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Immunology

Dietary fat affects immune response, production of antiviral factors, and immune complex disease in NZB/NZW mice

(xenotropic virus/lipoproteins/mitogenic response/natural thymocytotoxic autoantibody/anti-DNA antibody)

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ABSTRACT **Autoimmune-prone (NZB × NZW)F_1 (B/W) mice fed three nearly isocaloric diets with varied fat content showed a marked difference in their spontaneous development of immune complex disease and their immune responses. Those animals receiving the diets high in either unsaturated or saturated fats had more severe immune complex nephritis and died earlier than mice on the low-fat diet. Endogenous production of the mouse xenotropic virus was unaffected by dietary fats, but the serum lipoproteins associated with antiviral activity were increased to levels as high as 1:600,000 in the B/W mice on the high-fat diets. These lipoproteins may be partially responsible for the decreased mitogenic response of spleen cells from mice fed the two high-fat diets. The mice receiving a diet high in saturated fats produced substantially higher titers of natural thymocytotoxic autoantibody, an IgM class of antibody, than did the mice maintained either on the high-unsaturated-fat or low-fat diet. In contrast, the mice receiving the diet high in unsaturated fats made significantly greater levels of antibodies to double-stranded DNA, an IgG, than did the mice kept on the two other diets. These results suggest that the type of fat in the diet could affect the serum level of different immunoglobulin classes. The data provide further evidence that the amount of dietary lipids alone can influence cellular and humoral immune responses and the spontaneous development of immune complex disease.**

New Zealand Black (NZB) mice and their New Zealand White (NZW) hybrid (NZB × NZW)F_1 (B/W) progeny develop a disease complex characterized by hyperactive B cells which produce various autoantibodies (1, 2). Among these are antibodies to double-stranded (ds) DNA (2) and natural thymocytotoxic autoantibody (NTA) (3). This B-cell abnormality leads to fatal immune complex (IC) disease or lymphoid neoplasias in most animals (1, 2, 4). These mouse strains also have a high production of the endogenous xenotropic (X-tropic) mouse type C virus (MuLV) (5, 6). Although not directly involved in the disease syndrome, this virus may have a role as a cofactor in some of the autoimmune sequelae (7–10). We and others have demonstrated that female B/W mice can be "cured" of their autoimmune disease by a diet deficient in protein or calories (10, 11). We found that these dietary restrictions did not markedly alter the production, in the mice, of endogenous MuLV, serum lipoproteins that specifically neutralize the X-tropic virus (12–14), or antinucleic acid antibodies (10). These data indicated that diet-induced prevention or delay of IC disease in B/W mice and the prolonged longevity were unrelated to any influence upon production of these factors.

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We have determined that the serum factor responsible for X-tropic virus neutralization is an apolipoprotein associated primarily with mouse triglyceride-rich lipoproteins and high density lipoproteins (HDL) (12). Mice fed a high-fat diet for 24 hr have a 10- to 20-fold increase in this virus-neutralizing activity (12). In this paper we report the effect of a prolonged intake of high-fat meals on the levels of neutralizing factor (NF) in female B/W mice and on other factors that could be involved in the development of autoimmunity in these mice.

MATERIALS AND METHODS

Mice and Diets. The female B/W mice were raised at the animal care facility at the University of Southern California (Los Angeles). Parental female NZB and male NZW mice were obtained from the animal care facility at the National Institutes of Health (Bethesda, MD). After weaning, mice were fed a rodent Purina Chow for about a week before they were placed on one of three different dietary regimens (Table 1). The vitamin levels (including vitamin E) of all diets were adequate and sufficient for maintaing antioxidant activity (J. Januss, Teklab Test Diets, Madison, WI, personal communication). Moreover, protein and minerals were given in equal and adequate amounts so that the only variable was dietary fat. One group of mice received a diet rich in saturated fats, another group received a diet rich in unsaturated fats, and the third group received a diet low in fat. The caloric content of the low-fat diet differed by only 10% from that of the diet high in unsaturated fat and by about 20% from the diet high in saturated fat. The two fat diets differed by 10% in caloric value. Food was given ad lib. No difference in amount of food eaten by each group was noted. Mice were weighed before sacrifice to determine any effect of the diets on size and weight; no substantial differences were noted among the three diet groups. During the first 2–3 months, mice fed a low-fat diet gained 10% less weight than did mice on the high-fat diets. Subsequently, the mice in all three groups had essentially the same weight. All mice surviving at 8 months were sacrificed and their kidneys were examined microscopically (for IC disease). Tissues from mice dying earlier were also studied histopathologically.

Abbreviations: NZB, New Zealand Black; NZW, New Zealand White; B/W, (NZB × NZW)F_1; IC, immune complex; ds, double-stranded; NTA, natural thymocytotoxic autoantibody; X-tropic, xenotropic; MuLV, murine leukemia virus, mouse type C virus; HDL, high density lipoproteins; NF, neutralizing factor; FA, immunofluorescence assay; Con A, concanavalin A; PGE_2, prostaglandin E_2; LP, lipoproteins; ME, mouse embryo.

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Table 1. Diets used for study of B/W mice

	Composition, g/kg of diet		
	Diet I	Diet II	Diet III
Casein	200	200	200
Sucrose	508.6	613.6	691.6
Lard	180	0	0
Cholesterol	10	0	0
Corn oil	0	90	12
Cholic acid	5	0	0
Non-nutritive fiber	50	50	50
Mineral mix*	35	35	35
Vitamin mix†	10	10	10
Dry vitamin A palmitate (500,000 units/g)	0.01	0.01	0.01
Dry vitamin E acetate (500 units/g)	0.16	0.16	0.16
Choline chloride	0.31	0.31	0.31
Inositol	0.9	0.9	0.9
Caloric value, kcal/kg	4502	3978	3588
Linoleic acid, % of total fat	18	47.7	6.4

Diets obtained from Teklad Test Diets (Madison, WI). Diet I, high in saturated fat; diet II, high in unsaturated fat; diet III, low in fat.
* Williams-Briggs, Mod. (cat. no. 170911).
† Teklad (cat. no. 40060).

Histopathology. Sections of both kidneys and other major visceral organs (excluding bone marrow and central nervous system) were prepared for light microscopy by fixation in 10% neutral buffered formalin, and 5-μm sections were stained with hematoxylin and eosin (10). Kidney was scored on a scale of 1–4 according to extent of hypercellularity, enlargement of the glomeruli, and thickening of the mesangial matrix and capillary basement membranes. Immunoperoxidase staining has shown that this thickening is associated with an increase in immunoglobulin deposition (10). Specimens with a score of 3–4 were considered severely affected; those with scores less than 2 represented minimal disease.

Assays for Infectious Virus and Serum Antiviral Activity. X-tropic MuLV production by cells cultivated from B/W thymus, spleen, and kidneys was measured by the mink S+L− assay (15), by immunofluorescence (FA) testing in mink lung (American Type Culture Collection CCL 64) and D17 (dog) cells which had been grown with the mouse cells (16), and by a cocultivation procedure in which the B/W cells were mixed with non-virus-producing murine sarcoma virus-infected NRK (rat) cells. Seven-day supernatants from the cocultivated cells were subsequently assayed for focus formation on NRK cells (17). Ecotropic (mouse-tropic) MuLV was detected by the XC plaque assay (18) with secondary NIH Swiss mouse embryo (ME) cells. Virus content in tissues was assessed directly by inoculating 10% (wt/vol) extracts of frozen samples on ME or mink lung cells (16). Virus was detected by the FA or XC procedures.

Virus neutralization assays were conducted with the C57L or the NZB strain of X-tropic MuLV in the mink S+L− assay (14, 15). Mouse sera were screened by 1:10 dilutions and then retested at 1:2 dilutions. The titer of neutralizing activity is expressed as the reciprocal of the end dilution of serum that gave 66% suppression of focus formation by the virus. When 50% of the foci were prevented by a serum dilution, the titer is expressed as ±. Neutralization of ecotropic virus was measured by the XC assay in ME cells with the Moloney strain of MuLV.

Mitogenic Response of Spleen Cells and Prostaglandin Production. Single-cell suspensions of spleens from the B/W mice were prepared and exposed to the T-cell mitogen concanavalin A (Con A) by standard techniques (19). T-cell response was assessed by incorporation of radioactively labeled thymidine.

Prostaglandin E_2 (PGE_2) was measured by a radioimmunoassay (20). The PGE_2 antibody was provided by the Pasteur Institute (Paris, France). Nonradioactive PGE_2 was obtained from John E. Pike (Upjohn); radioactive PGE_2 was purchased from New England Nuclear. Marc Goldyne (San Francisco) helped in these assays.

Autoantibody Production. Measurement of NTA was performed by a cytotoxicity assay with BALB/c thymocytes (21). Anti-ds-DNA antibodies were detected by a modification of the procedures of Farr (22). *Escherichia coli* ds [^{14}C]DNA was prepared as described (22). The results are expressed as a percentage of ds [^{14}C]DNA precipitated by 0.01 ml of heat-inactivated (56°C, 30 min) serum. Statistical analyses of the results on autoantibody production were performed by using the two-sample *t* test.

RESULTS

Effects of Diet on Development of Disease and Longevity of the Mice. B/W female mice fed the high-fat diets succumbed to IC disease at an earlier age than did mice fed a low-fat diet. The mice had severe glomerulonephritis by 5 months of age and nearly 30% of them had died by 8 months of age (Fig. 1; Table 2). Two of the 28 mice fed a diet high in saturated fat developed generalized lymphomas at 5 months of age. In contrast, most of the mice fed a low-fat diet were living at 8 months of age. Those sacrificed at 5 months of age had no lymphomas and only 8 of 30 animals had significant glomerulonephritis. Mice in both high-fat-diet groups also showed fatty livers and more lymphoid hyperplasia and metastatic calcifications than did those receiving a low-fat diet.

Endogenous Virus Production. The extent of infectious X-tropic virus production by mice fed any one of the three diets

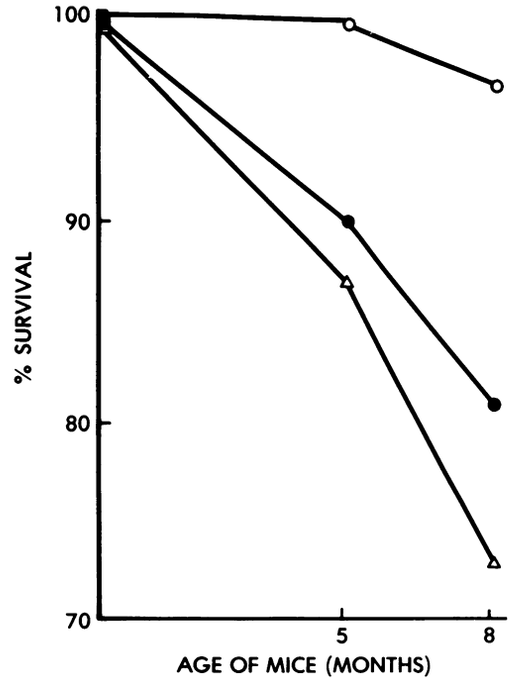

FIG. 1. Effect of dietary fat upon survival of female B/W mice. All mice were sacrificed at 8 months of age. At 1 month of age they were placed on various diets as follows: ●, high in saturated fat; ○, low in fat; △, high in unsaturated fat. Data from 50 mice in each group are presented.

Table 2. Effect of dietary fat on glomerulopathy and T-cell mitogenic response in female B/W mice

Diet	Live mice examined at 5 months	Mice with substantial glomerulopathy*	Splenocyte Con A response, cpm†
Saturated high fat	28	28	7,000
Unsaturated high fat	26	25	11,000
Unsaturated low fat	30	8	98,000

* See text for histologic evaluation. Mice on the low-fat diet had less severe glomerulopathy than did those on a high-fat diet.

† Mean of two animals. [^{3}H]Thymidine uptake by splenocytes 65 hr after exposure to 0.37 μg of Con A per culture.

was not substantially different. In all cases, approximately 100 infectious particles were detected per ml of supernatant in cultures containing 10^6 B/W cells. In general, stromal cells from the thymus released lower titers of virus than did cells derived from the kidney and spleen. Tissue extracts from representative mice at 4 and 8 months of age also showed no difference in X-tropic virus expression. Ecotropic virus was detected in about 30% of tissues from mice in all three groups. Its titer was approximately 1000–10,000 infectious particles per ml of culture fluid. No difference in expression of this endogenous virus was noted among the three groups. Similar results were obtained with B/W mice on regular and protein deficient diets (10).

Levels of NF. Remarkable differences were noted in the amount of NF in sera of mice maintained on the high-fat diets compared to those on the low-fat diet (Fig. 2). At 4 weeks of age, all mice had levels of 40–400, a titer frequently seen in 1- to 2-month-old B/W mice fed a conventional mouse chow (7). Those mice maintained on a low-fat diet for 7 months had levels of NF commonly found at that age in NZB and B/W mice, 1000–4000 (7). In contrast, mice fed high-fat diets had significantly higher levels of NF. Titers as high as 300,000–600,000 were found in sera from some mice maintained for 7 months on the high-fat diets. In general, the serum titers were 80,000–160,000; the highest titers were observed in mice receiving the diet high in saturated fat. No neutralization of the endogenous ecotropic MuLV occurred with any mouse serum, even at a dilution of 1:10.

To determine which lipoproteins (LP) were responsible for this virus neutralization, mouse LP were separated by standard techniques (13, 14) from a pool of sera from mice fed a diet high in saturated fat. Most of the neutralizing activity was found in the triglyceride-rich LP fraction (at a level of 100,000) but activity was also detected in the HDL (10,000). In order to study whether the high levels of antiviral activity were associated with an equivalent increase in total serum LP, the phospholipid content of the mouse LP was measured (23) (experiments were done by D. Hardman and J. Kane, University of California, San Francisco.) These studies indicated that the level of total LP in sera of mice on the high-fat diet was 10 times greater than that of the mice maintained on a low-fat diet. However, the NF levels were 100- to 600-fold higher in the mice receiving this dietary regimen (Fig. 2). Thus, these results suggest that high-fat diets can specifically enhance serum levels of the LP containing the anti-X-tropic virus NF.

Mitogenic Response of T Cells. Spleen cells removed from mice fed for 7 months either high-fat or low-fat diets were examined for their response to Con A (Table 2). The mitogen-treated spleen cells from mice fed a high-fat diet incorporated much less [^{3}H]thymidine than expected for spleen cells from normal mice of comparable age (24). Such a result has been reported for spleen cells from adult NZB and B/W mice (1, 2). In contrast, spleen cells from mice fed a low-fat diet exhibited a substantial blastogenic response to Con A. These results in-

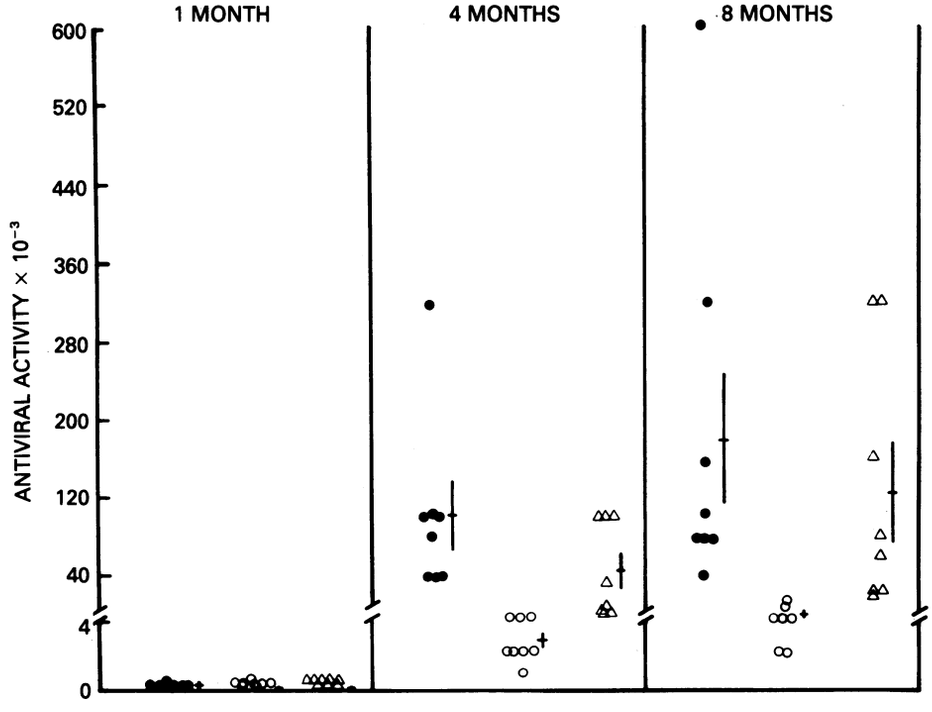

FIG. 2. Effect of dietary fat on serum levels of anti-X-tropic virus NF. The antiviral activity is expressed as the reciprocal of the end dilution of serum that suppressed 50–66% of infectious X-tropic MuLV. The assay was focus formation in mink S+L− cells (14). Data are given for sera from mice at 1 month of age (when they first received the diet) and at 4 and 8 months of age. Each symbol represents a single mouse. ●, Diet high in saturated fat; ○, diet low in fat; △, diet high in unsaturated fat; horizontal line, mean; vertical line, ± SEM.

Table 3. *In vitro* Con A response of female B/W mice maintained on diets differing in fat content

Diet	Mice, no.	Duration of diet, mo.	Indomethacin (2.8 μM)	Splenocyte response to Con A, cpm*	PGE_2, pg†
Saturated high fat	2	4	−	37,443	270
	2	4	+	40,052	163
	3	6	−	37,298	300
	3	6	+	38,528	168
Unsaturated high fat	2	3	−	165,864	340
	2	3	+	278,296	153
	4	6	−	29,255	241
	4	6	+	42,768	138
Unsaturated low fat	2	4	−	101,269	335
	2	4	+	218,728	142
	3	6	−	114,022	273
	3	6	+	130,101	155

* Mean [^{3}H]thymidine uptake by splenocytes (4×10^5 per culture) 65 hr after exposure to 0.37 μg of Con A per culture. The mean of three samples was used for each mouse.
† Measured by radioimmunoassay (20); mean value is given.

dicated that the low-fat diet maintained a normal mitogenic response of T cells in these autoimmune-prone animals which usually show a reduced T-cell response (1, 2).

The splenocyte response to Con A was also measured in spleen cells removed from the mice at 3, 4, and 6 months on their diets. The marked reduction in mitogenic response was first observed in the spleens of mice maintained on high-fat diets for 4 months (Table 3). Mice fed the low-fat diet, as noted above, had a normal mitogenic response. To evaluate the possible role of PGE_2 in this decreased splenocytic response, indomethacin (2.8 μM) was added at the time of the spleen cell culture and PGE_2 levels were measured in the supernatants. Indomethacin did not substantially increase the T-cell response in any of the mice after the reduction in splenocyte response had taken place (4–6 months). Moreover, although decreased in the cell cultures receiving the drug, PGE_2 levels did not differ remarkably for the three groups examined. The results therefore suggested that PGE_2 was not a major cause of the suppression of T-cell response in the mice fed the high-fat diets.

Autoantibody Production. NTA production was not high in any of the mice at 1 month of age, when they were begun on their test diets, or at 4 months of age, after they had been on their diets for 3 months (Fig. 3). These observations are consistent with the interval before the appearance of detectable disease in this strain (1, 2). At 8 months of age, however, marked differences in the production of NTA were observed in the three groups. The mice receiving the diet high in saturated fat had significantly higher levels of NTA than did mice receiving the low-fat diet ($P < 0.02$ for undiluted sera). In some mice, this NTA was detectable in sera at a dilution of 1:5 and 1:10. In contrast, mice maintained on a diet high in unsaturated fat had lower serum levels of NTA than did mice receiving the high-saturated-fat diet; they were significantly lower at serum dilutions of 1:5 and 1:10 ($P < 0.02$). Moreover, the titers of NTA in the sera of mice fed the diet high in unsaturated fat and the low-fat diet were not significantly different ($P > 0.05$).

When the anti-ds-DNA antibodies were measured, a different result was obtained (Fig. 4). As noted for NTA, no substan-

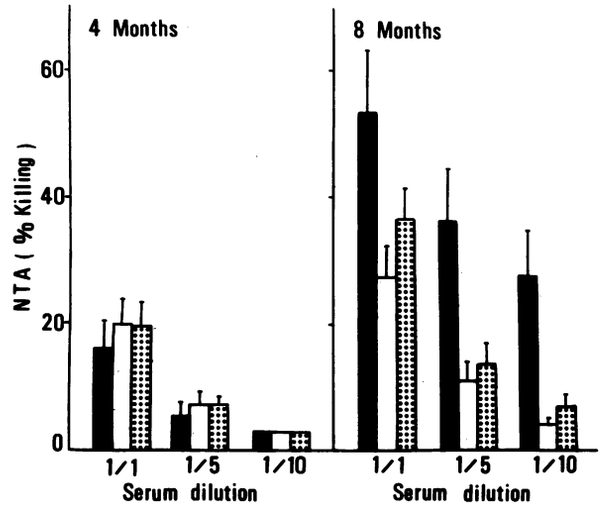

FIG. 3. Effect of dietary fat on spontaneous production of NTA. Data from 15–18 serum samples from each diet group at each time period are presented. The height of the bar represents the mean; vertical line represents SEM. ■, Diet high in saturated fat; □, diet low in fat; ⊞, diet high in unsaturated fat.

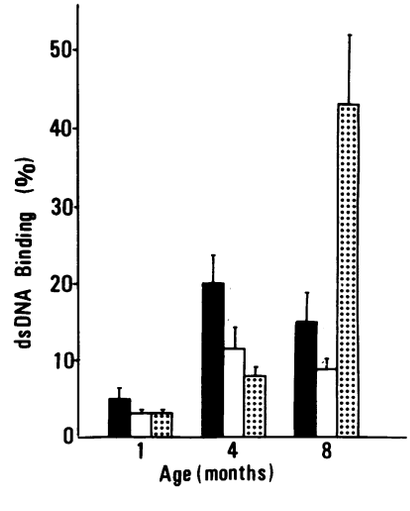

FIG. 4. Effect of dietary fat on spontaneous production of antibodies to ds DNA. Data from 15–18 serum samples from each diet group at each time period are presented. The height of the bar represents the mean; vertical bar represents SEM. ■, Diet high in saturated fat; □, diet low in fat; ⊞, diet high in unsaturated fat.

tial production of anti-ds-DNA antibody was found in any of the groups of mice at 1 and 4 months of age as has been reported (1, 2). At 8 months of age, however, after the mice had been on their diets for 7 months, those receiving a diet rich in unsaturated fat exhibited significantly higher levels of antibodies to ds DNA than did those that were kept on the high-saturated fat or low-fat diet ($P < 0.02$ and < 0.001, respectively). Notably, the sera from mice on the diet high in saturated fat, which contained the highest titers of NTA, had low levels of anti-ds-DNA which did not differ significantly from those found in the sera of mice on the low-fat diet ($P > 0.05$).

DISCUSSION

The results of this study indicate that, aside from their caloric value, dietary fats can greatly enhance the spontaneous development of autoimmune disease in the B/W strain of mice. They also influence both the cellular and humoral immune responses of the host. The data extend further the observation by Fernandes *et al.* (25, 26) who showed that diets rich in fat increased the severity of IC disease in NZB mice and had a detrimental effect on the cellular and humoral immune responses. However, because the caloric content of the diets varied, a direct effect of dietary lipids on the immune system could not be ascertained by their studies. Our results suggest that a marked reduction in dietary lipid intake itself, irrespective of calories, can decrease the incidence and severity of the disease, maintain near-normal immune responses, and prolong the life of the animal.

The absence of differences in X-tropic virus expression in tissues from the mice on all three diets mirrors the results of previous diet studies (10) and supports the conclusion that the X-tropic virus alone is not responsible for the autoimmune disease and neoplasia in the strain (7–10). Nevertheless, its interaction with lipoproteins and with antibodies to viral antigens may contribute to the IC disease (7, 9).

The effect of high-fat diets on levels of anti-X tropic virus NF is noteworthy. Previous studies demonstrated a 10- to 20-fold increase in titer of NF in mice receiving high-fat diets for 24–48 hr (14). We have now observed that mice fed for several months a diet rich in fat have a 100- to 600-fold enhancement in the serum level of this antiviral factor, and this increase cannot be explained solely by an increase in total mouse LP. A selected increase in NF synthesis seems to occur. The role of the antiviral LP is not known, but perhaps it is involved in the markedly decreased T-cell blastogenesis we and others (27) have observed in mice fed high-fat diets. NF could interact with X-tropic viral antigens on the surface of T cells and affect their mitogenic response (7, 14); mouse LP have been shown to suppress T-cell blastogenesis (28, 29).

We examined whether the suppressed spleen cell blastogenic response observed in the B/W mice receiving the high-fat diets was due to the large amount of polyunsaturated fatty acids present in the diets (see linoleic acid levels, Table 1). Mertin and Hunt (30) have suggested that, as precursors for prostaglandins, these fatty acids decrease immunity by increasing the production of these immunoinhibitory substances. However, our measurement of PGE_2 levels with and without the use of indomethacin suggests that this substance is not a major cause of the suppression of mitogenesis.

Finally, marked differences were observed in the autoantibody production by the three groups of mice. First, the small amount of autoantibodies in mice fed a low-fat diet suggests that this is a factor in the IC disease of B/W mice. Second, the quantity of each type of autoantibody made was different for the mice receiving each of the two high-fat diets. The NTA, an IgM autoantibody, was highest in mice receiving saturated fats. In contrast, substantial production of anti-ds-DNA, an IgG autoantibody, was associated with the intake of unsaturated fats. Similar observations on the preferential production of IgG, and not IgM, antibodies were made in studies of prenatal and postnatal mice receiving diets rich in polyunsaturated fats (27).

Further studies are required to determine if the type of fat in the diet can influence the serum levels of IgM and IgG antibodies. However, the data do permit the speculation that differences in fat content in the diet could affect the type of antibody produced by stimulated B cells. They provide additional evidence that dietary fats can play a substantial role in the regulation of the immune response in the host.

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