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Effect of Unsaturated Fatty Acids and Antioxidants on Immunoglobulin Production by Mesenteric Lymph Node Lymphocytes of Sprague-Dawley Rats[1]

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The effect of UFA on Ig production by rat MLN lymphocytes was examined to clarify their influence on allergic reactions. A 4-h treatment at 1 mM inhibited the production of IgA, IgG, and IgM by the lymphocytes, but stimulated IgE production. The IgE production-stimulating activity became stronger with increasing number of carbon atoms and/or double bonds. However, no saturated fatty acid with carbon numbers from 12 to 18 affected IgE production by the lymphocytes. Hydrogen peroxide exerted Ig production-regulating activity similar to that of UFA, suggesting that the effect of UFA is at least partly due to oxidation products. Thus, the effect of antioxidants on the Ig production-regulating activity of arachidonic acid was examined. α-Tocopherol and BHT annulled the stimulation of IgE production by arachidonic acid, but ascorbic acid was not effective. The IgE production-enhancing activity of UFA was closely related to their oxidation rate in culture medium. These results suggest that UFA enhance the allergic reaction through the stimulation of IgE production and the inhibition of IgA production, and that hydrophobic antioxidants are partially effective to annul the adverse effect of UFA.

Key words: food allergy, IgA antibody, IgE antibody, α-tocopherol, unsaturated fatty acid.

Among 4 types of allergic reactions, type I allergy plays an important role in the occurrence of allergies against food components and airborne antigens (*1*). In the type I allergy, induction of allergen-specific IgE is an essential step and allergen-specific IgA inhibits the allergic reaction through the inhibition of allergen absorption (*1*). Thus, class-specific regulation of Ig production, the inhibition of IgE production and the stimulation of IgA production, may alleviate allergic reactions. Such class-specific regulation of Ig production is induced by lymphokines such as IL-4, IL-5, IFN-α, and IFN-γ (*2-6*). Similar regulation can be induced by biomaterials, as observed with bile acids or lectins (*7-9*). For example, high concentrations of free and conjugated bile acids (around 400 to 500 μM) act as allergy-enhancing factors by stimulating IgE production and by inhibiting IgA production of rat MLN lymphocytes (*9*). The MLN lymphocytes belong to the gut immune system and play a key role in the inhibition of allergic reactions through the production and secretion of allergen-specific IgA. Thus, studies on the interaction between food components and MLN lymphocytes are important for dietary regulation of allergy development.

Bile acids are Ig production-regulating materials (*7-9*), which are synthesized from cholesterol in the liver and secreted into the duodenum to facilitate intestinal absorption of lipidic food components. The stimulation of IgE production and inhibition of IgA production by bile acids observed in cultured MLN lymphocytes suggested that lipidic materials enhance allergic responses (*9*). Among lipidic components in foodstuffs which are absorbed with the aid of bile acids, UFA have been reported to affect immune reactions by inhibiting lymphocyte proliferation (*10-12*), but their effect on the humoral immune system has not been demonstrated. In the present study, we examined the effect of fatty acids on Ig production of rat MLN lymphocytes to estimate their activities to modify the humoral immune system.

MATERIALS AND METHODS

Materials—Sodium salts of saturated and unsaturated fatty acids, Lau, Pal, Ste, OA, LA, ALA, GLA, AA, EPA, and DHA were purchased from Sigma Chemical (St. Louis, MO), and Myr from Tokyo Kasei Kogyo (Tokyo). Each was more than 99% pure. The antioxidants AsA and BHT were purchased from Sigma and Toc from Wako Pure Chemicals

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Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; AsA, ascorbic acid; BHT, butyrated hydroxytoluene; DHA, docosahexaenoic acid; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; Lau, lauric acid; MLN, mesenteric lymph node; Myr, myristic acid; OA, oleic acid; Pal, palmitic acid; PBS, phosphate buffered saline; PUFA, polyunsaturated fatty acids; Ste, stearic acid; TBARS, thiobarbituric acid reactive substance; Toc, α-tocopherol; UFA, unsaturated fatty acids.

(Osaka). Fatty acids and AsA were dissolved in PBS (pH 7.4) and added to the medium of lymphocyte cultures. BHT and Toc were dissolved in DMSO and one-thousandth volume of the DMSO solution was added to the culture medium. For ELISA of rat Ig, 0.05% Tween 20 dissolved in PBS was used for rinsing, and Block Ace (Dainihon Pharmaceutical, Osaka) for blocking and antibody dilution, as described previously (*8, 9*).

Cells and Cell Culture—Rat MLN lymphocytes were prepared and cultured as described previously (*9*). In short, MLN was excised from 9- to 10-week-old male Sprague-Dawley rats under light diethyl ether anesthesia and lymphocytes were isolated by using Lympholyte-Rat (Cederlane, Hornby, Canada). The cells were cultured in the RPMI 1640 medium supplemented with 10% fetal bovine serum (Intergen, NY) and the Ig content of the culture supernatant was measured by ELISA (*13*), as described previously (*8, 9*). To estimate the oxidation rate of UFA, the amount of TBARS in the medium in which MLN lymphocytes had been cultured for 4 h in the presence of 1 mM UFA was measured using an assay kit (Test Wako Kit, Wako Pure Chemicals), according to the method recommended by the supplier.

Statistical Analyses—Data were analyzed by Duncan's new multiple range test to evaluate the significance of differences (*14*).

RESULTS

Time Course of Immunoglobulin Production by Rat Mesenteric Lymph Node Lymphocytes—When rat MLN lymphocytes were cultured in 10% FBS/RPMI 1640 medium, IgA was accumulated rapidly in the medium during the first 24 h and then reached a plateau (Fig. 1). The accumulation of IgG continued during the first 72 h at a rate slower than in the case of IgA. For IgE, the maximum level was attained within 4 h after inoculation and then the level decreased quickly. The accumulation of IgM proceeded rapidly during 12-72 h, after a lag period during the first 12 h, and reached a plateau thereafter. Thus, the cells were cultured for 4 h in the presence of food components to examine Ig production-regulating activities.

Effect of Unsaturated Fatty Acids on Immunoglobulin Production by Rat Mesenteric Lymph Node Lymphocytes—In a preliminary experiment, MLN lymphocytes were treated with three UFA (OA, LA, and AA) at concentrations of 0.1 μM to 1 mM for 4 h and the Ig contents of the culture supernatants were measured by ELISA. Their effect on Ig accumulation in the medium was very small at concentrations below 0.1 mM, but they strongly inhibited the accumulation of IgA, IgG, and IgM, and stimulated IgE accumulation at 1 mM (data not shown). Thus, the cells were treated with 1 mM UFA in triplicate. As shown in Fig. 2, these UFA significantly inhibited IgA, IgG, and IgM accumulation. The inhibitory effect on IgA and IgM accumulation was strongest with LA, while there was no difference among UFA in the effect on IgG accumulation. They significantly stimulated IgE accumulation and the magnitude of the stimulating effect was in the order of AA > LA > OA. This suggested that the IgE production-stimulating activities of UFA are related to the number of carbon atoms and/or double bonds.

Effect of Saturated Fatty Acids on Immunoglobulin Production by Rat Mesenteric Lymph Node Lymphocytes—To clarify whether the carbon number of UFA is related to Ig accumulation-regulating activity, the effect of saturated fatty acids with carbon numbers from 12 to 18 was examined. Since all the saturated fatty acid exerted strong toxicity at 1 mM and Ste exerted toxicity even at 100 μM (data not shown), their effects were compared at 10 μM. As shown in Fig. 3, all the fatty acids weakly inhibited IgA accumulation irrespective of carbon number, but showed no significant effect on the accumulation of IgE, IgG, and IgM.

Effect of Hydrogen Peroxide on Immunoglobulin Production by Rat Mesenteric Lymph Node Lymphocytes—UFA, in particular PUFA, are easily oxidized by molecular oxygen and active oxygens such as hydroxyl radical, hydroperoxide, and hydrogen peroxide are produced. Thus, the effect of hydrogen peroxide, the only active oxygen which can pass through the cell membrane, on Ig accumulation by MLN lymphocytes was examined. As shown in Fig. 4, hydrogen peroxide strongly inhibited the accumulation

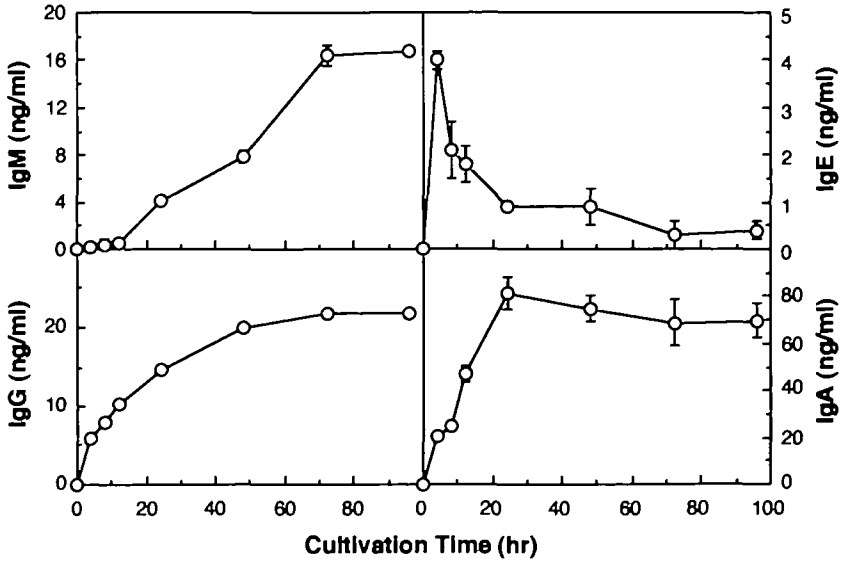

Fig. 1. **Time course of immunoglobulin production by rat mesenteric lymph node lymphocytes.** Cells (2×10^6 cell/ml) were cultured for 72 h and Ig contents of culture supernatants were measured by ELISA. Data are means ± SE of triplicate cultures.

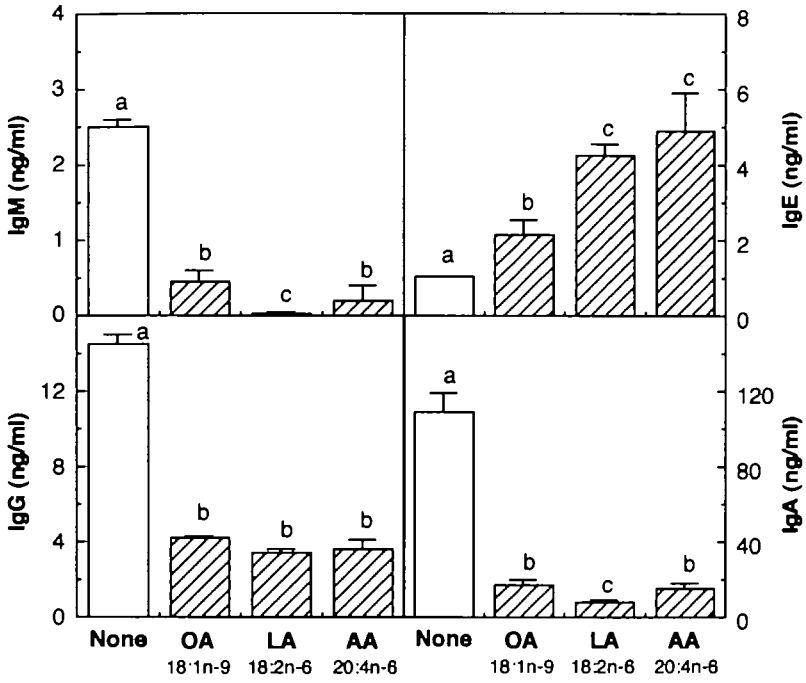

Fig. 2. **Effect of unsaturated fatty acids on immunoglobulin production by rat mesenteric lymph node lymphocytes.** Cells were cultured for 4 h in the presence of 1 mM OA, LA, or AA. Data are means±SE ($n=3$) and $^{a-c}$values not sharing a common letter are significantly different at $p<0.05$.

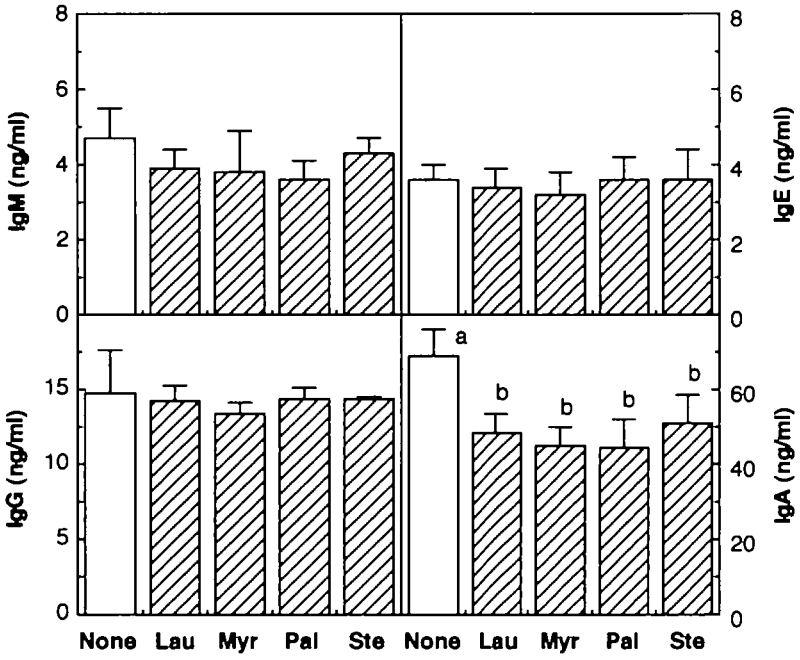

Fig. 3. **Effect of saturated fatty acids on immunoglobulin production by rat mesenteric lymph node lymphocytes.** Cells were cultured for 4 h in the presence of 10 μM Lau, Myr, Pal, or Ste. Data are means±SE ($n=3$) and ^{a,b}values not sharing a common letter are significantly different at $p<0.05$.

of IgA, IgG, and IgM at concentrations above 3 ppm. On the other hand, it stimulated IgE accumulation only at a very high concentration, 3,000 ppm. These results suggest that UFA enhance type I allergic response through the stimulation of IgE production and the inhibition of IgA production, and that their oxidation products might be partially responsible for the adverse reaction.

Effect of Antioxidants on Immunoglobulin Production-Regulating Activity of Arachidonic Acid—To examine whether oxidation products of UFA are responsible for the immunoregulatory activity, MLN lymphocytes were treated with 1 mM AA in the presence of antioxidants such as AsA, Toc, and BHT. In the case of hydrophilic AsA, cells were treated with 0.01 to 1 mM antioxidant. As shown in Fig. 5, AsA did not affect Ig production below 0.1 mM, but showed a strong inhibitory effect on IgA, IgG, and IgM accumulation at 1 mM. In contrast, AsA had no significant effect on IgE accumulation at the concentrations examined. Though AsA slightly weakened the inhibitory effect of AA on IgM accumulation, it did not modify the effect of AA on other Igs.

Hydrophobic Toc and BHT were dissolved in DMSO, and one-thousandth volume of the DMSO solution was added to the culture medium to avoid the undesirable effect of the

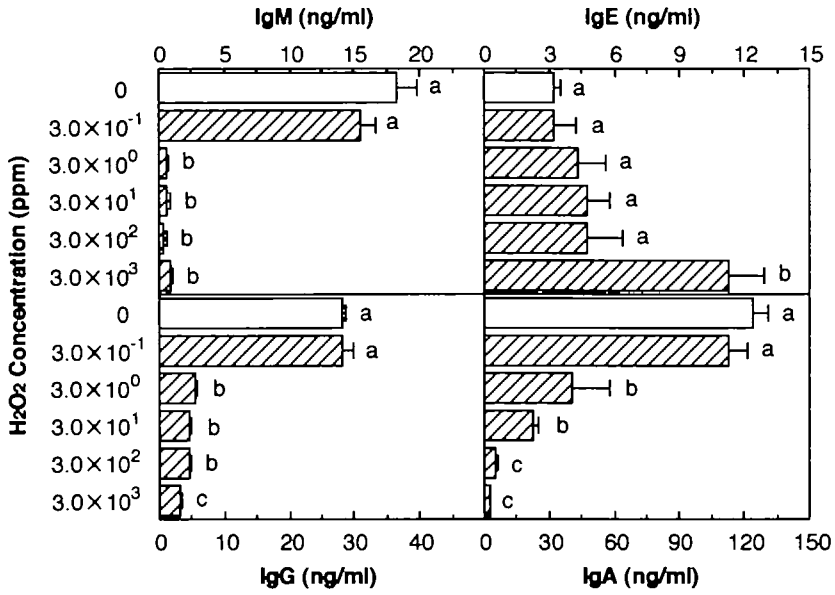

Fig. 4. **Effect of hydrogen peroxide on immunoglobulin production by rat mesenteric lymph node lymphocytes.** Cells were cultured for 4 h in the presence of various concentrations of hydrogen peroxide. Data are means±SE ($n=3$) and $^{a-c}$values not sharing a common letter are significantly different at $p<0.05$.

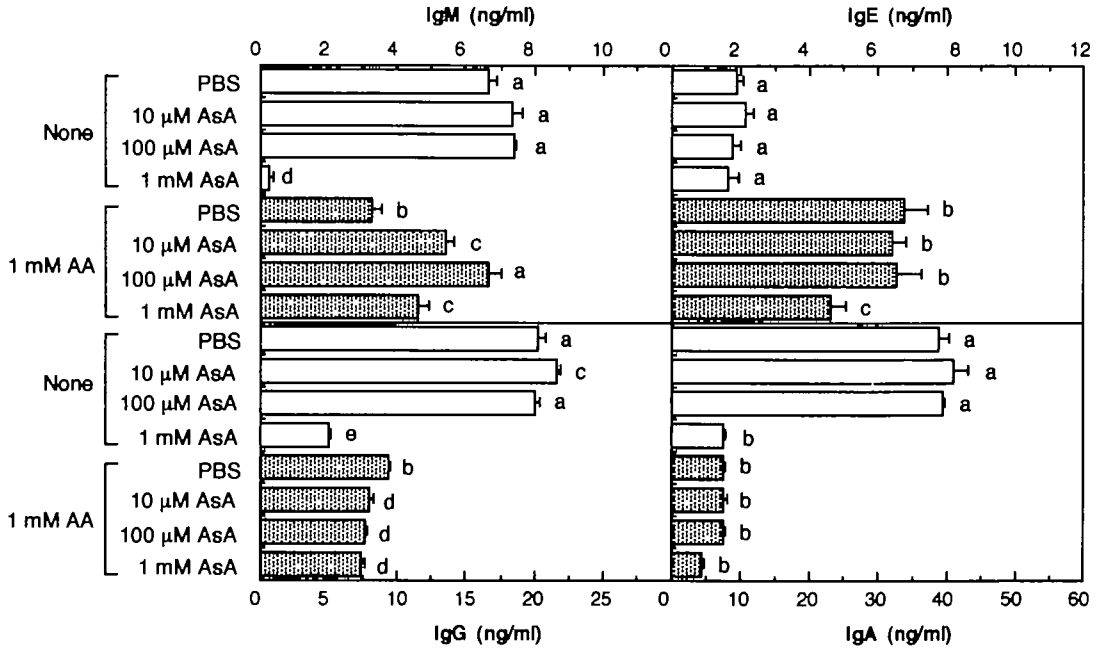

Fig. 5. **Effect of ascorbic acid on immunoglobulin production-regulating activity of arachidonic acid.** Rat MLN lymphocytes were cultured for 4 h in the presence of 0.01 to 1 mM AsA with (screened bars) or without (white bars) 1 mM AA. Data are means±SE ($n=3$) and $^{a-e}$values not sharing a common letter are significantly different at $p<0.05$.

solvent itself. As shown in Fig. 6, 0.1% DMSO slightly enhanced IgM accumulation, but did not affect accumulation of the other Igs. Because of the solubility of Toc in DMSO, the highest concentration adopted was 100 μM. Though Toc itself did not affect accumulation of any Ig, it completely annulled the IgE accumulation-stimulating activity of 1 mM AA even at a concentration as low as 1 μM. However, Toc could not annul the inhibitory effect of the UFA on the accumulation of IgA, IgG, and IgM. A synthetic antioxidant, BHT, also annulled the stimulatory effect of AA on IgE accumulation at the same concentration as observed with Toc, but it exerted weak IgA accumulation-inhibiting activity at 100 μM (data not shown). These results suggested that Toc is a more effective anti-allergic factor than BHT.

Relationship between IgE Production-Stimulating Activity and Oxidation Rate of Polyunsaturated Fatty Acids— UFA are classified into three groups, *n*-9, *n*-6, and *n*-3 series, depending on the position of the first double bond from the carboxyl end, and some of them have been reported to be anti-allergic (*29, 30*). However, all UFA enhanced IgE production of MLN lymphocytes and the enhancement was suppressed in the presence of Toc (Table I). The IgE production-enhancing activity of PUFA in-

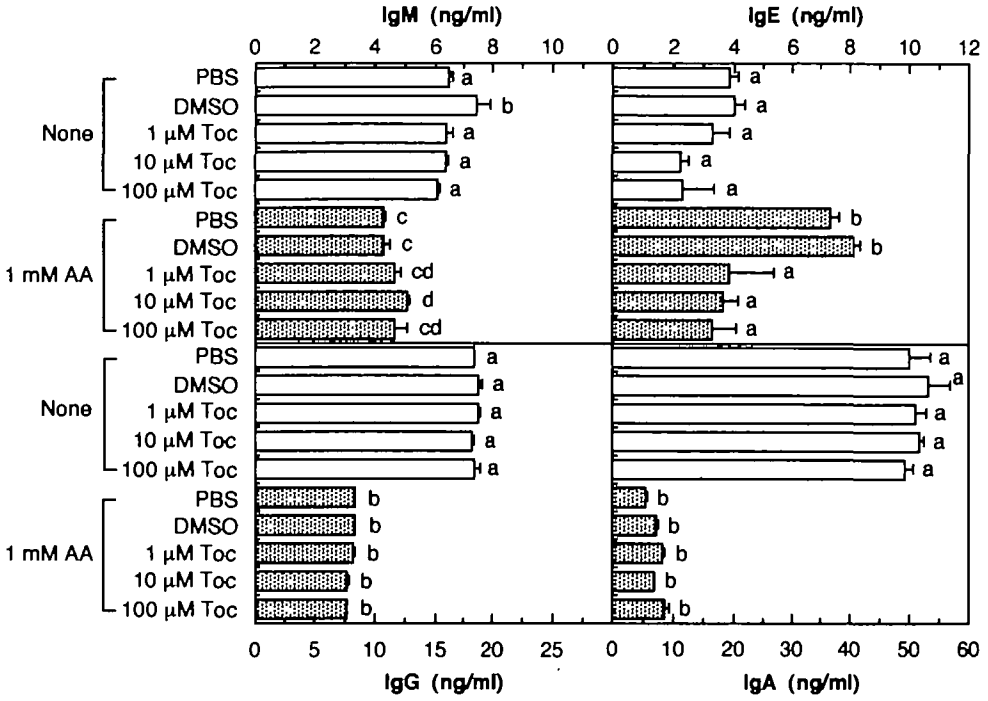

Fig. 6. **Effect of α-tocopherol on immunoglobulin production-regulating activity of arachidonic acid.** Rat MLN lymphocytes were cultured for 4 h in the presence of 1 to 100 μM Toc with (screened bars) or without (white bars) 1 mM AA. Data are means ± SE ($n=3$) and $^{a-d}$values not sharing a common letter are significantly different at $p<0.05$.

TABLE I. **Relationship between IgE production-stimulating activity and oxidation rate of unsaturated fatty acids.**

Fatty acid	IgE (ng/ml)		TBARS (nmol/ml)	
	Toc (−)	Toc (+)	Toc (−)	Toc (+)
None	0.23 ± 0.06^{a}	0.35 ± 0.07^{a}	0.45 ± 0.04^{a}	0.24 ± 0.01^{a}
OA (18:1*n*-9)	0.67 ± 0.05^{ab}	0.39 ± 0.04^{a}	0.73 ± 0.09^{a}	0.28 ± 0.02^{a}
LA (18:2*n*-6)	0.79 ± 0.13^{b}	0.84 ± 0.06^{ac}	2.14 ± 0.21^{b}	0.37 ± 0.04^{a}
ALA (18:3*n*-3)	1.60 ± 0.09^{c}	0.77 ± 0.27^{ac}	4.18 ± 0.52^{c}	0.89 ± 0.08^{ac}
GLA (18:3*n*-6)	4.90 ± 0.25^{d}	1.29 ± 0.32^{bc}	2.69 ± 0.11^{bf}	0.76 ± 0.15^{a}
AA (20:4*n*-6)	2.21 ± 0.18^{e}	0.81 ± 0.04^{ac}	5.52 ± 0.70^{d}	1.73 ± 0.36^{bc}
EPA (20:5*n*-3)	1.68 ± 0.12^{c}	0.44 ± 0.04^{a}	3.71 ± 0.08^{cf}	2.65 ± 0.63^{b}
DHA (22:6*n*-3)	4.73 ± 0.25^{d}	1.66 ± 0.16^{b}	8.86 ± 0.67^{e}	2.35 ± 0.44^{b}

MLN lymphocytes were cultured with 1 mM UFA for 4 h, in the presence or absence of 0.1 mM Toc, and culture supernatants were recovered to measure IgE and TBARS contents. Data are mean ± SE ($n=3$) and $^{a-f}$values not sharing a common superscript letter are significantly different at $p<0.05$.

creased in the order of OA<LA<ALA=EPA<AA<DHA=GLA. In the presence of UFA, an increase of TBARS level was also observed and the increase was inhibited by the addition of Toc simultaneously as well as the enhancement of IgE production. The TBARS value without Toc increased in the order of OA<LA<GLA<EPA<ALA<AA<DHA.

DISCUSSION

Allergies against foodstuffs or airborne allergens are mainly induced by the type I allergy system, in which induction of allergen-specific IgE is the key step (*1*). Most allergy patients exhibit a high serum IgE level (*15-17*). It has been shown that some biomaterials such as bile acids and lectins regulate IgE production of rat lymphocytes (*9*), in addition to lymphokines such as IL-4, IL-5, IFN-α, and IFN-γ (*2-6*). In the present study, we showed that UFA enhanced IgE production by MLN lymphocytes at 1 mM, the highest concentration at which no UFA exerted cytotoxicity. A similar enhancement of IgE production has been reported in rats fed a diet enriched in EPA (*18*). Thus, UFA may exert allergy-enhancing activity through the stimulation of IgE production.

Among the UFA used here, IgE production-stimulating activity became stronger with increase of the number of carbon atoms and/or double bonds. However, none of the saturated fatty acids with carbon numbers from 12 to 18 exerted IgE production-stimulating activity at 10 μM, the highest concentration at which no cytotoxicity was exerted by any of them. Double bonds in UFA are readily oxidized by molecular oxygen and produce various active oxygens, which exert various physiological effects (*19, 20*). In addition, there is evidence suggesting the participation of active oxygens in allergic diseases; enhancement of radical production by eosinophils or neutrophils (*21, 22*), increased serum level of lipid peroxidation product (*23*), and high breath hydrogen peroxide level (*24*). Thus, we examined the Ig production-regulating activity of hydrogen peroxide, the only active oxygen which can pass through the cell membrane, and found that IgE production by MLN lymphocytes was enhanced by hydrogen peroxide at 3,000 ppm (around 110 mM). Hydrogen peroxide has also been reported to enhance degranulation of rat peritoneal mast cells at 0.05 to 1 mM (*25, 26*) and that of RBL-2H3 cells at 2 to 20 mM (*27*). These results suggest that UFA and active oxygens enhance allergic responses by stimulating the production of IgE and release of chemical mediators.

Antioxidants such as AsA and Toc serve to prevent an adverse effect of active oxygens produced in foodstuffs. Among three antioxidants examined here, hydrophilic AsA could not annul the IgE production-stimulating activity of AA, but hydrophobic Toc and BHT could do so. This supports the above hypothesis that the oxidation products of UFA are partially responsible for the IgE production-

stimulating activity. Furthermore, the effectiveness of hydrophobic antioxidants in negating the IgE production-stimulating activity of UFA and the inability of a hydrophilic antioxidant to do so imply that the process proceeds in a hydrophobic environment such as the cell membrane, to which Toc and BHT have access.

In contrast to the enhancement of IgE production, UFA inhibited the production of the other antibodies, including IgA. Such reciprocal regulation of IgE and other Ig production is also observed with bile acids (*9*). These results suggest that the production of IgE is regulated in a different manner from that of other Igs. IgA plays an important role in the prevention of allergic reaction by inhibiting allergen absorption (*1*). Thus, it is important for allergy prevention to maintain IgA productivity at a sufficient level. Our results suggest that high concentrations of UFA act as allergy-enhancing factors, not only by IgE production stimulation, but also by IgA production inhibition, as observed with bile acids (*9*). The observation that antioxidants could not annul the IgA production-inhibiting activity, but did negate IgE production-stimulating activity of AA also supports the view that UFA regulate the production of IgE by a system different from that of other Igs, including IgA.

The above results suggest that the enhancement of IgE production by UFA is coupled with the oxidation of UFA in the culture medium. The oxidation rate of UFA, judged as TBARS value, showed an increasing tendency with increasing number of double bonds, though this was not entirely clear-cut. The IgE production-enhancing activity of UFA was more closely related to the TBARS value than to the number of double bonds. Therefore, the strength of the IgE production-enhancing activity of UFA was mainly related to their oxidation rates in the culture medium, which do not parallel the number of double bonds. In addition, other factors may affect the process, because the IgE production-enhancing activity and oxidation rate of UFA did not correlate precisely.

To prevent food allergies, removal of food allergens by excluding all foodstuffs containing the allergen(s) or by disruption of allergen(s) using proteases has been employed. Though allergen-specific digestion with a protease worked well in the preparation of low-allergenic rice (*28*), it is often difficult to destroy allergenicity without adversely affecting the nutritive value, taste and rheological properties of foods. In the present study, we showed that foods contain both allergy-enhancing and inhibiting factors, in addition to allergens. In this context, PUFA such as ALA, GLA, EPA, and DHA have been reported to be allergy-inhibiting factors (*29, 30*), but the exact mechanism by which they alleviate allergic response remains to be clarified. Reduction of allergy-enhancing factors and/or enrichment of allergy-inhibiting factors may provide a new approach to diminish the allergenicity of various foodstuffs. Clarification of the mechanism underlying modification of allergic reactions by food components and optimization of the intake of allergy-modifying factors should be helpful for alleviation of the allergenicity of conventional foods and prevention of incidents of allergic response.

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