Dietary fat influences Ia antigen expression, cytokines and prostaglandin E_2 production of immune cells in autoimmune-prone NZB × NZW F1 mice

BY BI-FONG LIN[1]*, CHAO-CHI HUANG[1], BOR-LUEN CHIANG[2]
AND SU-JEN JENG[1]

[1] *Laboratory of Nutritional Chemistry, Department of Agricultural Chemistry, College of Agriculture, and* [2] *Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan 10764, Republic of China*

To elucidate further the influences of dietary fat on autoimmune diseases, two groups of NZB/W F1 mice were fed with diets containing 200 g dietary fat/kg and 50 g dietary fat/kg (control) respectively. The difference in energy intake between these two groups was compensated with carbohydrate. Mice were bled regularly every month and some of them were killed for *in vitro* experiments after 5 months experimental diets. Higher immunoglobulin (Ig)M and IgG anti-double stranded DNA antibody levels, shortened life span and worsened proteinuria were noted in mice fed on the high-fat diet compared with those fed on 50 g dietary fat/kg. Phenotypic analyses of spleen cells and peritoneal exudate cells showed that the percentage of CD5$^+$ B cells and the mean fluorescent intensity of major histocompatibility molecules on the surface of both types of cells were higher in mice fed on the high-fat diet. In general, higher type 2 T-helper cell activity was noted in mice fed on the high-fat diet. In addition, cytokines such as interleukin-6, tumour necrosis factor-α and prostaglandin E_2 (PGE$_2$) produced by lipopolysaccharide-stimulated peritoneal exudate cells were also higher in the high-dietary-fat group. These studies suggest that high dietary fat and its related PGE$_2$ level might have a critical effect on the frequency of CD5$^+$ B cells, cytokine production, macrophage function and subsequent autoimmune regulation in autoimmune mice.

Systemic lupus erythematosus: Autoimmunity: CD5$^+$ B cells: Cytokines: Prostaglandins

Murine lupus models like NZB × NZW F1 (NZB/W F1), NZB.H-2^{bm12}, NZB × SWR F1 (SNF1), MRL.*lpr/lpr* and BXSB mice have provided much insight into the understanding of the pathogenic mechanisms of lupus (Theofilopoulos & Dixon, 1981; Steinberg *et al.* 1987; Yoshida *et al.* 1990). All these mice develop immunoglobulin G (IgG) anti-double stranded DNA (dsDNA) antibody, a characteristic of lupus, and the mice die of uraemia in early life. Although the underlying mechanism is yet to be defined, several possible mechanisms such as increased CD5$^+$ cell population, decreased macrophage and natural killer (NK) cell activity have been implicated in the immune regulation of the autoimmune process (Farinas *et al.* 1990; Takeda & Dennert, 1993). The amount and degree of saturation of dietary fat might affect the disease course in autoimmune mice. Low-fat and low-energy diets and fish oil decrease autoantibody production and prolong the life span in murine lupus models such as NZB × NZW F1 and MRL.*lpr/lpr* mice (Morrow *et al.* 1985; Robinson *et al.* 1986; Alexander *et al.* 1987; Ogura *et al.* 1989).

Most recently, one study suggested that different proportions and saturation of dietary fat might determine the degree of expressed Ia antigen which plays an important role in

* For reprints.

immune responses (Huang *et al.* 1992). Increased class I and II major histocompatibility (MHC) antigen expression on the cell surface of the immune cells could contribute to possible development of autoreactive immune cells and inflammatory effector cells in autoimmune diseases. In addition, the degree of Ia expression could also determine the magnitude of T-cell activation and subsequent cytokine production (Matis *et al.* 1983; Janeway *et al.* 1984). One most recent study showed that the degree of expressed MHC class II molecules with antigenic peptide might determine the development of T cells and cytokine secretion (Secrist *et al.* 1995). Abnormal cytokine production could further contribute to dysregulatory immune responses such as decreased NK cell activity, increased CD5$^+$ B cell population and the autoimmune process. Furthermore, evidence suggests that macrophage function could be modulated by the content of the dietary fat (Hubbard *et al.* 1991; Watanabe *et al.* 1993). Products such as interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and prostaglandin E$_2$ (PGE$_2$) of macrophages have been connected with the pathogenesis of murine lupus (Jacob & McDevitt, 1988; Alarcon-Riquelme *et al.* 1993).

To elucidate further the effects of dietary fat on autoimmunity, autoimmune-prone NZB × NZW F1 mice were fed with different concentrations of fat and immunological changes were followed, including the frequency of CD5$^+$ B cells, class I and II molecule density, cytokine production by T cells and macrophages. The present study describes the effect of high dietary fat on long-term follow-up immune responses of autoimmune-prone NZB/W F1 mice.

MATERIALS AND METHODS
Experimental animals

Four-week-old female NZB × NZW F1 (B/W F1) mice were purchased from Charles River Japan (Tokyo, Japan) and maintained in the Department of Agricultural Chemistry at National Taiwan University College of Agriculture. The mice were housed and fed individually. This animal room was on a 12 h light–12 h dark cycle, with a constant temperature (25° ± 2°) and humidity. Each group consisted of sixteen mice. Each mouse was fed with experimental diets starting from 1 month old, and six to eight mice from each group were killed for *in vitro* experiments after 5 months' feeding. The rest of the mice were maintained in our facility for subsequent studies of proteinuria and lifespan.

Diet

The composition of the diets given is shown in Table 1. As illustrated, the high-fat group's feed contained 200 g fat/kg and the control group's feed contained 50 g fat/kg. The fat was composed of equal amounts of lard and soyabean oil to mimic ordinary human dietary fat consumption. The difference in energy content was compensated for by carbohydrate. The mice were given free access to feed, weighed twice weekly, and feed consumption was measured every 2–3 d.

Reagents

Appropriate FITC (fluorescein) or PE (phycoerythrin)-conjugated monoclonal antibodies such as FITC-anti-mouse IgM (B cells, clone R6-60.2), FIT-anti-H-2K^d (class I-positive cells, clone AF3-12.1), PE-anti-CD5 (subset of B cells, clone 53-7.3) and FITC-anti-I-A^d (class II-positive cells, clone AMS-32.1) (PharMingen, San Diego, CA, USA) were used for phenotypic analyses of peritoneal exudate cells and spleen cells isolated from NZB/W F1 mice. Double staining with FITC-anti-mouse IgM and PE-anti-CD5 antibodies was used to identify CD5$^+$ B cells. For anti-dsDNA antibody ELISA, horseradish peroxidase (*EC* 1.11.1.7)-conjugated goat anti-mouse IgG (γ-chain specific, Jackson Inc., PA, USA) and anti-mouse IgM (μ-chain specific, Jackson Inc.) were used as secondary antibodies.

Table 1. *Composition of the experimental diets (g/kg diet)*

Ingredient	Low-fat diet	High-fat diet
Fat*	50	200
Casein†	200	200
Methionine	3	3
Sucrose	325	250
Maize starch	325	250
Cellulose	50	50
Choline	2	2
AIN-76 mineral mixture‡	35	35
AIN-76 vitamin mixture‡	10	10
Energy (kJ/kg)	16 170	19 320

* Composed of equal amounts of lard (Ya-Sern Company, Taipei, Taiwan, China) and soyabean oil (Taiwan Sugar Company, Taipei, Taiwan, China).

† Ingredient sources: casein, methionine and choline, Sigma Chemical Co. (St Louis, MO, USA); sucrose, Taiwan Sugar Company; maize starch, Roquette, Paris, France; α-cellulose, ARBOCEL®, type BE 600/300, J. Bettenmaier, & Söhke, Elwangen-Holzmühle, Germany.

‡ American Institute of Nutrition (1977).

Determination of proteinuria and life span

Starting from 4 months old, mice were evaluated for proteinuria and life span every 2 weeks. Proteinuria was measured with tetrabromophenol paper (Eiken Chemical Co., Tokyo, Japan) on freshly voided urine samples collected by massage of the bladder. This colorimetric assay, relatively specific for albumin, was graded 1+ to 4+ and the approximate protein concentrations were as follows: 1+ about 300 mg/l, 2+ about 1000 mg/l, 3+ about 3000 mg/l, and 4+ ⩾ 10000 mg/l. High grade proteinuria was defined as ⩾ 2+(⩾ 1000 mg/l). The results of the proteinuria test were graded by two different individuals in the laboratory.

Determination of anti-DNA antibodies

Mice were bled retro-orbitally every month and sera were collected for determination of anti-DNA antibodies. Sera were collected and stored at −70° until assay. Levels of anti-dsDNA antibodies were quantitated by ELISA on Immunolon I. For the assay of anti-dsDNA antibodies, plates were initially coated with 100 μl/well of 10 μg/ml methylated bovine serum albumin (BSA). After overnight incubation at 4°, plates were washed with phosphate-buffered saline (PBS) and 100 μl/well of 2·5 μg/ml calf thymus DNA in PBS was added. Plates were incubated overnight, drained, and 200 μl/well of PBS containing 1 mg gelatin/ml was added. After overnight incubation, plates were washed three times with PBS containing 0·5 ml Tween 20/l. Sera to be tested were diluted 1/100 or 1/200 in PBS containing 1 mg gelatin/ml and 5 μg bovine γ-globulin/ml and 100 μl of the diluted serum was added to the appropriate wells. After 2 h incubation at room temperature, plates were washed and 100 μl goat anti-mouse IgG or IgM–horseradish peroxidase diluted in PBS–gelatin–bovine-γ-globulin was added to each well. After 2 h at room temperature, plates were washed and 100 μl substrate solution/well was added. After 30 min incubation at room temperature, 50 μl of 50 g/l SDS was added to each well to stop the reaction. Absorbance was determined at 415 nm (Microplate, Bio-Tek Instrument Inc., VT, USA). The specificity of the assays was further evaluated with mAb 742H.7D (Cawley *et al.* 1991), specific for dsDNA (gift from Dr M. E. Gershwin, University of California at Davis, Davis, CA, USA).

Flow microfluorometric (FMF) analysis

Six to eight mice from each group were killed by CO_2 asphyxiation. Spleens were removed and single cells were isolated by lysing erythrocytes with NH_4Cl-Tris buffer. Peritoneal exudate cells were isolated by peritoneal lavage and washed three times with Hank's solution before use. Phenotypic analyses of spleen cells and peritoneal exudate cells were done by FMF. Portions of cells ($2 \cdot 5$–5×10^5 cells) were suspended in $0 \cdot 1$ ml PBS with 1 g/l sodium azide and incubated at 4° for 30 min with a predetermined optimal concentration of appropriate FITC- or PE-conjugated monoclonal antibodies. The cells were washed and resuspended in $0 \cdot 5$ ml PBS with 1 g/l sodium azide and subjected to FMF analysis. A total of 10 000 cells were counted and the frequency and mean density of each cell surface marker were determined using appropriate software (FACScan, Becton Dickinson, Mountain View, CA, USA). Controls were cells suspended in medium. Mean fluorescent intensity of positively stained cells for both class I and II MHC antigen was also monitored. The relative staining density was represented as log mean channel, measuring the average intensity of staining and expressed as mean fluorescent intensity.

Cytokine assay

Spleen cells, at a concentration of 1×10^6 cells/ml, were set up in twenty-four-well plates in RPMI 1640 medium supplemented with 100 g/l fetal calf serum (FCS) 4 mm-L-glutamine, 25 mm-HEPES, 5×10^{-5} M-2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin and $0 \cdot 25$ mg/ml amphotericin in the presence of concanavalin A (ConA; 20 μg/ml, Sigma, St Louis, MO, USA). After 24 h incubation the supernatant fraction was collected and assayed for cytokines. To determine the concentration of cytokine, sandwich-ELISA was used. Predetermined concentrations of anti-IL-2, γ-IFN, IL-4 and IL-10 antibodies (PharMingen, San Diego, CA, USA) were coated to ELISA and incubated at 4° overnight. After two washes with PBS containing $0 \cdot 5$ ml Tween 20/l, supernatant fraction was added to the plates and incubated at room temperature for 2 h. Biotin-conjugated anti-IL-2, γ-IFN, IL-4 and IL-10 antibodies and horseradish peroxidase-conjugated streptavidin were added subsequently. Absorbance determination was similar to that described previously.

Isolation and stimulation of peritoneal exudate cells

Peritoneal exudate cells were isolated by peritoneal lavage and washed three times with Hank's solution before use. Peritoneal exudate cells were stimulated with 5 μg/ml lipopolysaccharide (LPS) and incubated for 24 h. The supernatant fraction was collected and used for determination of cytokine production. Cytokines such as IL-6 and TNF-α were determined with sandwich-ELISA, and PGE_2 was determined with an EIA kit (ELISA Technologies Inc., KY, USA).

Statistical analysis

The two-sided non-paired Student's t test of the SAS program system was used to analyse the data from this study.

RESULTS
Feed intake and growth

The growth curve and feed intake of mice are shown in Fig. 1. Although feed intake (g/d) was a little lower in the high-fat diet group, the daily energy intakes were similar between mice fed with 50 and 200 g fat/kg diet for the first several months, so was body weight. However, the feed intake and body-weight gain decreased in mice of the high-fat group starting from 8 months old. Decreased feed intake and body weight in mice fed with 200 g fat/kg diet were probably related to the disease course.
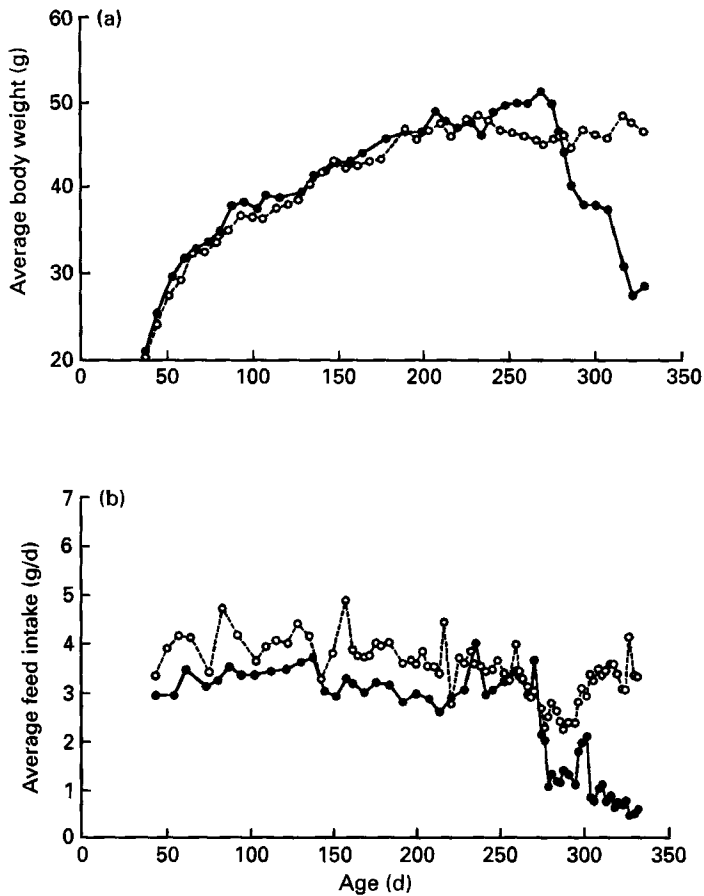
Fig. 1. (a) Growth curve and (b) daily feed intake of mice fed on diets containing 50 (○) or 200 (●) g fat/kg. Results are means of sixteen mice from each group except that the number of mice decreased when mice started dying after 6 months of age.

Anti-DNA antibodies

The isotypes of anti-dsDNA antibodies were studied by ELISA using goat anti-mouse IgG (γ-chain specific)–horseradish peroxidase or goat anti-mouse IgM (μ-chain specific)–horseradish peroxidase as secondary antibodies respectively. The level of IgG and IgM anti-dsDNA antibodies was followed up regularly every month (Fig. 2). Both IgG and IgM anti-dsDNA antibodies increased with age gradually and peaked at 7–8 months. The results suggested higher levels of both IgG and IgM anti-dsDNA antibodies in mice fed with 200 g fat/kg compared with those in mice of the 50 g fat/kg diet group ($P < 0.05$ at 6 months).

Phenotypic analysis and major histocompatibility antigen expression of spleen and peritoneal exudate cells

The results of phenotypic analysis are summarized in Table 2. There were no significant differences in the percentages of Ia$^+$ spleen and peritoneal exudate cells between the two groups. Also examined was the effect of dietary fat on CD5$^+$ B cells: the data showed that B cells with CD5$^+$ surface marker in the peritoneal exudate cells of the 200 g fat/kg diet group were significantly higher in percentage compared with those in the 50 g fat/kg diet

(a)

Absorbance at 415 nm

1.0 — 0.8 — 0.6 — 0.4 — 0.2 — 0.0

1 2 3 4 5 6 7 8 9

(b)

Absorbance at 415 nm

0.4 — 0.3 — 0.2 — 0.1 — 0.0
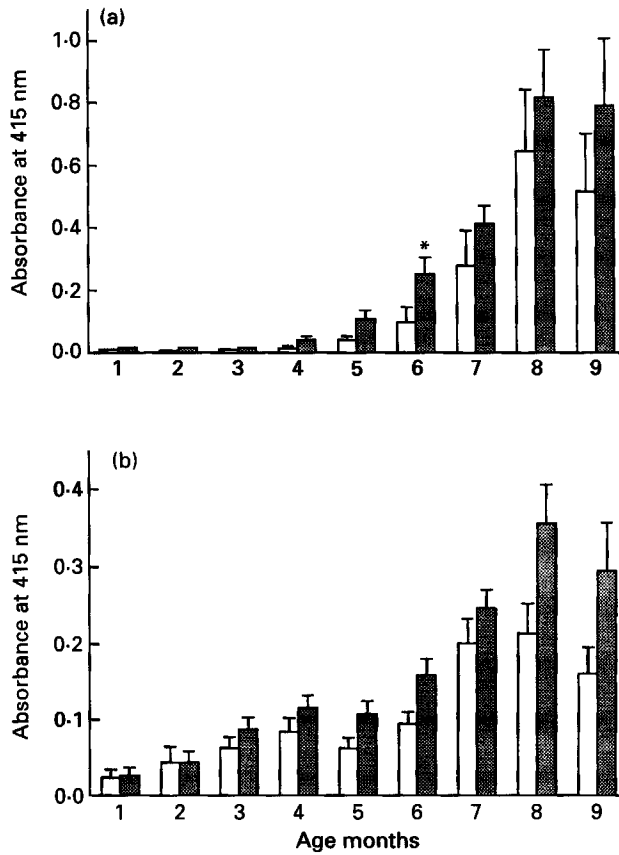
1 2 3 4 5 6 7 8 9

Age months

Fig. 2. (a) Serum anti-double-stranded DNA immunoglobulin G levels and (b) serum anti-double-stranded DNA immunoglobulin M levels of NZB/W female mice fed on diets containing 50 (□) or 200 (▨) g fat/kg. Values are means with their standard errors for samples from sixteen rats. Each serum sample was diluted 400-fold. * Mean value was significantly different from that for the 50 g fat/kg diet group ($P = 0.0162$; Student's t test).

Table 2. *Flow cytometric analysis of Ia antigen expression and immune cell populations in the spleen and peritoneum of NZB/W female mice fed on diets with different contents of fat*

(Mean values with their standard errors for six rats per dietary group)

Dietary group*	Ia+ Mean	SE	IgM+ Mean	SE	CD5+ Mean	SE	CD5+B Mean	SE
Peritoneal cells (%)								
50 g fat/kg	76·5	6·7	37·4	6·4	64·8	7·0	21·4	4·0
200 g fat/kg	82·6	1·6	54·6	5·8	75·1	4·4	37·7	5·2
Student's t test	NS		$P = 0.0766$		NS		$P = 0.0411$	
Spleen cells (%)								
50 g fat/kg	47·5	4·3	38·9	7·7	45·7	3·7	5·6	1·2
200 g fat/kg	46·7	8·9	39·0	10·6	60·8	5·7	10·5	5·1
Student's t test	NS		NS		$P = 0.0545$		NS	

* Mice were fed for 5 months with the low-fat diet containing 50 g fat and 650 g carbohydrate/kg or the high-fat diet containing 200 g fat and 500 g carbohydrate/kg.
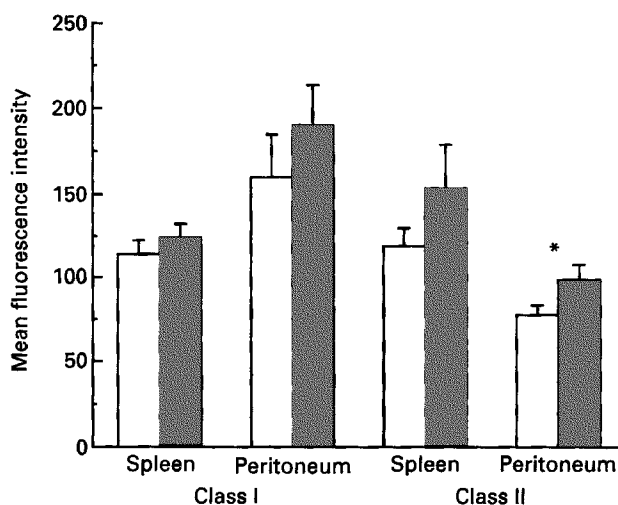
Fig. 3. The mean fluorescence intensity of major histocompatibility (MHC) class I and II on the surface of spleen cells and peritoneal cells isolated from mice fed on diets containing 50 (□) or 200 (▨) g fat/kg (*n* 5 for low-fat diet group; *n* 6 for high-fat diet group). Values are means with their standard errors indicated by vertical bars. The density of MHC class I and II on spleen cells and peritoneal cells of mice fed with the high-fat diet tended to be higher than on those of the low-fat diet group (* $P = 0.0935$, NS, Student's *t* test).

group. In addition, the percentage of peritoneal B cells in the high-fat group tended to be higher ($P = 0.0766$), than that of the low-fat diet group. The percentage of $CD5^+$ splenic cells in the high-fat group tended to be higher ($P = 0.0545$), than that of low-fat diet group. Mean fluorescent intensity of both class I and II MHC antigens on peritoneal exudate cells and spleen cells are shown in Fig. 3. The mean fluorescent intensity of Ia antigens of peritoneal exudate cells from mice of the high-fat-diet group tended to be higher ($P = 0.0935$) than that of the control group.

Proteinuria and life span

Proteinuria in mice fed with the high-fat diet was worse than that in mice fed with 50 g fat/kg diet (Fig. 4a). In addition, life span (Fig. 4b) in mice fed with the high-fat diet (285 (SD 60) d) was shorter than that in mice fed with the low-fat diet (389 (SD 97) d, $P = 0.0439$). All these data suggested increased severity of disease activity in mice of the high-dietary-fat group.

Cytokine production by mitogen-stimulated T cells

Cytokines such as IL-2, γ-IFN, IL-4 and IL-10 produced by mitogen-stimulated T cells were determined, and the results are summarized in Fig. 5. Cytokines such as IL-2 and γ-IFN (Fig. 5a) produced by type 1 T-helper cells were higher in mice fed with 50 g dietary fat/kg, although this was not statistically significant. In contrast, IL-4 and IL-10 (Fig. 5b) produced by type 2 T-helper cells were higher in mice of the high-dietary-fat group than in those of the 50 g dietary fat/kg group.

Cytokines and PGE_2 produced by peritoneal exudate cells

The data shown in Table 3 suggested that IL-6 and TNF-α produced by LPS-stimulated peritoneal exudate cells were significantly higher in mice fed with the high-fat diet ($P < 0.05$). In addition, PGE_2 production by peritoneal exudate cells of mice fed with the high-fat diet was significantly higher than that of mice fed on the low-fat diet.
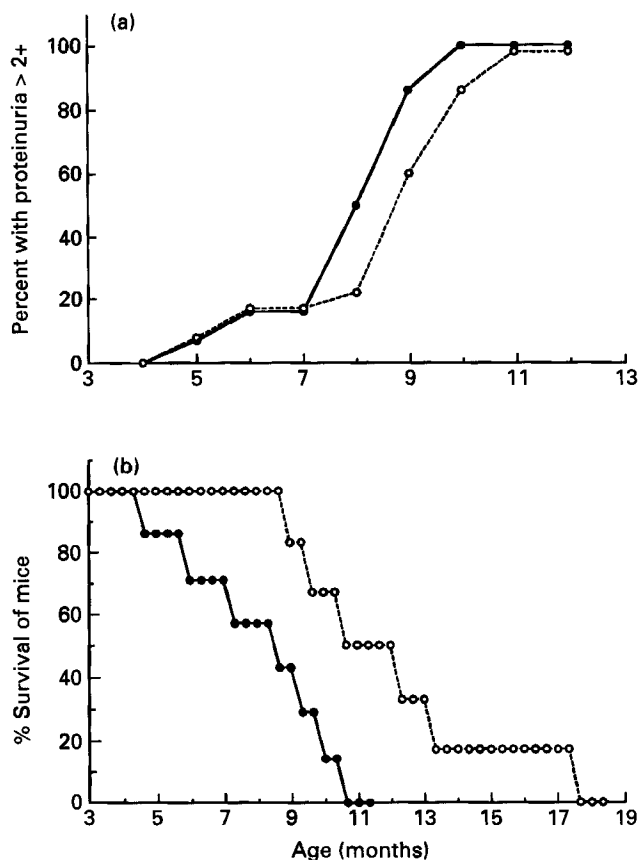
Fig. 4. (a) Proteinuria and (b) life span of NZB/W female mice fed on diets containing 50 (○) or 200 (●) g fat/kg. Life span of mice fed with the high-fat diet (285 (SD 60) d, n 7) was significantly shorter than that of mice fed with the low-fat diet (389 (SD 97) d, n 6; $P = 0.0439$).

DISCUSSION

Change of diet habit in recent years has had an impact on the nutritional status of both healthy and disease-plagued populations. The influence of a high-fat diet on immune regulation has been a special focus of nutritional research in recent years (Maki & Newberne, 1992; Berger *et al.* 1993). Certain diets such as a low-fat diet, fish oil and Zn deprivation have been documented to have some beneficial effects on improvement of the clinical course of the autoimmune diseases in murine lupus (Beach *et al.* 1981; Morrow *et al.* 1985; Robinson *et al.* 1986; Alexander *et al.* 1987; Ogura *et al.* 1989). However, the real mechanisms of the effects of fatty acids on autoimmunity are still not well defined. One study showed that low-energy and low-fat diets can decrease Ly-1 B cell frequency and prolong the life span of several strains of autoimmune-prone mice (Ogura *et al.* 1989). Fish oil, which produces arachidonic acid metabolites differing from the other oils, also reduces autoantibody production (Snyder *et al.* 1982; Santoli & Zurier, 1989).

To elucidate further the effect of dietary fat on the disease course and pathogenesis of autoimmunity, two groups of mice were fed with different amounts of fat. The difference in energy content of these two groups was compensated with carbohydrate in our study. Previous studies have suggested that energy-restriction may play a more important role in the modulation of autoimmunity in NZB/W F1 mice and significantly prolong the life span
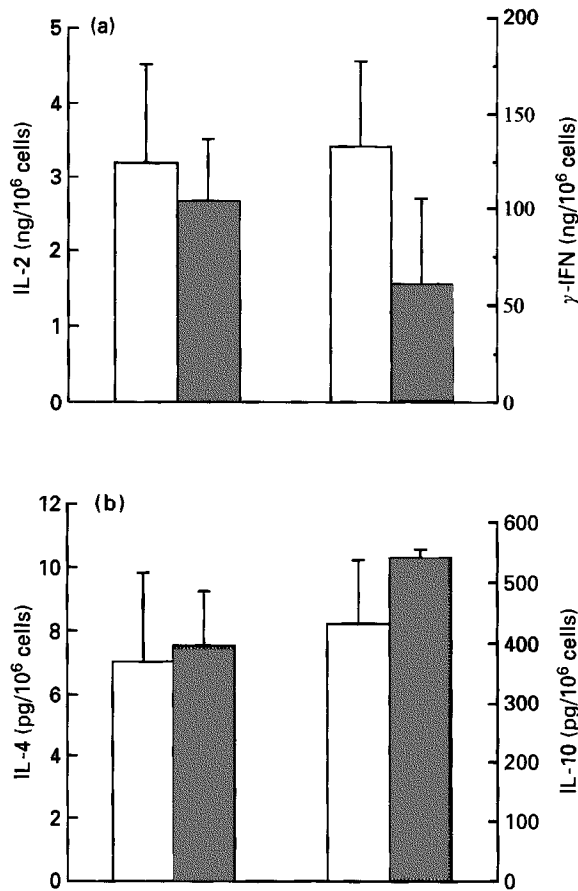
Fig. 5. Cytokines (a) interleukin-2 (IL-2) and γ-interferon (γ-IFN); (b) IL-4 and IL-10 produced by mitogen-stimulated spleen cells of mice fed on diets containing 50 (□) or 200 (▨) g fat/kg (n 5 for low-fat diet group, n 6 for high-fat diet group). Cells were incubated with medium in the presence of 20 μl/ml concanavalin A for 24 h. In general, higher type 2 T-helper cell activity was noted in mice of the high-dietary-fat group. Values are means with their standard errors indicated by vertical bars.

Table 3. *Cytokines and prostaglandin E_2 produced by lipopolysaccharide (LPS)-stimulated peritoneal exudate cells of NZB/W female mice fed on diets with different contents of fat*[*][†]

(Mean values with their standard errors for four rats per dietary group)

Dietary group	IL-6 (ng/10^6 cells)		TNF-α (pg/10^6 cells)		PGE$_2$ (pg/10^6 cells)	
	Mean	SE	Mean	SE	Mean	SE
50 g fat/kg	58·63	18·33	94·91	9·18	448·74	53·38
200 g fat/kg	134·35	24·04	311·66	70·25	903·18	203·09
Student's t test	$P = 0.0232$		$P = 0.001$		$P = 0.0122$	

IL-6, interleukin-6; TNF-α, tumour necrosis factor-α; PGE$_2$, prostaglandin E$_2$.

[*] Mice were fed for 5 months with a low-fat diet containing 50 g fat and 650 g carbohydrate/kg or a high-fat diet containing 200 g fat and 500 g carbohydrate/kg.

[†] Peritoneal exudate cells were incubated with medium only or in the presence of LPS at a final concentration of 5 μg/ml for 24 h. Values are shown as the increase caused by LPS stimulation.

of the mice (Fernandes & Good, 1984; Kubo *et al.* 1984). A low-energy diet, regardless of source of energy, played a more important role in the pathogenesis of the disease than a low-protein diet in murine lupus (Fernandes *et al.* 1976). The lower daily feed intake in the high fat diet group was compensated by the higher energy content of the diet in our experiment (19 320 kJ/kg in high-fat diet *v.* 16 170 kJ/kg in control diet). Therefore, the daily energy intakes were similar between these two groups. In addition, the body-weight gains were very similar between the two groups before the onset of disease. Both immunological and pathological changes noted in mice of the high-fat group were most probably caused by the higher fat intake. The present study showed a higher production of both IgM and IgG anti-dsDNA antibodies in the sera of mice fed with the high-fat diet. In addition, life span and proteinuria in mice of the high-dietary-fat group were worse than those of the control group. More interestingly, increased expression of class I and II antigens was noted on spleen cells and peritoneal cells of mice fed with high dietary fat. This finding is similar to a previous report on non-autoimmune mice (Huang *et al.* 1992), although the real effect of increased MHC molecules on immune effector cells needs to be further investigated. It has been suggested that the effect of Ia antigen on immune function is not only qualitative but also quantitative (Matis *et al.* 1983; Janeway *et al.* 1984). Increased MHC molecules on the surface of immune effector cells might contribute to abnormal expansion of autoreactive cells and production of autoantibodies. Our studies showed increased CD5$^+$ B cells and Ia antigen expression of peritoneal exudate cells in mice fed with high fat. Increased Ia expression might be related to the population of CD5$^+$ B cells which have been thought to be important in the pathogenesis of autoimmune diseases (Farinas *et al.* 1990). However, the relationship between dietary fat and CD5$^+$ B cells still needs to be clarified.

Cytokines such as IL-5 derived from TH$_2$ cells were found to stimulate B cells to produce autoantibodies (Umland *et al.* 1989; Cawley *et al.* 1993). This finding, along with decreased production of IL-2 in the autoimmune strains of mice like MRL.*lpr/lpr*, NZB/NZW F1 (Dauphinee *et al.* 1981), suggests possible dysregulation between TH$_1$ and TH$_2$ cells. The imbalance between these two types of T helper cells may contribute to the disease development in NZB and NZB-derived mice. Recent findings showed that PGE$_2$ and PGE$_1$ could increase intracellular cAMP level and negatively regulate TH$_1$ development (Betz & Fox, 1991; Gold *et al.* 1994). Increased intake of dietary fat could result in increased prostaglandin production and subsequent diminished IL-2 production. In addition, increased TH$_2$ cell activity might accompany increased production of IL-10, which might be important for CD5$^+$ B cell growth (Velupillai & Harn, 1994). This notion is supported by evidence of decreased peritoneal CD5$^+$ B cells after *in vivo* treatment with anti-IL10 antibodies (Ishida *et al.* 1992).

The effect of fat nutrition on macrophage function has been well documented (Maki & Newberne, 1992). In the present study increased production of IL-6, TNF-α and PGE$_2$ by LPS-stimulated peritoneal exudate cells was noted in mice fed with high dietary fat. Other studies also suggest that both essential fatty acids and fish oil play an important role in the functions and cytokine production of macrophages (Hubbard *et al.* 1991; Watanabe *et al.* 1993). Activated macrophages and their products are actively involved in the inflammatory process which is predominant in the late stage of lupus nephritis. One study also suggested that IL-6 produced by macrophages might have an effect on the production of anti-DNA autoantibodies (Alarcon-Riquelme *et al.* 1993). In addition, PGE$_2$ produced by macrophages, potential antigen-presenting cells, might be important for the development of autoreactive T cells and CD5$^+$ B cells.

The present results showed increases in peritoneal CD5$^+$ B cells, Ia antigen expression and cytokines such as IL-6, TNF-α and PGE$_2$ produced by LPS-stimulated peritoneal

exudate cells, in mice fed with 200 g dietary fat/kg. It will be interesting to investigate further the effect of the individual fatty acid intermediates on immune regulation of autoimmunity and explore the feasibility of dietary therapy for autoimmune diseases.

REFERENCES

Alarcon-Riquelme, M. E., Moller, G. & Fernandez, C. (1993). Macrophage depletion decreases IgG anti-DNA in cultures from (NZB × NZW) F1 spleen cells by eliminating the main source of IL-6. *Clinical Experimental Immunology* **91**, 220–225.

Alexander, N. J., Smythe, N. L. & Jokinen, M. P. (1987). The type of dietary fat affects the severity of autoimmune disease in NZB/NZW mice. *American Journal of Pathology* **127**, 106–121.

American Institute of Nutrition (1977). Report of the American Institute of Nutrition *ad hoc* committee on standards for nutritional studies. *Journal of Nutrition* **107**, 1340–1348.

Beach, R. S., Gershwin, M. E. & Hurley, L. S. (1981). Nutritional factors and autoimmunity. I. Immunopathology of zinc deprivation in New Zealand mice. *Journal of Immunology* **126**, 1999–2006.

Berger, A., German, J. B., Chiang, B.-L., Ansari, A. A., Keen, C. L., Fletcher, M. P. & Gershwin, M. E. (1993). Influence of feeding unsaturated fats on growth and immune status of mice. *Journal of Nutrition* **123**, 225–233.

Betz, M. & Fox, B. S. (1991). Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *Journal of Immunology* **146**, 108–113.

Cawley, D., Chiang, B.-L., Ansari, A. & Gershwin, M. E. (1991). Ionic binding characteristics of monoclonal autoantibodies to DNA from NZB.H-2^{bm12} mice. *Autoimmunity* **9**, 301–309.

Cawley, D., Chiang, B.-L., Naiki, M., Ansari, A. A. & Gershwin, M. E. (1993). Comparison of the requirements for cognate T cell help for IgG anti-dsDNA antibody production *in vitro*: T helper-derived lymphokines replace T cell help for B cells from NZB.H-2^{bm12} but not from B6.H-2^{bm12} mice. *Journal of Immunology* **150**, 2467–2477.

Dauphinee, M. J., Kipper, S. B., Wofsey, D. & Talal, N. (1981). Interleukin-2 deficiency is a common feature of autoimmune mice. *Journal of Immunology* **127**, 2483–2487.

Farinas, M. C., Stall, A. M., Solovera, J. J., Tarlinton, D. M., Herzenberg, L. A. & Strober, S. (1990). Ly-1 B cells and disease activity in (NZB × NZW) F1 mice. *Arthritis Rheumatism* **33**, 553–562.

Fernandes, G. & Good, R. A. (1984). Inhibition by restricted-calorie diet of lymphoproliferative disease and renal damage in MRL/lpr mice. *Proceedings of the National Academy of Sciences USA* **81**, 6144–6148.

Fernandes, G., Yunis, E. J. & Good, R. A. (1976). Influence of protein restriction on immune functions in NZB mice. *Journal of Immunology* **116**, 782–790.

Gold, K. N., Weyand, C. M. & Goronzy, J. J. (1994). Modulation of helper T cell function by prostaglandins. *Arthritis Rheumatism* **37**, 925–933.

Huang, S.-C., Misfeldt, M. L. & Fritsche, K. L. (1992). Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. *Journal of Nutrition* **122**, 1219–1231.

Hubbard, N. E., Somers, S. D. & Erickson, K. L. (1991). Effect of dietary fish oil on development and selected functions of murine inflammatory macrophages. *Journal of Leukocyte Biology* **49**, 592–598.

Ishida, H., Hastings, R., Kearney, J. & Howard, M. (1992). Continuous anti-IL-10 antibody administration depletes mice of Ly-1 B cells but not conventional B cells. *Journal of Experimental Medicine* **175**, 671–682.

Jacob, C. O. & McDevitt, H. O. (1988). Tumor necrosis factor-α in murine autoimmue 'lupus' nephritis. *Nature* **331**, 356–358.

Janeway, C. A. Jr, Bottomly, K., Babich, J., Conrad, P., Conzen, S., Jones, B., Kaye, J., Katz, M., McVay, L., Murphy, D. B. & Tite, J. (1984). Quantitative variation in Ia antigen expression plays a central role in immune regulation. *Immunology Today* **5**, 99–104.

Kubo, C., Johnson, B. C., Day, N. K. & Good, R. (1984). Calorie source, calorie restriction, immunity and aging of (NZB/W) F1 mice. *Journal of Nutrition* **114**, 1884–1899.

Maki, P. A. & Newberne, P. M. (1992). Dietary lipid and immune function. *Journal of Nutrition* **122**, 610–614.

Matis, L. A., Glimcher, L. H., Paul, W. E. & Schwartz, R. H. (1983). Magnitude of response of histocompatibility-restricted T cell clones is a function of the product of concentrations of antigen and Ia molecules. *Proceedings of the National Academy of Sciences USA* **80**, 6019–6023.

Morrow, W. J. W., Ohashi, Y., Hall, J., Pribnow, J., Hirose, S., Shirai, T. & Levy, J. A. (1985). Dietary fat and immune function in (NZB × NZW) F1 mice. *Journal of Immunology* **135**, 3857–3863.

Ogura, M., Ogura, H., Ikehara, S. & Good, R. A. (1989). Influence of dietary energy restriction on the numbers and proportions of Ly-1 + B lymphocytes in autoimmunity-prone mice. *Proceedings of the National Academy of Sciences USA* **86**, 4225–4229.

Robinson, D. R., Prickett, J. D., Makoul, G. T., Steinberg, A. D. & Colvin, R. B. (1986). Dietary fish oil reduces progression of established renal disease in (NZB × NZW) F1 mice and delays renal disease in BXSB and MRL/1 strains. *Arthritis Rheumatism* **29**, 539–546.

Santoli, D. & Zurier, R. B. (1989). Prostaglandin E precursor fatty acid inhibit human IL-2 production by a prostaglandin E-independent mechanism. *Journal of Immunology* **143**, 1303–1309.

Secrist, H., Dekruyff, R. H. & Umetsu, D. T. (1995). Interleukin-4 production by CD4 + T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *Journal of Experimental Medicine* **181**, 1081–1089.

Snyder, D. S., Beller, D. I. & Unanue, E. R. (1982). Prostaglandins modulate macrophage Ia expression. *Nature* **299**, 163–165.

Steinberg, A. D., Klinman, D. M., Kastner, D. L., Seldin, M. F., Gause, W. C., Scribner, C. L., Britted, J. L., Siegel, J. N. & Mountz, J. D. (1987). Genetic and molecular genetic studies of murine and human lupus. *Journal of Rheumatology* **14**, 166–176.

Takeda, K. & Dennert, G. (1993). The development of autoimmunity in C57BL/6 lpr mice correlates with the disappearance of natural killer type 1-positive cells: evidence for their suppressive action on bone marrow stem cell proliferation, B cell immunoglobulin secretion and autoimmune symptoms. *Journal of Experimental Medicine* **177**, 155–164.

Theofilopoulos, A. N. & Dixon, F. J. (1981). Etiopathogenesis of murine SLE. *Immunology Review* **55**, 179–216.

Umland, S. P., Go, N. F., Cupp, J. E. & Howard, M. (1989). Responses of B cells from autoimmune mice to IL-5. *Journal of Immunology* **142**, 1528–1535.

Velupillai, P. & Harn, D. A. (1994). Oligosaccharide-specific induction of interleukin 10 production by B220 + cells from schistosome-infected mice: a mechanism for regulation of CD4+ T-cell subsets. *Proceedings of the National Academy of Sciences USA* **91**, 18–22.

Watanabe, S., Onozaki, K., Yamamoto, S. & Okuyama, H. (1993). Regulation of dietary essential fatty acid balance of tumor necrosis factor production in mouse macrophages. *Journal of Leukocyte Biology* **53**, 151–156.

Yoshida, S., Castles, J. J. & Gershwin, M. E. (1990). The pathogenesis of autoimmunity in New Zealand mice. *Seminars in Arthritis Rheumatism* **19**, 224–242.

Printed in Great Britain