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Immunology

Inhibition by restricted-calorie diet of lymphoproliferative disease and renal damage in MRL/lpr mice

(lymphoproliferation/longevity/thymus/lymph nodes/spleen)

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ABSTRACT **Restriction of calorie intake from the time of weaning greatly prolongs life, and it inhibits development and expression of the lymphoproliferative syndrome, renal disease, and decline of certain immunologic functions with age in MRL/lpr mice. This dramatic influence of diet on mice of this short-lived autoimmunity-prone strain, while associated with decreased rate of growth, is not associated with debilitation or apparent disease in the MRL/lpr mice. The massive lymphadenopathy and splenomegaly that developed in the putatively well-fed animals was prevented by dietary restriction, as were histopathologic abnormalities of thymus, spleen, lymph nodes, and kidneys.**

Several studies in recent years have shown that diet can have profound influence in the development and expression of disease in short-lived autoimmunity-prone mice. In initial studies in this series, we showed that in NZB mice a diet low in protein and high in fat promoted thymic involution, immunologic involution, splenomegaly, and development of autoimmune hemolytic anemia and was associated with short life (1, 2). By contrast, diet low in fat and relatively high in protein favored longer life, inhibited involution of the thymus, inhibited development of autoimmune hemolytic anemia, and permitted prolonged maintenance of immunologic capacity (1, 2). Using diets of defined composition, we further showed that protein restriction had a significant influence on thymic involution, protected against development of splenomegaly, delayed involution of a number of humoral and cell-mediated immune responses, and inhibited autoantibody production but did not significantly prolong life of male or female mice of the autoimmunity-prone NZB strain (3). Dramatic results were also obtained with hybrid mice of the short-lived autoimmunity-prone (NZB × NZW)F_1 (B/W) lineage. When these hybrid mice were fed a diet low in calories from the time of weaning, life-span was more than doubled, while evidence of early immunological decline was inhibited (4, 5). Calorie restriction also delayed greatly development of renal disease and early involution of a variety of immunologic functions with age. Deposition of complement and immunoglobulin in a capillary distribution in glomeruli was also prevented (4, 5). With the B/W model, it was even possible to initiate the dietary calorie restriction as late as 4–5 months of age and prolong life and greatly inhibit the characteristic renal immunopathology that develops early in life in females of this strain (6). The influence of calorie restriction on the level of circulating immunocomplexes (CIC) was also notable, in that the CIC levels were markedly reduced in mice eating 10 Cal/day as compared to mice eating 20 Cal/day (1 Cal = 4.18 kJ) (7). Anti-DNA antibody production was reduced by such dietary manipulation (5). More recently, we have also shown that appearance of glycoprotein gp70 in serum as well as anti-gp70 and circulating gp70–anti-gp70 complexes [these complexes were found by Izui *et al.* (8) to be most intimately linked to glomerulonephritis] was inhibited by restricted diet in B/W mice.

Studies by others on B/W mice given low-calorie diets confirmed that life-span can be greatly lengthened by dietary management but that the amount of xenotropic RNA tumor virus in the tissues is not reduced by the dietary manipulations (9).

Additional studies of the *kd/kd* mutant mouse described by Lyon and Hulse (10) as having a form of progressive nephronothesis revealed that this mutation is also associated regularly with autoimmune hemolytic anemia. We found that, with this strain as well, low calorie intake very much lengthened life-span, inhibited the development of the autoimmune phenomena, and prevented progression of the renal pathology, including the progressive interstitial nephritis, glomerular destruction, and tubular atrophy (11). By contrast to caloric restriction, limitation of protein intake was not beneficial in this model. Ross (12) and Masoro *et al.* (13) have obtained similar results in rats and have shown that life-span of these animals, too, can be lengthened and renal lesions that normally occur with aging can be inhibited by life-long calorie restriction.

Two new strains of short-lived autoimmunity-prone mice have been developed at The Jackson Laboratory (14), and these strains have been rather extensively analyzed and compared to the NZB and B/W strains (15). One of these models of autoimmune disease, based on striking immunocomplex injury, is the MRL/*lpr/lpr* (MRL/l) mutant. Both males and females of this short-lived autoimmunity-prone strain develop an extraordinary lymphoproliferative disease, progressive glomerulonephritis, and characteristic necrotizing vasculitis early in life. Almost all animals of this strain have died by 6–8 months of age (16). Another of the models of autoimmunity is represented by mice of the BXSB strain, in which only the males develop autoimmune disease early in life (16).

It is the purpose of the present report to describe dramatic influences of diets low in calories on survival and development of the lymphoproliferative syndrome, glomerular lesions, and vasculitis in the MRL/l mice. We will show that each of these abnormalities is prevented or very much decreased by feeding the mice a diet low in calories from the time of weaning.

MATERIAL AND METHODS

Animals. Inbred MRL/l and MRL/n originally obtained from E. D. Murphy of The Jackson Laboratory were maintained as inbred strains at the inbred mouse colony of the Sloan–Kettering Institute. The breeding stocks are fed Pur-

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Abbreviations: CIC, circulating immunocomplexes; Cal, nutritionists' calorie (4.18 kJ); PHA, phytohemagglutinin; LPS, lipopolysaccharide B.

ina Lab Chow ad lib. To study the dietary influence, 3- to 4-week-old MRL/l mice of both sexes were placed on semi-purified diets.

Diets. Composition of diet, source of ingredients, methods of preparation, and feeding procedures as well as housing of animals were identical to those described previously (4). Briefly, the diet was (by weight) 22% casein, 33% dextrose, 33% starch, 5% corn oil, 4% mineral mixture, 2% vitamin mixture, and 1% agar. However, animals on the restricted (low-calorie) diet (10 Cal/day) were housed singly and were given an additional supplement of minerals and vitamins equal to half the amount given to the animals fed the normal diet (20 Cal/day). Exact amounts of food, either 5 g (approximately 20 Cal) or 2.5 g (approximately 10 Cal), were provided at the same time each day, between 10 and 12 a.m.

Animals were weighed weekly and moribund mice were killed and tissues fixed in Bouin's fluid for histopathology. In addition, mice on the dietary regimens were killed at 5 and 10 months for comparative histological study.

***In Vitro* Lymphocyte Stimulation Assays.** Details of these assays have been described (17). Nucleated spleen or lymph node cells (0.5×10^6), after being washed twice in RPMI 1640 medium, were cultured in RPMI 1640 medium containing 5% fetal calf serum and antibiotics in triplicate wells on Falcon 2040 Microtest II plates. Different dilutions of phytohemagglutinin (PHA) or concanavalin A (Con A) as T-cell mitogens and lipopolysaccharide B (LPS) as a B-cell mitogen were used. Sixteen hours prior to harvest, 0.5 μCi (1 Ci = 37 GBq) of [*methyl*-^{3}H]thymidine (New England Nuclear) was added. Cultures were harvested on glass filter papers and radioactivity was measured by scintillation counting using standard techniques.

Histology. Thymus, spleen, lymph nodes, heart, and kidneys were taken from mice sacrificed by bleeding followed by cervical dislocation. The tissues were fixed in 10% neutral Formalin and stained with hematoxylin and eosin in the usual way.

RESULTS

Fig. 1 compares survival curves on MRL/l female and male mice fed the two different defined diets. Mice of this strain fed 20 Cal/day of the semi-purified defined diet from the time of weaning developed profound lymphadenopathy, which first became noticeable at approximately 3 months of age. The mice began to sicken in the fifth month and all in our small series died by 6 months. By contrast, mice fed from weaning exactly ½ the Cal/day of the very same defined diet grew more slowly and did not develop lymphadenopathy, and all remained healthy up to 11 months of age.

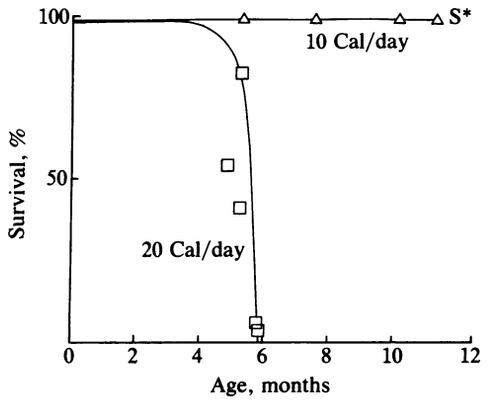

FIG. 1. Longevity of MRL/l male mice fed full-calorie (□) and restricted (△) defined diet. Note that life-span of mice fed restricted diet is greatly prolonged over life-span of full-fed MRL/l mice. *Survived to this point and then killed for histological analysis.

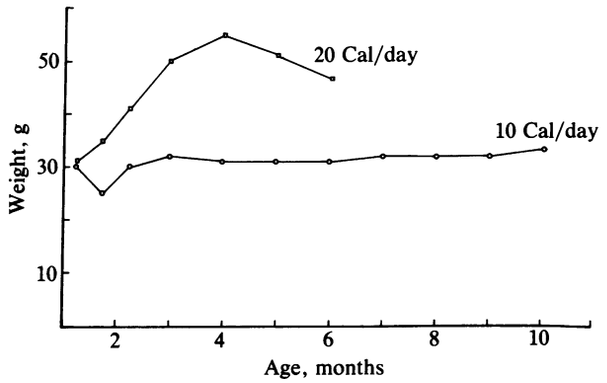

FIG. 2. Growth curves of mice fed restricted (○) or full-calorie (□) diets. Diets contained the same amount of minerals and vitamins. There were five male mice in each group. Note slower growth in the mice fed a restricted diet.

Other mice of this strain fed the low-calorie diet died after 12 months, either from infection with microorganisms present in the conventional environment or from renal disease. The mice with reduced calorie intake showed much less lymphoproliferative disease or renal disease, which regularly was a striking finding in the putatively well-fed controls, which died at about 6 months.

Growth curves for the mice fed the lower- and higher-calorie diets are presented in Fig. 2. Although the mice with the lower calorie intake grew more slowly than the mice with the higher calorie intake, they looked vigorous and healthy until very late in their lives. Fig. 3 compares representative calorie-restricted and putatively well-fed mice of this strain at 4 months of age. It will be seen from this figure that the calorie-restricted mice, although smaller than the animals fed the higher-calorie diet, looked healthy and vigorous. One aspect of the behavior of the mice fed the two different diets was strikingly different. The well-fed mice seemed to be less active than the calorie-restricted mice; indeed, the latter seemed almost constantly to be active as a consequence of their foraging for additional food.

Fig. 4 is a photograph of a dissection exposing lymph nodes, spleen, and thymus of representative mice of the two

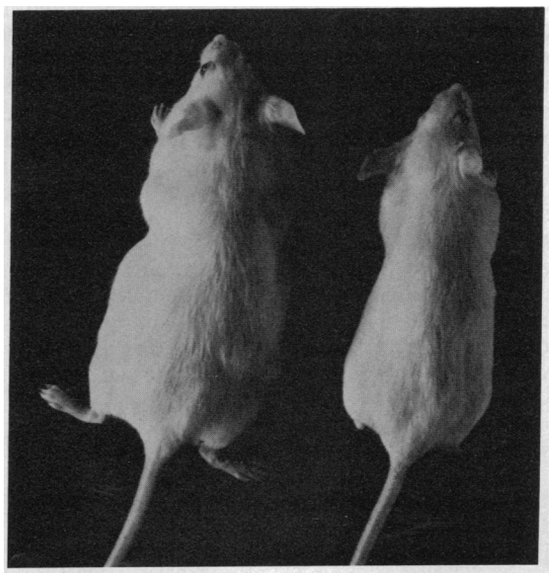

FIG. 3. MRL/l male mice fed 20 or 10 Cal/day. Note that mice fed restricted diet (right), although smaller, are sleek and healthy in appearance.

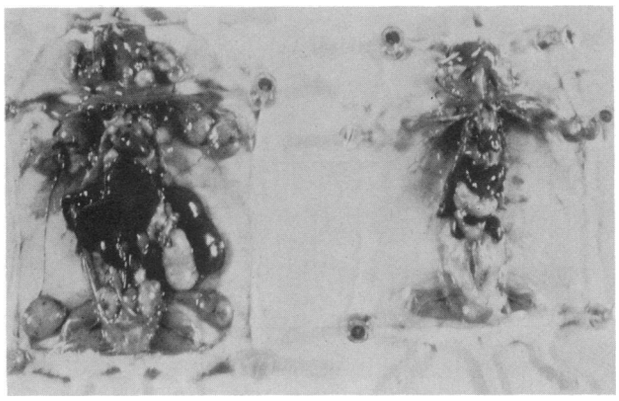

FIG. 4. Dissection of MRL/l mice fed 20 or 10 Cal/day. Note massive enlargement of submandibular, inguinal, and axillary lymph nodes in full-fed mouse (left) and absence of enlargement of these nodes and spleen in the mouse fed the restricted diet (right).

dietary groups at 5 months of age. It can be seen that although the thymuses of the mice on the two different diets were nearly comparable, the axillary, inguinal, submandibular, and mesenteric lymph nodes of the MRL/l mice fed the higher-calorie diet were massively enlarged. By contrast, all lymph nodes in each of these locations were small and appeared to be of normal size in the MRL/l mice fed the lower-calorie diet. Similarly, the massive splenomegaly that developed in all of the MRL/l mice on the higher-calorie ration did not develop in any of the mice fed the lower-calorie ration. The results observed in the mice fed the defined diet with higher calorie content were similar to those with MRL/l mice fed Purina Lab Chow ad lib.

Table 1 summarizes data on responses of spleen and lymph node cells to optimal concentrations of the two T-cell mitogens Con A and PHA and the B-cell mitogen LPS in MRL/l mice 5 months of age that had been fed from weaning either 10 or 20 Cal/day. It can be seen from the table that MRL/l mice fed the higher-calorie diet had strikingly lower proliferative responses of their spleen cells to PHA, Con A, or LPS than did the MRL/l mice fed 10 Cal/day. Although proliferative responses of lymph node cells to PHA and Con A were greater than those for spleen cells in the MRL/l mice fed 20 Cal/day, the proliferative responses of lymph node cells, like those of spleen cells, were greater in the 5-month-old MRL/l mice fed 10 Cal/day. Similarly, the B-cell mitogen, LPS, produced much lower proliferative responses in the mice fed the higher-calorie diet than in those fed the ration lower in calories. MRL/l mice fed lab chow ad lib showed, at 3 months, proliferative responses of both spleen and lymph node cells significantly lower than those of congenic MRL/n mice fed the same dietary regimen (data not shown).

Fig. 5 compares the histology of representative sections of spleen, lymph node, and thymus at 5 months of age from MRL/l mice fed 20 Cal/day. It can be seen from the illustration of the histology of the lymph nodes that the greatly expanded T zones encroach on B areas. Further, the thymus shows striking nodular medullary expansion in MRL/l mice fed 20 Cal/day that were either not present or much decreased in the MRL/l mice fed 10 Cal/day. Indeed, the histology of spleen, lymph nodes, and thymus at 5 months of age were virtually normal in the MRL/l mice fed the lower-calorie diet from weaning.

Table 1. Higher response to mitogens by spleen or lymph node cells from 5-month-old MRL/l mice fed 10 Cal/day

Diet, Cal/day	Cells	[^{3}H]Thymidine incorporation, cpm			
		Control	PHA	Con A	LPS
20	Spleen	995	4,300	3,000	3,600
	Lymph node	250	13,725	30,220	1,500
10	Spleen	812	35,600	39,030	28,000
	Lymph node	270	81,300	83,000	4,600

The glomeruli of the mice 5 months of age fed 20 Cal/day were uniformly inflamed and avascular. They were infiltrated with polymorphonuclear cells and showed striking vascular lesions, some of which were very similar to those seen in florid human lupus erythematosus. By contrast, at 5–6 months, the glomeruli of the mice fed 10 Cal/day from weaning regularly showed fine membranes, greater evidence of circulation of blood in the glomeruli, and almost no evidence of inflammation. Indeed, in most instances the glomeruli of these animals looked quite normal. Further, the severe periarteritis-like vasculitis (17) that was regularly seen in the renal vessels of the mice fed 20 Cal/day was almost completely absent at 6 months from the kidneys of mice fed 10 Cal/day (not illustrated).

DISCUSSION

As with NZB, B/W, and *kd/kd* mutant mice, all of which have a propensity to short life, autoimmunity, and early renal disease, MRL/l mice live much longer when fed a restricted diet. Indeed, the life-span of mice of this strain was approximately doubled when a restricted-calorie diet was provided from the time of weaning. Although the dietary restriction interfered with growth, it did not produce sickly animals. Instead, the MRL/l mice fed one-half the putative normal calorie intake were sleek and vigorous, and they were apparently protected from all major aspects of the disease that characterizes mice of this strain.

Perhaps most striking was the virtually complete suppression of the lymphoproliferative syndrome that usually appears in mice of this strain between 3 and 4 months of age. The flagrant lymphoproliferative syndrome never appeared in the mice fed low-calorie diet from the time of weaning. The renal lesions, another hallmark of the disease in MRL/l mice, were likewise greatly delayed, although renal lesions ultimately developed in some of the longer-lived MRL/l mice fed lower-calorie diets. Inflammatory and degenerative vascular lesions, infiltrations of lymphoid cells, and histological abnormalities in thymus, spleen, and lymph nodes were all minimized or inhibited altogether by calorie restriction.

Although the mechanism by which caloric restriction produces these profound influences on the complex syndrome that regularly develops in MRL/l mice must still be determined, it is of interest that the initial immunologic parameters investigated, namely the proliferative responses to phytomitogens and LPS endotoxin, each of which regularly declines sharply with aging in MRL/l mice, were maintained at high levels in the mice fed the restricted diet. Analyses of possible hormonal changes and other biochemical changes induced in MRL/l mice by diet, as well as extensive studies of immunoregulatory functions and immunologic parameters, seem very much in order.

Cantor and Gershon (15) have reported that MRL/l mice, like other mice prone to develop autoimmune phenomena and autoimmune diseases, have abnormalities in the function of the feedback regulatory T-cell circuits. It seems especially important to know whether inhibition of development of disease in these mice by calorie restriction can correct or beneficially influence these immunoregulatory mechanisms. By contrast, Theofilopoulos and Dixon (18) have emphasized that circulating immune complexes play a crucial pathogenetic role in MRL/l mice, as is also the case in B/W mice; the influences of diet on the levels of these complexes must also be assessed. Prior investigations have revealed that cal-

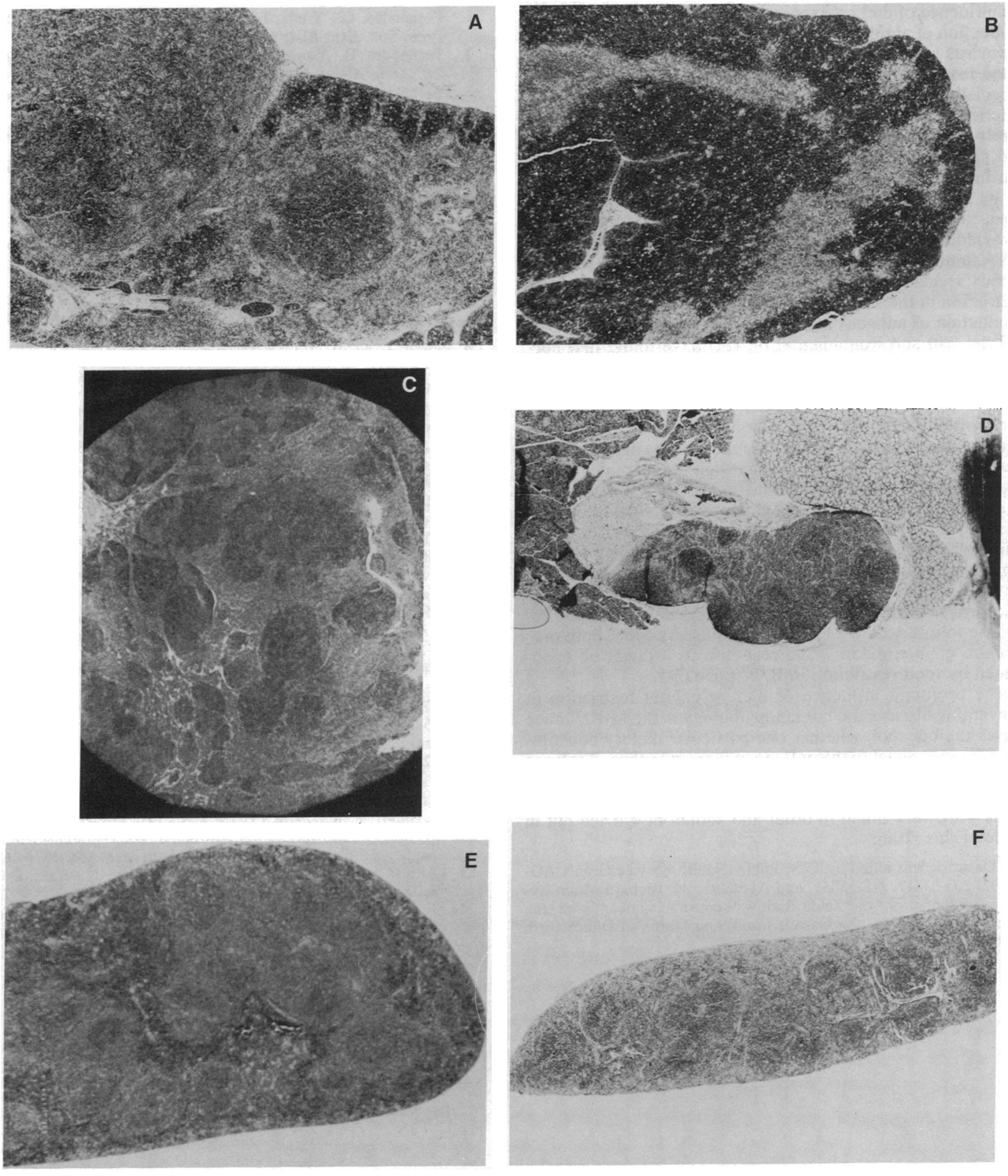

FIG. 5. Comparison of thymus, lymph nodes, and spleen of typical full-fed and calorie-restricted MRL/l mice at 5 month of age. (×25.) Notice that in the full-fed mice (*A*) the thymic architecture is distorted, with pathological accumulation of lymphoid cells, whereas in calorie-restricted mice (*B*) the thymus shows normal architecture. Similar evidence of lymphoproliferative disturbance is present also in lymph node (*C*) and spleen (*E*) of the full-fed mice. These abnormalities are absent from the lymph node (*D*) and spleen (*F*) of the calorie-restricted mice.

orie restriction from weaning lowers levels of CIC in B/W mice and that this lowering of CIC correlates with the improvement in the renal histology (7, 8).

Izui *et al.* (19) have emphasized that, in autoimmune mice, a fundamental abnormality of pathogenetic significance is an abnormality of the B-lymphocyte function reflected in increased spontaneous activation of B cells very early in life. Whether calorie restriction influences crucial functions of the B-cell or T-cell–B-cell interactions to result in inhibition of development of autoimmune disease must be determined. In addition, Theofilopoulos *et al.* (17) have recently shown that early thymectomy can prolong life for MRL/l mice.

Although the precise role played by virus or viruses in the lymphoproliferative syndrome, immune complex formation, and autoimmune disease in MRL/l mice is not known, it seems especially pertinent to measure in quantitative terms

the influence of diet on expression of virus or viruses, or on production of antigens such as the gp70 that are coded for in retrovirus genomes. It seems important also to measure immune responses to this antigen. This line of inquiry seems especially pertinent since, in B/W mice, similar dietary restriction prolongs life and prevents renal disease and has recently been shown to reduce dramatically formation of gp70 and gp70–anti-gp70 CIC in B/W mice (8). Along this line, Gardner *et al.* (9) have confirmed that B/W mice are protected by low calorie intake from autoimmunity and renal disease, but these investigators could not demonstrate that a low-calorie diet interfered with production of xenotropic RNA tumor virus or viruses in the tissues of the B/W mice. It thus seems especially important to know whether calorie restriction in the MRL/l mice decreases virus expression or production of antigens coded in the virus genome.

Talal (20) and Roubinian *et al.* (21) have found that hormonal manipulations profoundly influence expression of disease in B/W mice, which develops earlier in females than it does in males. Since restricted calorie intake prevents all aspects of autoimmunity, immunologic involution with aging, and development of the renal-vascular disease in mice of this the B/W strain, it has been thought that the diet might exercise an influence on hormonal make-up. MRL/l mice, however, develop disease with equal tempo and equal frequency in males and females. It will be of special interest to determine whether calorie restriction alters hormonal balance similarly in the mice of the two strains, B/W and MRL/l. Several autoimmune strains of B/W, MRL/l, and BXSB mice are also found to have defects in production of and response to interleukin 2 (22, 23). We observed that both production of interleukin 2 and response to it could be maintained by food restriction in B/W mice (24).

The profound influence of dietary calorie restriction in preventing disease and immunopathology in the MRL/l mice raises the question whether one particular dietary constituent is more crucial to this influence than any other. Analyses based on varying the intake of different major dietary constituents separately—e.g. fats, carbohydrates, and proteins—are very much in order and should be carried out in mice of this strain.

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