ReviewArticle

Lipids and immunology

Michihiro Sugano[1] PhD, Tamiho Koga[2] PhD and Kogi Yamada[1,3] PhD

[1]*Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto*
[2]*Laboratory of Food Processing, Nakamura Gakuen College, Fukuoka*
[3]*Laboratory of Food Science, Kyushu University School of Agriculture, Fukuoka, Japan*

Dietary fat plays an important regulatory role in the pathogenesis of a range of immune reactions. In food allergies, especially in type I allergic reactions, dietary fat can modulate the development of clinical symptoms through influencing the production of immunoglobulins (Ig), cytokines and chemical mediators. In general, polyunsaturated fatty acids (PUFA) of the *n*-3 family in relation to those of the *n*-6 family reduce the production of eicosanoids and hence, mitigate hypersensitivity. In this context, it is interesting that conjugated derivatives of linoleic acid (CLA) reduce the production of eicosanoids and regulate the production of Ig in a manner favourable to the prevention of allergic reactions. *Trans* monoene fatty acid (that is, elaidic acid), in relation to *cis* monoenoic fatty acid (that is, oleic acid), also behaves as an anti-allergic agent through interfering with the desaturation of linoleic acid. The information available indicates that different dietary fats influence differently the immune indices related to food allergic reaction. The effects appeared to be readily modified by the combination of food components, including dietary fats. Thus, an appropriate combination of a specific fat or fatty acid may be one approach to the regulation of allergic reaction.

Key words: chemical mediators, cytokines, food allergy, immunoglobulins, polyunsaturated fatty acids.

Mechanism of food allergy

Dietary fat influences immune functions through various metabolic events including modification of the production of immunoglobulins (Ig), cytokines and eicosanoids.[1–3] As food allergies are currently among the most prevalent immune disorders, we focused our study on the effect of dietary fat on these types of allergies. Food allergies[3] are induced by the invasion of food-specific allergens into the body. Once an allergen enters the body, a class of Ig is produced in response to the message carried by the allergen protein. Immunoglogulin E is the Ig responsible for the induction of the allergic reaction. In contrast, some Ig compete with IgE. Thus, IgA is anti-allergic in the intestinal immune system as it interferes with the absorption of allergen protein. Immunoglobulin G also exerts anti-allergic activity through competition with the binding of allergen protein with IgE, which binds on the surface of immune cells. Therefore, the class-specific regulation of the production of these Ig seems to be crucial for the regulation of allergic reaction.

Food protein-specific IgE thus formed then binds with the receptor on the surface of immune cells such as mast cells and basophils. In such a situation, when the same allergen enters again, it binds to IgE and stimulates a series of metabolic events in order to release a range of chemical mediators. Histamine is a preformed chemical mediator, and the production of eicosanoids is triggered by the allergen stimulation. These chemical mediators are responsible for the clinical symptoms of food allergies.

Effects of fatty acids on the production of immunoglobulins and chemical mediators *in vitro*

In this paper, we describe the results of our animal studies on the effects of different types of dietary fats such as *n*-3 polyunsaturated fatty acids (PUFA), conjugated isomer of linoleic acid (CLA) and *trans* monoene fatty acid on the immune indices in rats.

In order to attain preliminary information on the effect of different fatty acids on immune indices, we firstly investigated the effect of fatty acids on histamine release from a rat basophilic cell line, RBL-2H3.[4,5] As shown in Table 1, histamine release was slightly stimulated when the cells were incubated with unsaturated fatty acids, and the effect was marked in those having more double bonds.

The effect of fatty acids on Ig production was then studied using two origins of lymphocytes. When the lymphocytes from the mesenteric lymph node (MLN) were incubated with unsaturated fatty acids, there was a positive relationship between the number of double bonds of fatty acids and IgE production, and also thiobarbituric acid reactive substances (TBARS) (Fig. 1). Thus, the more double bonds that were present, the more IgE was produced. Consequently, a positive relationship was observed between IgE production and the TBARS level. However, somewhat different response patterns were observed in spleen lymphocytes, but again there was a positive relationship between IgE and TBARS levels.

When α-tocopherol was added to the incubation media, fatty acid-induced stimulation of the production of IgE was considerably lowered. α-Tocopherol also showed an alleviating effect on IgA production but it had no effect on IgG pro-

Correspondence address: Prof. Michihiro Sugano, Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Tsukide 3-1-100, Kumamoto 862—8502, Japan.
Tel: 81 96 383 2929; Fax: 81 96 384 6765
Email: suganomi@pu-kumamoto.ac.jp
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Table 1. Efects of long-chain fatty acids on histamine accumulation and release in RBL-2H3 cells *in vitro*

Fatty acid	Relative intracellular histamine content	A23187-stimulated histamine release (%)
None	1.00 ± 0.08^{ac}	88.3 ± 2.8^{a}
Lauric (12 : 0)	1.28 ± 0.01^{b}	87.5 ± 2.8^{a}
Myristic (14 : 0)	1.16 ± 0.01^{ab}	91.5 ± 1.9^{a}
Palmitic (16 : 0)	0.97 ± 0.04^{c}	84.7 ± 2.8^{a}
Stearic (18 : 0)	0.85 ± 0.07^{c}	88.3 ± 5.9^{a}
None	1.00 ± 0.02^{a}	78.3 ± 0.7^{a}
Oleic (18 : 1n-9)	1.27 ± 0.05^{bd}	77.8 ± 1.4^{a}
Linoleic (18 : 2n-6)	1.32 ± 0.02^{b}	86.1 ± 1.4^{b}
γ-Linolenic (18 : 3n-6)	1.14 ± 0.02^{ce}	89.0 ± 2.4^{bc}
α-Linolenic (18 : 3n-3)	1.07 ± 0.01^{c}	92.9 ± 3.5^{bc}
Arachidonic (20 : 4n-6)	1.32 ± 0.06^{b}	89.5 ± 4.5^{bc}
Eicosapentaenoic (20 : 5n-3)	1.20 ± 0.02^{de}	94.4 ± 1.4^{c}
Docosahexaenoic (22 : 6n-3)	1.16 ± 0.03^{ce}	94.6 ± 1.1^{c}

Mean ± SE of three incubations. Values without a common superscript letter are significantly different at $P < 0.05$.

duction (Fig. 2).[6,7] Thus, not all of the effects of unsaturated fatty acid on Ig and chemical mediator production can be explained by the oxidation alone.

Effects of dietary fats on immune indices related to food allergic reaction

In order to confirm these contrasting effects of PUFA *in vitro*, especially *n*-3 PUFA, we fed either eicosapentaenic acid rich (EPA) or docosahexaenoic acid rich (DHA) oils to rats and the effects on immune indices were compared with those of linoleic acid, a *n*-6 PUFA. Table 2 shows the fatty acid composition of these dietary fats. In order to avoid linoleic acid deficiency, an appropriate amount of safflower oil was added to *n*-3 oils. Sprague-Dawley rats were fed dietary fat at the 10% level for 3 weeks.

Analysis of serum Ig showed that in contrast to the results of *in vitro* study, DHA tended to decrease the serum IgE level. The EPA was not effective on this parameter (Table 3). Both EPA and DHA increased IgM level, suggesting an immuno-stimulating property of *n*-3 PUFA *in vivo*. Immunoglobulin A production by spleen lymphocytes tended to be reduced by *n*-3 PUFA, but IgG and IgM production were increased significantly. In addition, DHA reduced IgE production. Similar results were observed in MLN lymphocytes.

These observations suggest that *n*-3 PUFA did not stimulate IgE production *in vivo*; rather they may stimulate the immune defense system through an increase in IgM and IgG production. However, since IgA production by MLN lymphocytes was suppressed, *n*-3 PUFA may reduce the activity of gut immune system.

The release of leukotriene B_4 (LTB_4) by peritoneal exudate cells (PEC) was significantly reduced by *n*-3 PUFA, in particular by EPA (Fig. 3). Also, LTB_5 was detected in rats fed *n*-3 PUFA, and it was much greater in rats fed EPA than in those fed DHA.

In order to know the mechanism underlying the effect of *n*-3 PUFA on leukotriene production, the fatty acid composition of tissue lipids was analysed. There was a reduction of arachidonic acid by EPA and DHA, and the effect was more marked with EPA (Table 4). Thus, LTB_4 level was reflected by the arachidonic acid level.

The histamine data showed that *n*-3 PUFA reduced the content of histamine in peritoneal exudate cells (PEC). However, they tended to increase the release of histamine irrespective of activation, and the calculated rate of histamine release was higher with *n*-3 PUFA compared with linoleic

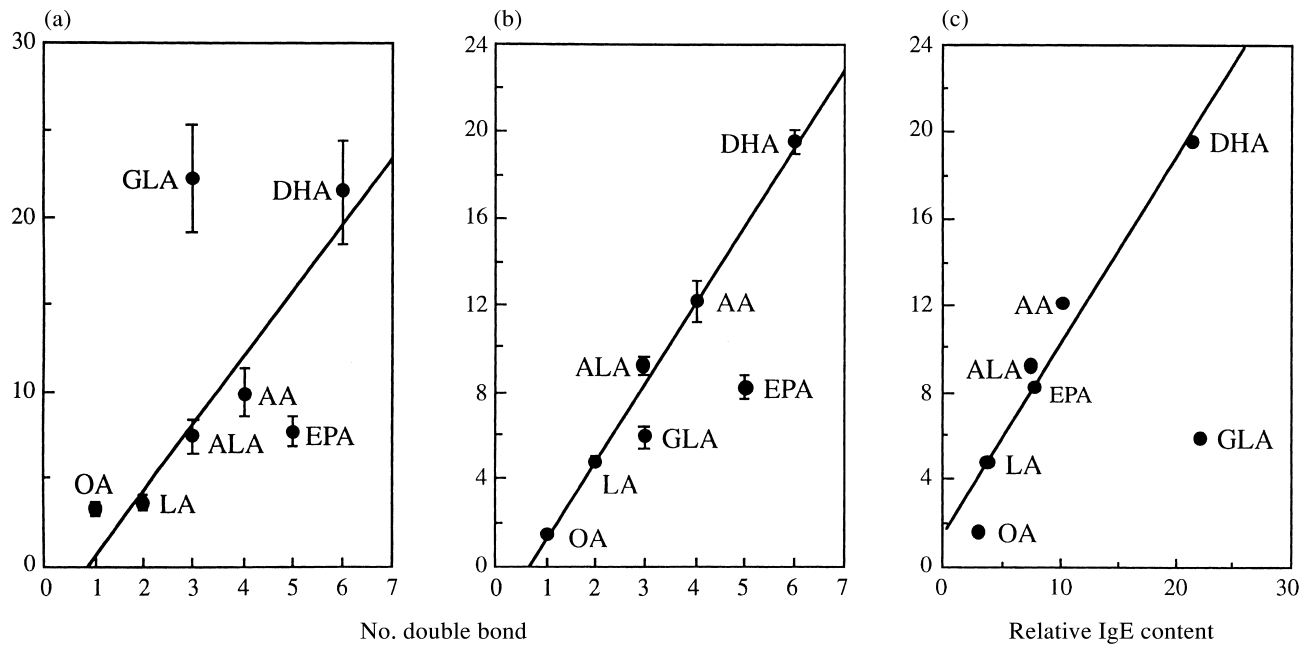

Figure 1. Correlation between relative immunoglobulin (IG) E content or lipid peroxidation and the number of double bonds of fatty acids in a mesenteric lymph node lymphocyte culture medium. Mean ± SE of three cultures. Values are relative to control cells incubated without fatty acids. (a) Relative IgE content ($\gamma = 0.965$); (b) relative thiobarbituric acid reactive substances (TBARS) ($\gamma = 0.999$); (c) relative TBARS ($\gamma = 0.898$).

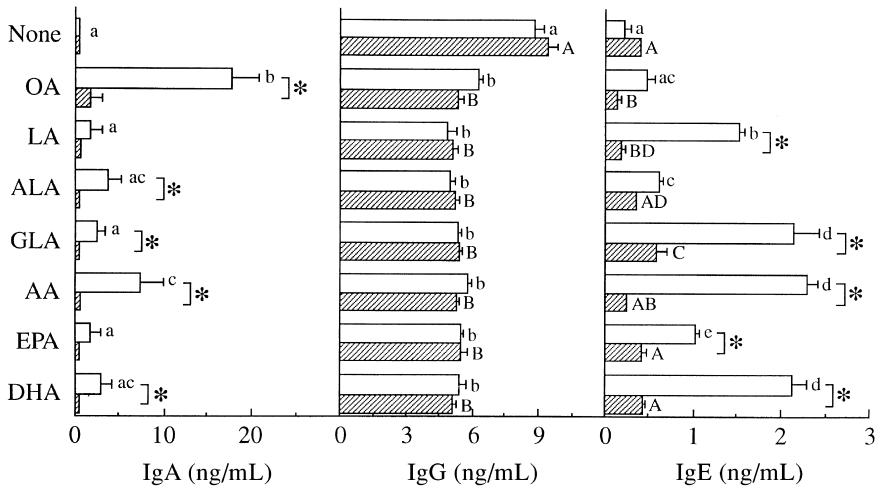

Figure 2. Effects of fatty acids and α-tocopherol on immunoglobulin levels in spleen lymphocyte culture medium. Mean ± SE of three cultures. (□) TOC–; (▨) TOC+. Bars without a common letter are significantly different at $P < 0.05$. *Significant difference between Toc– and Toc+.

Table 2. Fatty acid composition of dietary fat

Fatty acid	Safflower oil	EPA-rich oil	DHA-rich oil
		Weight (%)	
16 : 0	7.0	8.0	18.1
16 : 1	0.1	6.8	4.9
18 : 0	2.3	1.3	4.2
18 : 1	13.0	11.5	16.3
18 : 2n-6	76.9	18.9	13.9
18 : 3n-3	—	1.0	0.8
20 : 4n-6	—	1.3	1.9
20 : 5n-3	—	24.5	7.0
22 : 5n-6	—	0.2	1.6
22 : 5n-3	—	2.3	1.3
22 : 6n-3	—	10.1	22.7

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 3. Effects of dietary *n*-3 polyunsaturated fatty acid on serum immunoglobulin and thiobarbituric acid reactive substances (TBARS) levels of rats

Immunoglobulin	Dietary fat		
	Safflower oil	EPA-rich oil	DHA-rich oil
IgA (μg/mL)	68 ± 16	52 ± 6	79 ± 10
IgE (ng/mL)	5.3 ± 4.0	4.9 ± 2.1	2.1 ± 1.4
IgG (mg/mL)	6.6 ± 1.3	7.1 ± 1.2	7.7 ± 1.0
IgM (μg/mL)	175 ± 9[a]	201 ± 7[b]	226 ± 9[b]
TBARS (ng/mL)	1.74 ± 0.18	1.48 ± 0.21	2.06 ± 0.31

Mean ± SE of five rats. Values without a common superscript letter are significantly different at $P < 0.05$.

Table 4. Effects of dietary *n*-3 polyunsaturated fatty acids on unsaturated fatty acid composition of peritoneal exudate cell total lipids

Fatty acid	Dietary fat		
	Safflower oil	EPA-rich oil	DHA-rich oil
18 : 1n-9	11.4 ± 0.1[a]	13.8 ± 0.2[b]	13.6 ± 0.1[b]
18 : 2n-6	7.4 ± 0.1[a]	7.0 ± 0.1[b]	5.0 ± 0.1[c]
20 : 4n-6	12.7 ± 0.2[a]	5.2 ± 0.4[b]	9.0 ± 0.6[c]
20 : 5n-3	— [a]	5.9 ± 0.4[b]	2.0 ± 0.1[c]
22 : 6n-3	0.9 ± 0.1[a]	3.5 ± 0.2[b]	6.5 ± 0.3[c]

Mean ± SE of five rats. Values without a common superscript letter are significantly different at $P < 0.05$. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

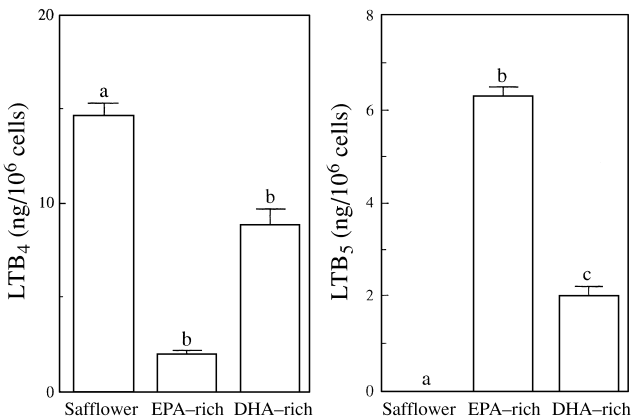

Figure 3. Effects of dietary *n*-3 polyunsaturated fatty acids on leukotriene release in peritoneal exudate cells of rats. Mean ± SE of five rats. Bars without a common letter are significantly different at $P < 0.05$.

acid. This suggests that *n*-3 PUFA increase histamine release through a modification of membrane fluidity.

In another trial, we studied the effect of dietary fat on immune functions of Brown-Norway rats by measuring other immune indices. When compared with rats fed either coconut oil or high-oleic safflower oil, those fed safflower oil or fish oil had higher serum antibody levels (Fig. 4). However, the serum level of rat chymase II, one of the indicators of intestinal immune function, was also lower in safflower oil, indicating possible immune defense activity of linoleic acid.[8]

In connection to the possible role of antioxidant on immune functions, we examined the effect of tea polyphenol on chemical mediator production.[9,10] Rats were fed different fats and the polyphenol. The LTB4 production by PEC was significantly lower in rats fed perilla oil than in those fed safflower oil or palm oil. Tea polyphenol feeding significantly reduced LTB4 production in all fat groups (Fig. 5).

Effects of dietary conjugated linoleic acid on immune indices

Conjugated isomers of linoleic acid (CLA) have recently been attracting much interest in lipid physiology.[11,12] CLA is produced from linoleic acid or α-linolenic acid in pasture by

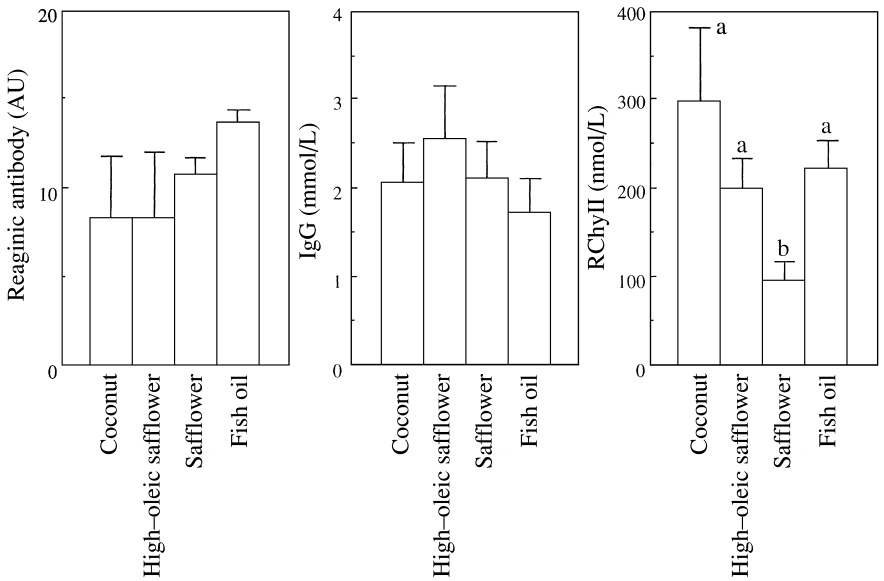

Figure 4. Effects of dietary fats on serum immunoglobulin and RChyII levels in Brown-Norway rats. Mean ± SE of five rats. Bars without a common letter are significantly different at $P < 0.05$. AU, arbitary unit.

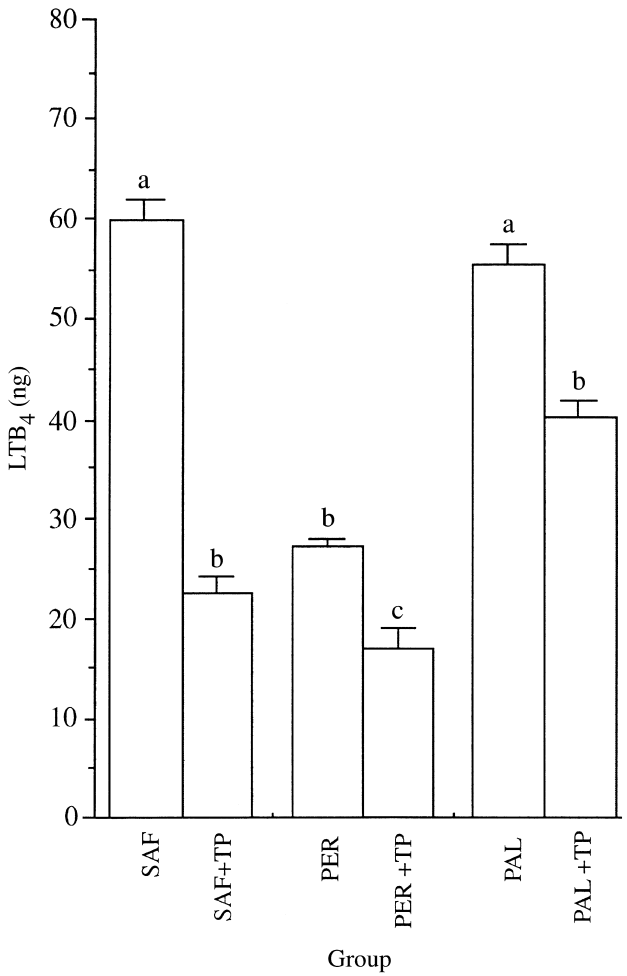

Figure 5. Effects of dietary fats and tea polyphenols (TP) on leukotriene B4 release from peritoneal exudate cells of Brown-Norway rats. Mean ± SE of five rats. Diets (10% fat and 1% TP) were fed for 3 weeks. SAF, safflower oil; PER, perilla oil; PAL, palm oil. Bars without a common letter are significantly different at $P < 0.05$.

the microorganisms living in ruminant lumens, and is incorporated in ruminant fats.

Conjugated isomers of linoleic acid are a unique fatty acid having a number of favourable physiological functions. The anticarcinogenic activity seems to be the most typical function. Since CLA is presumed to interfere with linoleic acid metabolism, its immunoregulatory functions are expected. In fact, Pariza, the pioneer of the CLA functionality, summarized some immunoregulatory functions of CLA from the standpoint of immune function.[13]

Conjugated isomers of linoleic acid were fed to rats at the dietary level of 0.5 and 1.0%, and several immune indices related to food allergy were measured. Conjugated isomers of linoleic acid reduced the concentration of plasma IgE, while increasing IgA and IgG, suggesting a possible favourable effect on the regulation of food allergic reactions (Fig. 6).[14] Results similar to those of serum were also observed in MLN Ig levels. The increased production of IgA by these lymphocytes indicates a possible stimulating effect of CLA on the intestinal immune system.

In addition to these preferable effects on Ig production, as shown in Fig. 7, CLA reduced the production of LTB_4 by the spleen and LTC_4 by the lung; both are the typical chemical mediators in these tissues. These changes in leukotriene production are at least in part attributed to the reduction of arachidonic acid by CLA in these tissues. Thus, the interfering effect of CLA with linoleic acid metabolism is indicated as a possible mechanism underlying the action of CLA.

It has been shown that some CLA are known to be elongated and desaturated like linoleic acid.[15] Therefore, there is an alternate possibility that CLA is more directly participating in the observed metabolic changes after being metabolized to eicosanoids.

Effects of dietary *trans* fatty acid on immune indices

Currently, *trans* fatty acids are also attracting attention with respect to several metabolic disorders.[16,17] Most of these effects seem to be related to the interfering action of *trans* acid on linoleic acid metabolism. It is therefore interesting to examine their effects on immune functions. In this experiment, we fed to rats either elaidic acid, which is a typical

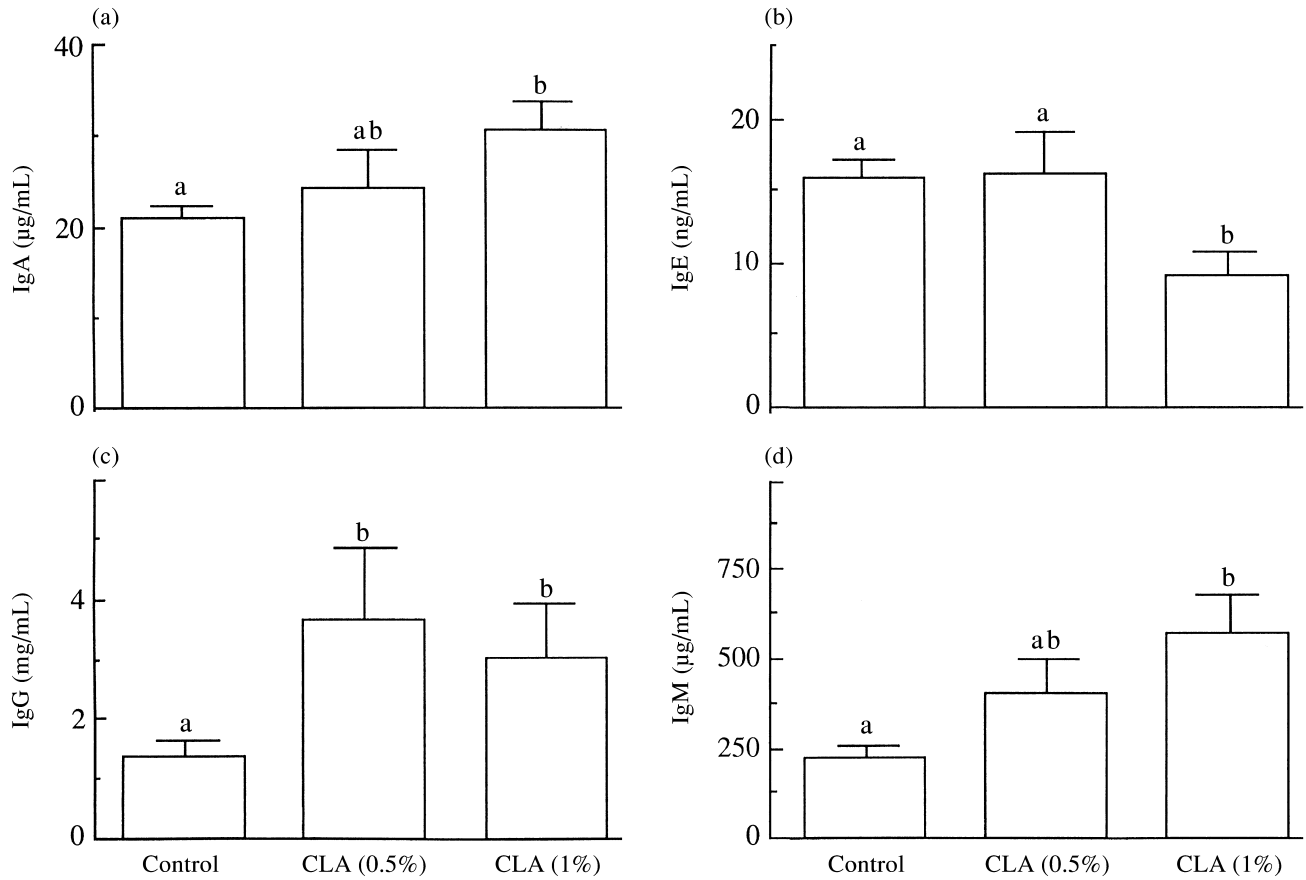

Figure 6. Effects of conjugated linoleic acid (CLA) on serum immunoglobulin levels in rats. Mean ± SE of five rats. Bars without a common letter are significantly different at $P < 0.05$.

trans monoene fatty acid, or oleic acid, the *cis* counterpart, and compared their interaction with *n*-6 and *n*-3 PUFA on immune indices.[18]

The results of plasma Ig levels showed that elaidic acid influenced Ig level somewhat differently compared with oleic acid (Table 5). Thus, in rats fed perilla oil, the level of IgG and IgM was higher in rats fed *trans* acid than in those fed *cis* acid. In contrast, IgA level was higher in *cis* acid. When safflower oil was the dietary fat, no such differences were observed.

Also, the effect of *trans* fatty acid on eicosanoid production was studied. As shown in Table 6, the serum level of prostaglandin E_2 (PGE_2) in rats fed safflower oil was lower when elaidic acid was fed than when oleic acid was fed. A similar response pattern was also observed in the splenic production of LTC_4.

Table 5. Interactions of elaidic acid with polyunsaturated fatty acids on plasma immunoglobulin levels in rats

	Plasma immunoglobulin			
Group	IgA (μg/mL)	IgE (ng/mL)	IgG (mg/mL)	IgM (ng/mL)
Perilla oil diet				
Oleic (*c*-18 : 1)	1.87 ± 0.14^{a}	17.8 ± 1.1^{a}	5.23 ± 0.56^{a}	93.1 ± 8.5^{a}
Elaidic (*t*-18 : 1)	1.47 ± 0.28^{b}	20.7 ± 1.5^{a}	6.90 ± 0.58^{b}	159 ± 11^{b}
Safflower oil diet				
Oleic (*c*-18 : 1)	34.2 ± 2.6^{c}	4.54 ± 0.78^{b}	1.19 ± 0.21^{c}	160 ± 23^{b}
Elaidic (*t*-18 : 1)	33.4 ± 4.8^{c}	4.84 ± 0.84^{b}	1.40 ± 1.10^{c}	188 ± 18^{b}

Mean ± SE of eight rats. Values without a common superscript letter are significantly different at $P < 0.05$.

Table 6. Interactions of elaidic acid with polyunsaturated fatty acids on splenic eicosanoid production

	Group			
Eicosanoid	Perilla oil		Safflower oil	
	Oleic	Elaidic	Oleic	Elaidic
Leukotriene C4 (ng/g)	49.7 ± 2.3	52.4 ± 10.5	432 ± 63	326 ± 25
Prostaglandin E2 (ng/g)	nd	nd	3.54 ± 0.94^{a}	1.31 ± 0.51^{b}

Mean ± SE of eight rats. Values without a common superscript letter are significantly different at $P < 0.05$. nd, not determined.

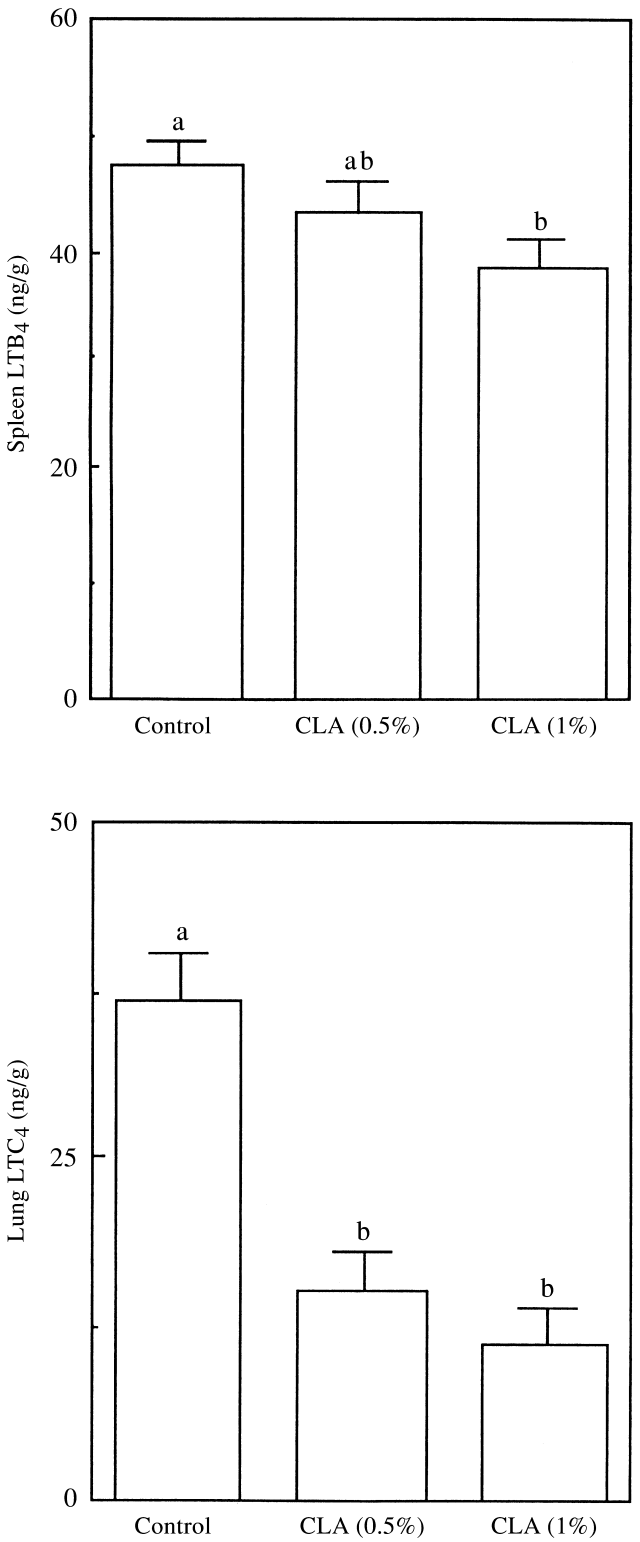

Figure 7. Effects of conjugated linoleic acid (CLA) on leukotriene production in spleen and lung of rats. Mean ± SE of five rats. Bars without a common letter are significantly different at $P < 0.05$.

The effect on eicosanoid production was attributed to the reduction of precursor fatty acid, arachidonic acid in this case, in tissue phospholipids. In addition, the incorporation of *trans* fatty acid in liver phospholipids was class-specific and it was higher in safflower oil fed rats (Table 7).

In summarizing, we showed that different dietary fats influence differently the immune indices related to food allergic reaction. The effects appeared to be readily modified by the combination with food components including dietary fats. Thus, an appropriate combination of a specific fat or fatty acid may be one approach to the regulation of allergic reaction.

Table 7. Effects of dietary polyunsaturated fatty acids on incorporation of *trans* fatty acids in liver phospholipids of rats

Phospholipid	Group	
	Perilla oil	Safflower oil
Phosphatidylcholine	7.2[a]	11.4[b]
Phosphatidylethanolamine	8.1[a]	12.7[b]
Phosphatidylinositol	8.0[a]	12.6[b]
Phosphatidylserine	4.8[a]	7.1[b]
Cardiolipin	—	—

Mean of eight rats. Values without a common superscript letter are significantly different at $P < 0.05$.

This study was conducted in accordance with the Guidelines of Animal Experiments approved by Kyushu University.

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