Dietary safflower oil, compared to coconut oil, differently affects splenocyte functions in ovalbumin-sensitized rats, leading to elevation of the circulatory IgE

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Dietary safflower oil, compared to coconut oil, differently affects splenocyte functions in ovalbumin-sensitized rats, leading to elevation of the circulatory IgE

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Prostaglandin E_2 (PGE_2) 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_2 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, Thl 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_2 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 23il ˚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) $Al(OH)_3$ (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 NH_4Cl solution (0.15 M NH_4Cl, 10 mM KHCO, 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 (2×10^8 cells) were immediately subjected to homogenization in 2 ml of cold homogenizing buffer (10 mM HEPES, pH 7.4 containing 0.25 mM sucrose, 2mM EDTA, 5mM 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 105,000x g for 60min 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 [^{32}P] 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 2x 10^6 cells/ml of culture medium. Each well contained 200μl 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 $NaHCO_3$, penicillin G (100 U/ml, Meiji Seika Co., Tokyo, Japan), streptomycin sulfate (100μg/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, 2.5μg/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% CO, and 95% air for 72 hr. Prior to termination of the incubation, the cells were incubated for 6 hr with 0.5μCi of methyl-[^{3}H] 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, Parckard). Results were expressed as mean net dpm (stimulated minus control) of triplicate-stimulated culture.

Secretion of PGE_2

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. PGE_2 content in the supernatants were determined by PGE_2 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 comparision 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 30min 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 IgG1 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[1].

	CO	SO
Reaginic Antibody (mm)	7.90 ± 0.65	11.5 ± 1.2[a]
IgG1 (μg/ml)	3.61 ± 1.11	0.986 ± 0.310[a]
IgG2a (μg/ml)	42.4 ± 12.9	77.7 ± 4.9[a]

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

Activity of PKC, Lymphoproliferation and PGE_2 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 PGE, 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,

Table 2. Effect of Dietary Fats on Activity of PKC, Lymphoproliferation and PGE_2 Production in Splenocytes'.

	CO	SO
Activity of PKC (U/mg protein)	308 ± 30	227 ± 13[a]
Incorporation of [^{3}H] Thymidine (x10^3 dpm/well)	7.03 ± 0.88	4.28 ± 1.44[a]
Splenocyte PGE_2 (pg/ml medium)		
Con A (−)	197 ± 8	305 ± 17[a]
Con A (+)	197 ± 15	208 ± 30[b]

[1] Values are means ± SE (n=5).
[a] Significantly different from CO at $p<0.05$.
[b] 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 PGE_2 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 PGE_2 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'

	Serum			Splenocytes		
Fatty acid	CO	SO	Pooled SE	CO	SO	Pooled SE
			(mol %)			
16:0	24.5	22.4	0.7	25.9	26.5	0.4
18:0	22.1	20.0	0.7	16.7	16.8	0.1
18:1	8.5	9.5	2.4	13.6	8.9[a]	0.7
18:2(n-6)	23.1	25.2	1.2	10.7	19.7[a]	1.4
18:3(n-3)	0.5	0.7	0.1	1.4	0.8[a]	0.1
20:3(n-6)	3.6	0.7'	0.4	2.9	1.8"	0.2
20:4(n-6)	13.1	18.8[a]	1.1	24.0	21.3"	0.4
22:4(n-6)	0.2	0.4[a]	0.0	2.2	2.88	0.1
22:5(n-6)	0.9	0.6[a]	0.1	0.7	0.5[a]	0.0
22:5(n-3)	0.3	0.1[a]	0.1	0.6	0.2[a]	0.1
22:6(n-3)	3.3	1.7[a]	0.3	1.5	0.7"	0.1

[1] Values are means of five rats per group.
[a] Significant difference from CO at $p<0.05$
CO, coconut oil; SO, safflower oil.

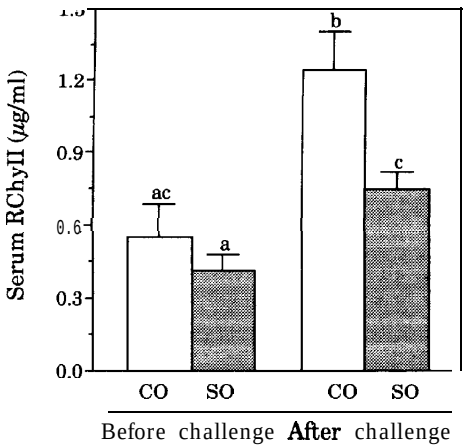

Figure Effect of Dietary Fats on the Levels of RChyII in Sera from Rats Prior and After Challenged with OVA.

Blood samples were obtained prior to and 3 hr after challenge. Each bar shows the mean ±SE for 5 rats per group. Each bar with different letters shows a significant difference, 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 RChyII prior to OVA challenge was similar between the groups; however, challenging orally OVA resulted in elevation of the RChyII 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.

Thl 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 Thl cells or other T cell subset (Levy *et al.,* 1982), it is shown that PGE_2 can act as an selective inhibitor for Thl cells, but not for Th2 cells (Betz and Fox, 1991). Therefore, increased production of PGE_2 derived from SO diet-fed rats may be involved in lowered proliferation of Thl cells, hence enhancing switch of Ig from IgM to IgE (Isakson *et al.,* 1982; Coffman and Carty, 1986).

PGE, 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 PGE, 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 PGE, 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 RChy II, 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 RChy II 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 PGE_2 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

Alayne, G. R., L. G. Kathy, J. H. Wilburta, N. M. Simin and L. P. Ronald 1991 Dietary arginine supplementation does not enhance lymphocyte proliferation or interleukin-2 production in young and aged rats. *J.* Nutr., 121: 1270-1278

Alexander, D. R. and D. A. Cantrell 1989 The role of kinases and phosphatases in human T-cell activation. Immunol. Today, 10: 200-205

Betz, M. and B. S. Fox 1991 Prostaglandin E_2 inhibits production of Thl lymphokines but not of Th2 lymphokines. *J.* Immunol., 146: 108-113

Cho, Y. and V. A. Ziboh 1995 Nutritional modulation of guinea pig skin hyperproliferation by essential fatty acid deficiency is associated with selective down regulation of protein kinase C-,3. *J. Nutr.*, 125: 2741-2750

Coffman, R. L. and J. Carty 1986 A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-y. J. Immunol., 136: 949-954

Duncan, D. B. 1955 Multiple range and multiple F tests. *Biometrics,* 11: l-42

Folch, J., M. Lees and G. H. Sloane Stanley 1957 A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem., 226: 497-506*

Fowler, K. H., R. S. Chapkin and D. N. McMurray 1993 Effects of purified dietary n-3 ethyl esters on murine T lymphocyte function. J. *Immunol.*, 151: 5186-5197

Groen, H., F. A. Klatter, A. S. van Petersen, J. M. Pater, P. Nieuwenhuis and J. Kampinga 1993 Composition of rat $CD4^+$ resting memory T-cell pool is influenced by major histocompatibility complex. *Transplant. Proc., 25: 2782-2783*

Ikeda, I., Y. Tomari and M. Sugano 1989 Interrelated effects of dietary fiber and fat on lymphatic cholesterol and triglyceride absorption in rats. *J. Nutr.,* **119: 1383-1387**

Imaizumi, K., K. Obata, I. Ikeda and M. Sakono 1995 Dissociation of protein kinase C activities and diacylglycerol levels in liver plasma membranes of rats on coconut oil and safflower oil diets. *J. Nutr. Biochem., 6: 528-533*

Isakson, P. C., E. Pure, E. S. Vitetta and P. H. Krammer 1982 T cell-derived B cell differentiation factor(s): effect on the isotype switch of murine B cells. J. *Exp. Med.,* **155: 734-748**

Ju, H.-R., I. Matsuura, K. Yamada, M., Sugano and K. Imaizumi 1995 Systemic release of mucosal mast-cell protease in primed Brown Norway rats after feeding with β-lactoglobulin. *Biosci. Biotech. Biochem., 59: 771-775*

Ju, H.-R., H.-Y. Wu, S. Nishizono, M. Sakono, I. Ikeda, M. Sugano and K. Imaizumi 1996 Effect of dietary fats and curcumin on IgE-mediated degranulation of intestinal mast cells in Brown Norway rats *Biosci. Biotech. Biochem., 60: 1856-1860*

Lessof, M. H. 1991 Mechanisms of food allergy and food intolerance. *In* "Food Allergy and Food Intolerance", ed. by J. C. Somogyi, H. R. Müler and Th. Ockhuizen, Karger, Tokyo, pp. 24-29

Levy, J. A., A. B. Ibrahim, T. Shirai, K. Ohta, R. Nagasawa, H. Yoshida, J. Estes and M. Gardner 1982 Dietary fat affects immune response and production of antiviral factors and immune complex disease

in NZB/NZW mice. *Proc. Nat. Acad.* Sci. *USA, 79:* 1974-1978

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

Metcalfe, D. D. 1991 Food allergy. *Curr. Opin. Immunol.*, 3: 881-886

Meydani, S. N. 1990 Dietary modulation of cytokine production and biologic functions. Nutr. *Rev.*, 48: 361-369

Meydani, S. N., R. J. Nicolosi and K. C. Hayes 1985 Effect of long-term feeding on corn oil or coconut oil diets on immune response and prostaglandin E_2 synthesis of squirrel and cebus monkeys. *Nutr. Res.*, 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 [^{3}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 lipids and immunity. *J. Nutr. Biochem.,* 5: 514-521

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

Pene, J., F. Rousset, F. Briere, I. Chretien, Y. Bonnéoy, H. Spits, T. Yokota, N. Arai, K. Arai, J. Banchereau 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 T_H1/T_H2 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

Sutton, B. J. and H. J. Gould 1993 The human IgE network. *Nature,* 366: 421-428