Dietary oxidized oil influences the levels of type 2 T-helper cell-related antibody and inflammatory mediators in mice

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The aim of this present study was to investigate the effect of amount and degree of oxidation of dietary oil on type 2 T-helper cell (TH)-related immune responses. Four groups of BALB/c mice were fed either 50 g soyabean oil/kg (50-S), 50 g oxidized oil/kg (50-O), 150 g soyabean oil/kg (150-S) or 150 g oxidized oil/kg (150-O). After 14 weeks consuming the experimental diets, the mice were immunized with ovalbumin (OVA) plus Al and antigen-specific immunoglobulin (Ig)E, IgG_1 and IgG_{2a}, inflammatory mediators such as prostaglandin (PG) E_2 and leukotriene (LT)B_4 were determined. Higher hepatic microsomal cytochrome P450 was noted in mice fed 150 g oxidized oil/kg compared with those of other groups. OVA-specific IgG_1 and IgE were higher in mice fed 150 g oxidized oil/kg compared with those of the other groups. The data suggested the interleukin (IL)-4: interferon (IFN)-γ ratio was higher in mice fed 50 g dietary oxidized oil/kg compared with that of the 50-S group. The IL-5:IFN-γ ratios were higher in the 150-S and 150-O groups than in the 50-S and 50-O groups. PGE_2 and LTB_4 produced by macrophages stimulated by lipopolysaccharide were highest in mice in the 150 g oxidized oil/kg group. The data suggested that an increased intake of oxidized oil might exert an unfavourable effect on the TH_2 response involved in allergic disease.

Oxidized oil: Immunoglobulin E: Asthma: Prostaglandin E_2: Leukotrienes

It has been well documented that environmental factors such as polluted air, tobacco, diet and infectious diseases play a critical role in the recently increasing prevalence of allergic diseases (Kimber, 1998). Several studies have demonstrated that both the quantity and quality of fat intake may play an important role in increasing the prevalence and severity of allergic diseases (Black & Sharpe, 1997; Hodge *et al.* 1998). Certain diets such as low-fat and fish-oil-containing diets have been shown to modulate the disease course of asthma or chronic lung disease (Arm *et al.* 1988; Smit *et al.* 1999; Schwartz, 2000).

Although the mechanism explaining how dietary fat affects immune response is still not well defined, several studies have suggested the involvement of a malfunction of macrophages and alterations in the production of proinflammatory mediators such as arachidonic acid, leukotrienes (LT), platelet activating factor, interleukin (IL)-1 and tumour necrosis factor-α (Sperling *et al.* 1987; Endres *et al.* 1989). In particular, the amount and composition of polyunsaturated fatty acids have been found to affect the synthesis of immune mediators such as prostaglandins (PG) and LT (Caughey *et al.* 1996). Much attention has been focused on polyunsaturated fatty acids of the *n*-3 class such as docosahexaenoic acid and eicosapentanoic acid, which are suggested to decrease the formation of these inflammatory mediators, including LTB_4, LTC_4, LTD_4 and LTE_4. Very few papers concerning the effect of oxidized oil by frying on immune responses have been reported; however, dietary oxidized oil may actually play an important role in health and in diseases (Lin *et al.* 1997). Dietary oxidized oil influences the hepatic and serum levels of triacylglycerol, cholesterol and hepatic cytochrome P450 (Lai & Lin, 1997; Lai *et al.* 1997). In addition, the degree of lipid oxidation can affect lymphoid organs and influence immune responses in normal mice (Oarada *et al.* 1989, 1991). It is believed that an increased dietary content of oxidized oil

Abbreviations: IFN, interferon; Ig, immunoglobulin; IL, interleukin; LT, leukotriene; OVA, ovalbumin; PG, prostaglandin; TH, T-helper cell.

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might result in the aggravation of such immune diseases as autoimmune diseases, allergic diseases and tumours (Lin *et al.* 1996).

No studies concerning the effect of oxidized oil on the immune response in animal models of allergic disease have been documented. This present study further investigated the effect of oxidized oil on serum levels of antigen-specific antibody, cytokine production pattern and inflammatory mediators.

Materials and methods

Preparation of oxidized oil and diets

The oxidized oil was prepared as follows: 5·5 kg soyabean oil (President Co., Tainan, Taiwan) was poured into a cast-iron wok (40 cm internal diameter, 10 cm central depth, 6·5 litre capacity) and heated on a gas stove which was adjusted to maintain the oil temperature at 205 ± 5°C. Wheat-flour dough sheets (12 × 4·5 × 0·15 cm, about 11 g in weight) were fried in the oil, one at a time. The wheat-flour dough was made by mixing together (g); high-gluten wheat flour 1500, table sugar 200, baking powder 5, water 600. The frying proceeded for 6 h/d and was repeated successively for 4 d according to the previously reported procedures (Huang *et al.* 1988; Lin *et al.* 1996). The resultant oxidized oil as well as the unfried fresh soyabean oil was stored at −20°C for the preparation of test diets. The fatty acid compositions of the fresh soyabean oil and the oxidized oil were measured according to the method described by Lee *et al.* (1990). The degree of oxidation was evaluated as acid value, absorbance at 233 nm, total polar compounds, and the non-urea-adductable fractions (Sallee, 1971). The quality of the soyabean oil declined after 24 h frying process (Table 1). The composition of experimental diets is summarized in Table 2. The casein content was increased in the 150 g oil/kg diets to provide the same % energy from protein as the 50 g oil/kg diet.

Animals and immunization

Female BALB/c mice between 6 and 8 weeks of age were purchased from the Animal Centre of the College of Medicine at National Taiwan University. The animal room had a 12 h light–dark cycle and a constant temperature (25 ± 2°C) and humidity. The mice were housed individually in stainless-steel wire cages and fed on a non-purified diet (Lab Rodent Chow; Ralson Purina, St Louis, MO, USA) before being fed on the experimental diets. Animal care and handling conformed to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985). Each mouse was fed on the experimental diet starting from 13-weeks-old. The four diets used contained either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S) or 150 g oxidized soyabean oil/kg (150-O). The mice were given free access to the test diets throughout the experiment. The mice were weighed twice per week, and food consumption was measured every 2–3 d. Each group included eight mice. In addition, eight mice per group were killed for lipid analysis, cytokine assay and proliferative study.

Table 1. The fatty acid composition (g/100 g total fatty acids) and the degree of oxidation of fresh soyabean oil and oxidized frying soyabean oil*

	Fresh soyabean oil	Oxidized soyabean oil
Fatty acid		
16:0	11·4	9·3
18:0	3·9	3·2
18:1	21·0	16·9
18:2	52·3	24·5
18:3	6·9	1·8
Unidentified	5·4	44·2
Acid value (mg KOH/g)	0·22	2·23
UV233 nm (absorbance/g)†	479	4912
Total polar compound (%)	6·27	69·1
NUAF (%)	1·35	15·0

NUAF, non-urea-adductable fraction.
* For details of procedures see p. 912.
† UV233 is the absorbance of light of wavelength 233 nm after proper dilution with *n*-hexane, expressed as absorbance (optical density)/g oil.

After 14 weeks of consuming the test diets (27-weeks-old), four groups of BALB/c mice were immunized by an intraperitoneal injection of 0·1 ml PBS solution containing ovalbumin (OVA, 2 μg) with AlOH as the adjuvant. The mice were immunized again, 2 weeks later, with 6 μg OVA plus the same adjuvant (Chuang *et al.* 1996). Blood was obtained at days 0, 14 and 28 from the retro-orbital venous plexus and centrifuged at 12 000 ***g*** for 10 min. Serum was collected and stored at −20°C before further assay.

Determination of serum anti-ovalbumin antibody levels

Serum anti-OVA IgE, IgG_1 and IgG_{2a} antibody titers were measured by ELISA. Briefly, ninety-six-well flat-bottomed microtiter plates (Costar, Cambridge, MA, USA) were coated with 10 μg OVA/ml $NaHCO_3$ buffer, pH 9·6. After overnight incubation at 4°C, the plates were washed three

Table 2. Composition of experimental diets (g/kg diet)

Ingredient*	50-S†	50-O†	150-S†	150-O†
Fresh soyabean oil	50	–	150	–
Fried soyabean oil	–	50	–	150
Casein	200	200	224	224
Methionine	3	3	3	3
Cornstarch	325	325	258	258
Sucrose	325	325	258	258
Cellulose	50	50	55	55
AIN-76 vitamin mix‡	10	10	11	11
AIN-76 mineral mix‡	35	35	39	39
Choline	2	2	2	2
Energy (kJ/kg)	16170	16170	18102	18102
% Energy from protein	20·8	20·8	20·8	20·8
% Energy from fat	11·7	11·7	31·3	31·3

* Ingredient sources: casein, methionine and choline, Sigma Chemical (St Louis, MO, USA); sucrose, Taiwan Sugar Company (Taipei, Taiwan); cornstarch, Roquatte (Paris, France); α-cellulose, ARBOCEL®, type BE 600/300, J. Bettenmaier & Söhne (Ellwangen-Holzmühle, Germany).
† 50-S, 50 g fresh soyabean oil/kg diet; 50-O: 50 g oxidised soyabean oil/kg diet; 150-S; 150 g fresh soyabean oil/kg diet; 150-O; 150 g oxidised oil/kg diet. For details of oxidation procedure, see p. 912.
‡ American Institute of Nutrition (1977).

times with PBS buffer and blocked with adult bovine serum (50 g/l) for 2 h at room temperature. After washes with PBS buffer, serum samples were diluted 1:10 in buffer-(10 g bovine serum albumin/l PBS) for overnight incubation at 4°C. Plates were then washed with PBS buffer containing 0·5 ml Tween 20/l. Either 0·1 ml biotin-conjugated anti-mouse IgG_1, IgG_{2a} or IgE, diluted in buffer (10 g bovine serum albumin/l PBS), was added. After 1 h, streptavidin-conjugated peroxidase was added. After a further 2 h the wells were washed with buffer (PBS buffer containing 0·5 ml Tween 20/l), 0·1 ml enzyme substrate solution (5·5 mg 2,2′-azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid (Sigma Chemical Co., St Louis, MO, USA) in 10 ml phosphate–citric buffer containing 0·01 ml H_2O_2/l) per well was added. After 30 min incubation at room temperature, 50 μl SDS (100 g/l) were added to each well to stop the reaction. Absorbance was determined at 415 nm (Microplate; Bio-Tek Instrument, Inc. Winooski, VT, USA). The antibody levels of samples were compared with the standard. The standard serum was a pool of serum collected from OVA-immunized mice with strong responses (usually optical density > 1). The concentration of standard serum was arbitrarily assigned 1 ELISA unit.

Liver cytochrome P450 activity

Livers were homogenized and centrifuged at 12 000 ***g*** for 20 min at 4°C. The supernatants were then further ultracentrifuged (105 000 ***g***; XL90, Beckman, Los Angeles, CA, USA) for 1 h and microsomes were isolated. The microsomal suspension was dissolved in buffer for further analysis. Cytochrome P450 content was determined by the dithionite–CO binding difference spectrum at 450 nm (Omura & Sato, 1964).

Ovalbumin-specific proliferative assays

To assay antigen-proliferative response further, spleen cells from OVA-immunized mice were plated in ninety-six-well round-bottomed plates with a concentration of 1×10^6 cells/ml in RPMI 1640 medium supplemented with 20 ml defined serum replacement/l (TCM™; Celox Co., Hopkins, MN, USA), 4 mM/L-glutamine, 25 mM-HEPES, 5×10^{-5} M2-mercaptoethanol, 100 U penicillin/ml, 100 μg streptomycin/ml and 0·25 mg amphotericin/ml in the absence or the presence of different concentrations of OVA (10 or 20 μg/ml) or anti-CD3 antibody (1 μg/ml). The cells were incubated at 37°C with 5 % CO_2 for 72 h. At 16 h before harvest, 37 kBq [^{3}H]thymidine was added to each well. The cells were harvested and radioactivity was measured with a β-counter (Beckman LS 5000 CE; Beckman). The data were expressed as the stimulation index: $(\text{sample}_{cpm} - \text{blank}_{cpm})/(\text{control}_{cpm} - \text{blank}_{cpm})$, where cpm is the counts/min.

Cytokine assay

Spleen cells, at a concentration of 5×10^6 cells/ml, were cultured with the medium described earlier in the absence or presence of OVA (20 μg/ml) for 48 h. Cytokine secretions by single cell suspensions of spleen cells were measured by sandwich-ELISA. Briefly, ninety-six-well flat-bottomed microtiter plates were coated with anti-cytokine antibody diluted in $NaHCO_3$ buffer, pH 9·6. After overnight incubation at 4°C, plates were washed three times and blocked with bovine serum albumin (30 g/l) at 37°C. After three washes with PBS containing 0·5 ml Tween 20/l, 0·1 ml sample was added for 2 h at 37°C. Plates were then washed with PBS buffer containing 0·5 ml Tween 20/l and biotin-conjugated anti-cytokine antibody diluted in buffer (10 g bovine serum albumin/l PBS) was added and incubated at 37°C for 1 h. After washes, streptavidin-conjugated peroxidase was added for an additional 1 h. After the wells were washed, 0·1 ml enzyme substrate of 2,2′-azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid solution was added to each well and the plate was left in a dark room for about 30 min. Plates were read in a microplate autoreader at 415 nm. The sensitivity of sandwich-ELISA used in our experiment is 15 pg/ml for IL-4, and 20 pg/ml for IL-5 and interferon (IFN)-γ.

Isolation and stimulation of peritoneal exudate cells

Peritoneal exudate cells were isolated by peritoneal lavage and washed three times with Hanks' solution before use. Peritoneal exudate cells with a concentration of $1{\cdot}5 \times 10^6$ cells/ml were set up in twenty-four-well plates in RPMI 1640 medium described earlier in the absence or presence of 5 μg lipopolysaccharide/ml and incubated for 24 h. The supernatant fraction was collected and used for determination of PGE_2 and LTB_4 productions. PGE_2 and LTB_4 were determined with an enzyme-linked immuno-assay kit (Cayman Chemical Co., Ann Arbor, MI, USA).

Statistical analysis

The significance of difference among four groups was analysed statistically by one-way ANOVA and Duncan's log multiple range test or Scheffe's multiple range test of the Statistical Analysis System program system (SAS/STAT version 6; SAS Institute Inc., Cary, NC, USA) throughout the study.

Results

Feed intake and growth

The feed intake and body weights of the mice are shown in Table 3. Although feed intake (g/d) was significantly lower in the 150 g fat/kg groups, the daily energy and protein intakes were only significantly lower in 150-S group. The feed efficiency was significantly lower in the oxidized oil groups. Therefore, gain in body weight and final body weights were the lowest in 150-O group due to its lowest feed efficiency though there was no significant difference in initial body weight before the dietary treatment. The results suggested that the increased content of oxidized oil in the diet did decrease the feed efficiency of mice.

Mice fed oxidized oil (50-O, 150-O) had significantly higher relative liver weight compared with the mice fed fresh oil (Table 4). In contrast, mice of the 150-O group had significantly lower relative heart weight than those of the

Table 3. The effect of experimental diets on body weight, gain in body weight, feed efficiency and total energy intake of ovalbumin-immunized BALB/c mice*

(Mean values with their standard errors for eight mice per dietary group)

	Dietary fat							
	50-S		50-O		150-S		150-O	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial body weight (g)	23·8	0·6	24·1	0·6	23·2	0·4	23·3	0·5
Final body weight (g)	29·8^{a}	1·0	29·2^{a}	0·6	27·2^{ab}	0·9	26·1^{b}	0·8
Gain in body weight (g)	7·0^{a}	0·5	5·2^{b}	0·4	5·1^{b}	0·6	3·0^{c}	0·4
Intake (g/d)	3·0^{a}	0·1	3·0^{a}	0·1	2·4^{b}	0·2	2·6^{b}	0·1
Feed efficiency (%)†	1·8^{a}	0·9	1·4^{b}	1·1	1·7^{ab}	1·8	0·9^{c}	1·1
Protein intake (g/d)	0·60^{ab}	0·02	0·61^{a}	0·03	0·54^{b}	0·04	0·58^{ab}	0·03
Energy intake (kJ/d)	48·1^{ab}	1·7	48·5^{a}	2·1	43·1^{b}	2·9	46·4^{ab}	2·1

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$; one way ANOVA and Scheffe's multiple range test).

* Mice were fed for 18 weeks on a diet containing either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S), or oxidized soyabean oil/kg (150-O). For details of diets and procedures, see p. 912 and Tables 1 and 2.

† Feed efficiency (%) = (gain in body weight (g)/total intake (g))×100.

50 g fat/kg 50-S and 50-O groups, and lower relative spleen weights than those of the fresh oil 50-S and 150-S groups. Mice of the 150-O group had a significantly higher hepatic thiobarbituric acid-reactive substance value (nmol/g organ) than that of the other groups (50-S, 0·97 (SE 0·11); 50-O, 0·85 (SE 0·10); 150-S, 1·28 (SE 0·12); 150-O, 1·73 (SE 0·28)). Although the thiobarbituric acid-reactive substance value of spleen was not determined in this study, our previous studies suggest no significant difference between mice fed the fresh oil or oxidized oil diets (Liu & Huang 1995; Lin *et al.* 1997).

It has been well documented that hepatic cytochrome P450 contents significantly increase in rats fed oxidized oil compared with those of rats fed fresh oil (Huang *et al.* 1988). Thus, cytochrome P450 is a direct indicator of the dietary effect of oxidized oil. As shown in Table 4, hepatic cytochrome P450 content significantly increased in OVA-immunized BALB/c mice of 150-O group. The effect of oil quantity and quality on cytochrome P450 content was more obvious when data were expressed per g body weight.

Serum anti-ovalbumin antibodies level

Immunoglobulin (Ig)E anti-OVA antibody tended to be higher in mice of 150-O group than those of the other groups ($P = 0·052$ *v.* 50-S group, $P = 0·084$ *v.* 50-O group and $P = 0·057$ *v.* 150-S group by non-paired Student's *t* test) (Table 5). Furthermore, the IgG_1 anti-OVA antibody level of mice fed 150 g oxidized oil/kg was also significantly higher compared with mice fed 50 or 150 g fresh oil/kg ($P < 0·05$). Both IgE and IgG_1 subclasses are affected by type 2 T-helper cell (TH)-related cytokines (Lee *et al.* 1999). In contrast, TH_1-related IgG_{2a} anti-OVA antibody was significantly lower in mice fed high dietary oxidized oil when compared with mice fed fresh oil. All these data suggest that a large amount of dietary oxidized oil could increase TH_2-related antigen-specific antibody production and subsequently cause more serious inflammatory response.

Antigen-specific proliferative responses and cytokine secretion of spleen cells

The results of T cell response to stimulation of OVA antigen are illustrated in Table 5. OVA antigen-specific proliferative response of spleen cells of mice fed dietary frying oil was lower, although statistically not significant, compared with those of the other groups. Furthermore, anti-CD3 antibody stimulated proliferative response was significantly lower in mice fed 150 g oxidized oil/kg. The proliferative response tended to be lower in mice fed with higher amount of dietary oil (150-S and 150-O) compared

Table 4. Relative tissue weight (g/kg body weight) and liver microsomal cytochrome P450 contents of immunized BALB/c mice*

(Mean values with their standard errors for eight mice per dietary group)

	50-S		50-O		150-S		150-O	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Relative tissue weight (g/kg body weight)								
Liver	67·0^{b}	4·1	89·2^{a}	4·3	56·2^{b}	3·1	80·8^{a}	5·1
Spleen	7·6^{a}	0·4	6·7^{ab}	0·6	8·1^{a}	0·4	5·8^{b}	0·7
Hepatic microsomal cytochrome P450								
nmol per mg protein	1·2^{c}	0·1	1·5^{bc}	0·1	1·6^{b}	0·1	3·0^{a}	0·2
nmol per g liver	3·5^{b}	0·2	5·0^{b}	0·3	5·1^{b}	0·5	9·4^{a}	0·9
nmol per liver	6·4^{b}	0·4	10·5^{b}	0·8	7·7^{b}	1·0	20·1^{a}	3·1
nmol per kg body weight	208^{c}	11	399^{b}	24	291^{c}	36	841^{a}	87

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$; one way ANOVA and Scheffe's multiple range test).

* Mice were fed for 18 weeks on a diet containing either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S), or 150 g oxidized soyabean oil/kg (150-O). For details of diets and procedures, see p. 912 and Tables 1 and 2.

Table 5. Immunological variables of ovalbumin-immunized BALB/c mice fed on 50 g or 150 g fresh oil or oxidized oil/kg diet*
(Mean values with their standard errors for eight mice per dietary group)

	50-S		50-O		150-S		150-O	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
OVA-specific immunoglobulin (ELISA units)								
IgG1	$2{\cdot}1^{b}$	0·3	$2{\cdot}5^{ab}$	0·4	$2{\cdot}1^{b}$	0·2	$3{\cdot}1^{a}$	0·4
IgG2a	$1{\cdot}0^{a}$	0·1	$0{\cdot}9^{ab}$	0·1	$1{\cdot}1^{a}$	0·1	$0{\cdot}7^{b}$	0·1
IgE	0·9	0·2	0·9	0·2	0·9	0·2	1·9	0·7
Proliferative response (stimulation index)†								
OVA (10 μg/ml)	2·0	0·7	3·7	1·6	3·0	1·9	1·1	0·2
OVA (20 μg/ml)	2·6	0·8	4·2	1·5	5·0	3·4	1·2	0·2
Anti-CD3 Ab (1 μg/ml)	$265{\cdot}8^{a}$	136·3	$175{\cdot}7^{a}$	106·1	$104{\cdot}8^{a}$	35·7	$48{\cdot}9^{b}$	19·5
Cytokine levels (ratio)								
IL-4:IFN-γ	$9{\cdot}1^{b}$	0·3	$12{\cdot}3^{a}$	0·5	$10{\cdot}5^{ab}$	0·7	$9{\cdot}1^{b}$	0·5
IL-5:IFN-γ	$0{\cdot}5^{a}$	0·2	$0{\cdot}5^{a}$	0·1	$1{\cdot}7^{b}$	0·5	$1{\cdot}4^{b}$	0·2
Arachidonic acid metabolites (pg/1 × 10^6 cells)								
PGE_2	$0{\cdot}5^{b}$	0·1	$1{\cdot}7^{ab}$	0·5	$2{\cdot}9^{ab}$	0·9	$3{\cdot}6^{a}$	2·0
LTB_4	$49{\cdot}3^{a}$	21·9	$77{\cdot}2^{ab}$	40·6	$143{\cdot}7^{b}$	32·7	$392{\cdot}6^{c}$	83·9

OVA, ovalbumin; Ig, immunoglobulin; Ab, antibody; IL, interleukin; IFN, interferon; PG, prostaglandin; LT, leukotriene.
[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$; one way ANOVA and Scheffe's multiple range test).
* Mice were fed for 18 weeks on a diet containing either 50 g soyabean oil/kg (50-S), 50 g oxidized soyabean oil/kg (50-O), 150 g soyabean oil/kg (150-S), or 150 g oxidized soyabean oil/kg (150-O). For details of diets and procedures, see p. 912 and Tables 1 and 2.
† Stimulation index = $(\text{sample}_{cpm} - \text{blank}_{cpm})/(\text{control}_{cpm} - \text{blank}_{cpm})$, where cpm is the counts per minute.

with those of 50-S and 50-O groups. In addition, the levels of cytokines produced by spleen cells stimulated with OVA antigen are presented in Table 5. The results were summarized as the IL-4:IFN-γ and IL-5:IFN-γ ratios. The data suggested the IL-4:IFN-γ ratio was higher in mice fed 50 g dietary oxidized oil/kg compared with that of 50-S group. In contrast, there was no difference in the IL-4:IFN-γ ratio between the 150-S and 150-O groups. Furthermore, the IL-5:IFN-γ ratio in mice of both 150-S and 150-O groups was higher than those of 50-S and 50-O groups.

Prostaglandin E_2 and leukotriene B_4 level

PGE_2 and LTB_4 produced by lipopolysaccharide-stimulated peritoneal exudate cells tended to be higher in mice fed with a high dietary frying oil (Table 5). PGE_2 and LTB_4 were not detectable when cells were cultured without lipopolysaccharide stimulation. PGE_2 production of peritoneal exudate cells from mice in the 150-O group was also significantly higher than that in the 50-S group. Furthermore, LTB_4 level in mice of 150-O group was significantly higher compared with those of the other groups. These data suggested inflammatory mediators were higher in mice fed high dietary oxidized oil.

Discussion

Increasing prevalence of certain allergic diseases such as allergic rhinitis and bronchial asthma has been documented (Aberg *et al.* 1995). Among the environmental factors, air pollution and dietary habit change have been suggested to play the critical role (Barnes, 1994; Kimber, 1998). Changes in the amount and quality of dietary fat might have an important impact on immunological changes of these atopic diseases. Furthermore, fried food becomes an important fat source in the present-day industrialized dietary pattern, although the relative contribution is difficult to assess. Total energy contributed by dietary fat has been reported to have increased from 30 % during the 1910s to 36 % during the 1980s in the US diet (Committee on Diet and Health Food and Nutrition Board, 1989). The 150 g fresh oil or oxidized oil/kg diet used in the present study provides 31 % total energy from fat.

It has been well documented that diet plays a role in asthma and immune function (Delafuente, 1991; Greene, 1999; Fogarty & Britton, 2000). The data presented here are from one of the few studies to investigate the effect of oxidized oil on immunological changes in a murine model of asthma. Lower relative spleen weight was noted in OVA-immunized BALB/c mice fed on a diet containing 150 g oxidized oil/kg. In addition, lower mitogen-stimulated proliferative response of spleen cell was also noted in mice fed a high amount of oxidized oil, which has also been reported previously (Lin *et al.* 1997). These data suggested that high amount of oxidized oil intake results in impairment of spleen cells proliferative ability. It will be interesting to study the role of oxidative stress and related enzyme activity in the activation of immune cells further. In addition, it has been documented that TH_1 and TH_2 cells respond differently to distinct population of antigen-presenting cells (Weaver *et al.* 1988; Gajewski *et al.* 1991). The effect of antigen-presenting cells and accessory molecules on TH cells' functions are more pronounced on proliferative response rather than cytokine production ability. This may be the reason for the discrepancy between low proliferative response and active cytokine production in mice in the 150-O group. The data also showed higher IL-5:IFN-γ ratio of cytokine profile produced by T cells of mice fed high amount of dietary fat. Cytokines such as IL-5 derived from TH_2 cells were found to induce eosinophilia, which is critical in the late stage of inflammation of asthma (Marom *et al.* 1982). In addition, the IL-4:IFN-γ ratio was higher in mice fed 50 g dietary oxidized oil/kg compared with that of the 50-S group. Furthermore, OVA-specific

IgE and IgG_1 antibody production was not suppressed by dietary oxidized oil. Allergen-specific IgE and mast cells are the effector molecules and cells in triggering inflammatory responses in allergic diseases; however, the central theme of the pathogenic mechanisms involved in allergenic diseases is the role of allergen-specific T cells and related cytokines (Ishizaka, 1989; Chretien *et al.* 1990; Romagnanai, 1990). The result of the present study demonstrated increased TH_2-related antigen-specific IgE and IgG_1 antibody in mice fed high dietary oxidized oil. Since the IL-4:IFN-γ ratio was not increased in mice fed high dietary oxidized oil, the higher level of antigen-specific IgE might have resulted from the adjuvant effect of very high levels of PGE_2 and LTB_4 that have been suggested to enhance IgE production (Dugas *et al.* 1990; Yamaoka *et al.* 1994). All these data together suggested that both quantity and quality of dietary fat affected the production of antigen-specific IgE and IgG_1 and inflammation-related cytokines.

Inflammatory mediators such as histamine, LT, PG, platelet-activating factor and chemokines have been documented to play a critical role in inflammation of late stage of asthma (Abraham *et al.* 1983; Russi *et al.* 1984). Among them, PG, LT and platelet-activating factor are the major metabolites derived from lipids (O'Byrne & Manning, 1992). The present data demonstrated higher PGE_2 and LTB_4 levels in mice fed 150 g dietary oxidized oil/kg compared with those of the other groups. LTB_4, a potent chemotactic agent for the neutrophil, is important in evoking more serious inflammation during the late stage of asthma (Arm *et al.* 1988). The data also showed that PGE_2 and PGE_1 could increase intracellular cAMP level and negatively regulated TH_1 development (Santoli & Zurier, 1989; Betz & Fox, 1991; Gold *et al.* 1994). Increased PGE_2 and LTB_4 levels might not only aggravate the inflammatory process, but also increase antigen-specific IgE production. Increased intake of dietary oxidized oil could result in increased prostaglandin production and subsequent higher TH_2 activity. Higher cytochrome P450 content was noted in mice fed dietary oxidized oil, which is similar to the previous report (Huang et al. 1988). Although microsomal cytochrome P450 activity is more important for PG metabolism, certain enzymes such as PGH synthase implicated in the metabolism of arachidonic acid metabolism may also increase in mice fed oxidized oil (Raz *et al.* 1989). More studies are needed, however, as dietary oxidized oil may induce higher enzyme activity involved in the pathway of arachidonic acid metabolism and subsequently enhance the production of inflammatory mediators.

It is possible that unidentified fatty acids contained in the oxidized oil (442 g/kg) might play a critical role in the detrimental effect of allergic response. More studies on the identification of the possible components are needed. The formation of volatile and non-volatile products in dietary oxidized oil has been reported (Chang *et al.* 1978). The major compounds formed during the frying process and left in oxidized oil are non-volatile. These non-volatile compounds can only be identified as non-urea-adduct-forming esters. The polymers formed during deep-fat frying were essentially dimers and trimers, which made the identification of these components relatively difficult. However, it would be very informative if the active substance in oxidized oil responsible for the changes of inflammatory response could be identified in the future.

These present data demonstrated that increasing content of dietary oxidized oil in modern food could increase both IgE, the IL-4:IFN-γ or IL-5:IFN-γ ratio and inflammatory mediators such as PGE_2 and LTB_4, which are all hazardous for the disease severity of asthma.

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