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Prostaglandin E, (PGE,) and protein kinase C (PKC) affect proliferation of T cell and its subset which lead classical B cell isotype switching to immunoglobulin (Ig) E. In order to examine a mechanism(s) underlying different levels of circulating IgE in response to dietary saturated and polyunsaturated fats, production of PGE, and activity of PKC were measured in splenocytes of ovalbumin (OVA)-sensitized rats. Safflower oil (SO) diet-fed rats, compared to coconut oil diet-fed rats, elevated serum levels of OVA-specific IgE and IgG2a and lowered the IgG1. Activity of PKC and concanavalin (Con) A-induced T-lymphoproliferation were lowered in splenocytes from SO diet-fed rats. Production of PGE, was elevated splenocytes from SO diet-fed rats; however, this difference was disappeared when the cells were cultured with Con A. SO diet-fed rats increased proportion of linoleic acid but decreased the arachidonic acid in splenocyte phospholipids. We conclude that dietary polyunsaturated fatty acid is an important modulator of PGE, production and PKC activity in splenic immune cells and it may play a role in the circulatory level of IgE.

INTRODUCTION

Immediate type of hyperreactions mediated by food-antigen specific immunoglobulin (Ig) E take part in inflammatory symptoms among infants and younger children (Lessof, 1991; Metcalfe, 1991). Therefore, it should be primarily important to know the effect of dietary fats on circulatory levels of specific IgE. The prerequisites for class switch are largely known. B cells undergo class switch to IgE when they are activated and simultaneously interact with T-helper cells (Th2 cells) and interleukins produced by Th2 cells. In contrast, Th1 cells suppress the switch (Sutton and Gould, 1993). In vivo this interaction normally take places in the germinal center. Our previous study showed that rats fed safflower oil (SO) rich in linoleic acid, compared to rats fed coconut oil (CO) rich in saturated fatty acids, tended to increase the circulatory reaginic activity in antigen-primed Brown Norway (Ju et al., 1996).

IgE synthesis, dietary fat appears to mediate the effect through altering membrane structure, prostaglandin (PG), cytokines and protein kinase C (PKC) in T cells (Meydani, 1990; Peck, 1994). In this context Brown Norway rats used in our previous study (Ju et al., 1995 and 1996) may not be an appropriate animal model to examine a role of lymphocytes, in particular T cells, since this strain is lacking a population of suppressor T cells
(Groen et al., 1993; Peszkowski et al., 1994), hence showing a negligible response of proliferation of splenocytes and lymph node cells to concanavalin (Con) A (unpublished observation).

In the present study, we immunized Sprague-Dawley rats with ovalbumin (OVA), raised on saturated fatty acid- and linoleic acid-rich diet and measured mitogenic response to Con A, production of prostaglandin (PG) E<sub>2</sub> and activity of PKC in splenocytes.

MATERIALS AND METHODS

Animals and diet

Six wk old Sprague-Dawley rats, weaned to a diet containing bovine milk (CRF-1, Oriental Yeast Co., Tokyo, Japan), were obtained from Seiwa Experimental Animal Co. (Fukuoka, Japan). They were maintained on our animal facility in a controlled temperature at 23±1°C and acclimatized for 5 days on a commercial non-purified diet (NMF, Oriental Yeast Co.). Both commercial diets are free of OVA. Rats were then maintained on AIN-93G based diets (Reeves et al., 1993) containing soybean protein (Fujipro R, Fuji Oil Co., Osaka, Japan) as a source of dietary protein (20%) and coconut oil (CO) and safflower oil (SO) as a source of dietary fat (10%), as described previously (Ju et al., 1996). Rats were initially sensitized with an intraperitoneal injection of 500 μg of OVA (Sigma Chemicals Co., St. Louis, MO, USA) adsorbed onto 0.5 ml of 3% (w/v) Al(OH)<sub>3</sub> (Wako Pure Chemicals Co., Osaka, Japan), and sensitized again on days 7. On days 21, rats were killed by withdrawing blood from aorta under diethyl ether anesthesia. Spleen was removed aseptically. In a separate set of experiment, on days 21, rats received a gavage feed containing 100 mg OVA in 2 ml saline and killed 3 hr later as described previously (Ju et al., 1996). In order to measure a mucosal mast cell protease (rat chymase II: RChy II), blood was obtained from tail vein and aorta before and after the gavage feed, respectively. All aspects of the experiment were approved by the Kyushu University Animal Policy and Welfare Committee.

Preparation of splenocytes

Spleen was placed in 10 ml of PBS and disrupted with micro slide glasses as described previously (Alayne et al., 1991). Spleen cell suspensions were passed through nylon mesh sheet, and red blood cells were lysed in a modified isotonic NH₄Cl solution (0.15 M NH₄Cl, 10 mM KHCO₃, and 9.86 mM EDTA-2Na, pH 7.4) and then centrifuged. The splenocytes were resuspended in PBS. The number of viable cells was determined by making a 1:100 dilution of the cell suspension in 0.2% trypan blue and counted by use of a hemocytometer.

Determination of PKC activity

Freshly prepared splenocytes (2×10⁸ cells) were immediately subjected to homogenization in 2 ml of cold homogenizing buffer (10 mM HEPES, pH 7.4 containing 0.25 mM sucrose, 2 mM EDTA, 5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM β-mercaptoethanol and 10 μg/ml leupeptin), using a teflon homogenizer, as described previously (Parant and Vial, 1990; Imaizumi et al., 1995). The homogenate was cen-
trifuged at 105,000 x g for 60 min with using Beckman Ultracentrifuge (Type 50 Ti, Beckman, Tokyo, Japan) to separate the particulate fraction from the cytosol fraction (Imaizumi et al., 1995). The particulate fraction was extracted with the homogenizing buffer containing 0.1% Nonidet P-40 (Nacalai Tesque, Inc.), the resulting solution passed through DE-52 column (0.5 x 4 cm, Whatman, Maidstone, England), and the PKC activity determined by its H1 histone kinase activity in the presence of calcium, 1-oleoyl-2-acetylglycerol (Nacalai Tesque Inc.) and phosphatidylserine (Doosan Serdary Research Lab., Canada) (Parant and Vial, 1990; Imaizumi et al., 1995). Protein was determined by Bradford method according to the manufacturer (Bio-Rad Laboratory Inc., CA, USA.). Results were expressed as 1 unit of [32P] per mg of protein.

**Lymphocyte proliferation with Con A**

For lymphocyte proliferation assays, splenocytes were cultured in 96-well microtiter plates (Falcon, Nippon Becton Dickinson & Co., Tokyo, Japan) at a concentration of 2 x 10[^6^] cells/ml of culture medium. Each well contained 200 μl of the cell-containing medium. The culture medium consisted of RPMI-1640 (Nissui Co., Tokyo, Japan) containing 5 mg/ml 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), 1.93 mg/ml NaHCO3, penicillin G (100 U/ml, Meiji Seika Co., Tokyo, Japan), streptomycin sulfate (100 μg/ml, Meiji Seika Co.), 0.29 mg/ml L-glutamine and 10% heat inactivated fetal bovine serum (Rehatuin, Intergen Co., NY, USA) with Con A (Sigma Chemicals Co.). In the preliminary experiment, 2.5 μg/ml of Con A was optimum to induce a maximum lymphocyte proliferation. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO2 and 95% air for 72 hr. Prior to termination of the incubation, the cells were incubated for 6 hr with 0.5 μCi of methyl-[3H] thymidine (740 GBq/mmol, New England Nuclear, Boston, MA, USA). The incorporation of the radioactivity into DNA was estimated by disrupting the cells with 10% trichloroacetic acid (Flower et al., 1993). Radioactive DNA was solubilized in tissue solubilizer (NCS, Amersham Japan, Tokyo, Japan) and the radioactivity was counted in Aquasol 2 (Packard, Tokyo, Japan) with using a liquid scintillation counter (Tri-curb 2250, Packard). Results were expressed as mean net dpm (stimulated minus control) of triplicate-stimulated culture.

**Secretion of PGE2**

Splenocytes were prepared as described above and incubated for 48 hr with or without addition of Con A. The supernatants from each culture were centrifuged to eliminate remaining cells and then stored at -40°C. PGE2 content in the supernatants were determined by PGE2 enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA).

**Determination of reaginic antibody, IgG subclass, RChy II and fatty acid**

Reaginic antibody was assessed by passive cutaneous anaphylaxis assay (PCA) as described previously (Ju et al., 1996; Ovary, 1965). Briefly, recipient rats were injected intradermally with 0.1 ml of the appropriately diluted test sera. Forty-eight hr later, each rat was injected intravenously with 1 ml dye-antigen saline solution containing OVA (1 mg/ml) and Evans blue (0.45%) (Nacalai Tesque, Inc.). The reaction was estimated by measuring the diameter of the blue spot on the inner surface of the skin.

Concentration of serum IgG, IgG1 and IgG2a specific to OVA was determined by
enzyme-linked immunosorbent assay (ELISA) method as described previously (Ju et al., 1995 and 1996). Quantitation of RChy II in the serum was performed by ELISA kit (Moredum, Institute, Edinburgh, UK) as described previously (Ju et al., 1996). Total lipids were extracted from the serum and splenocytes by the method of Folch et al. (1957). Total phospholipid fraction was separated by thin-layer chromatography and their fatty acid methyl esters were determined by gas liquid chromatography on a SILAR 10C column (Chromato TEC, Osaka, Japan) as previously described (Ikeda et al., 1989).

**Statistical analyses**

Differences between two groups were assessed by Student’s t-test. Duncan’s multiple range test was used for comparison of more than two group (Duncan, 1955). In all cases, they were considered as significant when the calculated p value was less than 0.05.

**RESULTS**

Food intake and body weight gain were not influenced by the type of dietary fats (data not shown).

**Concentration of serum reaginic antibody and IgG subclass**

As shown in Table I, reaginic antibody (IgE) was higher in SO group than in CO group. Heat-treatment at 56 °C for 30 min of these antisera destroyed the reaginic activity. Serum total IgG specific to OVA was not influenced by the dietary fats (data not shown). However, the level of IgGl was lower in SO group than in CO group, whereas IgG2a was higher in SO group.

| Table 1. Effect of Dietary Fats on Serum Reaginic Antibody and IgG Subclass. |
|-----------------|-----------------|-----------------|
|                 | CO              | SO              |
| Reaginic Antibody (mm) | 7.90 ± 0.65      | 11.5 ± 1.2*     |
| IgGl (μg/ml)       | 3.61 ± 1.11      | 0.986 ± 0.310*  |
| IgG2a (μg/ml)      | 42.4 ± 12.9      | 77.7 ± 4.9*     |

*1 Values are means ± SE (n=5).
*2 Significantly different from CO at p<0.05.
CO, coconut oil; SO, safflower oil.

**Activity of PKC, Lymphoproliferation and PGE2 Production in splenocytes**

As shown in Table II, activity of PKC in the membrane fractions freshly prepared from the splenocytes was lower in SO group than in CO group.

Lymphocyte proliferation induced by Con A for 72 hr was lower in SO group than in CO group.

Accumulation of PGE2 in the splenocyte medium, which was incubated without supplementation of Con A for 48 hr, was higher in SO group than in CO group. However,
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Table 2. Effect of Dietary Fats on Activity of PKC, Lymphoproliferation and PGE₂ Production in Splenocytes'.

<table>
<thead>
<tr>
<th></th>
<th>CO</th>
<th>SO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity of PKC (U/mg protein)</td>
<td>308 ± 30</td>
<td>227 ± 13(^*)</td>
</tr>
<tr>
<td>Incorporation of ([\text{H}]) Thymidine (x10(^3) dpm/well)</td>
<td>7.03 ± 0.88</td>
<td>4.28 ± 1.44(^*)</td>
</tr>
<tr>
<td>Splenocyte PGE₂ (pg/ml medium)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con A (-)</td>
<td>197 ± 8</td>
<td>305 ± 17(^*)</td>
</tr>
<tr>
<td>Con A (+)</td>
<td>197 ± 15</td>
<td>208 ± 30(^*)</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SE (n=5).
\(^*\) Significantly different from CO at p<0.05.
\(^\dagger\) Significantly different from Con A (-) at p<0.05.

CO, coconut oil; SO, safflower oil.

no difference was observed between the group when incubated in the presence of Con A. Con A in the medium lowered the PGE₂ production in the lymphocytes from SO-fed rats, but not from CO-fed rats. In a preliminary experiment with rats fed commercial non-purified diet (NMF), Con A-containing medium, compared to Con A-free medium, lowered PGE₂ production of splenocytes (117 ± 2.3 vs 232 ± 36.9, p<0.05).

Fatty acid composition of phospholipids in serum and splenocytes

Table III shows the effect of dietary fat on the fatty acid composition of phospholipids

Table 3. Effect of Dietary Fats on Fatty Acid Composition of Serum and Splenocytes Phospholipids'.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Serum</th>
<th>Splenocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO</td>
<td>SO</td>
</tr>
<tr>
<td>16:0</td>
<td>24.5</td>
<td>22.4</td>
</tr>
<tr>
<td>18:0</td>
<td>22.1</td>
<td>20.0</td>
</tr>
<tr>
<td>18:1</td>
<td>8.5</td>
<td>9.5</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>23.1</td>
<td>25.2</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>3.6</td>
<td>0.7*</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>13.1</td>
<td>18.8(^*)</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>0.2</td>
<td>0.4*</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>0.9</td>
<td>0.6*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.3</td>
<td>0.1*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>3.3</td>
<td>1.7*</td>
</tr>
</tbody>
</table>

\(^1\) Values are means of five rats per group.
\(^*\) Significant difference from CO at p<0.05.

CO, coconut oil; SO, safflower oil.
in the serum and splenocytes. In the serum, proportions of linoleic, arachidonic and docosatetraenoic acid were higher in SO group than in CO group, whereas the proportions of eicosatrienoic, docosapentaenoic (n-6, n-3) and docosahexaenoic acid were lower in SO group. In the splenocytes, the proportion of linoleic acid was higher in SO group than in CO group, whereas arachidonic acid was lowered in SO group. Decreased proportions of oleic, linolenic, docosapentaenoic (n-6, n-3) and docosahexaenoic acid were accompanied in SO group.

**Concentration of serum RChy II**

As shown in Figure, serum level of RChy II prior to OVA challenge was similar between the groups; however, challenging orally OVA resulted in elevation of the RChy II from both dietary groups and the extent was greater in CO group than in SO group.

**DISCUSSION**

In the present study as well as previous study with Brown Norway rats (Ju et al., 1996), dietary SO, compared to CO, consistently elevated the circulatory level of antigen-
specific IgE. In addition, dietary fats differently influenced the level of antigen-specific IgG2a and IgG1. Although these Ig levels are influenced by clearance of these Ig, factors influencing synthesis of Ig, particularly IgE, were examined in the present study.

Present studies showed that dietary SO, compared to CO, lowered Con A-dependent T-lymphoproliferation. These results are coincided with the previous results by others, who showed that diets supplemented with safflower oil or corn oil resulted in suppression of lymphoblastogenesis (Meydani et al., 1985; Newberne, 1981; Locniskar et al., 1983). Reduction of Con A-dependent T cell proliferation in SO group appeared to be partly explained by the altered activity of PKC in the splenocytes. In fact, phorbol 12-myristated 13-acetate-treated T cells accelerated T cell proliferation (Alexander and Cantrell, 1989). In addition, Cho and Ziboh (1995) showed that essential fatty acid deficiency resulted in an elevated epidermal hyperproliferation and increased expression and activity of epidermal PKC-α and β-isozymes in guinea pigs, whereas these elevations were lowered when animals were refed SO for 2 wk. Therefore, dietary fats appear to differentially regulate PKC activity, and hence influencing proliferation of tissue or cell.

Th1 cell-derived interferon (IFN)-γ suppresses classical B cell isotype switching to IgE (Pene et al., 1988). Although it was not determined in the present study whether suppression of Con A dependent proliferation of specific T cell from SO diet-fed rats was due to Th1 cells or other T cell subset (Levy et al., 1982), it is shown that PGE2 can act as an selective inhibitor for Th1 cells, but not for Th2 cells (Betz and Fox, 1991). Therefore, increased production of PGE2 derived from SO diet-fed rats may be involved in lowered proliferation of Th1 cells, hence enhancing switch of Ig from IgM to IgE (Isakson et al., 1982; Coffman and Carty, 1986).

PGE2 is a major product of arachidonic acid metabolism, and is derived from macrophage and non-macrophage in the splenocytes. Proportion of arachidonic acid in the splenocyte-phospholipid was rather lower in SO diet-fed rats than in CO diet-fed rats, although it was reversed in the serum counterpart. Therefore, increased production of PGE2 in splenocytes from SO diet-fed rats could not be simply attributed to the proportion of arachidonic acid in the splenocytes. Although we did not measure the fatty acid composition of splenic macrophage which is a minor population in splenocytes, it remains a possibility that dietary fats differently influenced the arachidonic acid proportion in the macrophage.

In the present experiment, addition of Con A to incubation medium resulted in lowered production of PGE2 in splenocytes from SO diet-fed rats, but not from CO diet-fed rats. This might result from altered signal transduction at cell surface from Con A. Therefore, the biophysical characteristics of splenocyte-plasma membrane may be primarily responsible for the presently observed effects on T cell mitogenesis, PKC activity and eicosanoid production. In fact, Peck et al. (1995) showed that anisotropy of splenic mononuclear cells was lowered in mice fed safflower or fish oil diets than in those fed saturated fatty acid- or monounsaturated fatty acid-rich diet.

In the present study, we confirmed our previous study (Ju et al., 1996) that SO diet-fed rats, compared to CO diet-fed rats, lowered the level of RChyII, an indicator of degranulation of mucosal mast cells, although the circulatory level of IgE was lower in CO-fed rats. We have no direct evidence to explain why SO diet resulted in decreased release of RChyII from the mucosal mast cells. It is warrant for further study how dietary
SO regulates IgE-mediated mucosal mast cell degranulation.

In summary, present study showed that dietary polyunsaturated fatty acid is an important modulator of PGE₂ production and PKC activity in splenic immune cells and it may play a role in the circulatory level of IgE.

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REFERENCES


Dietary Fats and Immune Response

Locniskar, M., K. M. Nauss and P. M. Newberne 1983 The effect of quality and quantity of dietary fat on
the immune system. *J Nutr., 113: *951-961
Meydani, S. N., R. J. Nicolosi and K. C. Hayes 1985 Effect of long-term feeding on corn oil or coconut oil
5:* 993-1002
Newberne, P. M. 1981 Dietary fat, immunological response, and cancer in rats. *Cancer Res., 41:* 3783-
3785
Ovary, Z. 1965 PCA reaction and its elicitation by specific immunoglobulin species and fragments. *Fed.
Proc., 24: *94-97
Parant, M. R. and H. J. Vial 1990 Rapid and serial determination of protein kinase C activity and of the
associate [*H]PDBu binding using a 96-well microtiter plate and a cell harvester. *Anal. Biochem.,
184:* 283-290
Peck, M. D. 1994 Interactions of lipids with immune function II: experimental and clinical studies of
Peck, M. D., Li, Z., Han, T. and Pressman, B. C. 1995 Diets high in polyunsaturated oils decrease
lymphocyte membrane anisotropy and B-cell mitogenesis. *Nutrition, 11:* 755-757
and J. De Vries 1988 IgE production by normal human lymphocytes is induced by interleukin 4 and
suppressed by interferons α and γ and prostaglandin E,. *Proc. Nat. Acad. Sci. USA, 85:* 6880-6884
Peszkowski, M. J., G. Warfvinge and Åke Larsson 1994 Allergic and irritant contact responses to DNFB in
BN and LEW rat strains with different T1/T2 profiles. *Acta Derm. Venereol., 74:* 371-374
Reeves, P. G., F. H. Nielsen and G. C. Fahey 1993 AIN-93 purified diets for laboratory rodents: final report
of the american institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A
rodent diet. *J Nutr., 123:* 1939-1951