Influence of diet on vascular lesions in autoimmune-prone B/W mice

(atherosclerosis/fat/autoimmunity/immune complexes/lipid-proliferative intima)

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ABSTRACT Autoimmune-prone B/W mice, which are known to develop severe glomerulonephritis and vasculitis, are also found to develop arteritis and proliferative and fatty-proliferative lesions of the aorta and its branches as well as renal inflammatory lesions. High intake of saturated fat in the diet enhances the development of these atherosclerotic and autoimmune lesions significantly in female mice, whereas restriction of dietary calories and fat inhibits their development. Ad lib feeding of laboratory chow, high in fiber and low in fat, does not foster development of vascular lesions but does permit the development of autoimmune renal disease.

Recent analysis of the influence of diet on immunity, longevity, and development of diseases of aging in experimental systems has generated new information (1–3). Mice of the New Zealand strains NZB and NZW and their hybrid (NZB × NZW)/F1; (B/W) have been used by many investigators as models of autoimmune disease. B/W mice have a disease that is analogous to human systemic lupus erythematosus (4, 5).

We showed that fat restriction in NZB mice delayed development and decreased severity of autoimmunity, inhibited immunologic and thymic involution, and prolonged life (6). NZB mice develop sarcomas, hepatomas, and lymphomas in high frequency (7, 8). Restriction of protein inhibited hemolytic anemia, delayed thymic involution, decreased splenomegaly, prolonged function of the T-cell immune system, and inhibited development of malignancies (9).

When total calories were restricted in the shorter-lived, autoimmune-prone B/W mice, which die of renal disease between 7 and 14 months of age, life-span was regularly more than doubled (10), a finding confirmed by Gardner et al. (11). Prolongation of life was associated with decrease of circulating immune complexes and retrolateral envelope glycoprotein-gp70 antibody, decreased deposition of immunoglobulins and complement in the glomerular capillaries, and prolongation of balanced immunologic function (12, 13). Compared to calorie-restricted mice, well-fed animals showed earlier involution of the thymus, earlier development of T-cell dysfunction, spontaneous development of suppressor T cells that depress B-cell functions, decline of interleukin 2 levels, and earlier onset and more severe autoimmune and renal disease, including several cardiovascular lesions (14, 15, 16).

Maintenance of immune functions, prolongation of life, and inhibition of immunologic injury was achieved in the short-lived, autoimmune-prone B/W mice when dietary restriction of calories was started as late as 3–5 months of age, a time when autoimmunity has already appeared (16). Others showed that survival of the long-lived C57BL/6 mice, a strain generally free of tumors and autoimmune diseases, is increased by calorie restriction (17). In the latter model, dietary restriction delayed maturation and significantly delayed involution of immunologic function (18).

Andrews et al. (19) found that 25–30% of the mice of NZB, B/W, MRL/1, and BXSB strains showed evidence of acute myocardial infarction and considered this a major factor in early death of these mice. Acute polyarteritis-like lesions in renal and coronary arteries were observed (20). Furthermore, NZB mice are known to develop hypertension and hypertensive vascular disease (21).

We fixed heart tissue for histopathologic examination during our prior investigations of the influence of different diets on length of life, immune function, and autoimmune disease (10, 14, 16). We report herein histopathological findings observed in mice on five different diets.

MATERIALS AND METHODS

Animals. Inbred male and female B/W mice, produced at the Good/Old mouse colony at Sloan–Kettering Institute by mating NZB and NZW strains, were used. The mice were housed under standard animal care conditions that included controlled humidity and temperature and altered light and dark cycles.

Diets. The basic formula of the defined diets, by weight, was 22% casein, 33% dextrose, 33% starch, 5% fat (corn oil), 4% mineral mixture, and 2% vitamin mixture (7). When fat proportion was increased the carbohydrate proportion was decreased proportionately. Group I was fed Purina Lab Chow (22% crude protein and 3–4% fat) ad lib; group II, 22% casein and 5% fat (corn oil), 20 cal/day (1 cal = 4.184 kJ); group III, 22% casein and 5% corn oil, 10 cal/day; group IV, 20% corn oil, 20 cal/day; and group V, 20% lard, 20 cal/day. Thus, mice were fed normal calories/low fat, low calories/low fat, or normal calories/high saturated fat diets. Preparation of food and feeding procedures have been described (7). Mice of diet groups II, IV, and V (high in calories) had life-spans of 6–14 months; however, when the calorie intake was restricted to 50%, the life-span of the mice was at least doubled. This prolongation of life was observed regardless of whether life-span was calculated by median survival time, mean survival time, life-span of the 10% longest survivors, or life-span of the very longest survivor.

Histology. Heart tissue, including the aorta and its major branches, was collected at the time of sacrifice from terminally ill animals and age-matched mice on the low calories/low fat

Abbreviation: cal, the nutritionist's calorie = 1 kcal = 4.184 kJ.

diet to permit histological and immunological comparisons with mice fed the high calorie diets. A block of tissue containing the heart, aorta, and the proximal portions of the aortic branches was fixed in 10% neutral formalin and was stained with hematoxylin/eosin, as is routine in our laboratories. Sections of aorta were mostly longitudinal, and aortic branches were usually cut in cross section. For mice on the several diets, comparisons were made of the aorta, subclavian and renal vessels, as well as the coronary vessels.

RESULTS

General Histology. Arterial lesions were defined as areas of intimal proliferation characterized by at least three cell layers above the elastic lamina. Arterial lesions were classified according to the method of Minick et al. (22). An arterial lesion was considered to be fatty proliferative if some or all of the intimal cells had a foamy cytoplasm or if histologically characteristic cholesterol clefts were observed (Fig. 1A, B, and D). A proliferative arterial lesion lacked evidence of lipid deposition (Fig. 1C). In addition, an arterial lesion was classified as arteritic if it showed fibrinoid necrosis and inflammatory infiltrates.

In confirmation of the findings of Andrews et al. (19), myocardial scars were observed in a significant proportion of mice in groups I and II (17%). These lesions also were seen in mice of groups IV and V, but not in mice of group III under 15 months of age.

Table 1 compares the incidence and the average number of arterial lesions per mouse among the five groups. Female mice fed Purina Lab Chow (group I) and those fed 10 cal/day containing 5% unsaturated fat (group III) had the lowest incidence

FIG. 1. (A) Aorta of B/W mouse fed a 20% corn oil diet (group IV) showing marked fatty proliferative intimal thickening. (Hematoxylin/eosin, x125.) (B) Higher magnification of boxed area in A. Thickened intima contains pools of cholesterol crystals (arrows). (Hematoxylin/eosin, x320.) (C) Section through the proximal aspect of a major coronary artery of a B/W mouse fed laboratory chow, illustrating a proliferative lesion characterized by fibromuscular intimal thickening devoid of visible lipid. (Hematoxylin/eosin, x320.) (D) Occlusive fatty proliferative intimal lesion of B/W mouse fed 20% corn oil. The lumen of this artery, a major branch of the abdominal aorta (probably the superior mesenteric artery), is reduced to a slit. Lipid is present deep in the intima near the internal elastic lamina. (Hematoxylin/eosin, x165.)
of arterial lesions, with overall comparisons of these two with the mice on high calorie or high fat diets (groups II, IV, and V) being statistically significant at \( P < 0.001 \) and \( P < 0.015 \), respectively, by the \( \chi^2 \) test for contingency tables.

The comparisons of groups I and III vs. II, IV, and V were not statistically significant among male mice because the incidence of arterial lesions was not as highly elevated in the high calories/high fat diets as it was for females. The distribution of types of lesions (fatty proliferative, proliferative, and arteritis) was comparable on all diets, as was the average number of lesions among mice having at least one lesion. Differences between males and females on all diets were significant at the \( P < 0.001 \) level.

**Life-Span.** The B/W female mice fed the low calories/low fat diet had life-spans ranging from 7 to 20 months; B/W males on the same diet had life-spans ranging from 11 to 34 months. The females, which have the more severe autoimmune disease and the shortest life-spans, had significantly more vascular lesions than the males. By contrast, in mice fed high calories/high fat diets the life-span of males ranged from 8 to 15 months and in females it ranged from 6 to 12 months.

**DISCUSSION**

In prior studies, we and others (4, 5, 14, 16) found that the laboratory chow diet permitted development of autoimmune renal lesions and involvment of immunologic function with age in B/W mice. In the present study, ad lib feeding of the same laboratory chow diet, which is high in fiber and relatively low in fat, was associated with a low frequency of atherosclerotic and arteriosclerotic lesions as well as low serum cholesterol levels (unpublished data), compared to more completely defined diets that were lower in fiber content and higher in animal protein and fat.

Because this study was not designed to perform a thorough morphometric evaluation of the arterial lesions observed, only a rough grading of the lesions and semiquantitative assessment was possible. Nevertheless, these findings indicate that short-lived B/W mice may represent a model of atherosclerosis and arteriosclerosis. Because the mice of this strain regularly develop vascular lesions based on deposition of immune complexes, the findings also raise the question of whether immunological mechanisms play a role in the pathogenesis of atherosclerosis and arteriosclerosis, as suggested by Minick et al. (22). These results also show that calorie and fat restriction prevents development of both renal lesions and cardiovascular lesions in B/W mice, whereas diets high in fat and high in calories seem to aggrivate the cardiovascular lesions.

These findings agree with those of Minick (23) and Alonso et al. (24), which indicate that hyperlipidemia enhances the development of atherosclerosis induced by immunologic arterial injury. A recent study (25) in retired breeder Sprague–Dawley rats, showing an association between immune complex deposition, arteriosclerosis, and depressed T-cell functions, is consonant with our findings. Further, Kritchevsky et al. (26) showed that casein is more cholesterolemic and atherogenic to rabbits than is vegetable protein; our mice, which developed atherosclerosis while on the defined diets, had been given a diet containing casein.

We have observed in the past that calorie and fat intake can influence T-cell function, interleukin 2 production, immune complex formation, and immune complex deposition in autoimmune-prone B/W mice (14, 15, 44). The present studies, in which the laboratory chow diet, which is relatively free of casein, was associated with a low incidence of vascular lesions and the defined diets, even those lower in fat, were linked to a higher incidence of vascular lesions, suggest that components of semipurified diets other than fat may influence the development of atherosclerosis and arteriosclerosis in B/W mice. Studies to address this issue directly in mice prone to develop autoimmune and cardiovascular-renal disease seem to be in order.

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