Dietary Docosahexaenoic Acid Can Alter the Surface Expression of CD4 and CD8 on T Cells in Peripheral Blood

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The effect of dietary docosahexaenoic acid (DHA) on T cell states in peripheral blood was investigated. Weanling male C57Bl/6N mice were kept on one of three 10% fat diets containing various amounts of DHA and linoleic acid for 4 weeks. Changing the concentration of dietary DHA did not alter the proportion of T cells expressing CD4 or CD8. However, increasing the concentration of dietary DHA lowered the expression of CD4 and CD8 on the cell surface. The decreased expression of these surface molecules involved in T cell proliferation has serious implications in the role of DHA as an immunosuppressant.

Keywords: *Docosahexaenoic acid; CD4; CD8; T cells; surface expression; peripheral blood*

INTRODUCTION

Over the past 20 years, *n*−3 polyunsaturated fatty acids (PUFA) have received attention as suppressors of hyperimmune response (Kromann and Green, 1980; Prickett et al., 1981; Kelley et al., 1985). In contrast to the pronounced usefulness of diets enriched with *n*−3 PUFA in the treatment of inflammatory disorders, such disorders appear more frequently with diets rich in *n*−6 PUFA. Recently, some investigators have examined how dietary *n*−3 PUFA can affect both the phenotypic composition of lymphocyte and the expression of T cell surface molecules (Berger et al., 1993; Shapiro et al., 1994; Yaqoob et al., 1994; Jenski et al., 1995; Jeffery et al., 1996; Sasaki et al., 1999). Especially, the conditions surrounding surface expression of CD4 and/or CD8 are important in intercellular interactions involving antigen presenting cells (APC) for antigen recognition, which is indispensable in the activation of T cells. However, conflicting results were obtained in these investigations. Some of them are attributed to the levels of dietary fat given to the animals. In addition, the effect of dietary *n*−3 PUFA on the T cell response in the peripheral blood is not fully understood (Wu et al., 1996; Sasaki et al., unpublished results). Therefore, the present study is designed to investigate the effect of various dietary concentrations of docosahexaenoic acid (DHA) as a source of *n*−3 PUFA on T cells in peripheral blood. DHA was used in this study because it has been suggested that it has a more specific biological effect as compared with other *n*−3 PUFA (Fowler et al., 1993; Ikeda et al., 1994).

EXPERIMENTAL PROCEDURES

Thirty-six 3-week-old C57Bl/6N male mice (CLEA Japan, Tokyo, Japan) were housed in individual cages and kept in an air-conditioned room (22 ± 1 °C and 50 ± 10% humidity) with a 12-h light/dark cycle. The mice, which had approximately the same body weight, were assigned to one of three experimental groups. Semisynthetic diets were formulated daily by adding fats and then given to the mice at 6:00 p.m. The food was removed at 9:00 a.m. the following morning to avoid the ingestion of lipid peroxides. The mice received water ad libitum. Major ingredients of the diet were 20% casein, 0.3% DL-methionine, 45% sucrose, 15% cornstarch, 3.5% AIN-76 mineral mix, 1.0% AIN-76 vitamin mix, 0.2% choline bitartrate, 5% cellulose, and 10% dietary fat (w/w). The dietary fats were prepared as a mixture of DHA ethyl ester (97%; Harima Chemical, Ibaragi, Japan), linoleic acid ethyl ester (92%; Tokyo Kasei Kogyo, Tokyo, Japan), and palm oil (Hayashi Chemicals Co., Tokyo, Japan). The fatty acid compositions of the dietary fat were determined using gas chromatography (Sasaki et al., 1998) and are shown in Table 1. The *n*−6/*n*−3 PUFA ratios of individual dietary fats were adjusted to 173 (diet I), 4.78 (diet II), and 2.02 (diet III). To minimize oxidation, 0.02 g/100 g *tert*-butylhydroquinone was added to the dietary fats, which were divided into aliquots for daily use. After the 4-week feeding period, the mice were sacrificed under anesthesia with sodium pentobarbital (Abbott Laboratories, North Chicago, IL).

Cell suspensions of peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood using Percoll (Sigma Chemical, St. Louis, MO) gradient centrifugation (Moriguchi et al., 1987). Heparinized blood samples from two mice on the same diet were pooled. Both the number and viability of leukocytes were studied microscopically by using trypan blue dye exclusion test. Fluorescein isothiocyanate or phycoerythrin-conjugated monoclonal antibodies [CT-CD4 (rat anti-CD4; Caltag Laboratories, San Francisco, CA) and YTS-169.4 (rat anti-CD8; Caltag Laboratories)] were used for immunohistology staining. The cell suspensions in phosphate-

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Table 1. Fatty Acid Compositions of Dietary Fat[a]

	diet		
fatty acid	I	II	III
C14:0	0.7	0.7	0.7
C16:0	31.8	32.2	32.1
C16:1	0.1	0.1	0.2
C18:0	2.9	3.1	3.0
C18:1 *n*−9	29.0	28.6	28.3
C18:2 *n*−6	34.4	28.6	23.5
C20:5 *n*−3	−	0.3	0.5
C22:6 *n*−6	−	5.5	11.0
SFA	35.5	36.0	35.9
MUFA	29.4	29.0	28.5
PUFA	35.1	35.0	35.6
PUFA/SFA	0.99	0.97	0.99
MUFA/SFA	0.83	0.81	0.79
n−3 PUFA	0.2	6.0	11.7
n−6 PUFA	34.5	28.7	23.6
n−6/*n*−3	173	4.78	2.02

[a] Prepared as mixtures of DHA ethyl ester (97%), linoleic acid ethyl ester (92%), and palm oil. −, not detected.

Table 2. Body Weight Gain and States of PBMC in Mice Fed Experimental Diet[a]

	diet		
	I	II	III
body wt gain (g)	12.0 ± 0.3	11.7 ± 0.7	12.5 ± 0.4
PBMC			
cell numbers (× 10^5/mL)	7.18 ± 1.07	7.63 ± 0.77	7.63 ± 1.26
$CD4^+CD8^-$ (%)	42.7 ± 3.5	42.3 ± 2.9	42.3 ± 4.9
$CD4^-CD8^+$ (%)	29.8 ± 1.2	32.8 ± 2.1	30.1 ± 2.0
expresn of surf. molecules			
CD4	58.2 ± 1.2[a]	53.3 ± 0.8[ab]	51.9 ± 0.4[b]
CD8	59.8 ± 2.6[a]	50.2 ± 3.7[ab]	45.0 ± 4.2[b]

[a] Cells were stained with fluorescein-conjugated monoclonal antibodies. Cells were then analyzed by flow cytometry as described under Experimental Proceduress. Data shown are mean ± SE of six samples. Different letters denote significant differences ($P < 0.05$). See Table 1 for details of the dietary fatty acid composition.

buffered saline containing 0.1% sodium azide (modified-PBS, pH 7.2) were incubated with an optimal dilution of these monoclonal antibodies as described previously (Sasaki et al., 1999). The stained cells were washed with modified-PBS and fixed with 2% paraformaldehyde in PBS (Sigma Chemical). Lymphocyte phenotype was studied with EPICs Elite (Coulter, Miami, FL) after the exclusion of dead cells with forward- and side-light scatters. Fluorescence intensity was represented on a four-decade logarithmic as the relative intensity obtained by subtracting the median intensity value for the control, stained with the isotype control (fluorescein-conjugated rat IgG).

Data are expressed as mean ± SE of six samples. The significance of differences between values was analyzed using one-factor ANOVA, followed by Duncan's new multiple-range test performed on SPSS software (Statistical Package for the Social Sciences, Inc., Chicago, IL). Probability values of <0.05 were considered to be significant.

RESULTS AND DISCUSSION

No differences were found in body weight gain and PBMC cell numbers among the groups (Table 2). Furthermore, it was found that the differences in the concentration of dietary DHA had little or no effect on the ratio of $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells. In contrast, we found in another investigation on mice fed a 10% fat diet for 4 weeks that the dietary DHA showed a tendency to lower the proportion of splenic T cells (Sasaki et al., 1999). Accordingly, the findings in the previous and current study reveal that the ability of dietary DHA to lower the proportion of peripheral T cells might be tissue dependent. This speculation is supported by another investigation we conducted showing that feeding rats a diet enriched in *n*−3 PUFA (*n*−6/*n*−3 ratio of 0.99) for 9 weeks resulted in the reduction of T cells in the spleen but not in either the PBMC or thymus (Sasaki et al., unpublished results). However, Wu et al. reported that the consumption of a diet containing 3.3% of caloric intake in the form of eicosapentaenoic acid (EPA) plus DHA (*n*−6/*n*−3 ratio of 1.1) for 14 weeks significantly decreased the proportion of T cells in the PBMC of cynomolgus monkeys (Wu et al., 1996). Although further investigation will be needed to resolve this discrepancy between their results and ours, it may illustrate that the effect of *n*−3 PUFA on lymphocyte phenotype depends on the experimental conditions, including the species of animal used and/or feeding period.

The present study demonstrated that dietary DHA dose-dependently down-regulated expression of CD4 and CD8 on T cells in PBMC (Table 2). We previously found the suppressive effects of dietary DHA on surface expression in splenic T cells but not on all differentiation stages of lymphocytes in the thymus (Sasaki et al., 1999). Jenski et al. (1995) observed an increased DHA incorporation into splenocytes and a decrease in the expression of a CD8 epitope when the cells were cultured in the presence of DHA. Recently, Hughes et al. (1996a,b) demonstrated that both dietary supplementation with *n*−3 PUFA and cultivation with EPA or DHA can reduce the surface expression of major histocompatibility complex (MHC) class II molecules and some adhesion molecules on human monocytes activated with interferon-γ. Although the mechanism that underlies the decrease of the expression of these surface molecules is still unclear, both Hughes's data and ours suggest that consumption of DHA may impede intercellular interaction involved in antigen recognization by T cells and APC. In support of this view, the incubation of cytotoxic T cell clones with DHA inhibited the ability of CD8 to bind to the immobilized MHC class I molecule (Annel et al., 1995). On the basis of the above evidence, it was suggested that the modulation of the expression of surface molecules involved in T cell activation may cause the immunosuppressant effect of DHA. Although the immunosuppressive effect of DHA can provide a valuable adjunct to alleviation of allograft rejection (Sasaki et al., 1996), it is questioned whether these changes of immune states might increase susceptibility against opportunistic infection. To address this question, Halvorsen et al. (1997) reported that human monocytes sustain their phagocytic potential, a vital protective function against infection, after daily supplementation of 3.6 g of DHA. Therefore, dietary DHA seems to serve as a safe and efficient means to prevent and/or improve some diseases characterized by hypersensitive immunoreactions.

ABBREVIATIONS USED

APC, antigen presenting cells; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MHC, major histocompatibility complex; modified PBS, phosphate-buffered saline containing 0.1% sodium azide; PBMC, peripheral blood mononuclear cells; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.

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