Dietary Effect of EPA-rich and DHA-rich Fish Oils on the Immune Function of Sprague-Dawley Rats

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The dietary effect of fish oils (FOs) rich in eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) on the immune function of Sprague-Dawley rats was compared with that of safflower oil. After 3 weeks of feeding at the 10% level of a dietary fat, the IgG and IgM production by splenocytes and IgG production by mesenteric lymph node (MLN) lymphocytes were significantly higher in the FO-fed rats, while no significant difference was found in IgA or IgE productivity by both the spleen and MLN lymphocytes. In the FO-fed rats, peritoneal exudate cells released a lower amount of LTB4, reflecting their lower arachidonic acid level, and a higher amount of LTB4, reflecting their higher EPA level in phospholipids. On these EPA-rich FO exerted a stronger effect than DHA-rich FO immune functions.

Key words: fish oil; fatty acid; histamine; immunoglobulin; leukotriene

Allergic reactions are usually classified into 4 types,1) of which the type I allergy plays an important role in the occurrence of allergies against food components and airborne antigens.2) With this type of allergy, the induction of allergen-specific IgE and release of such chemical mediators as histamine or LT are essential steps. On the other hand, allergen-specific IgA inhibits the reaction by inhibiting allergen absorption in the gut, and IgG, through the competition with IgE. Thus, regulation of Ig production and chemical mediator release may alleviate the type I allergic reaction.

In fact, various food components have been reported to affect the above reactions. Enhancement of IgE production and inhibition of other Ig production by rat MLN lymphocytes have been reported for bile acids and lectins,3) polyunsaturated fatty acids (PUFAs),4,5) hydrogen peroxide,6) and some food colorings.6) The IgE production-enhancing activity of PUFAs becomes stronger with the increasing number of double bonds,5) while some antioxidants have been reported to exert an anti-allergic effect.4,7–9) For example, α-tocopherol inhibited the expression of IgE production-enhancing activity of PUFA and epigallocatechin gallate (EGCG) enhanced IgA productivity7) in rat MLN lymphocytes. In addition, the Ig production-regulating activity of tocopherol has also been reported in feeding experiments on Brown Norway and Sprague-Dawley rats.9) These results suggest that the Ig production-regulating activity of PUFAs is may be related to their oxidation.

In addition, chemical mediator release from mast cells or basophils was suppressed by various food components such as PUFAs,10–12) tea polyphenols13) and flavonoids.14–16) PUFAs with more than 3 double bonds inhibited LTB4 release from rat PEC, the inhibitory effect becoming stronger with increasing number of double bonds.12) In addition, dietary EPA and/or DHA have been reported to affect LT productivity by rat leukocytes15) and human neutrophils.16)

These findings suggest that these PUFAs could serve as effective anti-allergic components in foodstuffs, although they enhanced IgE production by rat lymphocytes in vitro.4,5) In the present study, we compared the effect of two FO preparations with different EPA and DHA contents on Ig productivity by lymphocytes and LT productivity by PEC with Sprague-Dawley rats to clarify which PUFA was the more effective anti-allergic agent in feeding experiments. In addition, we study here the role of lipid peroxidation in expressing the immunoregulatory function of these PUFAs.

Materials and Methods

Materials. SAF was donated by Rinoru Oil Mill Co. (Nagoya, Japan), and EPA- and DHA-rich FOs were prepared by Sagami Chemical Research Center (Sagamihara, Japan). EPA-rich FO contained 1.6% LA, 31.8% EPA and 13.1% DHA, while DHA-rich FO contained 2.7% LA, 7.0% EPA and 26.7% DHA. To
avoid any LA shortage, the original fish oils were each mixed with SAF. The final compositions of LA, EPA and DHA, and the n-3/n-6 ratio in the SAF diet, respectively, were: 76.9, 0, 0, and 384.5; in the EPA-rich diet were: 18.9, 24.5, 10.1, and 0.6; and in the DHA-rich diet were: 13.9, 7.0, 22.7, and 0.6.

A thiobarbituric acid test was purchased from Wako Pure Chemical Industries (Osaka, Japan), while mouse anti-rat CD4-FITC and CD2-RPE were purchased from Serotec (Oxford, England).

Animals and diets. Four-week-old male Sprague-Dawley rats were obtained from Seac Yoshitomi (Yoshitomi, Japan) and housed individually in a room with a controlled temperature of 20–23°C and light cycle of 08.00 to 20.00 h. After acclimatizing for 7 days, the animals were divided into 3 groups of ten rats each and free access was provided to the experimental diets and to deionized water. The diets were prepared according to the recommendation of the American Institute of Nutrition AIN-93G, and contained 37.9% cornstarch, 20% casein, 13.2% α-cornstarch, 10% sucrose, 10% dietary fats, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 0.3% L-cystine, 0.25% cholesterol bitartrate, and 0.0014% tert-butylhydroquinone. Fresh diets were prepared each day and fed during 16.00 to 10.00 h to minimize lipid oxidation. After 3 weeks of feeding, 5 rats in each group were killed by withdrawing blood from the abdominal aorta under diethyl ether anesthesia. The liver and spleen of each were immediately excised and weighed. Lymphocytes were isolated from the spleen and MLN lymphocytes were isolated using Lympholyte-rat (Cedarlane, Hornby, Canada) as described previously. The lymphocytes were cultured for 24 hr in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), and Ig the content in the culture medium was measured by ELISA as described previously.

Measurement of Ig productivity. MLN and spleen lymphocytes were isolated by using Lympholyte-rat (Cedarlane, Hornby, Canada) as described previously. The lymphocytes were cultured for 24 hr in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), and Ig the content in the culture medium was measured by ELISA as described previously.

Flow cytometry. The spleen lymphocytes were prepared as described previously, and cell concentration was adjusted to 2 × 10⁶ cells/100 µl, before 5 µl of either mouse anti-rat CD4-FITC or mouse anti-rat CD2-RPE was added to label surface antigen molecules CD4 and CD2, respectively. After rinsing and fixing with 2% para-formaldehyde, the cells were subsequently analyzed for surface phenotype by an Epics Profile II flow cytometer (Coulter Corp., Hialeah, FL, U.S.A.).

Measurement of chemical mediator-releasing activity. PEC were stimulated with calcium ionophore A23187 (Sigma Chemicals, St. Louis, MO, U.S.A.) as described previously. The amount of histamine released from PEC was measured according to the fluorometric assay of Shore et al., with slight modifications. LTB₄ and LTB₅ were measured by the method of Powell et al., using high-performance liquid chromatography (HPLC), with slight modifications.

Lipid analysis. Tissue and serum lipids were extracted by the method of Folch et al., PC and PE were separated by thin-layer chromatography, and the fatty acid composition of the lipid fractions was analyzed by gas chromatography with a SILAR 10C column.

Statistics. Data were analyzed by Duncan's new multiple-range test to evaluate the significance of differences.

Results

Effect on serum TBARS and Ig levels

Table 1 shows the dietary effect of FOs on the growth of the rats. After 3 weeks of feeding, there was no significant difference in weight gain, food intake or food efficiency. Among the groups, no significant difference was apparent in the serum TBARS, IgA, IgE or IgG levels (Table 2). On the other hand, the IgM level was significantly higher in the rats fed with EPA- or DHA-rich FO than in those fed with SAF.

Effect on Ig productivity by spleen and MLN lymphocytes and on the splenic T-cell population

The foregoing result suggests that dietary fats affected the Ig productivity by rat lymphocytes. Thus, the effect of FOs on Ig productivity by the spleen and MLN lymphocytes was examined. As shown in Fig. 1, IgA productivity by the spleen lymphocytes tended to be lower in the rats fed with FOs than in those fed with SAF, especially in the rats fed with EPA-rich FO. IgE productivity was significantly higher in the rats fed with EPA-rich FO than in those fed with DHA-rich FO, and was intermedi-

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Body weight (g)</th>
<th>Total food intake (g/20 days)</th>
<th>Food efficiency (g/g) (weight gain/food intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Weight gain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAF</td>
<td>154 ± 5</td>
<td>160 ± 8</td>
<td>441 ± 12</td>
</tr>
<tr>
<td>EPA-rich</td>
<td>156 ± 3</td>
<td>151 ± 8</td>
<td>420 ± 8</td>
</tr>
<tr>
<td>DHA-rich</td>
<td>153 ± 3</td>
<td>155 ± 7</td>
<td>426 ± 11</td>
</tr>
</tbody>
</table>

Each data value is expressed as the mean ± SE (n = 10).

Table 2. Effect of Dietary Fats on Serum TBARS and Ig Levels

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>FTBARS (ng/ml)</th>
<th>IgA (µg/ml)</th>
<th>IgE (µg/ml)</th>
<th>IgG (mg/ml)</th>
<th>IgM (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAF</td>
<td>1.7 ± 0.2</td>
<td>68 ± 16</td>
<td>5.3 ± 4.0</td>
<td>6.6 ± 1.3</td>
<td>175 ± 9*</td>
</tr>
<tr>
<td>EPA-rich</td>
<td>1.5 ± 0.2</td>
<td>52 ± 6</td>
<td>4.9 ± 2.1</td>
<td>7.1 ± 1.2</td>
<td>201 ± 7b</td>
</tr>
<tr>
<td>DHA-rich</td>
<td>2.1 ± 0.3</td>
<td>79 ± 10</td>
<td>2.1 ± 1.4</td>
<td>7.7 ± 1.0</td>
<td>226 ± 9b</td>
</tr>
</tbody>
</table>

Each data value is expressed as the mean ± SE (n = 5). Values not sharing a common superscript letter are significantly different at p < 0.05.
ate in the SAF-fed rats. On the contrary, IgG and IgM productivity by the spleen lymphocytes was significantly higher in the rats fed with FOs than in those fed with SAF. The increase in IgG and IgM productivity was significantly higher in the rats fed with DHA-rich FO than in those fed with EPA-rich FO. For the MLN lymphocytes, a similar tendency with IgA and IgE productivity was observed in the rats fed with EPA- or DHA-rich FO (Fig. 2), although there was no significant difference in IgE productivity. IgG productivity was highest in the rats fed with EPA-rich FO and lowest in those fed with DHA-rich FO. Differences in these groups were significant. In addition, IgM productivity tended to be lower in the rats fed with FOs than in those fed with SAF.

To clarify the effect of dietary fats on Ig productivity by spleen lymphocytes, a T cell subset analysis was executed. As shown in Table 3, there was no significant difference in the proportion of T-cell subsets nor in the ratio of CD4⁺/CD8⁺.

**Effect on the chemical mediator-releasing activity and fatty acid composition of PEC**

Table 4 shows the effect of dietary fats on the histamine-releasing activity of PEC. The histamine content of the cells was significantly lower in the rats fed with FOs, especially in those fed with EPA-rich FO. When the cells were incubated for 20 min in the absence of A23187, a fairly large amount of histamine leaked. This spontaneous leakage (without stimulation by A23187) was in the order of DHA-rich, SAF and EPA-rich, and the difference among the groups was significant. When the cells were stimulated with A23187, the amount of histamine released into the medium was almost doubled in the cells of the SAF group, while the magnitude of the increase of histamine release was small in the cells isolat-
ed from the rats fed with FOs. On the contrary, the release rate, the ratio of (A23187-stimulated release—spontaneous release)/(total histamine content—spontaneous release) of histamine tended to be higher in the rats fed with FOs, a significant difference being observed in EPA-rich FO-fed rats.

A significant decrease of LTB₄-releasing activity was observed in PEC isolated from rats fed with FOs, especially with EPA-rich FO (Fig. 3). LTB₄ synthesized from EPA was detected only in the FO-fed groups, the amount of LTB₄ being much higher in the EPA-rich FO group than in the DHA-rich FO group.

To clarify the cause of the change in LT-producing activity, the fatty acid composition of PEC total lipid was measured. As shown in Table 5, the proportion of arachidonic acid (AA) was significantly lower in the rats fed with FOs, especially in the rats fed with EPA-rich FO. In addition, EPA was detected only in the rats fed with FOs, the level being dependent on the dietary content. A similar result was obtained for liver PC and PE, although the level of AA was much higher in liver phospholipids than in the PEC total lipids, while the EPA level between PEC and liver was comparable in these samples. In contrast, the level of DHA was much higher in the liver phospholipids, especially for PE, than in PEC.

**Discussion**

EPA and DHA have been reported to exert immunoregulatory activity. Many researchers have reported that these PUFAs suppressed the proliferation of lymphocytes stimulated with lectins in rats, mice, and humans. In addition, EPA and DHA regulate Ig production by rat lymphocytes. Lim et al. have reported that α-linolenic acid, EPA and DHA suppressed IgA, IgG and IgM production by spleen lymphocytes in Sprague-Dawley rats. In addition to the inhibition of Ig production, all PUFAs exerted IgE production-stimulating activity by rat spleen and MLN lymphocytes. Although anti-allergic effects have been reported for some PUFAs such as α-linolenic acid, such stimulation of IgE production and inhibition of IgA and IgG production may enhance the type I allergic reaction. Since the IgE production-enhancing activity of PUFAs became stronger with increasing number of double bonds and was weakened in the presence of antioxidants, lipid peroxidation would seem to participate in the reaction. Thus, we compared the dietary effect of FOs rich in EPA (20:5n-3) or DHA (22:6n-3) with SAF rich in LA (18:2n-6) on serum TBARS and Ig levels, as well as on Ig productivity by spleen and MLN lymphocytes.

We added SAF and two FOs at the 10% level. Despite the abnormally high n-3 PUFA content, there was no significant difference in serum TBARS and IgE levels between the SAF and FO groups. In addition, the IgE level in culture supernatants of both spleen and MLN lymphocytes isolated from the rats that had been fed with FOs was not significantly different from the SAF-fed rats. Thus, it appears that EPA and DHA may not enhance IgE productivity by the lymphocytes or serum IgE level, even in fairly high doses.

FOs tended to exert decreased IgA productivity by both spleen and MLN lymphocytes, although there was no decrease in the serum IgA level. In addition, FOs significantly enhanced the serum IgM level and the productivity of IgG and IgM by spleen lymphocytes compared with the effect of SAF. The magnitude of IgA and IgM increases was higher in the DHA-rich FO group than the EPA-rich FO group. The serum IgG level was also increased in the order of DHA>EPA>SAF, although the difference was not significant. These results suggest that FOs, especially DHA-rich FO, stimulate the systemic immune system by enhancing IgG and IgM productivity by splenocytes. However, decreased IgA productivity, especially in the gut immune system, may weaken the biodefensive ability, since secretory IgA inhibited the intrusion of such xenobiotics as allergens, viruses and bacteria. Thus, an excessive intake of FOs

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**Table 5. Effect of Dietary Fats on the Fatty Acid Composition of PEC Total Lipids and Liver Phospholipids**

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Fatty acid composition (%)</th>
<th>PEC</th>
<th>Liver PC</th>
<th>Liver PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2n-6 (LA)</td>
<td>20:4n-6 (AA)</td>
<td>20:5n-3 (EPA)</td>
<td>22:6n-3 (DHA)</td>
<td>n-6/n-3 ratio</td>
</tr>
<tr>
<td>SAF</td>
<td>7.4±0.1₁ 12.7±0.2₁ 0.9±0.1₁ 33.7±2.1₁</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA-rich</td>
<td>7.0±0.1₁ 5.2±0.4₁ 5.9±0.4₁ 3.5±0.2₁ 0.8±0.0₁</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHA-rich</td>
<td>5.0±0.1₁ 9.0±0.6₁ 2.0±0.1₁ 6.5±0.3₁ 1.5±0.0₁</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| SAF           | 10.8±0.1₁ 35.4±0.9₁ 0.1±0.0₁ 3.4±0.1₁ 13.8±0.6₁ |       |       |       |
| EPA-rich      | 10.1±0.4₁ 19.8±0.6₁ 4.9±0.3₁ 11.4±0.3₁ 1.7±0.1₁ |       |       |       |
| DHA-rich      | 7.2±0.7₁ 25.7±0.4₁ 1.5±0.1₁ 12.8±0.6₁ 2.3±0.1₁ |       |       |       |

| SAF           | 5.4±0.7 33.2±1.0₁ |       |       |
| EPA-rich      | 4.8±0.4 4.4±0.5₁ 7.0±0.4₁ 25.9±0.3₁ 0.6±0.0₁ |       |       |
| DHA-rich      | 4.2±1.2 19.6±0.8₁ 2.2±0.2₁ 26.0±2.6₁ 0.9±0.1₁ |       |       |

Each data value is expressed as the mean±SE (n=5). Values not sharing a common superscript letter are significantly different at p<0.05. n.d.: not detected.
should be avoided to maintain the gut immune system at an appropriate level.

FO feeding affected the chemical mediator-releasing activity of PEC more dramatically. In the rats fed with FOs, the intracellular histamine level was significantly lower than in those fed with SAF, but intracellular histamine leaked more extensively in the rats fed with DHA-rich FO. Since the response to A23187 stimulation was less marked with FOs than with SAF, it seems likely that n-6 and n-3 PUFAs affected the degree of degradation differently. Dietary EPA and DHA are easily incorporated into lipid fractions and increase membrane fluidity. We also found that the EPA and DHA contents were increased, even in PEC after feeding FOs. The lower histamine content and leakage of histamine from PEC may have been due to an increase in membrane fluidity.

The dietary enrichment of n-3 PUFA usually leads to a decrease in AA, the starting material for 4-series LT synthesis that induces the type I allergy. The decrease in AA may reduce LTB4 and LTC4 release, since substrate availability is a crucial factor regulating eicosanoid production. In the PEC total lipids, the AA content was 12.7% in the SAF group, 5.2% in the EPA group and 9.0% in the DHA group (100:41:71, for SAF, EPA and DHA, respectively). On the other hand, the effect of dietary fats on LTB4-releasing activity of PEC was more marked (100:13:60). EPA is oxidized with lipoxygenase to give LTB4. The relative releasing activity of LTB4 (100:32 for EPA and DHA, respectively) was almost in parallel with the EPA content (100:34). These results suggest that LTB4 production was mainly dependent on the level of EPA, but that an unknown factor other than the AA level was related to LTB4 production by PEC.

LTB4 is a strong inducer of the type I allergy, and LTB4 suppresses the reaction by competing with LTB4. The decreased LTB4 release and increased LTB4 release may effectively alleviate the type I allergic reaction. Although DHA was a more effective suppressor of LTB4 release than EPA in vitro, EPA was more effective than DHA in feeding experiments. These PUFAs are easily oxidized with molecular oxygen to produce active oxygen that induces various degenerative diseases. Although the oxidation rate of PUFAs in lymphocyte culture medium has a linear relationship with the number of double bonds in most PUFAs, EPA was oxidized at a much lower rate than expected from the number of double bonds. Thus, EPA in relation to DHA not only has higher anti-allergic activity but also lower disease-inducing ability.

References


20) Tsang, W. M., Weisman, C., and Smith, A. D., Effect of fatty acid mixtures on phytolhemaglutinin-stimulated lymphocytes of


