Dietary fat influences Ia antigen expression, cytokines and prostaglandin E₂ production of immune cells in autoimmune-prone NZB × NZW F₁ mice

BY BI-FONG LIN*, CHAO-CHI HUANG¹, BOR-LUEN CHIANG² AND SU-JEN JENG³

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

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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 E₂ (PGE₂) 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 PGE₂ 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-2bm¹, 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

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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 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-\(\alpha\) (TNF-\(\alpha\)) 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 \(\times\) 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 \(\times\) 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° \(\pm\) 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-Ad (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 (\(\gamma\)-chain specific, Jackson Inc., PA, USA) and anti-mouse IgM (\(\mu\)-chain specific, Jackson Inc.) were used as secondary antibodies.
Table 1. Composition of the experimental diets (g/kg diet)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Low-fat diet</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat*</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>Casein†</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Methionine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>325</td>
<td>250</td>
</tr>
<tr>
<td>Maize starch</td>
<td>325</td>
<td>250</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Choline</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>AIN-76 mineral mixture‡</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN-76 vitamin mixture‡</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Energy (kJ/kg)</td>
<td>16170</td>
<td>19320</td>
</tr>
</tbody>
</table>

* 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. Bettemmaier, & Söke, Elwangen-Holzmühle, Germany.


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°C 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°C, 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 CO₂ asphyxiation. Spleens were removed and single cells were isolated by lysing erythrocytes with NH₄Cl-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 (2·5–5 × 10⁶ cells) were suspended in 0·1 ml PBS with 1 g/l sodium azide and incubated at 4°C for 30 min with a predetermined optimal concentration of appropriate FITC- or PE-conjugated monoclonal antibodies. The cells were washed and resuspended in 0·5 ml PBS with 1 g/l sodium azide and subjected to FMF analysis. A total of 10000 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 × 10⁶ 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 0·25 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°C overnight. After two washes with PBS containing 0·5 ml Tween 20/1, 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 PGE₂ 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 intake 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.
**Anti-DNA antibodies**

The isotypes of anti-dsDNA antibodies were studied by ELISA using goat anti-mouse IgG (\(\gamma\)-chain specific)–horseradish peroxidase or goat anti-mouse IgM (\(\mu\)-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 group.
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 (C) or 200 (D) 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)

<table>
<thead>
<tr>
<th>Dietary group*</th>
<th>Ia+ Mean ± SE</th>
<th>IgM+ Mean ± SE</th>
<th>CD5+ Mean ± SE</th>
<th>CD5+B Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal cells (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 g fat/kg</td>
<td>76.5 ± 6.1</td>
<td>31.4 ± 6.4</td>
<td>45.8 ± 7.0</td>
<td>21.4 ± 4.0</td>
</tr>
<tr>
<td>200 g fat/kg</td>
<td>82.6 ± 1.6</td>
<td>54.6 ± 5.8</td>
<td>75.1 ± 4.4</td>
<td>37.7 ± 5.2</td>
</tr>
<tr>
<td>Student’s t test</td>
<td>NS</td>
<td>*P = 0.0766</td>
<td>NS</td>
<td>*P = 0.0411</td>
</tr>
<tr>
<td>Spleen cells (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 g fat/kg</td>
<td>47.5 ± 4.3</td>
<td>38.9 ± 7.7</td>
<td>45.7 ± 3.7</td>
<td>5.6 ± 1.2</td>
</tr>
<tr>
<td>200 g fat/kg</td>
<td>46.7 ± 8.9</td>
<td>39.0 ± 10.6</td>
<td>60.8 ± 5.7</td>
<td>10.5 ± 5.1</td>
</tr>
<tr>
<td>Student’s t test</td>
<td>NS</td>
<td>NS</td>
<td>*P = 0.0545</td>
<td>NS</td>
</tr>
</tbody>
</table>

* 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.
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 class I MHC antigens on spleen cells and peritoneal cells of mice fed with the high-fat diet tended to be higher than the low-fat diet group (*$P = 0.0935$, NS, Student's $t$ test). 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 those of the low-fat diet group (*$P = 0.0935$, NS, Student's $t$ test). 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 class I MHC antigens on spleen cells and peritoneal cells of mice fed with the high-fat diet 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, $\gamma$-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 $\gamma$-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-\(\alpha\) 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.
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 E₂ 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)

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>IL-6 (ng/10⁶ cells)</th>
<th>TNF-α (pg/10⁶ cells)</th>
<th>PGE₂ (pg/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>50 g fat/kg</td>
<td>58.63±18.33</td>
<td>94.91±9.18</td>
<td>448.74±53.38</td>
</tr>
<tr>
<td>200 g fat/kg</td>
<td>134.35±24.04</td>
<td>311.66±70.25</td>
<td>903.18±203.09</td>
</tr>
<tr>
<td>Student's t test</td>
<td>P = 0.0232</td>
<td>P = 0.001</td>
<td>P = 0.0122</td>
</tr>
</tbody>
</table>

IL-6, interleukin-6; TNF-α, tumour necrosis factor-α; PGE₂, prostaglandin E₂.

* 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 TH2 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 TH2 and TH1 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 PGE2 and PGE1 could increase intracellular cAMP level and negatively regulate TH1 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 TH2 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 PGE2 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, PGE2 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 PGE2 produced by LPS-stimulated peritoneal
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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 and explore the feasibility of dietary therapy for autoimmune diseases.

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