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Effect of Unsaturated Fatty Acids and α-Tocopherol on Immunoglobulin Levels in Culture Medium of Rat Mesenteric Lymph Node and Spleen Lymphocytes[1]

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Mesenteric lymph node (MLN) and spleen lymphocytes of Sprague-Dawley rats were cultured with 1 mM unsaturated fatty acids (UFAs) with or without 100 μM α-tocopherol (Toc), and the immunoglobulin content and thiobarbituric acid (TBA) value of the culture media were measured to clarify the relationship between lipid peroxidation and the IgE level in the culture medium. The increase in the IgE content and TBA value induced by UFAs was alleviated in the presence of Toc in both lymphocytes, and was correlated well with their oxidation rates in most cases. γ-Linolenic acid enhanced the IgE level much more than would be expected from its oxidation rate in both lymphocytes, and linoleic acid showed similarly high activity only in splenocytes. These results suggest that lipid peroxidation is partly responsible for the enhancement of IgE level induced by UFAs.

Key words: immunoglobulin, lipid peroxidation, α-tocopherol, unsaturated fatty acids.

In allergies against food components and airborne antigens, the induction of allergen-specific IgE is an essential step, and allergen-specific IgA suppresses the allergic reaction through the inhibition of allergen absorption (*1*). Thus, the class-specific regulation of Ig production, inhibition of IgE production, and stimulation of IgA production, may alleviate allergic reactions. Such class-specific regulation of Ig production was induced by cytokines (*2-5*) and some biomaterials such as lectin (*6*), bile acids (*7, 8*), and UFAs (*9, 10*).

UFAs have also been reported to affect immune reactions through the inhibition of lymphocyte proliferation (*11, 12*) and the modulation of antigen-presenting cell function (*13*). Administration of fish oil rich in EPA and DHA (*14*) or plant lipids rich in GLA (*15*) have been shown to reduce inflammation and pain in patients with rheumatoid arthritis. It has also been reported that *n*-3 PUFA are highly immunosuppressive (*16, 17*) and therefore are useful in treatment of autoimmune and inflammatory disorders (*18, 19*). In addition, PUFAs inhibit the release of inflammatory agents from mast cells (*20*). On the other hand, UFAs may enhance allergic symptom through the enhancement of IgE production and the inhibition of IgA and IgG production (*10*). Interestingly, the enhancement of IgE production induced by UFAs was suppressed in the presence of lipophilic antioxidants such as Toc (*10*). This suggests that the oxidation process of UFAs plays a crucial role in the expression of the Ig production-regulating activity. Thus, we studied here the relationship between Ig production-regulating activity and oxidation rate of UFAs in the culture medium of rat lymphocytes in the presence or absence of Toc.

MATERIALS AND METHODS

Materials—Sodium salts of UFAs with purities over 95% were purchased from Sigma Chemical (St. Louis, MO) and dissolved in PBS (pH 7.4). Toc and the TBA test kit were purchased from Wako Pure Chemical Industries (Osaka). Toc was dissolved in DMSO (Nacalai Tesque, Kyoto), and 0.1% (by volume) of the DMSO solution was added to the culture medium.

For ELISA of Ig, 0.05% Tween 20 in PBS (TPBS) was used for rinsing, and Block Ace (Dainihon Pharmaceutical, Osaka) for blocking and antibody dilution. Control rat IgE, IgG, IgM, and goat anti-rat IgE were purchased from Bethyl (Montgomery, TX), biotin-conjugated mouse anti-rat IgE, mouse anti-rat IgA, and HRP-mouse anti rat IgA from Zymed (California, USA), POD-conjugated avidin from Dako (Glostrap, Denmark). Goat affinity-purified $F(ab')_2$ fragment to rat IgG, goat affinity purified $F(ab')_2$ fragment to rat IgM, POD-conjugated goat affinity purified $F(ab')_2$ fragment to rat IgG, and POD-conjugated goat affinity purified $F(ab')_2$ fragment to rat IgM were purchased from Cappel (West Chester, PA). Substrate solution for

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Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; DHA, docosahexaenoic acid; DMSO, dimethyl sulfoxide; EPA, eicosapentaenoic acid; ELISA, enzyme-linked immunosorbent assay; GLA, γ-linolenic acid; Ig, immunoglobulin; LA, linoleic acid; MLN, mesenteric lymph node; OA, oleic acid; POD, peroxidase; PBS, phosphate-buffered saline; PUFA, polyunsaturated fatty acid; TBARS, thiobarbituric acid-reactive substance; Toc, α-tocopherol; UFA, unsaturated fatty acid; ABTS, 2,2′-azino-bis 3-ethylbenz-thiazoline sulfonic acid.

ELISA was a 10 : 9 : 1 (by volume) mixture of 0.006% H_2O_2 dissolved in 0.2 M citrate buffer (pH 4.0), distilled water, and 6 mg/ml of ABTS (Wako).

Cells and Cell Culture—MLN and spleen were excised from male 9-week-old Sprague-Dawley rats under diethyl ether anesthesia and lymphocytes were squeezed out into the RPMI 1640 medium (Nissui Pharmaceutical, Tokyo). After incubating for 30 min at 37°C to remove fibroblasts, 5 ml of the cell solution was layered on 4 ml of Lympholyte-rat (Cedarlane, Hornby, Canada) and centrifuged at 1,500 × *g* for 30 min. The lymphocyte band at the interface was recovered and the cells were rinsed three times with the medium. Then cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (Intergen, NY) with or without 1 mM UFA and 100 μM Toc. Ig contents of the culture supernatants were then measured by ELISA, and cell number was counted using a electronic cell counter (Coulter Electronics, England).

Measurements of Immunoglobulins—Ig production was quantified by sandwich ELISA, as described previously (*6*). Briefly, 96-well ELISA plates were coated with 100 μl per well of goat anti-rat IgA, IgE, IgG, or IgM diluted (1,000 times) with 50 μM carbonate-bicarbonate buffer (pH 9.6) for 1 h at 37°C to fix the antibodies. After blocking with 300 μl of the blocking solution at 4°C overnight, each antibody was allowed to react with 50 μl of the culture supernatant for 1 h at 37°C. Bound Igs were then treated with 100 μl of POD-conjugated goat anti-rat IgA, biotin-conjugated mouse anti-rat IgE, POD-conjugated goat anti-rat IgG, or POD-conjugated goat anti-rat IgM for 1 h at 37°C. For IgE, wells were treated with 100 μl of POD-conjugated avidin. Wells were rinsed four times for IgE determination or three times for other Igs with TPBS between each step. After incubating at 37°C for 15 min with the substrate solution, the reaction was stopped by adding 100 μl of 1.5% oxalic acid and the absorbance at 415 nm (A_{415}) was measured with a MPR A_{41} micro plate reader (Tosoh, Tokyo).

Measurements of Lipid Peroxidation Products—Lymphocytes were cultured for 4 h with or without UFA and Toc as described above, and culture supernatants were collected to quantify TBARS contents using a Test Wako kit according to the method given by the supplier. Briefly, 100 μl each of sample was mixed well with 4 ml of 1/12 N

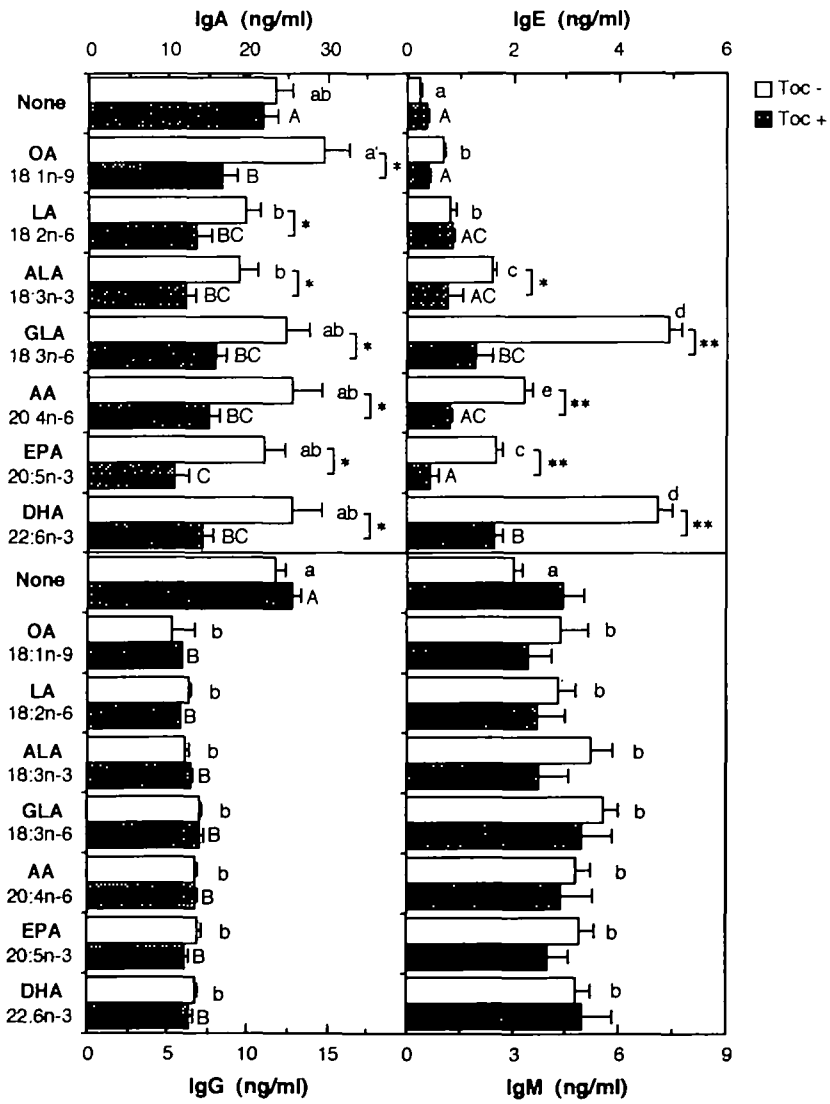

Fig. 1. Effect of fatty acids and α-tocopherol on Ig levels in culture medium of mesenteric lymph node lymphocytes of Sprague-Dawley rats. Cells (2×10^6 cells/ml) were cultured for 4 h (for determination of IgE) and 24 h (for determination of IgA, IgG, IgM) with or without 1 mM UFA, in the presence or absence of 100 μM Toc. Control cells were cultured with PBS. Data are means ± SE ($n = 3$ cultures). Values not sharing a common letter are significantly different at $p < 0.05$. Differences within a set of cultures for each Ig were analyzed by Student's *t* test, $^*p < 0.05$; $^{**}p < 0.01$.

sulfuric acid and 0.5 ml of 10% phosphotungstic acid, and then centrifuged for 10 min at 3,000×*g*. The precipitate was washed once with the mixture of 2 ml of 1/12 N sulfuric acid and 0.3 ml of 10% phosphotungstic acid, and then dissolved in 4 ml of distilled water. After adding 1 ml of TBA solution, the mixture was heated for 1 h in boiling water. TBA pigment produced by the reaction of TBA with malondialdehyde was extracted with 5 ml of *n*-butanol, and its fluorescent intensity (excitation at 515 nm and emission at 553 nm) was then measured using a Shimadzu spectrofluorophotometer RF-500 (Kyoto, Japan).

Statistical Analysis—Data were analyzed by Duncan's new multiple range test (*21*). Differences within a set of cultures for each Ig were analyzed by Student's *t* test.

RESULTS

Effects of Unsaturated Fatty Acids and Tocopherol on Immunoglobulin Levels in Culture Medium of Mesenteric Lymph Node Lymphocytes—MLN lymphocytes were cultured with 1 mM UFA in the presence or absence of 100 μM Toc for 4 h to measure IgE and for 24 h for IgA, IgG, and IgM. As shown in Fig. 1, apart from a slight enhancement by OA, the IgA level was not significantly influenced by UFAs in the absence of Toc. In the presence of Toc, IgA concentration was decreased by all UFAs. All UFAs also decreased IgG level, irrespective of the presence or absence of Toc. In contrast, all UFAs significantly increased the IgM level in the absence of Toc, while no significant differences were observed in the presence of Toc.

In case of IgE, all UFAs, and particularly GLA and DHA, increased its level in the absence of Toc. This enhancement of IgE level by UFAs did not necessarily parallel with the numbers of carbon atoms or double bonds of the UFAs. In the presence of Toc, the enhancement was reduced. The inhibition rate (%) of Toc was: 41.8±5.8 (OA), 3.8±3.8 (LA), 53.1±14.3 (ALA), 74.1±5.6 (GLA), 63.0±1.4 (AA), 73.7±0.3 (EPA), and 65.0±2.5 (DHA).

Effects of Unsaturated Fatty Acids and Tocopherol on Immunoglobulin Levels in Culture Medium of Splenocytes—The effects of UFAs and Toc on Ig levels in culture medium of spleen lymphocytes are showed in Fig. 2. Spleen lymphocytes expressed a much lower IgA level than MLN lymphocytes, and OA markedly enhanced the level of IgA

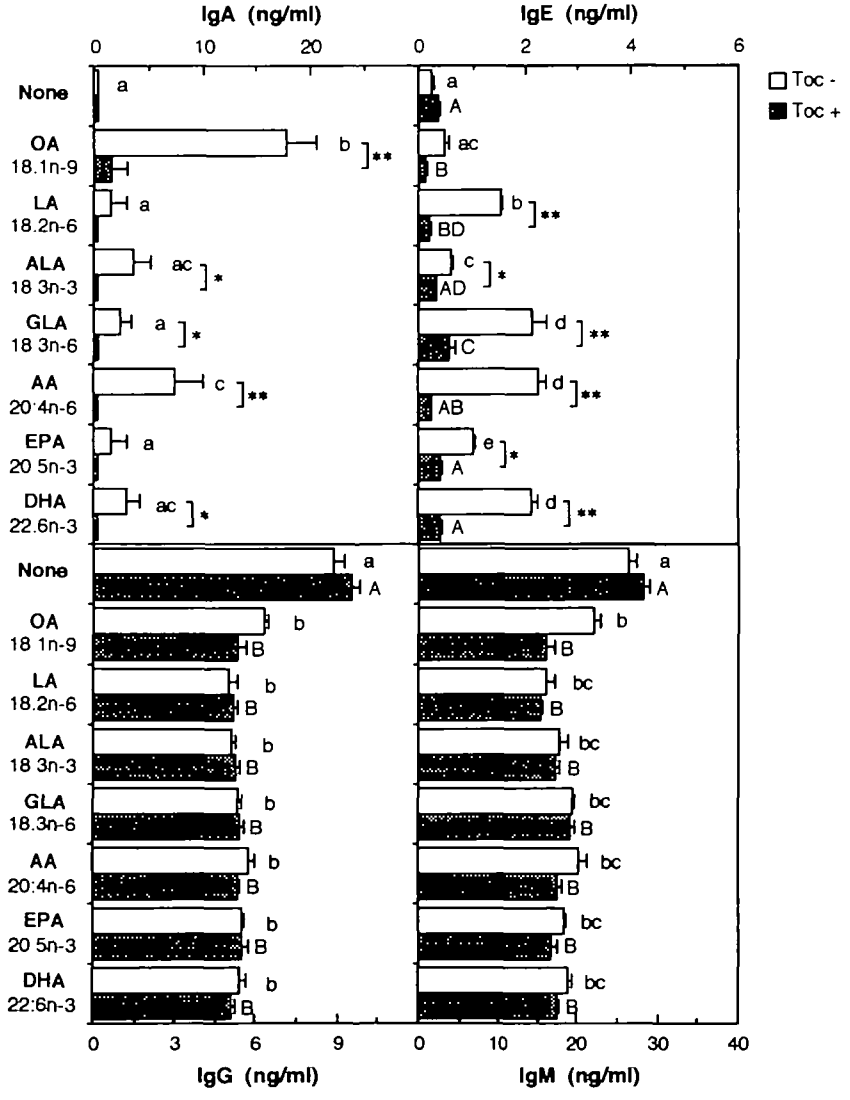

Fig. 2. **Effect of fatty acids and α-tocopherol on Ig levels in culture medium of spleen lymphocytes of Sprague-Dawley rats.** Cells (2×10^6 cells/ml) were cultured for 4 h (for determination of IgE) and 24 h (for determination of IgA, IgG, IgM) with or without 1 mM UFA, in the presence or absence of 100 μM Toc. Control cells were cultured with PBS. Data are means± SE ($n=3$ cultures). Values not sharing a common letter are significantly different at $p<0.05$. Differences within a set of cultures for each Ig were analyzed by Student's *t* test, $^*p<0.05$; $^{**}p<0.01$.

in the absence of Toc. Other UFAs also enhanced IgA level, but the effect was suppressed in the presence of Toc. Irrespective of the presence or absence of Toc, all UFAs decreased the level of IgG, as was also seen in MLN lymphocytes. Toc did not influence this effect of UFA, again as in the case of MLN lymphocytes. The IgM level was also decreased by UFA, in contrast to the case of MLN lymphocytes. However, Toc did not influence the IgM level.

As in the case of MLN lymphocytes, all UFAs enhanced the level of IgE. Marked enhancement was observed by LA and AA in addition to GLA and DHA, which exerted a prominent effect in MLN lymphocytes. Toc suppressed the increase of IgE levels induced by UFA as was seen in IgA. The inhibition rate (%) of Toc was: 72.1±2.76 (OA), 85.5±2.1 (LA), 49.3±1.2 (ALA), 73.7±1.1 (GLA), 88.9±0.3 (AA), 86.8±3.1 (EPA), and 81.9±0.9 (DHA). The inhibitory effect of Toc was greater in spleen lymphocytes than in MLN lymphocytes.

Cytotoxic Effect of Unsaturated Fatty Acids—To clarify the mechanism by which changes were induced in the Ig levels in culture medium of lymphocytes, the cytotoxic effect of UFAs was examined after 4 h cultivation. As summarized in Table I, all UFAs except LA exerted a strongly toxic effect against both lymphocytes. OA exerted stronger toxicity than LA, and UFAs having three or more double bonds exerted much stronger toxicity than LA. However, the cytotoxic effect of the latter was independent of the number of double bonds. In addition, the protective effect of Toc was very limited except in the case of splenocytes cultured with ALA or GLA. These results suggest a low correlation between the IgE level and the cytotoxic effect of UFA.

Lipid Peroxide Contents in Fatty Acid Samples and Culture Medium—The inhibitory effect of Toc on the enhancement of IgE level by UFA suggests that the oxidation of UFA is involved in the enhancement. Thus, we measured the TBA values of UFA samples and of the media cultured with UFA for 4 h. The UFA samples were prepared by dissolving UFA in PBS at 1 mM, and the TBA values were measured immediately. As shown in Table II, TBA values of UFA samples other than EPA were higher for UFAs with more double bonds.

The TBA values of the culture media were measured after incubating MLN and spleen lymphocytes for 4 h with 1 mM UFA in the presence or absence 100 μM Toc. In the control culture without UFA, MLN lymphocytes gave a higher TBA value than the splenocytes irrespective of the presence or absence of Toc. TBA values in culture media of both MLN and spleen lymphocytes were markedly increased in the presence of UFA, especially DHA and AA. The TBA values were usually higher in MLN lymphocytes than in splenocytes. In the presence of Toc, the increase in the TBA value was suppressed. No significant difference was seen in the inhibition rate by Toc between the two types of lymphocytes (data not shown).

Correlation of IgE and TBARS Levels in Culture Medium—To clarify the relationship between lipid peroxidation and the increase in IgE level in these lymphocytes, correlations between relative IgE content, relative TBA value, and the number of double bond were examined.

In the case of MLN, a linear relationship was observed between relative IgE content and the number of double bonds for all UFAs except GLA and EPA, GLA with three double bonds enhanced IgE level as strongly as DHA with

TABLE I. **Cytotoxic effect of unsaturated fatty acids.**

Fatty acids	Cell number×10⁴ (cells/ml)			
	MLN		Spleen	
	Toc(−)	Toc(+)	Toc(−)	Toc(+)
None	23.28±0.66[a]	16.58±0.55[a]	18.32±0.47[a]	15.04±1.75[a]
OA (18:1*n*-6)	6.59±0.68[b]	5.91±0.03[b]	7.94±0.59[b]	7.56±0.34[bd]
LA (18:2*n*-6)	18.79±0.85[c]	18.74±0.82[c]	14.97±0.53[c]	16.30±1.03[a]
ALA (18:3*n*-3)	2.45±0.24[d]	3.65±0.57[d]	3.03±0.28[d]	9.16±0.93[b]
GLA (18:3*n*-6)	2.06±0.57[d]	2.24±0.30[d]	2.27±0.28[d]	7.79±0.79[bd]
AA (20:4*n*-6)	1.19±0.08[d]	2.30±0.12[d]	2.15±0.20[d]	4.46±1.68[cd]
EPA (20:5*n*-3)	1.68±0.10[d]	2.99±0.12[d]	2.58±0.23[d]	3.20±0.58[c]
DHA (22:6*n*-3)	1.96±0.27[d]	2.25±0.23[d]	2.47±0.15[d]	2.65±0.54[c]

Cells were cultured for 4 h with or without 1 mM UFA in the presence or absence of 100 μM Toc, then cell number was counted. Data are means±SE ($n=3$). Values not sharing a common letter are significantly different at $p<0.05$.

TABLE II. **Effect of fatty acids and α-tocopherol on lipid peroxidation in cell culture medium.**

Fatty acids	UFA sample	TBARS value (nmol MDA/ml)			
		MLN		Spleen	
		Toc(−)	Toc(+)	Toc(−)	Toc(+)
None	—	0.45±0.04[a]	0.24±0.01[a]	0.27±0.03[a]	0.20±0.00[a]
OA (18:1*n*-9)	0.03±0.02[a]	0.73±0.09[a]	0.28±0.02[a]	0.87±0.15[ab]	0.33±0.06[a]
LA (18:2*n*-6)	0.03±0.01[a]	2.14±0.21[b]	0.37±0.04[a]	0.67±0.03[ab]	0.30±0.06[a]
ALA (18:3*n*-3)	0.11±0.01[b]	4.18±0.52[c]	0.89±0.08[ac]	1.10±0.15[bd]	0.50±0.06[a]
GLA (18:3*n*-6)	0.12±0.01[bd]	2.69±0.11[bf]	0.76±0.15[a]	1.17±0.20[bd]	0.40±0.10[a]
AA (20:4*n*-6)	0.17±0.01[cd]	5.52±0.70[d]	1.73±0.36[bc]	3.07±0.37[c]	0.93±0.03[b]
EPA (20:5*n*-3)	0.10±0.03[b]	3.71±0.08[cf]	2.65±0.63[b]	1.60±0.06[b]	0.83±0.07[b]
DHA (22:6*n*-3)	0.20±0.01[c]	8.86±0.67[e]	2.35±0.44[b]	3.07±0.44[c]	1.47±0.03[c]

Cells (2×10^6 cells/ml) were cultured for 4 h with or without 1 mM UFA in the presence or absence of 100 μM Toc. Culture supernatants were collected to measure peroxide content by the TBARS method. Data are means±SE ($n=3$). Values not sharing a common letter are significantly different at $p<0.05$.

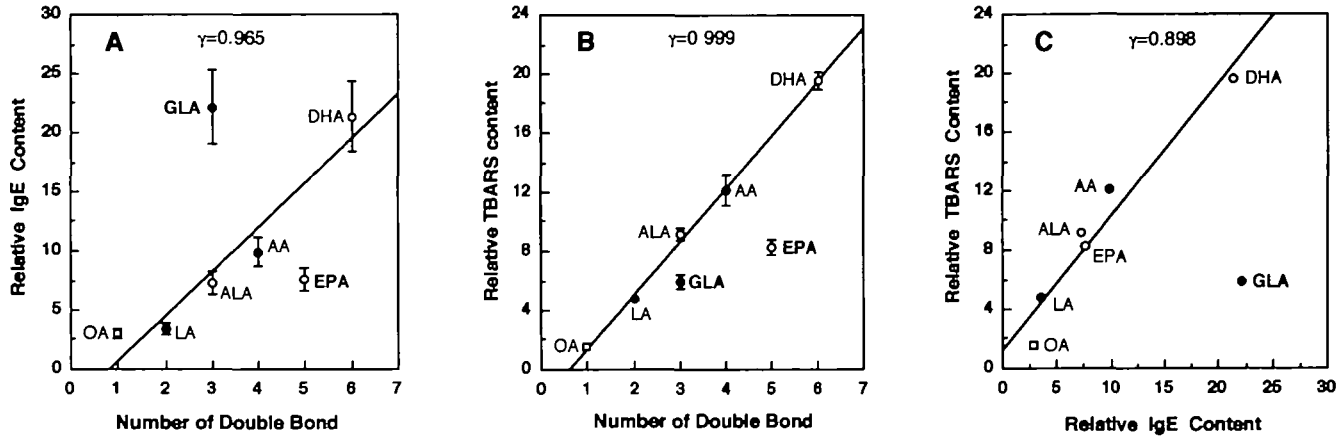

Fig. 3. **Correlation between relative IgE or relative lipid peroxidation in MLN lymphocyte culture medium and the number of double bonds of UFA.** Cells (2×10^6 cells/ml) were cultured for 4 h with or without 1 mM UFA. Supernatants of the cell culture were collected to measure IgE content (ELISA) and peroxide content (TBARS method). Results were expressed as the ratio of IgE or TBARS content of the samples treated with UFA relative to control cells. Data are means±SE ($n=3$ cultures).

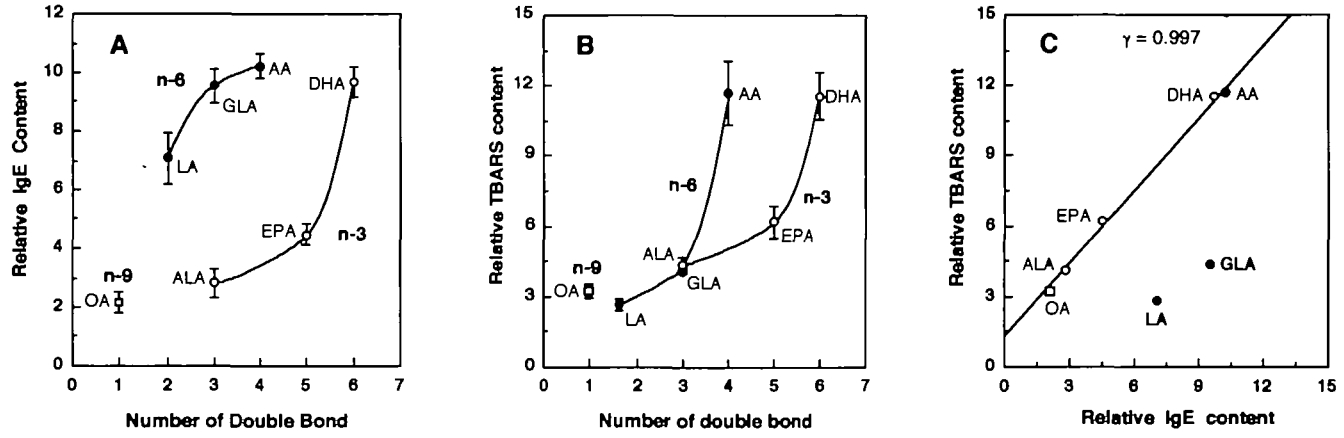

Fig. 4. **Correlation between relative IgE or relative lipid peroxidation in spleen lymphocyte culture medium and the number of double bonds of UFA.** Cells (2×10^6 cells/ml) were cultured for 4 h with or without 1 mM UFA. Supernatants of the cell culture were collected to measure IgE content (ELISA) and peroxide content (TBARS method). Results were expressed as the ratio of IgE or TBARS content of the samples treated with UFA relative to control cells. Data are means±SE ($n=3$ cultures).

six double bonds, while EPA with five double bonds enhanced IgE level as weakly as ALA with three double bonds (Fig. 3A). The highest correlation was observed between relative TBARS content and the number of double bonds for all UFAs, except GLA and EPA (Fig. 3B). In this case, both GLA and EPA gave lower TBARS values than those anticipated. A linear relationship was also observed between relative TBARS content and relative IgE content for all UFAs except GLA (Fig. 3C). GLA enhanced IgE level markedly more than would be anticipated from its TBARS content.

In the case of splenocytes, *n*-6 PUFAs enhanced IgE level more markedly than *n*-3 PUFAs, though the enhancing effect was augmented with the increase in the number of double bonds in both PUFA families (Fig. 4A). In addition, the oxidation rate of AA was much faster than that expected from the number of double bonds (Fig. 4B). A linear relationship was observed for all UFAs between TBARS and IgE except LA and GLA, which enhanced the IgE level more than would be expected from their oxidation rates (Fig. 4C).

DISCUSSION

In allergies against foodstuffs or airborne allergens, the induction of allergen-specific IgE is a key step (*1*) and most allergy patients exhibit a high serum IgE level (*22, 23*). We have reported that bile acids and UFAs enhance IgE level in culture medium of rat MLN lymphocytes (*8, 10*), while lipophilic antioxidant Toc suppresses the expression of the enhancing effect (*10*). In the present study, we found that UFAs enhanced IgE level in rat spleen lymphocytes in addition to MLN lymphocytes, and that in most cases their enhancing effect exhibited a linear relationship with their oxidation rate in the culture medium. These results suggest that the oxidation of UFAs is somehow involved in the enhancement of IgE level.

Spleen lymphocytes belong to the systemic immune system and MLN lymphocytes to the gut immune system. These lymphocytes contain different proportions of $CD4^+$ and $CD8^+$ cells and exert different Ig production patterns (*3*). Though UFAs enhanced IgE level in both lymphocytes,

some differences were observed depending on the source of lymphocytes. Oxidation rates of GLA and EPA were slower than would be expected from the number of double bonds in MLN lymphocytes. In addition, GLA enhanced the IgE level more than would be expected from its oxidation rate in MLN lymphocytes, while GLA and LA had the same effect in splenocytes. These results suggest that the lymphocytes are able to distinguish between UFAs.

Toc is thought to be an important structural component of biological membranes and is believed to act as a free radical scavenger in lipid peroxidation (*24*). It protects PUFAs from oxidation in cell membranes through its free radical-quenching activity in biomembranes at an early stage of free radical attack (*25*). Some *in vivo* experiments indicated that Toc accumulates in membranes rich in PUFAs and that relatively low concentrations of Toc are required to prevent lipid peroxidation of a large number of PUFA molecules (*26*). The inhibition by Toc of the increase in the IgE level induced by UFAs in MLN and spleen lymphocytes suggests the possibility that Toc plays a significant role in the alleviation of allergic symptoms.

Several lines of evidence indicate that PUFAs have anti-inflammatory and immunomodulatory effects. GLA and EPA reduced inflammation and repaired tissue injury in an animal model (*27*). Administration of fish oil rich in EPA and DHA (*14*) or plant lipids rich in GLA (*15*) reduces inflammation and pain in patients with rheumatoid arthritis. Ingestion of several grams per day of very long chain *n*-3 fatty acids may provide considerable health benefits in relation to inflammatory diseases (*14, 28*).

In contrast, we reported that PUFAs with three or more double bonds suppressed the release of LTB_4 from rat peritoneal exudate cells and that the inhibitory effect increased with the number of double bonds (*20*). This indicates that the oxidation process may be involved in the expression of the anti-allergic effect. In the present study, PUFAs behaved as allergy-enhancing factors through the enhancement of IgE production. The enhancement of IgE level by UFAs was weakened by a lipophilic antioxidant Toc (*10*), and Toc did not suppress the expression of LTB_4 release-inhibiting activity of PUFAs (*20*). These results suggest that simultaneous intake of PUFAs and Toc is prerequisite for the expression of their anti-allergic effects.

In conclusion, UFAs increased the IgE content and TBA value, and the increases were alleviated in the presence of Toc in both lymphocytes. The IgE production-enhancing activity of UFAs was correlated well with their oxidation rates, indicating the involvement of lipid peroxidation. GLA exerted a much higher IgE production-enhancing activity than would be expected from its oxidation rate in both MLN and spleen lymphocytes, suggesting a special role of GLA in allergic regulation. To clarify the mechanism by which Ig levels are regulated by UFA and Toc, the interactions between UFAs and cellular components are under investigation.

REFERENCES

1. Metcalfe, D.D. (1991) Food allergy. *Curr. Opin. Immunol.* **3**, 881-886
2. Pene, J., Rousset, F., Briere, F., Chretien, I., Wideman, J., Bonnefoy, J.Y., and De Vries, J.E. (1988) Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of soluble CD23 antigen. *Eur. J. Immunol.* **18**, 929-935
3. Gauchat, J.F., Gascan, H., Roncarolo, M.G., Rousset, F., Pene, J., and De Vries, J.E. (1991) Regulation of human IgE synthesis: the role of $CD4^+$ and $CD8^+$ T cells and the inhibitory effects interferon-α. *Eur. Respir. J.* **4**, 31s-38s
4. Ochel, M., Vohr, H.W., Pfeiffer, C., and Gleichmann, E. (1991) IL-4 is required for the IgE and IgG1 increase and IgG1 autoantibody formation in mice treated with mercury chloride. *J. Immunol.* **146**, 3006-3011
5. Rousset, F., Robert, J., Andary, M., Bonnin, J.P., Souillet, G., Chretien, I., Briere, F., Pene, J., and De Vries, J.E. (1991) Shift in interleukin-4 and interferon-γ production by T cells of patients with elevated serum IgE level and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J. Allergy Clin. Immunol.* **87**, 58-69
6. Lim, B.O., Yamada, K., and Sugano, M. (1994) Effect of bile acids and lectins on immunoglobulin production in rat mesenteric lymph node lymphocytes. *In vitro Cell. Dev. Biol.* **30**, 407-413
7. Yamada, K., Lim, B.O., and Sugano, M. (1993) Suppression of immunoglobulin production of rat lymphocytes by bile acids. *In vitro Cell Dev.* **29A**, 840-841
8. Lim, B.O., Yamada, K., and Sugano, M. (1994) Inhibition of immunoglobulin production in human namalwa cells and rat spleen lymphocytes by bile acids. *Biosci. Biotech. Biochem.* **58**, 1107-1111
9. Drevon, C.A. (1992) Marine oils and their effects. *Nutr. Rev.* **50**, 38-45
10. Yamada, K., Hung, P., Yoshimura, K., Taniguchi, S., Lim, B.O., and Sugano, M. (1996) Effect of unsaturated fatty acids and antioxidants on immunoglobulin production by mesenteric lymph node lymphocytes of Sprague-Dawley rats. *J. Biochem.* **120**, 138-144
11. Calder, P.C. and Newsholme, E.A. (1992) Polyunsaturated fatty acids suppress human peripheral blood lymphocyte proliferation and interleukin-2 production. *Clin. Sci.* **82**, 695-700
12. Soyland, E., Nenseter, M.S., Brathen, L., and Drevon, C.A. (1993) Very long chain *n*-3 and *n*-6 polyunsaturated fatty acids inhibit proliferation of human T-lymphocytes in vitro. *Eur. J. Clin. Invest.* **23**, 112-121
13. David, A., Hughes, S.S., and Andrew, C.P. (1996) *n*-3 polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes *in vitro*. *J. Nutr.* **126**, 603-610
14. Kremer, J.M., Jubiz, W., Michalek, A., *et al.* (1987) Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blinded, controlled, crossover study. *Ann. Intern. Med.* **106**, 497-503
15. Leventhal, L.J., Boyce, E.G., and Zurier, R.B. (1993) Treatment of rheumatoid arthritis with gammalinolenic acid. *Ann. Intern. Med.* **119**, 867-873
16. Goetzl, E.J., Wong, M.Y.S., Payan, D.G., Chernov-Rogan, T., Pikett, W.C., and Blake, V.A. (1986) Effects of eicosapentaenoic acid on immune responses and inflammation in humans in *Health Effects of Polyunsaturated Fatty Acids in Seafood* (Simopoulos, A.P.S., Kifer, R.R., and Martin, R.E., eds.) pp. 239-246, Academic Press, Orlando, FL
17. Lewis, R.A., Lee, T.H., and Austen, K.F. (1986) Effects of omega-fatty acids on the generation of products of the 5-lipoxygenase pathway in *Health Effects of Polyunsaturated Fatty Acids in Seafood* (Simopoulos, A.P.S., Kifer, R.R., and Martin, R.E., eds.) pp. 227-238, Academic Press, Orlando, FL
18. Burton, J.L. (1989) Dietary fatty acids and inflammatory skin disease. *Lancet* **i**, 27-30
19. Robinson, D.R., Colvin, R.B., Hirai, A., and Steinberg, A.D. (1986) Dietary marine lipids modify autoimmune disease in *Health Effects of Polyunsaturated Fatty Acids in Seafood* (Simopoulos, A.P.S., Kifer, R.R., and Martin, R.E., eds.) pp. 247-259, Academic Press, Orlando, FL
20. Yamada, K., Mori, M., Matsuo, N., Shoji, K., Ueyama, T., and Sugano, M. (1996) Effect of fatty acids on accumulation and secretion of histamine in $RBL\text{-}2H_3$ cells and leukotriene release from peritoneal exudate cells isolated from Wistar rats. *J. Nutr.*

Sci. Vitaminol. **42**, 301-311
21. Duncan, D.B. (1955) Multiple range and multiple F test. *Biometrics* **11**, 1-42
22. Somville, M.A., Machiels, J., Gilles, J.G.G., and Saint-Remy, J. M.R. (1989) Seasonal variation in specific IgE antibodies of grass-pollen hypersensitive patients depend on steady state IgE concentration and is not related to clinical symptom. *J. Allergy Clin. Immunol.* **83**, 486-494
23. Wadee, A.A., Boting, L.E., and Rabson, A.R. (1990) Fruit allergy: Demonstration of IgE antibody to a 30 kd protein present in several fruits. *J. Allergy Clin. Immunol.* **85**, 801-807
24. Kawamura, T., Oshisa, Y., Abe, Y., Ishimori, A., Shineha, R., and Yokota, K. (1992) Plasma lipid peroxides in the operation of esophageal cancer (in Japanese). *Rinsho-Byori* **40**, 881-884
25. Van Gossum, A., Kurian, R., Whitwell, J., and Jeebhoy, K.N. (1988) Decrease in lipid peroxidation measured by breath pentane output in normal after oral supplementation with vitamin E. *Clin. Nutr.* **7**, 54-57
26. Buttriss, J.L. and Diplock, A.T. (1988) The relationship between α-tocopherol and phospholipid fatty acids in rat liver subcellular membrane fractions. *Biochim. Biophys. Acta* **962**, 81-90
27. Tate, G.A., Mandell, H.R., Karmari, R.A., Laposata, M., Barker, D.G., and Schumacher, H.R. (1988) Suppression of monosodium urate crystal-induced acute inflammation by diets enriched with gamma linolenic acid and eicosapentaenoic acid. *Arthritis Rheum.* **31**, 1543-1551
28. Bjorneboe, A., Soyland, E., Bjorneboe, G.-E., Rajka, G., Drevon, C.A. (1987) Effect of dietary supplementation with eicosapentaenoic acid in the treatment of atopic dermatitis. *Br. J. Dermatol.* **117**, 463-469