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Immunology

Modification of age-related immune decline in mice dietarily restricted from or after midadulthood

(aging/underfeeding/cellular immunity/cancer)

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ABSTRACT **Although weaning-initiated dietary restriction of rodents is known to increase maximum survivorship and inhibit spontaneous late-life disease and immunologic aging, restriction begun in adulthood has been much less thoroughly evaluated. In the present studies, male mice of a long-lived F_1 hybrid strain were gradually restricted dietarily beginning at 12 mo or older until their body weights stabilized at 60–70% of controls. Underfeeding decreased the number of nucleated cells per spleen but increased the percentage of T cells. For mice restricted at 12, 17, or 22 mo and tested at various ages thereafter, the [^{3}H]thymidine uptake of spleen cells after phytohemagglutinin stimulation significantly exceeded values for age-matched unrestricted controls. Restriction did not, however, alter either splenocyte responses to concanavalin A or to B-cell mitogens or phytohemagglutinin responses of peripheral lymph node cells. In the splenic plaque-forming cell response to injected sheep erythrocytes, restricted and control mice differed more clearly in response kinetics than in peak levels. The splenic cell-mediated lymphocytotoxic response to alloantigens was comparable in old mice (27–29 mo) restricted since 12 mo of age with that of young (5- to 6-mo) controls and was greater than that of age-matched old controls. Spontaneous tumors were observed less frequently in 19- to 25-mo-old mice restricted at 12 mo of age than in mice restricted at 17 mo or in controls. Our results indicate that appropriate food restriction initiated in adulthood influences immunosenescence and spontaneous tumor incidence in a fashion not unlike its weaning-initiated counterpart.**

Dietary restriction imposed on rats and mice beginning at weaning (3–6 wk old) extends survival (1–3) more than any other procedure tested in rodents (4–6) and is the only strategy known to slow age-related increases in mortality rates in homeotherms (7). Restriction also inhibits or delays (or both) the occurrence of many late-life diseases (8–11) and retards the development of immunosenescence (12–16). The most successful restriction diets limit caloric intake while apparently providing adequate quantities of other required nutrients, amounting to "undernutrition without malnutrition."

Underfeeding started in adulthood has received far less attention than weaning-initiated dietary restriction (WDR), despite the fact that adult-initiated dietary restriction (ADR) might be a feasible option for human use. One reason for this comparative neglect could be an earlier report of shortening of the lifespan of ADR rats (17). However, these rats did not consume a nutrient-enriched diet and restriction was not gradually imposed. Even those past studies (18–21) showing a positive effect of ADR on survival are not entirely convincing. They also have all used relatively abrupt as opposed to gradual restriction. In recent longevity studies involving gradual reduction in food intake with nutrient enrichment of the diets in two strains of mice [(C57BL/10Sn × C3H/HeDiSn)F_1 and C57BL/6J] restricted beginning at 12 to 13 mo of age, we observed 10–20% increases in mean and maximum lifespans as well as cancer inhibition (ref. 22; unpublished data). ADR has been reported to inhibit breast cancer in mice (8) and renal disease in mice (23) and rats (24). With regard to immunologic effects, only mouse splenocyte mitogen-induced T-cell proliferative responses (which are known to decline with age) have been looked at. These were increased in 16- to 17-mo-old mice restricted from 12 mo of age (14) and in 22-mo-old mice restricted from 17 mo of age (25).

The present studies, done with male mice of the long-lived (C3H.SW/Sn × C57BL10.RIII/Sn) F_1 strain (C3B10RF_1), were aimed at providing further information on the impact of gradually imposed adult-initiated diet restriction on immunologic aging and spontaneous cancer incidence.

MATERIALS AND METHODS

Mice. C3B10RF_1 mice bred in our animal facilities were studied. Those fed Purina lab chow ad lib have a mean lifespan of ≈28 mo, and those restricted at weaning have a mean lifespan of ≈40 mo. Lifespan was not determined in the present experiments as all mice were killed and studied for immune parameters and cancer incidence. Stimulator cells in the cell-mediated lymphocytotoxicity (CML) study came from DBA male mice (*H-2*^{d}; Jackson Laboratories).

Diet Strategies. Three hundred male mice weaned at 3 to 4 wk of age were housed in groups of 4–6 with free access to Purina lab chow. At 12 mo, mice were individually caged and randomly assigned to one of two diet categories. (*i*) Purina lab chow (designated LC mice; $n \approx 200$): Continuing to eat ad lib, these mice served as controls and a source to be either restricted or switched to a semipurified control diet later in life. Mice younger than 12 mo old and consuming Purina lab chow ad lib are also designated in this report as LC mice. Purina lab chow has a guaranteed analysis (minimum) of 23% protein and 4.5% fat. (*ii*) Restricted (designated R_{12}, restricted beginning at 12 mo of age; $n = 72$): These mice were gradually restricted, using diets 2 and 3 of Table 1. To guard against malnutrition, the three diets of Table 1 differed in level of enrichment of casein, salts, vitamin mixture, and brewer's yeast, with the diet fed in the most restricted amounts (diet 3) being richest in these materials. Semipurified diets were prepared once a month and stored at 4°C.

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Abbreviations: WDR, weaning-initiated dietary restriction; ADR, adult-initiated dietary restriction; C3B10RF_1, (C3H.SW/Sn × C57BL10.RIII/Sn) F_1 mice; LC (mice), mice fed Purina lab chow ad lib; R_{12}, R_{17}, and R_{22} (mice), mice dietarily restricted at 12, 17, and 22 mo of age, respectively; SCD (mice), mice fed a semipurified control diet; LMA, late middle age; PHA, phytohemagglutinin; Con A, concanavalin A; PPD, purified protein derivative; LPS, lipopolysaccharide; CML, cell-mediated lymphocytotoxicity; PFC, plaque-forming cell; DPFC, direct PFC; IPFC, indirect PFC; SRBC, sheep erythrocytes.

Table 1. Diet composition and feeding strategies

Constituents	Amount, g of ingredient/kg of diet		
	Diet 1	Diet 2	Diet 3
Casein, vitamin-free test*	200.0	280.0	350.0
Cornstarch	260.8	192.6	157.6
Sucrose	260.8	192.6	157.6
Corn oil (Mazola)	135.0	135.0	135.0
Nonnutritive fiber	56.4	51.9	40.0
Salt mixture, USP XIV	60.0	102.0	110.0
Vitamin mixture	23.0	39.0	42.2
Brewer's yeast	4.0	6.9	7.4
Zinc oxide	0.05	0.08	0.10

Diet 1 was a semipurified control fed as daily 6.5- to 7.0-g feedings on Mondays–Thursdays and a 13- to 14-g Friday portion (155–165 kcal/wk). This level was found in preliminary studies to support body weight similar to that of LC mice. Diet 2 was fed to restricted mice for the first month of restriction. It was a 28% casein diet, enriched in salt and vitamin mixtures, brewer's yeast, and zinc oxide relative to diet 1 and fed via a 6.5- to 7.0-g feeding on Mondays, Tuesdays, and Wednesdays, followed by an 11.0- to 11.5-g feeding on Fridays (120–130 kcal/wk). Diet 3 was fed to restricted mice after the first month of restriction. It was a 35% casein diet, the most enriched of the three diets in salt and vitamin mixtures, brewer's yeast, and zinc oxide and fed as a 6.5- to 7.0-g feeding on Monday and Wednesday with a 10.0- to 10.5-g portion on Friday (90–100 kcal/wk). The three diets were nearly isocaloric, providing ≈4 kcal/g diet.

* Casein, fiber, salt mixture, vitamin mixture, and brewer's yeast were purchased from ICN Pharmaceuticals. Other ingredients were purchased locally.

Diet restriction was also started at 17 and 22 mo of age. Animals from the LC population were restricted in the same way as the R_{12} mice, thereby forming groups R_{17} (n = 32) and R_{22} (n = 21). Other 17-mo-old LC mice were fed nearly ad lib amounts of a semipurified control diet (Table 1, diet 1), forming the SCD group (n = 6). We also measured splenic T-cell levels using other LC mice, with restriction starting at 10 to 11 mo and rendered more severe than for R_{12}, R_{17}, or R_{22} by feeding 90–100 kcal (1 cal = 4.18 J) of diet 3 for the first month and 75–85 kcal thereafter.

Mitogen Responses. Mice were killed by cervical dislocation, autopsied, only used in immune experiments if grossly normal, and studied individually. Techniques for measuring mitogen-induced [^{3}H]thymidine uptake as an index of DNA synthesis and microscopic quantitation of phytohemagglutinin (PHA)-stimulated blast cells are detailed elsewhere (15). Other mitogens used were concanavalin A (Con A), purified protein derivative (PPD), and *Escherichia coli* lipopolysaccharide (LPS). Optimal mitogen concentrations have not been found to differ between restricted and control mice (25). Cultures were carried out in triplicate and results are calculated as mean cpm in stimulated cultures − mean cpm in control cultures. PHA-induced blast cells were quantified after 48 hr of culture by classifying 100 Wright-stained cells as blasts or nonblasts. Results are reported as % blast cells in PHA-stimulated cultures − % blast cells in unstimulated cultures.

Plaque-Forming Cell (PFC) Assay. Mice were injected intraperitoneally with sheep erythrocytes (SRBC; 0.2 ml of 10% solution), and killed 4, 8, or 12 days later. Splenocytes were assayed for levels of direct PFC (DPFC) and indirect PFC (IPFC) (26).

CML. Spleen cell effectors were generated in a 4-day mixed lymphocyte reaction using irradiated DBA/2 splenocytes as stimulators. Various ratios of effector to ^{51}Cr-labeled P815 target cells (mastocytoma of DBA/2 origin) were incubated for 4 hr. Percent cytotoxicity was calculated as [(experimental − spontaneous)/(maximum − spontaneous)] × 100. Procedural details have been reported (27).

T-Cell Quantitation. Indirect immunofluorescence was carried out by using monoclonal anti-mouse Thy 1.2 (α-Thy 1.2, a mouse IgM from New England Nuclear), goat anti-mouse IgM [F(ab')$_2$ fragment; Cappel Laboratories, Cochranville, PA), and fluorescent microspheres (Covaspheres MX, Covalent Technology, San Jose, CA). Splenocytes were incubated with either α-Thy 1.2 or phosphate-buffered saline (controls), washed, incubated with anti-mouse IgM-coated Covaspheres, and wet mounted. About 200 cells per coded slide were examined and those with >4 Covaspheres were brightly fluorescent and scored as positive. The "specific fluorescence" was calculated as % positive cells in presence of α-Thy 1.2 plus labeled Covaspheres − % positive cells for control.

Statistics. Intergroup differences for all except tumor incidence data were analyzed by the two-tailed t statistic for two means. Intergroup differences in tumor incidence were evaluated by the test for significance of difference between two proportions.

RESULTS

Body and Organ Weights. Body weights for mice are shown in Fig. 1. The LC mice gained weight rapidly, reaching ≈45 g by 6 mo of age and averaging 48 to 49 g at the onset of underfeeding at 12, 17, or 22 mo of age. Many adult LC mice (≈50%) appeared moderately obese. Patterns of weight loss were similar among the R_{12}, R_{17}, and R_{22} groups. Stable weights of 32–35 g were gradually reached after ≈5 mo restriction of R_{12} and R_{17} mice. R_{22} mice were studied after 3 to 4 mo of underfeeding and presumably had not reached this stable level (Fig. 1). At 19 to 20 mo of age, R_{12} organ weights were lower ($P < 0.01$) than in SCD mice for spleen (55% decrease), liver (44%), heart (21%), and kidney (13%). R_{12} mice had lower organ weight/body weight ratios for spleen (32% decrease, $P < 0.01$) and liver (15%, $P < 0.02$), but higher ratios ($P < 0.01$) for kidney (33% increase) and heart (21%).

Mitogen Responses. Levels of PHA responses of splenocytes from 19- to 20-mo-old R_{12}, SCD, and LC mice ranked R_{12} > LC > SCD (Table 2). Con A and PPD responses did not differ

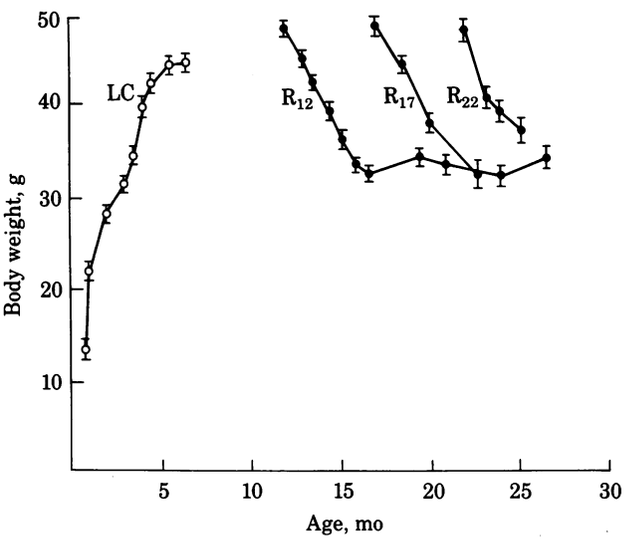

FIG. 1. Body weights of male C3B10RF$_1$ mice. Values are mean ± SEM. For LC mice, n = 30–48; for R_{12} mice, n = 61–72, except for the two oldest groups for which n = 16–28; for R_{17} mice, n = 34–36, except at 23 mo for which n = 11; for R_{22} mice, n = 18–21. The first value for each restricted mouse group would also be for LC mice at that age, since restriction was begun then.

Table 2. Spleen cell mitogen-induced proliferative responses in 19- to 20-month-old R_{12} and control (SCD and LC) mice

Group	*n*	PHA	Con A	PPD
R_{12}	8	35,702 ± 3218*	90,506 ± 7,643	21,507 ± 4921
SCD	6	14,360 ± 2336†	75,977 ± 11,162	21,748 ± 4090
LC	8	23,515 ± 2787	78,529 ± 4,433	18,212 ± 2275

Values represent mean ± SEM for individual mice (1 or 2 mice per group tested in each experiment). Cultures were done in triplicate and are expressed as cpm of [^{3}H]thymidine corrected for unstimulated uptake (which ranged from 4000 to 9000 cpm and was not influenced by diet).

* Significantly different from SCD and LC; $P < 0.01$.

† Significantly different from LC; $P < 0.02$.

among groups (Table 2). Based on these results, LC mice were used thereafter as controls. Spleens of R_{12} mice contained 20–40% of the total number of nucleated cells and 50–60% of the number of cells per mg of spleen compared with LC or SCD mice (data not presented). Similar reductions in spleen weight and cell yield were observed in restricted mice in all subsequent experiments.

Effects of ADR started later in life were studied by comparing splenocyte PHA, Con A, and LPS responses of 20- to 22-mo-old R_{12}, R_{17}, R_{22}, and LC mice and of 2- to 3-mo-old LC mice. A second series measured reactivity to PHA and Con A of 25- to 28-mo-old R_{12}, R_{17}, R_{22}, and LC mice and of 5- to 6-mo-old LC mice. Intergroup differences in LPS responses were not found (data not shown). PHA and Con A responses from both series are summarized in Fig. 2. Age-related decreases in PHA and Con A reactivity occurred in all groups. At 20–22 mo of age, PHA responses of R_{12} and R_{17} mice exceeded those of age-matched LC mice (R_{12}, $P < 0.01$; R_{17}, $P < 0.05$) while not differing from those of 2- to 3-mo-old LC mice. Con A reactivity of 20- to 22-mo-old R_{12} (but not R_{17}) mice exceeded that of age-matched LC mice ($P < 0.05$). In the second series, PHA responses of 25- to 28-mo-old R_{12} and R_{22} mice were greater than those of age-matched LC mice (R_{12}, $P < 0.01$; R_{22}, $P < 0.05$); however, R_{17} mice did not show a significant increase ($0.10 > P > 0.05$). Con A responses did not differ significantly among 25- to 28-mo-old LC, R_{12}, R_{17}, and R_{22} mice. The rank order of PHA reactivity in this second series, young LC > R_{12} > old LC mice, was also reflected in the levels of blast cells induced. Spleens of three mice per group were assayed for levels of blast cells and [^{3}H]thymidine uptake. Young LC mice showed the greatest response [blasts, 40 ± 7%; cpm, 31,828 ± 2697], R_{12} were intermediate (33 ± 4% and 14,074 ± 3024 cpm), and old LC mice showed the least response (21 ± 4% and 1616 ± 1175 cpm).

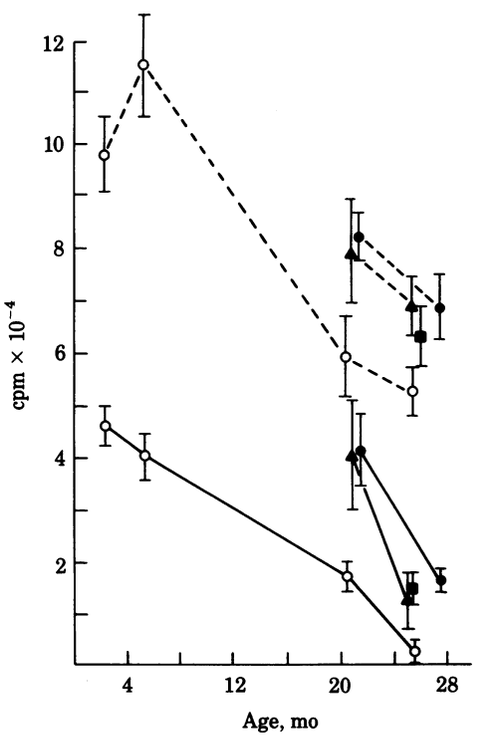

FIG. 2. Splenic lymphocyte mitogen-induced proliferative responses for ADR mice. Values are mean ± SEM for n = 6–13 mice. Triplicate cultures were carried out and results are expressed as cpm of [^{3}H]thymidine corrected for unstimulated uptake (which was 3000–10,000 cpm and not influenced by age or diet). Responses: ——, PHA; – – – –, Con A. Mice: ○, LC; ●, R_{12}; ▲, R_{17}; ■, R_{22}.

Responses of cells from pooled peripheral lymph nodes of 20- to 23-mo-old individual R_{12} and LC mice were determined using four mice from each group. No differences in PHA reactivity were observed between R_{12} (46,215 ± 2200 net cpm) and LC (46,123 ± 4453) mice. Cell yields were much less for R_{12} (3–10 × 10^6 cells per pool) than for LC mice (30–40 × 10^6).

PFC Responses to SRBC. Young and late-middle-age LC mice (LMA-LC) were compared with late-middle-age R_{12} and R_{17} for splenic DPFC and IPFC levels after primary immunization with SRBC (Table 3). Generally, restriction affected response kinetics but not peak PFC levels. The day 4 DPFC response of LMA-LC mice was ≈20% of the value for young LC mice. DPFC responses of young LC mice peaked on day 4, whereas LMA-LC mice responded well at 4 or 8 days. At day 4, R_{12} and R_{17} mice generated only low (or no) DPFC; however, unlimited Purina lab chow given to R_{12} mice for the 8 days before assay, increasing body weights from 31.3 ± 1.2 to 39.5 ± 1.5 g in the six mice tested, led to day 4 DPFC responses equal to those of LMA-LC mice. The highest DPFC levels for day 8 were found in R_{12} mice (which peaked at this day). R_{17} mice produced few DPFC on any day. Lowest DPFC levels on day 12 were found in young LC mice. IPFC responses on day 8 ranked young LC > R_{12} > LMA-LC, R_{17}. Significant intergroup differences in IPFC levels on day 12 were not observed.

CML. Young (5- to 6-mo-old) and old (25- to 28-mo) LC mice were compared with old (27- to 29-mo) R_{12} mice for allogeneic CML following a 4-day *in vitro* sensitization (Table 4). The level of response of old R_{12} mice equaled or approached that of young LC mice, whereas old LC mice demonstrated various degrees of decline. Old controls gave more variable responses but always responded less than young LC or R_{12} mice. No significant differences were found between young LC and old R_{12} animals at any effector/target ratio.

Splenic T-Cell Levels. Ten- to 11-mo-old LC mice (n = 7) were restricted for 2 to 3 mo and their spleens were compared with those of age-matched unrestricted LC mice (n = 7) for organ weights, cell yields, and T-cell levels. The restricted group had body weights of 46.4 ± 0.7 g when the study began and 32.9 ± 1.1 g at sacrifice. Restricted mice had lighter spleens (0.049 ± 0.004 mg versus 0.093 ± 0.006) with fewer cells (21 ± 4 × 10^6 versus 88 ± 10 × 10^6) and an increased percentage of T cells as shown by specific fluorescence (31 ± 2% versus 18 ± 2%; $P < 0.01$). Background levels of fluorescence were not significantly influenced by diet. Absolute levels of splenic T cells were higher for unrestricted compared with restricted mice (16 ± 2 × 10^6 versus 6 ± 1 × 10^6; $P < 0.01$).

Spontaneous Tumor Incidence. Tumor incidences in sacrificed R_{12}, R_{17}, and LC mice screened for use in the mitogen and SRBC studies are given in Table 5. Only 10% of the R_{12} mice had a tumor versus 30–35% for R_{17} and LC mice ($P < 0.05$).

Table 3. Influence of food restriction in adulthood on responses to SRBC

Group	*n*	DPFC/10^6			IPFC/10^6	
		Day 4	Day 8	Day 12	Day 8	Day 12
R_{12}	6–8	22 ± 8	255 ± 37	59 ± 8	257 ± 68	329 ± 119
Refed R_{12}	6	154 ± 53	ND	ND	ND	ND
R_{17}	3–5	18 ± 5	69 ± 49	48 ± 5	18 ± 12	205 ± 65
LMA-LC	5–8	182 ± 72	109 ± 39	49 ± 15	47 ± 20	333 ± 108
Young LC	6–8	843 ± 105	59 ± 9	12 ± 2	464 ± 167	246 ± 44

Values represent mean ± SEM for individual spleens. Mice were 21–25 mo old [except young LC (3–5 mo)] when injected intraperitoneally with SRBC. Splenic DPFC levels were measured 4, 8, or 12 days after immunization; IPFC levels were measured on days 8 and 12. The refed R_{12} group of mice consisted of 23- to 24-mo-old R_{12} mice switched to Purina lab chow ad lib 4 days before SRBC injection and assayed after an additional 4 days of LC feeding. For DPFC on day 4, young LC > all other groups ($P < 0.01$), LMA-LC > R_{12}, R_{17} ($P < 0.05$), refed R_{12} > R_{12} ($P < 0.02$); for DPFC on day 8, R_{12} > young LC ($P < 0.01$) and LMA-LC, R_{17} ($P < 0.02$); for DPFC on day 12, young LC < LMA-LC ($P < 0.02$) and R_{12}, R_{17} ($P < 0.01$); for IPFC on day 8, young LC > LMA-LC, R_{17} ($P < 0.01$) and R_{12} ($P < 0.05$), R_{12} > LMA-LC ($P < 0.02$) and R_{17} ($P < 0.01$). ND, not determined.

Hepatomas were the most common. Of six mice with lymphoma, only one (an R_{12}) was diet restricted in adulthood.

DISCUSSION

Our findings indicate that dietary restriction gradually imposed on previously unrestricted 12-mo-old male mice can decrease rates of age-related decline in T-dependent immune responses (spleen cell PHA, CML, and PFC) and inhibit the occurrence of spontaneous cancer. Restriction starting at 17 mo of age increased PHA reactivity but not PFC responses (CML not tested). Hepatoma was not inhibited in R_{17} mice. Restriction initiated at 22 mo of age also increased splenic PHA reactivity (other responses not tested). Not significantly influenced by ADR were (*i*) splenic Con A, LPS, or PPD responses and (*ii*) PHA responses of peripheral lymph node cells. Only 20–40% as many nucleated cells could be harvested from the spleens of restricted mice; however, ≈30% of these were T cells, compared with ≈20% for nonrestricted mice. Likewise, ≈30% of the spleen cells of restricted mice appeared to be blast cells 48 hr after PHA stimulation, compared with ≈20% in unrestricted (LC) mice. These findings suggest that ADR increases PHA responses in spleen cell suspensions at least in part by increasing the proportion of PHA-responsive T cells.

In previous immunologic studies of ADR mice, only splenic mitogen responses were examined. Mann (25) observed that PHA and Con A responses particularly, and LPS responses to a lesser degree, were increased in 22-mo-old female CBA/J mice fed only every other day for 5 mo, compared with controls fed ad lib. In our earlier studies, we found elevated spleen cell PHA and Con A responses in 16- to 17-mo-old male (C57BL/10Sn × C3H/HeDiSn) F_1 mice restricted for 4 to 5 mo (14). We did not find responses to PPD, LPS, pokeweed mitogen, or to alloantigens in the mixed lymphocyte reaction to be significantly influenced by ADR. [CBA is a nonobese mouse strain and (C57BL/10Sn × C3H/HeDiSn) F_1 mice are less prone to obesity than the mice used in the present study. Therefore, the effects of the present study do not seem to depend merely on inhibiting obesity.]

WDR has been much more widely studied immunologically than ADR. WDR mice of several strains have shown increased spleen cell PHA and Con A responses both early in life (11, 14) and in mid and late adulthood (12, 15). We recently compared young or middle-aged C3B10RF$_1$ females, restricted since weaning, to age-matched control mice fed the semipurified control diet (15). Restricted mice showed changes similar to those reported here, including reduction in numbers of spleen cells, increased percent of splenic T cells, increased splenic (but not lymph node) PHA (but not Con A) responses, and an increased percent of PHA-induced blast cells. Primary splenic PFC responses to injected SRBC were lower for WDR mice than for controls in young C3H mice (11) and higher than for controls in middle age-to-old (NZB × NZW) F_1 mice (28) and in C57BL/6J mice (12). Splenic CML responses of 10-mo-old (NZB × NZW) F_1 WDR mice exceeded those of controls (28). Thus, there are many similarities between immunologic effects of WDR and ADR.

Food restriction imposed on adult rodents inhibits the occurrence of spontaneous diseases. Breast cancer incidence in 20-mo-old DBA female mice was sharply reduced by underfeeding begun at 2, 5, or 9 mo of age (8). The autoimmune nephropathy of (NZB × NZW) F_1 mice was less advanced, and serum anti-DNA antibody titers were reduced by food restric-

Table 4. Influence of age and food restriction beginning in adulthood on CML

Group	% lysis at effector/target ratio			
	10:1	5:1	2.5:1	0.5:1
R_{12}	98 ± 3	87 ± 3	63 ± 4	27 ± 3
Old LC	78 ± 8	61 ± 10	40 ± 9	16 ± 4
Young LC	95 ± 2	90 ± 3	69 ± 6	30 ± 4

R_{12} and old LC mice were 25–29 mo old when tested; young LC mice were 5 to 6 mo old. Values represent mean ± SEM % specific lysis in a ^{51}Cr release assay after a 4-day *in vitro* sensitization with DBA/2 spleen cells. Target cells used were P815 mastocytoma cells. Mice were tested individually with one per diet group studied in each of five experiments. For an effector/target ratio of 10:1, R_{12} > old LC ($P < 0.05$); for ratios of 5:1 and 2.5:1, R_{12}, young LC > old LC ($P < 0.05$); for a ratio of 0.5:1, young LC > old LC ($P < 0.05$).

Table 5. Spontaneous tumor incidence in sacrificed mice dietarily restricted beginning in adulthood

Group	*n*	Age at autopsy, mo	Tumor incidence			
			Hepatoma		Lymphoma	
			No.	%	No.	%
R_{12}	50	19–25	4	8*	1	2
R_{17}	26	21–23	8	31	0	—
LC	48	19–23	12	25	5	10

Results summarize gross autopsy findings for mice used (or not used if tumor bearing) in the mitogen and SRBC studies. Only hepatoma and lymphoma were observed. Four lymphomas were splenic and two others (both in LC mice) originated in the lower gastrointestinal tract.
* Significantly different than R_{17} ($P < 0.01$) and LC ($P < 0.05$) mice.

tion begun at 4 to 5 mo of age (23). Dietary restriction imposed on the mutant *kdkd* mouse at 2–2.5 mo of age strikingly inhibited renal disease and improved survivorship (29). Incidences of cardiac and renal disease were reduced in rats restricted beginning at 3 mo of age to 60% of those of ad lib-intake animals (30). ADR of rats during the second year of life delayed age-related alterations in kidney function and reduced the incidence and severity of renal lesions (24).

The present findings suggest that dietary restriction of mice beginning in midadulthood can substantially decrease many age decrements in immune responses and inhibit spontaneous cancer. Encouragingly, these influences obtain despite insufficient knowledge about optimization of restricted dietary regimes (e.g., type and content of fats or proteins, antioxidant additions). Influences of fats on survival and tumor incidence in mice have been discussed by Fernandes *et al.* (31). ADR differs from WDR in that neither growth rates nor the time of onset of puberty can be altered by the former. ADR would appear the only practical mode of diet restriction potentially adaptable to human use.

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