

Dietary Perilla Oil Inhibits Proinflammatory Cytokine Production in the Bronchoalveolar Lavage Fluid of Ovalbumin-Challenged Mice

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Abstract To evaluate the anti-inflammatory effects of different dietary oils on ovalbumin-sensitized and -challenged mice. Experimental BALB/c mice were fed with different diets containing 5% corn oil [rich in linoleic acid, 18:2n-6 polyunsaturated fatty acids (PUFA), as a control diet], 5% perilla oil (rich in α -linolenic acid, 18:3n-3 PUFA) or 5% compound oil containing 50% corn oil and 50% perilla oil, for 5 consecutive weeks. The leukocyte count, inflammatory mediators, and cytokine levels, including proinflammatory and Th1/Th2 cytokines in the bronchoalveolar lavage fluid (BALF) from the mice were determined. The results showed that 5% compound oil administration significantly ($P < 0.05$) decreased eosinophilic infiltration. Dietary perilla oil could not significantly ($P > 0.05$) decrease the eosinophil accumulation or the secretions of inflammatory mediators such as prostaglandin E_2 (PGE₂), histamine, nitric oxide and eotaxin. However, dietary perilla oil significantly ($P < 0.05$) reduced proinflammatory cytokine (TNF- α , IL-1 β and IL-6) and Th1 cytokine (IFN- γ and IL-2) production. The production of Th2 cytokine IL-10, but not IL-4 and IL-5, was also significantly inhibited by perilla oil administration. The results suggest that dietary perilla oil might alleviate inflammation via decreasing the secretion of pro-inflammatory cytokines in BALF, but failed to regulate the

Th1/Th2 balance toward Th1 pole during the Th2-skewed allergic airway inflammation.

Keywords Perilla oil ·
n-3 Polyunsaturated fatty acids (PUFA) ·
Proinflammatory cytokines · Th1/Th2 balance ·
Ovalbumin-sensitized and -challenged mice

Introduction

In the modern Western dietary pattern there is an increase in the consumption of total fat and n-6 polyunsaturated fatty acids (PUFAs), but a decrease in n-3 PUFAs and antioxidants. The changes in dietary lipid patterns are potent promoters of many chronic diseases [1]. Asthma, one of the chronic inflammatory airway disorders may be related to dietary lipids [2]. Reasonably, daily modification of the dietary lipid pattern may alleviate asthmatic damage.

Asthma is recognized as a T-helper type 2 (Th2)-skewed allergic disease [3]. Th2-type cells are important in humoral immunity and defense against extracellular pathogens [4] via eosinophilic infiltration, as well as IgE and IgG1 production in vivo [5]. Th2-type cytokines include interleukin (IL)-4, IL-5, IL-6, IL-9, IL-10, and IL-13. IL-4 and IL-13 are implicated in isotype switching of B-cells to produce IgE. IL-5 promotes the differentiation, recruitment, and survival of eosinophils [6]. In contrast to the Th2 cells, Th1 cells are characterized by the production of IL-2, interferon (IFN)- γ and tumor necrosis factor (TNF)- α . Th1 cells promote cell-mediated immunity to destroy intracellular pathogens [4], inhibit eosinophilic infiltration, IgE and IgG1 production, and strengthen IgG2a production in vivo [5]. Th1-skewed immune responses are generally pro-inflammatory and may result in autoimmune

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and chronic inflammatory diseases. However, Th2-skewed immune responses may cause asthma and allergy [7]. Whether it is possible to maintain the Th1/Th2 balance through dietary modification, especially the dietary lipid pattern, is an important issue in immunology.

Many animal experiments and clinical intervention studies indicate that long-chain n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from fish oil have anti-inflammation effects [8]. However, arachidonic acid (ARA, 20:4n-6), which is a component of phospholipids in the cell membrane, may produce ARA-derived eicosanoid inflammatory mediators, e.g. leukotriene (LT) B₄ and prostaglandin (PG) E₂ [9]. It has been found that EPA (n-3 PUFA) supplementation can replace ARA as an eicosanoid substrate to inhibit ARA metabolism and decrease inflammation status resulting from inflammatory eicosanoids [10].

In contrast to linoleic acid (18:2n-6), which is abundant in vegetable oils such as corn oil, and converted to ARA in animal cells [11], α -linolenic acid (ALNA, 18:3n-3), which exists in green leafy vegetables, as well as some seed and vegetable oils such as flaxseed oil and perilla oil, is a possible precursor of EPA and DHA. However, ALNA use to replicate the effects of EPA or DHA is still not properly justified in vivo. Perilla oil is obtained from the seeds of *Perilla frutescens*, and exhibits high n-3 fatty acid ALNA bioavailability [12]. Perilla oil consists of about 50–60% ALNA in the form of triglycerides. ALNA has been recognized as an essential fatty acid in nutrition [13]. Increase uptakes of ALNA have similar effects as EPA and DHA on health and chronic disease control [14]. Weanling NIN wistar rats that consumed 10% perilla seed oil in their diet for 18 weeks suffered no toxicological effects [15]. Generally, perilla oil is valuable more for its medicinal benefit than its flavor, such as uses for chemopreventive effects on tumors [16] and plasma triacylglycerol level [17]. Recently, Kim and Choi [18] indicated that dietary perilla oil showed effects similar to that of fish oil on EPA and DHA accumulation in the hepatic membrane fraction of male Sprague Dawley rats. Perilla seeds are widely used to treat asthma in traditional Chinese medicine. However, the medicinal effects of dietary perilla oil administration on asthmatic airway inflammation have been scarcely discussed. As perilla oil is rich in ALNA, we hypothesized that it has physiological effects, especially in immunomodulation, like EPA and DHA. Thus, perilla oil was selected for study.

This study evaluated the anti-inflammatory and immuno-modulatory potential of perilla oil (rich in n-3 PUFA) using ovalbumin (OVA)-sensitized and -challenged allergic inflammatory murine models. The diet consisted of 5% corn oil (rich in linoleic acid, 18:2n-6) as the control diet. The immune responses in the bronchoalveolar lavage

fluid (BALF) from the experimental mice were determined to evaluate the effects of perilla oil administration on airway inflammation and allergic characteristics.

Methods

Materials, Experimental Animals and Feeds

The experimental feed was prepared according to the recommendation of the American Institute of Nutrition AIN-76 [19] and varied only in lipid composition. The basic composition of each feed, expressed in g/100 g, contained 40 g sucrose, 25 g corn starch, 20 g casein, 5 g fiber, 3.5 g mineral mixture, 1 g vitamin mixture, 0.3 g DL-methionine, 0.2 g choline bitartrate and 5 g lipid. The components of each feed were thoroughly mixed and stored at -20°C . There were three kinds of lipid used in the experiment. Perilla oil (PER) rich in α -linolenic acid ($>60.5\%$) was purchased from MasterAsia Marketing Co. (Taipei, Taiwan, ROC) as the source of n-3 fatty acid. Corn oil (COR) rich in linoleic acid but poor in α -linolenic acid ($<2\%$) was purchased from Chang Chi Foodstuff Factory Co. (Taichung, Taiwan, ROC) as a control and the source of n-6 fatty acids. Compound oil (COR-PER) was a mixture containing 50% corn oil and 50% perilla oil.

Female BALB/c ByJNarl mice (6 weeks old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, ROC and maintained at the Department of Food Science and Biotechnology at National Chung Hsing University College of Agriculture and Natural Resources in Taichung, Taiwan, ROC. The animal room was kept on a 12-h light and 12-h dark cycle. Constant temperature ($25 \pm 2^{\circ}\text{C}$) and humidity were maintained. The mice were housed and kept on a chow diet (laboratory standard diet) to acclimatize for 2 weeks before feeding the experimental diet. After this equilibrium period, the mice were divided into four groups varied by oil source and sensitized treatments: non-sensitized control (PBS/COR, $n = 12$), dietary control (OVA/COR, $n = 8$), OVA/COR-PER ($n = 8$) and OVA/PER ($n = 9$). Each group was fed with the specified experimental diets for 5 consecutive weeks. Mice food intake and body weight were measured twice a week during the study period. There were no differences in food intake, feed efficiency and body weight gain between the four groups.

OVA-Sensitized and -Challenged Allergic Inflammation Murine Model

To test the effects of n-3 and/or n-6 fatty acids on airway inflammation, the mice (8 weeks old) were sensitized and

challenged to induce allergic airway inflammation. The mouse allergic airway inflammation model was manipulated as described by Lin et al. [20] and Ye et al. [21]. In brief, the mice were sensitized using an intraperitoneal injection (i.p.) of 0.2 ml alum-precipitated antigen containing 8 µg of ovalbumin (OVA, albumin chicken egg grade III, Sigma A-5378, MO, USA) and 2 mg Al(OH)₃ to induce primary immunity after supplementation of the specified experimental diets for 1 week. Two booster injections of this alum-OVA mixture were given 7 and 14 days later, respectively. Non-sensitized control mice received alum-phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.2–7.4, 0.2 µm filtered) only. One week later, the mice were then challenged by aerosolized OVA at a concentration of 5 mg OVA per milliliter PBS for 60 min, twice at 3 day intervals and repeated twice in 24 h. The aerosolized OVA were produced using an ultrasonic nebulizer (sw918, Shinmed). Non-sensitized control mice received PBS only. Two days later, the animals were anesthetized with diethyl ether, exsanguinated using retro-orbital venous plexus puncture and immediately euthanized using CO₂ inhalation. The bronchoalveolar lavage fluid (BALF) was collected and assayed for cytokines and other inflammatory mediators. The experimental design is given in Fig. 1.

Collection of BALF and Cellular Differential Counts

The BALF collection and differential cell counts were modified from the methods described by Ye et al. [21]. The mice were anesthetized with diethyl ether, exsanguinated using retro-orbital venous plexus puncture and immediately euthanized using CO₂ inhalation. Using a cannula, their lungs were immediately lavaged through the trachea with 5 aliquots of 0.6 ml Hank's balanced salts solution (HBSS) which was free of ionized calcium and magnesium

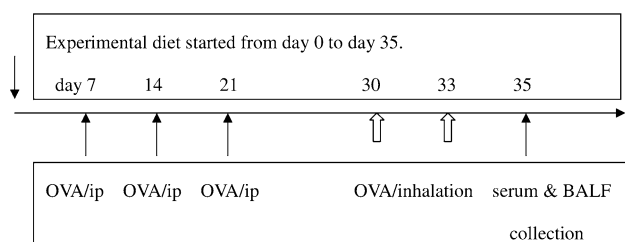


Fig. 1 Sensitization and challenge protocols for inducing allergic airway inflammation. Mice were sensitized with OVA/alum at days 7, 14, 21. The sensitized mice were then challenged twice with aerosolized OVA for 60 min at days 30, 33. The challenge was administered twice a day. All experimental mice were sacrificed at day 35 and their bronchoalveolar lavage fluids (BALF) were collected and analyzed

(HyClone). The bronchoalveolar lavage fluid (BALF) was centrifuged at 400g for 10 min at 4°C. The supernatant (BALF) volume was determined and stored at –70°C for future assay. The cell pellet was resuspended in minimum essential medium (MEM, HyClone) containing 10% bovine serum albumin (HyClone) and the final cell density was 1×10^6 cells/ml. Total cells counts were determined with a hemocytometer using the trypan blue dye exclusion method. Cytoцентрифугed preparations were stained with Liu's stain for differential cell counts. Based on standard morphologic criteria, a minimum of 200 cells were counted and classified as macrophages, lymphocytes, or eosinophils.

Assay Inflammatory Mediators in BALF

Histamine

The BALF histamine level was determined using the Histamine-ELISA kit (Cat. No. A05890, SPI-BIO). The procedure was carried out according to the manufacturer's instructions for use.

Prostaglandin E₂ (PGE₂)

The BALF PGE₂ level was determined using the competitive enzyme immunoassay method (Prostaglandin E₂ Express EIA Kit, Cat. No.500141, Cayman).

Nitric Oxide (NO)

Aliquots of 80 µl BALF samples and standards (0–100 µM sodium nitrite (Sigma S-2252) dissolved in double distilled water) were pipetted into the 96-microplate wells (Nunc). Aliquots of 160 µl Griess reagent were then added into each well to develop the color. The Griess reagent was freshly prepared from Reagent A and B at a ratio of 1:1 (Reagent A: 2% (w/v) sulfanilamide (Sigma S-9251) dissolved in 2.5% (v/v) phosphoric acid; Reagent B: 0.2% (w/v) N-1-naphthylethylene diamide dihydrochloride (Sigma N-9125) dissolved in 2.5% (v/v) phosphoric acid). After incubation for 10 min, the plate was read on a plate reader (ELISA reader, ASYS/HITECH Jupiter) at 540 nm. Using the standard curve, the NO concentration for each unknown sample was determined.

Protein

The BALF protein content was analyzed using the Bio-Rad protein assay dye reagent concentrate (Cat. no. 500-0006), according to the accompanying instructions, using a 96-well micro-titre plate.

Measurement of Cytokine and Chemokine Levels in BALF by an ELISA

IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IFN- γ and TNF- α

Cytokine (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IFN- γ , TNF- α) levels in BALF were determined using sandwich ELISA kits, respectively. The IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IFN- γ and TNF- α concentrations were assayed according to the cytokine ELISA protocol of manufacturer's instructions (mouse DuoSet ELISA Development system, R&D Systems). The sensitivity of these cytokine assays was <15.6 pg/ml.

Eotaxin

The BALF eotaxin concentration was determined using the mouse eotaxin sandwich ELISA kit (Quantikine M murine, R&D Systems). The eotaxin concentration was assayed according to the manufacturer's instructions. The sensitivity of this assay was <15.6 pg/ml.

Statistical analysis

Data were expressed as means $\pm$ SD. Data within OVA-sensitized treatment are analyzed using analysis of variance (ANOVA) followed by Duncan's test. Data between the dietary control and non-sensitized control are analyzed using unpaired Student's *t*-test. Differences between the dietary control and non-sensitized control or among OVA-sensitized groups were considered statistically significant if $P < 0.05$.

Results

The OVA-Sensitization and -Challenge Effects on Differential Indicators in Bronchoalveolar Lavage Fluid (BALF) from BALB/c Experimental Mice

The OVA-sensitization and -challenge effects on differential indicators in BALF from BALB/c experimental mice are shown in Table 1. The results showed that sensitization and challenge with OVA significantly ($P < 0.05$) increased an influx of total leukocytes (increase from $5.7 \pm 3.4 \times 10^5$ to $21.5 \pm 14.4 \times 10^5$ cells/mouse) into the lungs and airways. Challenge with OVA significantly increased an infiltration of eosinophils (increase from 1.9 ± 1.1 to $65.9 \pm 12.4\%$) but decreased the monocytes/macrophages (decrease from 86.3 ± 11.7 to $32.2 \pm 11.5\%$) infiltration into the lungs and airways. Challenge with OVA also significantly increased PGE₂ (increase from 1.18 ± 0.80 to 6.75 ± 3.95 ng/mouse)

Table 1 Effects of OVA-sensitization and -challenge on differential indicators in bronchoalveolar lavage fluid (BALF) from BALB/c experimental mice

Indicators in BALF	Treatments	
	PBS/COR	OVA/COR
Cellularity		
Total cells ($\times 10^5$ /mouse)	5.7 ± 3.4	$21.5 \pm 14.4^*$
Monocytes/macrophages (%)	86.3 ± 11.7	$32.2 \pm 11.5^*$
Lymphocytes (%)	11.7 ± 11.3	1.87 ± 1.69
Eosinophils (%)	1.9 ± 1.1	$65.9 \pm 12.4^*$
Inflammatory mediators		
Protein (μ g/mouse)	266 ± 96	368 ± 139
Eotaxin (pg/mouse)	270 ± 108	356 ± 107
PGE ₂ (ng/mouse)	1.18 ± 0.80	$6.75 \pm 3.95^*$
Histamine (pmol/mouse)	10.40 ± 3.94	9.79 ± 3.55
Nitric oxide (nmol/mouse)	5.10 ± 1.57	5.68 ± 2.83
Pro-inflammatory cytokines		
IL-1 β (pg/mouse)	34.3 ± 26.5	29.6 ± 19.6
TNF- α (pg/mouse)	280 ± 223	216 ± 128
IL-6 (pg/mouse)	289 ± 204	257 ± 134
Th1-type cytokines		
IFN- γ (pg/mouse)	118.6 ± 104.8	77.6 ± 56.5
IL-2 (pg/mouse)	155 ± 92	167 ± 46
Th2-type cytokines		
IL-4 (pg/mouse)	161 ± 90	161 ± 84
IL-5 (pg/mouse)	265 ± 196	222 ± 110
IL-10 (pg/mouse)	548 ± 315	418 ± 190

Data are presented as means $\pm$ SD ($n = 6-9$). Asterisk (*) means significantly ($P < 0.05$) different from the PBS/COR group within the same row analyzed using unpaired Student's *t*-test

secretion into the lungs and airways. However, the other measured BALF indicators did not significantly change after OVA sensitization and challenge (OVA/COR group) compared to the sensitization control (PBS/COR) group.

Effects of Different Dietary Oil on Cellularity of BALF from OVA-Sensitized and -Challenged Mice

Effects of different dietary oils on cellularity of BALF from OVA-sensitized and -challenged mice are shown in Table 2. Total leukocyte count in BALF from experimental mice that consumed the diet containing perilla oil (OVA/COR-PER, OVA/PER) did not significantly change compared to the dietary control (OVA/COR). Among the OVA-sensitized and -challenged groups, dietary compound oil (COR-PER) significantly increased the percentage of monocytes/macrophages (increase from 32.2 ± 11.5 to $52.7 \pm 18.8\%$), whereas decreased that of eosinophils

Table 2 Effects of different dietary oil on total leukocytes and cell distributions in bronchoalveolar lavage fluid (BALF) from OVA-sensitized and -challenged BALB/c mice

Groups	Total cells ($\times 10^5$ /mouse)	Monocytes/macrophages (%)	Lymphocytes (%)	Eosinophils (%)
OVA/COR	21.5 $\pm$ 14.4	32.2 $\pm$ 11.5 ^a	1.87 $\pm$ 1.69	65.9 $\pm$ 12.4 ^b
OVA/COR-PER	17.0 $\pm$ 9.7	52.7 $\pm$ 18.8 ^b	0.71 $\pm$ 0.82	46.6 $\pm$ 18.1 ^a
OVA/PER	34.1 $\pm$ 16.6	29.1 $\pm$ 14.8 ^a	2.69 $\pm$ 2.90	68.3 $\pm$ 16.8 ^b

Data are presented as means $\pm$ SD ($n = 6-9$). Data within the same column not sharing the same superscript letters are significantly different ($P < 0.05$) from each other analyzed using analysis of variance (ANOVA) followed by Duncan's test

Table 3 Effects of different dietary oil on protein and eotaxin levels in bronchoalveolar lavage fluid (BALF) from OVA-sensitized and -challenged BALB/c mice

Groups	Protein (μ g/mouse)	Eotaxin (pg/mouse)
OVA/COR	368 $\pm$ 139	356 $\pm$ 107
OVA/COR-PER	412 $\pm$ 142	371 $\pm$ 124
OVA/PER	478 $\pm$ 63	442 $\pm$ 174

Data are presented as means $\pm$ SD ($n = 6-10$). Data within the same column are analyzed by analysis of variance (ANOVA) followed by Duncan's test. There is no significant difference among groups. The sensitivity of eotaxin kit was about <7.8 pg/ml

(decrease from 65.9 ± 12.4 to $46.6 \pm 18.1\%$) in BALF. The percentages of lymphocytes in BALF did not significantly differ among groups.

Effects of Different Dietary Oil on Inflammatory Mediator Levels in BALF from OVA-Sensitized and -Challenged Mice

Effects of different dietary oil on eotaxin and protein levels in BALF are shown in Table 3. Effects of different dietary oil on inflammatory mediator levels in BALF are shown in Table 4. The results showed that perilla oil administration did not significantly affect protein, eotaxin, and inflammatory mediator levels in BALF.

Table 4 Effects of different dietary oil on inflammatory mediators of bronchoalveolar lavage fluid (BALF) from OVA-sensitized and -challenged BALB/c mice

Groups	PGE ₂ (ng/mouse)	Histamine (pmol/mouse)	Nitric oxide (nmol/mouse)
OVA/COR	6.75 $\pm$ 3.95	9.79 $\pm$ 3.55	5.68 $\pm$ 2.83
OVA/COR-PER	8.21 $\pm$ 6.65	10.15 $\pm$ 6.95	5.41 $\pm$ 1.47
OVA/PER	10.91 $\pm$ 5.04	12.28 $\pm$ 3.80	5.87 $\pm$ 1.70

Data are presented as means $\pm$ SD ($n = 6-11$). Data within the same column are analyzed by analysis of variance (ANOVA) followed by Duncan's test. There is no significant difference among groups. The detection limit of the kits used in this study: PGE₂ 36 pg/ml; Histamine 0.5 nM

Table 5 Effects of different dietary oils on the levels of pro-inflammatory cytokines IL-1 β , TNF- α and IL-6 in bronchoalveolar lavage fluid (BALF) from OVA-sensitized and -challenged BALB/c mice

Groups	IL-1 β (pg/mouse)	TNF- α (pg/mouse)	IL-6 (pg/mouse)
OVA/COR	29.6 $\pm$ 19.6 ^b	216 $\pm$ 128 ^b	257 $\pm$ 134 ^b
OVA/COR-PER	24.7 $\pm$ 6.3 ^{ab}	178 $\pm$ 52 ^{ab}	227 $\pm$ 45 ^{ab}
OVA/PER	12.5 $\pm$ 7.1 ^a	108 $\pm$ 60 ^a	141 $\pm$ 53 ^a

Data are presented as means $\pm$ SD ($n = 6-11$). Data within the same column not sharing the same superscript letters are significantly different ($P < 0.05$) from each other analyzed using analysis of variance (ANOVA) followed by Duncan's test

Effects of Different Dietary Oil on the Levels of Pro-inflammatory Cytokines in BALF from OVA-Sensitized and -Challenged Mice

Table 5 shows the effects of different dietary oil on the levels of pro-inflammatory cytokines IL-1 β , TNF- α and IL-6 in BALF from OVA-sensitized and -challenged mice through 5 weeks feeding. Sensitization and challenge with OVA did not significantly influence the secretion of pro-inflammatory cytokines, but dietary perilla oil significantly decreased these pro-inflammatory cytokines levels in BALF of OVA-sensitized and -challenged mice.

Effects of Different Dietary Oils on the Levels of Th1/Th2 Cytokines in BALF from OVA-Sensitized and -Challenged Mice

To evaluate the effects of different dietary oils on the secretion of Th1/Th2 cytokines in the local tissues of lungs and airways, the amounts of Th1 cytokines (IFN- γ and IL-2) and Th2 cytokines (IL-4, IL-5 and IL-10) in BALF were measured (Tables 6, 7). Dietary perilla oil significantly decreased the levels of Th1 cytokines in BALF; IFN- γ level decreased from 77.6 ± 56.5 to 21.9 ± 27.5 pg/mouse and IL-2 level decreased from 153 ± 42 to 110 ± 28 pg/mouse (Table 6). The levels of Th2 cytokines IL-4, IL-5, and IL-10 in BALF from experimental mice

Table 6 Effects of different dietary oils on the levels of Th1-type cytokines in bronchoalveolar lavage fluid (BALF) from OVA-sensitized and -challenged BALB/c mice

Group	IFN- γ (pg/mouse)	IL-2 (pg/mouse)
OVA/COR	77.6 $\pm$ 56.5 ^b	153 $\pm$ 42 ^{ab}
OVA/COR-PER	67.7 $\pm$ 19.7 ^b	175 $\pm$ 64 ^b
OVA/PER	21.9 $\pm$ 27.5 ^a	110 $\pm$ 28 ^a

Data are presented as mean $\pm$ SD ($n = 6-11$). Data within the same column not sharing the same superscript letters are significantly different ($P < 0.05$) from each other analyzed using analysis of variance (ANOVA) followed by Duncan's test

Table 7 Effects of different dietary oil on the levels of Th2-type cytokines in bronchoalveolar lavage fluid (BALF) from OVA-sensitized and -challenged BALB/c mice

Group	IL-4 (pg/mouse)	IL-5 (pg/mouse)	IL-10 (pg/mouse)
OVA/COR	161 $\pm$ 84	222 $\pm$ 110	418 $\pm$ 190 ^b
OVA/COR-PER	174 $\pm$ 34	206 $\pm$ 43	378 $\pm$ 84 ^{ab}
OVA/PER	121 $\pm$ 31	137 $\pm$ 55	242 $\pm$ 92 ^a

Data are presented as means $\pm$ SD ($n = 6-11$). Data within the same column not sharing the same superscript letters are significantly different ($P < 0.05$) from each other analyzed using analysis of variance (ANOVA) followed by Duncan's test

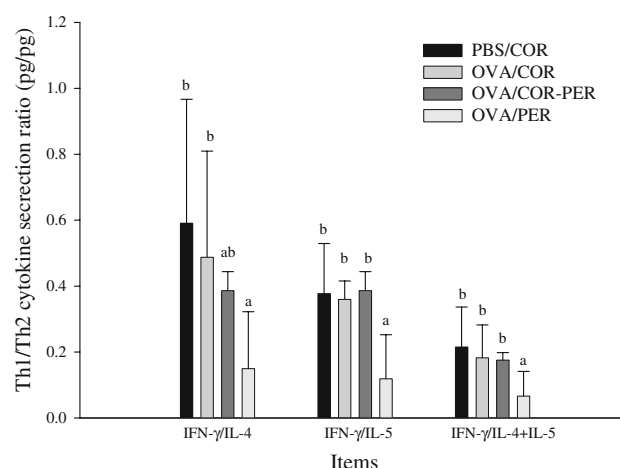
were decreased by dietary perilla oil administration (Table 7). Especially, IL-10 levels significantly decreased (decrease from 418 $\pm$ 190 to 242 $\pm$ 92 pg/mouse).

Effects of Different Dietary Oils on the Secretion Ratios of Th1/Th2 Cytokines in BALF from OVA-Sensitized and -Challenged Mice

To compare the secretion ratios between Th1 and Th2 cytokine levels in BALF, the secretion ratios of Th1/Th2 cytokine displayed as IFN- γ /IL-4, IFN- γ /IL-5 and IFN- γ /(IL-4 + IL-5) are shown in Fig. 2. The results showed that sensitization and challenge with OVA slightly decreased the secretion ratio of Th1/Th2 cytokine in BALF. However, dietary perilla oil administration significantly decreased the secretion ratios of Th1/Th2 cytokines in BALF (IFN- γ /IL-4, decrease from 0.49 $\pm$ 0.32 to 0.15 $\pm$ 0.17; IFN- γ /IL-5, decrease from 0.36 $\pm$ 0.06 to 0.12 $\pm$ 0.13; IFN- γ /(IL-4 + IL-5), decrease from 0.18 $\pm$ 0.10 to 0.07 $\pm$ 0.08).

Discussion

The purpose of this study was to evaluate the effects of perilla oil administration on airway inflammation using an

**Fig. 2** Effects of different dietary oils on Th1/Th2 cytokine secretion ratios in bronchoalveolar lavage fluid (BALF) from BALB/c mice in an OVA-sensitized and -challenged asthmatic murine model. Data are presented as mean $\pm$ SD ($n = 6-11$). Within the same Th1/Th2 cytokine item not sharing the same letters are significantly different ($P < 0.05$) from each other analyzed using analysis of variance (ANOVA) followed by Duncan's test

allergic asthma animal model. The established animal model significantly increased the flux of total leukocytes, especially eosinophils, as well as PGE₂ release into the bronchoalveolar tissues (Table 1), and slightly increased the Th2-skewed inclination in BALF (Fig. 2) compared to OVA/COR with the PBS/COR group. The levels of non-specific and anti-OVA specific IgE were significantly increased in the sera after OVA-sensitization and -challenge treatments (data not shown). Mice sensitized and challenged with OVA exhibited asthma-like symptoms [20], e.g. allergic airway inflammation, infiltration of differential cells, such as eosinophils, into the lungs and airways, and chemical mediator releases including inflammatory eicosanoids, histamine, Th1/Th2 cytokines, nitric oxide (NO), or eotaxin [3]. This study suggests that an intermediate airway inflammation mouse model was achieved. This airway inflammation mouse model (Fig. 1) might be suitable for evaluating the effects of different dietary lipids on the airway inflammation in vivo. Unfortunately, no significant up-regulation of cytokines occurred as expected in BALF in the established experimental animal model (Table 1). Undoubtedly, this might limit the results interpretation and relevance. Therefore, cytokine levels secreted by lymphocytes from the spleen or other lymphoid organs remain to be further clarified to compensate for the lack in the model.

In this study perilla oil rich in ALNA (18:3n-3) and corn oil rich in linoleic acid (18:2n-6) were selected and subjected to the allergic airway inflammation animal study. The compound oil consisted of n-3/n-6 fatty acid (PER/COR) exhibited the best anti-allergic effects via

significantly decreasing eosinophils, but increasing monocytes/macrophages infiltration into the airways (Table 2). Although lymphocytes in BALF did not significantly change, the decrease in eosinophilic infiltration suggested that compound oil (COR-PER) administration might remodel the inflammatory condition of the airways via decreasing vasodilation, mucus hypersecretion and bronchoconstriction [22, 23]. However, perilla oil administration alone could not significantly change the eosinophilic infiltration status in the airways. The anti-eosinophilic infiltration effect mechanism using a mixture of perilla and corn oils remains to be further clarified. To further clarify the effects of n-3 or n-6 PUFA, this study suggests that an ovalbumin sensitized group fed a diet supplemented with 5% of oil not rich in n-3 or n-6 fatty acids should be added. The added group will be helpful to further decipher the anti-inflammatory effects due to the anti-inflammatory effects of n-3 fatty acids or the lack of inflammatory effects of n-6 fatty acids.

The suppressive effects of n-3 PUFAs, such as EPA and DHA, on the production of arachidonic acid-derived eicosanoids have been reported [2, 24, 25]. Perilla oil was proposed to have anti-inflammatory potential for its high bioavailability of the n-3 fatty acid ALNA (18:3n-3) [12]. Contrary to our hypothesis, dietary perilla oil did not significantly affect the levels of inflammatory mediators in BALF such as protein, eotaxin, PGE₂, histamine, and nitric oxide (Tables 3, 4). Although the n-3 PUFAs such as EPA and DHA inhibit the production of inflammatory eicosanoids [24, 25], the synthesis of EPA and DHA from their precursor ALNA (18:3n-3) is low efficiency in cells [26, 27]. This study further hypothesized that ALNA (n-3, PUFA) from perilla oil having lower anti-eicosanoid effects than those of EPA and DHA (n-3, PUFA) from fish oil might result from its low conversion efficiency from ALNA to EPA and DHA in vivo. The low conversion efficiency from ALNA to EPA and DHA in vivo might decrease the anti-eicosanoid effect of perilla oil. To further clarify whether or not the ALNA in the perilla oil is converted to EPA or DHA and speculate the mechanisms by which EPA and DHA may cause immuno-modulation, the tissue fatty acid composition of experimental mice should be analyzed. Unfortunately, this study did not analyze the fatty acid composition in relevant cells or tissues. However, Kim and Choi (2005) have analyzed fatty acid composition in tissues and have demonstrated that both dietary corn oil (rich in C18:2n-6, 57.29%) and dietary perilla oil (rich in C18:3n-3, 61.49%) indeed reflected their fatty acid composition upon hepatic microsomal phospholipids of male Sprague–Dawley rats either through 4-day feeding or 4-week feeding [18]. They reported that total n-6 and n-3 fatty acid composition in hepatic microsomal phospholipids of the experimental rats, fed with 10% corn

oil and 10% perilla oil diet through 4-day feeding, were 46.85% (Σ n-6), and 4.47% (Σ n-3), as well as 32.57% (Σ n-6), and 22.58% (Σ n-3, including considerable EPA and DHA), respectively [18]. Comparatively the fatty acid composition of C20:4 in the analyzed tissue were 30.10 and 17.73%, respectively [18]. Although only 5% perilla oil diet was used in this study, this study suggests that the anti-inflammatory activity of dietary perilla oil might be partially via increasing n-3 fatty acid composition, but decreasing C20:4 (arachidonic acid) composition in vivo.

To further elucidate the effects of dietary perilla oil administration on cytokine secretions in BALF, pro-inflammatory cytokines including IL-1 β , IL-6 and TNF- α , and Th1/Th2 cytokines were determined. The results revealed that dietary perilla oil administration significantly inhibits the production of pro-inflammatory cytokines, IL-1 β , IL-6 and TNF- α , in BALF (Table 5). Undoubtedly, perilla oil administration exhibited anti-inflammatory potential via inhibiting the production of pro-inflammatory cytokines. Since the fatty acid composition (purity) in perilla oil was >99.9%, this study suggests that the effective components in perilla oil are fatty acids but not other components. However, the effective components in perilla oil and their immuno-regulatory mechanism should be further clarified.

Furthermore, this study showed that both Th1 (IFN- γ and IL-2) and Th2 (IL-10), especially Th1 cytokine secretions were significantly inhibited by dietary perilla oil, suggesting that perilla oil administration might also have immunosuppressive effects on Th1 cells from OVA-sensitization and -challenge mice (Tables 6, 7). IL-10 is generally synthesized in a lower level at the late stage of inflammation [28, 29] to inhibit Th1 cell and delayed-type hypersensitivity responses [6]. Thus, the decrease in Th1 cytokine production might also result in a significant decrease of IL-10 production in vivo. Recent evidences have indicated that lipid micro domains in the T-cell plasma membrane are remodeled using a dietary lipid consisting of n-3 PUFA, EPA and DHA [30–33]. Long-chain n-3 PUFAs suppress Th1-cell, but not Th2-cell, development [34, 35], and modulate the Th1/Th2 balance toward the Th2 pole [36–38]. The results from this study also exhibited that perilla oil, which is rich in ALNA (n-3 PUFA), regulated the Th1/Th2 balance toward to Th2-skewed inclination in the asthmatic model as compared to corn oil (rich in n-6 PUFA) administration (Fig. 2). The Th2-skewed immune response is disadvantage to allergic diseases.

In conclusion, the results from this study showed that perilla oil administration might alleviate bronchoalveolar inflammation by decreasing the secretion of pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6) and Th1 cytokines (IFN- γ and IL-2) into the local lung and airway

tissues, but failed to regulate the Th1/Th2 balance toward the Th1 pole during the Th2-skewed allergic airway inflammation.

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