Review

Structure-Activity Relationship of Immunoregulatory Factors in Foodstuffs

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Received February 12, 1998; Accepted April 1, 1998

The structure-activity relationship of allergy enhancing and suppressing factors in foodstuffs was summarized in this text. Polyunsaturated fatty acids (PUFA) enhance allergic response by enhancing IgE production and by suppressing production of other antibodies in rat lymphocytes. Because the expression of IgE production enhancing activity was inhibited by lipophilic antioxidants, lipid oxidation reaction seems to have participated in the activity. Xanthene dyes, a group of synthetic dyes, also exert IgE production enhancing effect, and the activity is dependent on the degree of halogenation. On the other hand, PUFA exert anti-allergic effect by suppressing the release of chemical mediators from rat peritoneal exudate cells, and the activity becomes stronger with the increase in the number of double bonds. In addition, antioxidants such as tea polyphenols (TP) and flavonoids exert anti-allergic effect mainly due to the suppression of chemical mediator release. Histamine release suppressing activity is observed in triphenol derivatives, but not in diphenols. On the contrary, LTB₄ release suppressing activity is observed in mono and diphenolic derivatives with antioxidative activity. These results suggest that various food components have immunoregulatory activity and that optimization of their intake is desirable.

Keywords: allergy, polyunsaturated fatty acids, xanthene dyes, tea polyphenols, flavonoids, IgE, histamine, LTB₄

Recently, there has been an increasing incidence of allergic disorders, in particular hypersensitivity to food or airborne antigens. Allergic reactions are usually classified into 4 types, and type I allergy plays an important role in the induction of allergies against these antigens (Metcalf, 1991). In this type of allergy, induction of allergen-specific IgE and release of chemical mediators such as histamine and leukotrienes (LT) from mast cells or basophils are critical steps. On the contrary, allergen-specific IgA suppresses the reaction through the inhibition of allergen absorption. In addition, allergen-specific IgG suppresses type I allergy through competition with IgE. Thus, class-specific regulation of immunoglobulin (Ig) production, such as inhibition of IgE production and stimulation of IgA or IgG production, may lead to the alleviation of allergic symptoms.

It has been reported that various food components affect the above immune functions and some of them exert anti-allergic effect. Elevation of the medium IgE level of rat lymphocytes is induced by bile acids (Lim *et al.*, 1994a, b), concanavalin A (Lim *et al.*, 1994b), unsaturated fatty acids (Yamada *et al.*, 1996a), and xanthene dyes (Kuramoto *et al.*, 1997). On the contrary, a decrease in IgE level is induced by various natural colorings (Kuramoto *et al.*, 1996) and the extracts of some vegetables (Kaku *et al.*, 1997) and teas (Yamada *et al.*, 1997). In addition, elevation of the IgA level is induced by triphenolic compounds such as gallic acid and epigallocatechin gallate (EGCG) (Yamada *et al.*, 1997). These findings suggest that foods contain both allergy-

enhancing and -suppressing factors. Control of these allergy-regulating factors makes it possible to produce anti-allergic foods which improve the symptoms of allergies, irrespective of the type of antigen.

In addition to the above Ig production regulating factors, chemical mediator release from mast cells or basophils is suppressed by various food components such as polyunsaturated fatty acids (PUFA) (Prescott, 1984; Thien *et al.*, 1993; Yamada *et al.*, 1996b), tea polyphenols (TP) (Matsuo *et al.*, 1996, 1997), flavonoids (Baumann *et al.*, 1980; Corvazier & Maclouf, 1985; Laughton *et al.*, 1991; Yamada *et al.*, 1996c, 1998) and some plant extract (Yamamoto *et al.*, 1993). It has also been reported that dietary enrichment of some PUFA affect chemical mediator production (Terano *et al.*, 1984; Lee *et al.*, 1985), and some dietary fats have been reported to improve allergic symptoms in allergic patients (Lovell *et al.*, 1981; Schalin-Karrila *et al.*, 1987). Thus, clarification of the mechanism by which they regulate allergic reaction may have great benefits in the prevention of allergies. We discuss here the structure-activity relationship of some immunoregulatory components present in foodstuffs.

Polyunsaturated Fatty Acids

PUFA regulate various types of biological functions including immune reactions. Arachidonic acid (AA, 20:4n-6) is a substrate of lipoxygenase and cyclooxygenase which generate 4-series LT and 2-series prostaglandins. Among them, 4-series LT such as LTB₄ and LTC₄ plays an essential

role in the induction of type I allergy. On the contrary, another n-6 γ -linolenic acid (GLA, 20:3n-6) has been reported to be anti-allergic (Lovell *et al.*, 1981; Schallin-Karrila *et al.*, 1987). In addition, eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids belonging to the n-3 series have been reported to inhibit the process of type I allergy (Prescott, 1984; Terano *et al.*, 1984; Lee *et al.*, 1985; Thien *et al.*, 1993).

The anti-allergic effect of PUFA is partly due to their inhibitory effect on the production of 4-series LT. As shown in Table 1, PUFA having more than 3 double bonds inhibit LTB₄ release from rat peritoneal exudate cells (PEC), and their inhibitory effect is increased with an increase in the number of double bonds (Yamada *et al.*, 1996b). In addition, orally administered EPA has been reported to suppress LTB₄ production and enhance LTB₅ production of leukocytes in rats (Terano *et al.*, 1984) and in humans (Thien *et al.*, 1993). LTB₅ which is generated from EPA can interfere with the production of 4-series LT. However, the effect of LTB₅ on the inhibition of LTB₄ production by rat PEC seems to be negligible, because DHA exert the highest inhibitory effect without LTB₅ production.

On the other hand, PUFA may enhance type I allergy by increasing the IgE production of B lymphocytes (Yamada *et al.*, 1996a; Hung *et al.*, 1997). PUFA increase the medium IgE level in the rat mesenteric lymph node (MLN) lymphocytes and decrease the level of IgA, IgG and IgM, as well as hydrogen peroxide which is produced after the oxidation of PUFA (Yamada *et al.*, 1996a). Because the increase in IgE level by PUFA is inhibited in the presence of a lipophilic antioxidant α -tocopherol but not in the presence of a hydrophilic antioxidant ascorbic acid, the oxidation of PUFA under lipophilic circumstances such as the cell membrane seems to be important in the stimulatory effect (Yamada *et al.*, 1996a). On the other hand, the inhibitory effect on the production of IgA, IgG and IgM could not be canceled in the presence of antioxidants. These results suggest that the production of IgE is regulated independently from the production of other Igs.

The increase in medium IgE level can be induced by n-3 PUFA as well as n-6 PUFA in rat MLN lymphocytes (Hung *et al.*, 1997). As shown in Fig. 1, their enhancing effect on the IgE level is almost parallel with the number of double bonds and their oxidation velocity in the culture medium, though there are some exceptions. Among them, EPA enhanced the level of IgE more mildly than the level expected from the number of double bonds, probably due to a slower oxidation rate than expected from the number of double bonds. In the case of GLA, it exerted a strong enhancing effect on the IgE level, though its oxidation velocity was much slower than that expected from the number of double bonds. In the case of splenocytes, n-6 PUFA enhanced the medium IgE level more markedly than n-3 PUFA. In addition, the oxidation velocities of AA and DHA were faster than those expected from the number of double bonds. In splenocytes, linoleic acid (LA, 18:2n-6) and GLA enhanced the IgE level more strongly than expected from their oxidation rate. These results suggest that the immunoregulatory effect of PUFA is highly structure-dependent.

Bile Acids

In jaundice patients, an elevation of serum bile acid and depression of the cellular immune system have been reported (Newble *et al.*, 1975; Lindblad *et al.*, 1977; Pennington *et al.*, 1977). Bile acids are synthesized from cholesterol in the liver and secreted into the duodenum in the taurine- or glycine-conjugated form to help the digestion and absorption of lipid components in foodstuffs. The majority of bile acids are reabsorbed from the ileum, and unabsorbed bile acids are de-conjugated and epimerized by intestinal bacteria. The de-conjugated bile acids exert stronger toxicity and suppress their IgM production more strongly than conjugated bile acids in human Namalwa cells (Lim *et al.*, 1994a).

In addition, de-conjugated bile acids inhibit IgG and IgM production of rat spleen lymphocytes more strongly than conjugated bile acids (Yamada *et al.*, 1993; Lim *et al.*, 1994a). Among free bile acids, deoxycholic (DCA) and chenodeoxycholic (CDCA) acids with two OH groups inhibit Ig production more strongly than cholic acid (CA) with three OH groups. These two dihydroxy bile acids are strong activators of neutrophils (Dahm *et al.*, 1988), and the glycine-conjugate of CDCA inhibits DNA synthesis by human lymphocytes more strongly than the glycine-conjugate of CA (Gianni *et al.*, 1980). These results suggest that dihydroxy bile acids exert stronger immunoregulatory effect than the trihydroxy derivatives.

The Ig production regulating activity of bile acid has also been reported in rat MLN lymphocytes which belong to the gut immune system (Lim *et al.*, 1994b). In this case, bile acids elevate the medium IgE level of the lymphocytes at concentrations over 3 mM in CDCA and 30 mM in CA and DCA. On the other hand, these bile acids exert a suppressive effect on IgA and IgG accumulation at the same doses. The increase in IgE level and the decrease in IgA and IgG levels may enhance the type I allergy reaction. Such class-specific regulation of Ig synthesis is induced by cytokines such as IL-4, IL-5, IFN- α , and IFN- γ (Pene *et al.*, 1988; Gauschat *et al.*, 1991; Ochel *et al.*, 1991; Rousset *et al.*, 1991). In addition to H₂O₂ and PUFA previously discussed, lectins regulate Ig production by MLN lymphocytes class-specifically (Lim *et al.*, 1994b). In this case, concanavalin A markedly elevated the medium IgE level of the lymphocytes, while pokeweed mitogen down-regulated the IgE level. These results suggest that Ig produc-

Table 1. Effect of unsaturated fatty acids on chemical mediator release from rat peritoneal exudate cells.

Fatty acids	Histamine release (%)	LTB ₄ release (ng/10 ⁶ cells)
None	78.3±0.7 ^a	27.6±2.3 ^{ab}
OA (18:1n-9)	77.8±1.4 ^b	22.2±0.9 ^{ae}
LA (18:2n-6)	86.1±0.3 ^b	30.8±2.4 ^b
ALA (18:3n-3)	92.9±3.5 ^{bc}	15.9±0.7 ^{ce}
GLA (18:3n-6)	89.0±2.4 ^{bc}	11.8±0.1 ^c
AA (20:4n-6)	89.5±4.5 ^{bc}	26.0±5.3 ^{ab}
EPA (20:5n-3)	94.4±1.4 ^c	4.1±0.1 ^d
DHA (22:6n-3)	94.6±1.1 ^c	Not detected ^d

Data are means±SE ($n=3$) and values without a common letter are significantly different at $p<0.05$. OA; oleic acid, LA; linoleic acid, ALA; α -linolenic acid, GLA; γ -linolenic acid, AA; arachidonic acid, EPA; eicosapentaenoic acid, DHA; docosahexaenoic acid.

tion of lymphocytes is subject to class-specific regulation by biological effectors including food components.

Food Colorings

Food additives have been suspected to be enhancers of allergies (Nish *et al.* 1991; Tarlo & Sussman, 1993; Weber, 1993; Wuhrich, 1993), but there is little evidence for the hypothesis. Thus, we examined the Ig production regulating activity of some food additives and found that xanthene dyes regulated Ig production by rat splenocytes class-specifically (Kuramoto *et al.*, 1997). In this case, halogenated dyes elevate medium IgE level, while they down-regulate the level of IgG and IgM. A similar reverse effect on the production of IgE

and other antibodies has been observed in bile acids (Lim *et al.*, 1994b), PUFA (Yamada *et al.*, 1996a), H₂O₂ (Yamada *et al.*, 1996a), and lectins (Lim *et al.*, 1994b), as previously described. These results mean that the production of IgE can be regulated independently from the production of other Igs.

Figure 2 shows the structure of xanthene dyes, and Table 2 shows their Ig production regulating activity. Among them, tetra-iodinated derivatives such as Rose Bengal and erythrosine elevated the IgE level most markedly, but the effect of diiodofluorescein was negligible (Kuramoto *et al.*, 1997). In addition, it was reported that iodotyrosines and thyroid hormones with 1 to 4 iodines exert no effect on the Ig production regulating activity (Kuramoto *et al.*, 1997). These results suggest that the activity of xanthene dyes is dependent on both basic structure and halogenation pattern. The enhancement of IgE level may deteriorate the symptoms of type I allergy, and the decrease in the IgG and IgM levels may injure defensive ability against various xenobiotics.

Fortunately, natural food colorings do not express such adverse effects. Most natural food colorings exerted the inverse effects, such as decrease in IgE level and increase in IgM level (Kuramoto *et al.*, 1996). Among them, curcumin has been reported to exert immunoregulatory (Sui *et al.*, 1993) and lipid metabolism regulating (Fujiyama-Fujiwara *et al.*, 1992) activities. The inhibition of $\Delta 5$ -desaturation by

Table 2. Effect of xanthene dyes on immunoglobulin production by rat spleen lymphocytes

	IgE (ng/ml)	IgG (ng/ml)	IgM (ng/ml)
None	2.7±0.3 ^a	49.3±0.8 ^a	193±4 ^a
Acid red	3.0±0.2 ^a	47.1±1.3 ^a	179±6 ^a
Phloxine	7.9±0.1 ^b	37.1±0.5 ^b	185±8 ^a
Erythrosine	9.2±0.1 ^c	38.4±1.5 ^b	182±2 ^a
Rose Bengal	11.3±0.3 ^d	24.9±0.3 ^c	140±4 ^b

Data are means±SE (*n*=3) and values without a common letter are significantly different at *p*<0.05.

Fig. 3. Structure of flavonoids and tea polyphenols.

Fig. 4. Effect of tea polyphenols on histamine and LTB₄ release from rat peritoneal exudate cells stimulated with calcium ionophore A23187. Peritoneal exudate cells isolated from Wistar rats were stimulated with 5 μ M A23187 in the presence of 100 μ M tea polyphenols. Data are means $\pm$ SE ($n=3$), and values without a common letter are significantly different at $p<0.05$.

curcumin reported by Fujiyama-Fujiwara *et al.* (1992) suggests that it alleviates allergic symptoms by inhibiting the production of AA, the starting PUFA for 4-series LT synthesis.

Tea Polyphenols

Green tea contains polyphenols such as epicatechin (EC), epicatechin gallate (ECg), epigallocatechin (EGC), and epigallocatechin gallate (EGCg). As shown in Fig. 3, epicatechin derivatives have diphenolic B ring and epigallocatechins have triphenolic B ring. These TP have been reported to exert diverse biological effects, such as anti-viral (Nakayama *et al.*, 1990), anti-mutagenic (Shimoi *et al.*, 1986), anti-cancer (Fujita *et al.*, 1989; Agarwal *et al.*, 1992; Taniguchi *et al.*, 1992; Mitsui *et al.*, 1995), and anti-allergic (Matsuo *et al.*, 1996, 1997; Yamada *et al.*, 1997) activities.

When rat PEC were stimulated with a calcium ionophore A23187 in the presence of these TP, those having the triphenol structure inhibited the release of histamine and LTB₄, but EC without the triphenol group did not, as shown in Fig. 4 (Matsuo *et al.*, 1996). Among them, EGCg with two polyphenol groups exerts the greatest inhibitory effect. In the case of other phenolic compounds, triphenolic pyrogallol (PG) and gallic acid (GA) inhibit histamine release from rat basophilic leukemia RBL-2H3 cells stimulated with A23187, but diphenolic catechol (CA), resorcinol (RE), and hydroquinone (HQ) did not, as shown in Table 3 (Matsuo *et al.*, 1997). These results suggest that the triphenol structure plays an important role in the inhibition of histamine release.

In the case of Ig production, PG, GA and EGCg enhance the medium IgA level of cultured rat splenocytes, but the activity of diphenols is negligible (Yamada *et al.*, 1997). Hu *et al.* (1992) also showed that triphenolic TP exert strong mitogenic activity on B cells, but not the diphenolic counterparts. In addition, EGC and EGCg exert selective toxicity against E1A-3Y1 cells, a rat 3Y1 fibroblast line transformed by the E1A gene of human oncogenic adenovirus, but EC does not (Mitsui *et al.*, 1995). It is also reported that EGCg exerts stronger bacteriocidal (Hattori *et al.*, 1990) and lipid bilayer damaging activities than EC (Ikigai *et al.*, 1993). These results suggest that the triphenolic group modifies various cellular activities more strongly than the diphenol group, probably through its stronger interaction with the cell

Table 3. Effect of phenolic compounds on chemical mediator release from rat peritoneal exudate cells.

	R1	R2	R3	R4	R5	R6	Relative histamine release (%)	Relative LTB ₄ release (%)
None							100 $\pm$ 4 ^a	100 $\pm$ 4 ^a
CA	OH	OH	H	H	H	H	102 $\pm$ 1 ^a	27 $\pm$ 6 ^b
RE	OH	H	OH	H	H	H	134 $\pm$ 2 ^b	92 $\pm$ 12 ^a
HQ	OH	H	H	OH	H	H	121 $\pm$ 2 ^c	45 $\pm$ 4 ^b
PG	OH	OH	OH	H	H	H	73 $\pm$ 1 ^d	22 $\pm$ 2 ^b
SA	COOH	OH	H	H	H	H	116 $\pm$ 8 ^c	94 $\pm$ 13 ^a
PA	COOH	H	OH	OH	H	H	125 $\pm$ 2 ^{bc}	76 $\pm$ 5 ^a
GA	COOH	H	OH	OH	OH	H	87 $\pm$ 3 ^e	99 $\pm$ 12 ^a

Data are means $\pm$ SE ($n=3$) and values without a common letter are significantly different at $p<0.05$. CA; catechol, RE; resorcinol, HQ; hydroquinone, PG; pyrogallol, SA; salicylic acid, PA; protocatechuic acid, GA; gallic acid.

membrane.

In the case of LTB₄ release, a different structure-activity relationship has been observed, as shown in Table 3. Diphenols with a high radical scavenging activity, such as CA and HQ, exert LTB₄ release inhibitory activity as well as triphenolic PG, but *m*-diphenolic RE with a low radical scavenging activity does not inhibit LTB₄ release from rat PEC stimulated with A23187 (Yamada *et al.*, 1998). This suggests that the antioxidative effect of phenolic compounds is essential for the inhibition of LTB₄ release.

Table 3 also shows that carboxylated compounds such as GA and protocatechuic acid (PA) do not inhibit LTB₄ release, though these compounds exert high radical scavenging activity (Yamada *et al.*, 1998). Kohiyama *et al.* (1997) also reported that carboxylated diphenols strongly inhibited lipoxygenase reaction in a cell free system, but not in intact rat platelet. In addition, carboxylated catecholamine does not exert cellular DNA breaking activity, though it exerts DNA breaking activity *in vitro* as strong as other noncarboxylated compounds which induce cellular DNA breakage (Yamada *et al.*, 1985). The addition of a highly hydrophilic carboxyl group may reduce the membrane permeability of phenolic compounds. The inability of carboxylated compounds in the inhibition of LTB₄ release suggests that incorporation of phenolic compounds into the cells is essential for the process.

Table 4. Effect of flavonoids on chemical mediator release from rat peritoneal exudate cells.

Flavonoids	Relative histamine release (%)	Relative LTB ₄ release (%)
None	100±4 ^a	100±4 ^a
Kaempferol	122±6 ^b	3±2 ^b
Quercetin	81±3 ^c	Not detected ^b
Myricetin	52±1 ^d	12±0 ^b
Luteolin	Not tested	Not detected ^b
Cyanidin	Not tested	67±7 ^d

Data are means±SE (*n*=3) and values without a common letter are significantly different at *p*<0.05.

Flavonoids

Flavonoids are another group of natural phenolic antioxidants which have been reported to inhibit chemical mediator release (Baumann *et al.*, 1980; Torel *et al.*, 1986; Husain *et al.*, 1987; Afanas'ev *et al.*, 1989). Among them, flavonols have an *m*-diphenolic A ring and a B ring having various numbers of OH groups, as shown in Fig. 3. When rat PEC were stimulated with A23187 in the presence of flavonols with mono-, di-, or tri-phenolic B ring, all compounds strongly inhibited LTB₄ release from the rat PEC stimulated with A23187, as shown in Table 4 (Yamada *et al.*, 1996c, 1998). Thus, some structure other than the B ring may have participated in the inhibition of LTB₄ release.

Among flavonoids with the diphenolic B ring, luteolin (flavone) and quercetin (flavonol) exert much stronger inhibitory effect than cyanidin (anthocyanidin) (Yamada *et al.*, 1996c). The only structural difference between these compounds is in the C ring. Flavonols have C3-OH and C4-carbonyl groups, flavones have C4-carbonyl but not C3-OH, and anthocyanidins have C3-OH but not C4-carbonyl groups. These results suggest that the C4-carbonyl group plays an important role in the inhibition of LTB₄ release. In addition, the activities of quercetin derivatives glycosylated at the C3-position are much weaker than those of quercetin (Yamada *et al.*, 1996c). This suggests that the C3-OH group is partly contributed to the inhibitory effect of flavonols. In conclusion, the inhibition of LTB₄ release by flavonols may be due to at least two structural groups: *o*-diphenolic or triphenolic structure in the B ring and the C4-carbonyl group conjugated with OH groups at the C3 or A5 position.

Dietary Effect

The previous results were obtained from *in vitro* assay systems including those using mammalian cells. However, the activity observed on the cellular level may not be always reproducible in animal experiments. Thus, feeding experiments are essential for the estimation of the anti-allergic effect of food components before its clinical application. In the case of PUFA, dietary EPA and/or DHA have been reported to affect eicosanoid production (Lee *et al.*, 1985; Terano *et al.*, 1984; Thien *et al.*, 1993). In the case of phenolic compounds, dietary TP has been reported to affect the development of various diseases (Gao *et al.*, 1994).

When rats were fed three different types of fats, safflower oil (SA) rich in LA, perilla oil (PE) rich in ALA, or palm oil (PA) rich in saturated palmitic acid and monounsaturated

oleic acid (OA, 18:1n-9), for three weeks at the 10% level, a decrease in the LTB₄ releasing activity in PEC was observed only in the rats fed PE, accompanied with a significant decrease in the proportion of AA in the total lipid fraction of the PEC (Yamada *et al.*, 1998). This suggests that the n-3 PUFA in PE decrease the production of 4-series LT by decreasing the level of AA.

On the contrary, TP decreased the LTB₄ releasing activity of PEC in all dietary fat groups without a decrease in AA content. However, TP feeding did not affect the histamine releasing activity of PEC. It has been reported that orally administered catechin enhances the blood phenol level in humans (Das, 1971). In addition, many reports on the physiological effects of dietary TP (Stensvold *et al.*, 1992; Gao *et al.*, 1994) support that some amount of orally administered TP can be absorbed from the gut and exert their diverse physiological activities. Thus, the decrease in the LTB₄ releasing activity of rat PEC may be due to the TP incorporated into PEC. Their inability to the histamine releasing activity suggests that TP suppress histamine release from the cells through the interaction between extracellular TP and cell membrane.

These results suggest that dietary fats and TP affect the immune reactions through different mechanism. In addition, it has been reported that dietary α -tocopherol and sesamin could not exert a significant effect on the LTC₄ productivity of rat lung tissue in individual feeding, but simultaneous feeding dramatically decreased LTC₄ productivity in the lung in a tissue-specific manner (Gu *et al.*, 1994, 1995). These results suggest that the synergism of food components should be clarified to construct designed foods with multifunctional activities, including the anti-allergic effect.

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