematopoietic and lymphoid systems, are closely linked to autoimmune phenomena, autoimmune disease, and autoantibody production in genetically short-lived mice (9–12). These cells are thought to develop from B lymphocyte precursors present in fetal or newborn liver or neonatal bone marrow, but not from precursors present in spleen or adult bone marrow (29). They may also be derived from precursors in adult peritoneum and are present in relatively high proportion in peritoneal cell populations (13). The Ly-1 B cells appear to contribute to production of autoantibodies, especially of the IgM class, and they may also produce putative natural antibodies important to immune defense. A corresponding subpopulation of B cells in man, the Leu-1 B cell subset, may be associated with production of rheumatoid factors and other autoantibodies (30, 31).

In previous studies we have shown that the proportions of Ly-1 B lymphocytes present among cells of the spleen, peritoneal exudate, and peripheral blood in autoimmune-prone B/W, NZB, MRL/l, and BXSB mice are dramatically reduced as a function of CEIR imposed at the time of weaning (19). The results we report herein confirm and extend our previous findings that CEIR can dramatically influence the pathogenesis of autoimmune disease in autoimmune-prone mice in general and in MRL/l mice and BXSB mice in particular, and further suggest that the reduction in the Ly-1 B cell subpopulation may be one crucial means by which CEIR prevents autoimmune disease and extends the life-span in genetically short-lived mice. The data show that CEIR decreases Ly-1 B cell proliferation not only in the spleen, PEC, and peripheral blood but also among cells of the mesenteric lymph nodes, thymus, and bone marrow.

Our findings are made especially relevant by recent observations of the propensity of Ly-1 B cells to develop monoclonal populations in autoimmune-prone NZB mice, and, perhaps even more significantly, in older mice of normal strains. Stall *et al.* (18) have detected Ly-1 B clones in the peritoneal cavity, and eventually in the lymphoid tissues of C57BL/6, BALB/c, and CBA mice more than 15 months of age. Raveche *et al.* (17) found that hyperdiploid Ly-1 B cells become a subpopulation in the spleens of NZB mice and suggest that the loss of immune responsiveness observed in older NZB mice may be due to the expansion of these abnormal Ly-1 B cells, which may have the capacity to suppress certain immune functions.

Furthermore, the findings of *Stall et al.* (18) add further support to the theory that hyperplastic or neoplastic Ly-1 B cells constitute a murine equivalent of human B-type chronic lymphocytic leukemia. The relationship between normal Ly-1 B cells and their diploid and hyperdiploid clones is still unclear. As Raveche suggests, it may be that the relatively long-lived cells of the Ly-1 B lineage accumulate alterations in DNA content which result in unregulated growth and clonal development. In light of our prior finding that CEIR can reduce the number of proliferating lymphoid cells, our observations of the powerful influence of undernutrition without malnutrition on Ly-1 B proliferation and, perhaps, on hyperplastic and neoplastic Ly-1 B clonal expansion presents an interesting departure point for further studies of dietary control of tumorigenesis

in mice of normal as well as genetically short-lived strains. Moreover, we as well as several other investigators have shown that CEIR can dramatically reduce the incidence of tumor development in both mice and rats (32–37 and G. Fernandes, personal communication), pointing to the need for further investigation of the effects of diet on cancer.

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