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Effects of dietary ω3 and ω6 lipids and vitamin E on chemokine levels in autoimmune-prone MRL/MpJ-*lpr/lpr* mice

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Abstract

Elevated levels of chemokines, such as Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES), Monocyte Chemotactic Protein-1 (MCP-1), Macrophage Inflammatory Protein-1α (MIP-1α), and Macrophage Inflammatory Protein-1β (MIP-1β) have been found in rheumatoid arthritis (RA) and juvenile arthritis (JA), and they may be associated with the pathogenesis of these diseases. These chemokines are implicated in the migration of specific leukocytes into the joints. Omega-3 (ω3) fatty acid rich-fish oil (FO) and vitamin E may delay the progress of certain autoimmune diseases. The present study was designed to understand the effects of dietary lipids (ω-6 and ω-3 fatty acids) and vitamin E on the production of chemokines in autoimmune-prone MRL/*lpr* (a mouse model for RA) and congenic control MRL/++ mice. The MRL mice were fed for 4.5 months ω-6 and ω-3 diets that varied in lipid sources (corn oil; CO and fish oil; FO) and vitamin E levels (269 I.U./kg and 694 I.U./kg diet). Spleen cells were isolated and cultured aseptically in the presence of PHA for 48 h at 37°C and the levels of chemokines (RANTES, JE/MCP-1 and MIP-1α) were determined in the cell-free supernatants. The levels of RANTES and JE/MCP-1 were significantly higher in MRL/*lpr* mice compared to MRL/++ mice. The FO had differential effect on RANTES and MCP-1 production by spleen cells. The production of RANTES and JE/MCP-1 by spleen cells in mice fed the FO diets was significantly lower than in mice fed the CO diets ($p < 0.0001$). The levels of vitamin E did not affect the production of RANTES and JE/MCP-1. The levels of vitamin E had a significant effect on MIP-1α as the spleen cells of mice fed diets containing 694 IU/kg diet of vitamin E produced significantly higher levels of MIP-1α compared to the group of mice fed the diets containing 269 IU of vitamin E ($p < 0.0001$). The data obtained from this study in MRL/*lpr* and MRL/++ mice suggest that FO diets containing ω-3 fatty acids are beneficial in decreasing the levels of certain pro-inflammatory chemokines (RANTES and MCP-1) thereby delaying the onset of and severity of autoimmune symptoms in MRL/*lpr* mouse model.

Keywords: Autoimmunity; Chemokines; Autoimmune mice; MCP-1; MIP-1α; RANTES; ω-3 lipids; Vitamin E

1. Introduction

Defective regulation of inflammatory responses and disordered immune mechanisms are central to the pathological processes encountered in certain autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). SLE pathogenesis involves complex interactions between multiple cytokines, chemokines, their receptors and other immunomodulatory factors. RA is an autoimmune disease with chronic joint inflammation. MRL/*lpr* mice, a model for RA, spontaneously develop arthritis, massive lymphadenopathy with hypergammaglobulinemia, autoantibodies, high levels of acute phase proteins, and immune complex glomerulonephritis, compared to congenic control MRL/++ mice [1]. They are homozygous for the lymphoproliferative (*lpr*) gene, which has been identified as a defective *fas* gene. Mutation of the *fas* gene results in both the accumulation of abnormal T cells in lymphoid tissue and many features of generalized autoimmune disease including immune complex glomerulonephritis. Alterations in proliferative response to lectins [2], abnormal levels of serum anti-DNA antibodies [3], imbalances in pro- and anti-inflammatory cytokines [4] and increases in the expression of certain proinflammatory cytokines and oncogenes [5] have been observed in RA and SLE.

Fish oil (FO), rich in ω-3 polyunsaturated fatty acids (PUFA) is reported to improve clinical symptoms and modulate the level of specific cytokines and inflammatory me-

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diators in RA patients [6,7]. The beneficial effects of dietary ω-3 PUFA and vitamin E may be a promising therapy in reducing the severity of inflammatory arthritis and may reduce medication dosages. Nutritional intervention with marine lipids containing long chain ω-3 fatty acids (EPA, DHA), have been reported to significantly increase the life span, and delay the onset of autoimmune disease, in autoimmune-prone mice [8–12].

Along with other factors, chemokines have been implicated in the pathogenesis of inflammatory arthritis. Chemokines and opioids cause chemotactic responses in immune cells [13]. Many chemotactic cytokines in the 8,000–11,000 molecular weight range have been shown to be potent activators and chemoattractants for leukocyte subpopulations *in vitro* and elicit the accumulation of inflammatory cells *in vivo.* Over expression of chemokines enhances the recruitment of leukocytes into the joints, resulting in synovial cell proliferation, pannus formation and bone erosion.

Chemokines are chemoattractant agents that attract mature immune effector cells, such as T lymphocytes and monocytes, to sites of injury or infection by the process of chemotaxis. Chemokines are produced and released by a variety of cells in response to injury, antibodies, allergens, or invading microorganisms. Their activity is mediated by binding to their receptors. This interaction results in the migration of leukocytes into various tissue sites. The recruitment of leukocytes into regions of inflammation involves a cascade of interactions between adhesion molecules and the endothelium. This cascade is comprised of selectin-mediated rolling on endothelium, cellular activation and trans-endothelial migration [14]. Chemokines regulate this process by enhancing the adherence of leukocytes to the vascular endothelium. Decreasing the levels of chemokines may alleviate clinical symptoms of the disease. Chemokines are considered one of the factors that can enhance inflammation thus decreasing the levels of chemokines may benefit arthritis patients.

The present study was designed to test the hypothesis that the diets containing ω-3 fatty acids and vitamin E decrease the levels of specific chemokines (RANTES, MCP-1 (JE/MCP-1) and MIP-1α), thereby may delay the progression of inflammatory arthritis in autoimmune-prone MRL/*lpr* and congenic control MRL/++ mice, while ω-6 lipids may increase the levels of specific chemokines.

2. Methods and materials

2.1. Experimental animals and diets

Weanling female MRL/*lpr* and congenic control female MRL/++ mice (10/group) purchased from Jackson Laboratories (Bar Harbor, ME) were used in this study. All diet ingredients were purchased from Dyets (Bethlehem, PA). The composition of the experimental diets and fatty acids of the two dietary oils are shown in Tables 1 and 2 respectively. Mice were fed semi-purified diets containing 10% (w/w) corn oil (CO, ω-6) (ICN, Irvine, CA) or 10% menhaden fish oil (FO, ω-3) (U.S. Department of Commerce, National Marine Fisheries Service (NMFS), Charleston, NC) with two levels of vitamin E (269 I.U./kg and 694 I.U./kg diet). The diets containing 269 IU vitamin E/kg diet consisted of 75 IU vitamin E from AIN-76 vitamin mixture and 193.7 from the oils. In the diets containing 694 IU vitamin E/kg diet, 75 IU vitamin E from AIN-76 vitamin mixture, 193.7 IU from the oils and 425 IU vitamin E from DL-α tocopheryl acetate purchased from *Dyets* (Bethlehem, PA). Both dietary oils contained equal amounts of antioxidant supplements. Fresh food was provided daily and precautions were taken to prevent the oxidation of lipids. The diets were prepared once a week, stored in airtight containers, and flushed with nitrogen every time before closing the containers.

The mice were maintained in plastic cages with a 12 h light/dark cycle. The body weights were recorded every 2

Table 1
Composition of the experimental diets

Ingredient	Percent (%)
Casein	20
Dextrose	45
Starch	16
Corn oil or fish oil*	10
Cellulose	3.5
AIN-76 salt mixture[+]	3.5
AIN-76 vitamin mixture[#]	1.5
DL-methionine	0.3
Choline	0.2

Fish oil diet was supplemented with 1% corn oil (fish oil = 9%, corn oil = 1%). Both dietary oils contained equal amounts of antioxidant supplements (Fish oil was a gift from U.S. Department of Commerce, National Marine Fisheries Service, Charleston, NC).

[#] Vitamins in the diets (ICN Biochemicals, mg/kg of diet): vitamin retinyl acetate (500,000 IU/g) = 27mg; vitamin D concentrate (850,000 IU/g) = 1.88mg; DL-α-tocopheryl acetate (250 IU/g) = 300mg; ascorbic acid = 67.5mg; inositol = 75mg; choline = 1125mg; menadione = 33.75mg; *p*-aminobenzoic acid = 75mg; niacin = 63.75mg; riboflavin = 15mg; pyridoxine hydrochloride = 15mg; thiamine hydrochloride = 15mg; calcium pantothenate = 45mg; biothin = 0.42mg; folic acid = 0.1.35mg; and vitamine B_{12} = 0.0195mg.

In the AIN-76 vitamin mixture, 99.5% of the vitamin E is present in the form of DL-α tocopheryl acetate (also known as all-rac-α-tocopherol; 1g is equivalent to 250 IU of vitamin E; D-α-tocopherol (RRR-α-tocopherol)—100mg/kg diet and D-γ-tocophyerol (RRR-γ tocopherol)—90mg/kg diet; TBHQ-16mg/kg diet. The diets containing 269 IU vitamin E/kg diet consisted of 75 IU vitamin E from AIN-76 vitamin mixture + 193.7 IU from the oils. The diets containing 694 IU vitamin E, consisted of 75 IU vitamin E from AIN-76 vitamin mixture + 193.7 IU from the oils + 425 IU from dl-α tocopheryl acetate (*Dyets* (Bethlehem, PA).

[+] Minerals in the diet (g/kg diet): calcium phosphate, dibasic 17.5g, sodium chloride 2.59g, potassium citrate monohydrate 1.82g, potassium sulfate 1.82g, magnesium oxide 0.123g; ferric citrate 0.21g, zinc carbonate (70% zinc oxide) 0.056g, cupric carbonate (53-55Cu) 0.011g, potassium iodate 0.00035g, sodium selenite 0.00035g, chromium potassium sulfate 0.0193g, sucrose 4.13g. The salt mixture was supplemented with 0.0023 mg/kg diet of sodium fluoride.

Table 2
Fatty acid composition of the oils*

Fatty acids	Corn oil (%)	Fish oil (%)
14:0	0.03	8.4
16:0	10.33	13.97
18:0	1.99	2.51
20.0	–	0.14
16:1	0.16	11.17
18:1	24.00	10.00
18:2ω-6	52.60	0.66
18:3	0.09	–
20:4ω-6	–	1.45
20:5ω-3	–	13.37
22:4ω-6	–	0.11
22:5ω-6	–	0.39
22:5ω-3	–	2.06
22.6ω-3	–	8.60
Others	10.8	27.20

* Source: Galloway, S.B. (1989). U.S. Department of Commerce, NOAA, National Marine and Fisheries Service. The fatty acid composition was analysed by gas chromatography (Personal Communication).

wks; the MRL/*lpr* mice were checked for enlarged lymph nodes. The investigators strictly followed National Institutes of Health guidelines as described in the guide for the care and use of laboratory animals. The mice were sacrificed at 4.5 m of age by cervical dislocation.

2.2. Preparation and culturing spleen cells

The spleens were aseptically collected free of connective tissue; single-cell suspensions were prepared. The spleens were minced gently in RPMI-1640 medium containing 5% fetal bovine serum (FBS, heat-inactivated), 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acids (Gibco-BRL, Grand Island, NY). The cell suspension was centrifuged at 100 × g at 4°C and then, washed three times in the same medium. Cell viability was determined by the Trypan blue exclusion test. The spleen cells (5×10^6 cells/ml) were cultured in 12 well-flat bottom tissue culture plates (Corning, NY) in RPMI-1640 culturing medium containing 5% FCS in the presence of optimal concentration of phytohemagglutinin (PHA, 10 μg/ml) for 48 h at 37°C in a CO_2 incubator. The spleen cells were cultured with various concentrations of PHA and the optimal concentration was selected (data not shown). Cell suspensions were centrifuged at 10,000 × g for 1 min. Cell free supernatants were collected and stored at −70°C for the analysis of chemokines.

2.3. Determination of chemokines in PHA stimulated spleen cell culture supernatants

Levels of chemokines (RANTES, JE/MCP-1, and MIP-1α) in the cell-free supernatants were determined by ELISA (enzyme linked immunosorbent assay) technique. The mouse chemokine kits were purchased from R & D Systems (Minneapolis, MN) and their protocols were followed. A monoclonal antibody specific for each mouse chemokine was pre-coated onto a 96-well microtiter plates. An assay diluent (50 μl) was added into each well, followed by standard, control or samples (50 μl). The plates were incubated for 2 h at room temperature and washed. 100-μl of specific mouse chemokine conjugate was added to each well. The plates were incubated for 2 h at room temperature. Following the washing process, a substrate solution (100 μl) was added to the wells. Due to the enzyme reaction, a blue color was formed, which then turned yellow after adding stop solution (100 μl). The plates were read in a micro plate reader set to 450 nm. The concentrations of chemokines in the samples were calculated from the standard curve generated by a Table Curve program (Jandel Scientific Inc., San Rafael, CA).

2.4. Statistical analysis

The data were analyzed by Statview 4.0/Super ANOVA software package (Abacus Concepts, Berkeley, CA USA) using ANOVA. The differences among the means ($p < 0.05$) were performed by Fisher's PLSD (Protected Least Significant Difference) test.

3. Results

3.1. Body weights, incidence of lymph nodes and survival

As the lymphoproliferative (*lpr*) gene is over-expressed in the MRL/*lpr* mice, these mice suffer from lymphadenopathy, enlarged lymph nodes and accelerated aging. The body weights of the mice fed CO and FO with 269- and 694-IU of vitamin E (for 4.5 m) are presented in Fig. 1. The body weights of MRL/lpr mice were significantly lower compared to the MRL/++ congenic control mice. In the MRL/++ congenic control mice, the body weights were similar except in the group fed CO-694 IU vitamin E diet had lower body weights compared to the other MRL/++ groups. The food consumption, as estimated by disappearance of food, was similar in all the groups, pair feeding was not necessary. When the MRL/*lpr* mice were nine wk old, all the mice were surviving but the mice fed the CO-269 IU-vitamin E and FO-694 IU vitamin E diets exhibited enlarged lymph nodes (Table 3). Fifty percent of the mice in the CO-269 IU-vitamin E diet had enlarged lymph nodes at nine wk while those in the 694 IU vitamin E diets did not exhibit visible signs of enlarged lymph nodes. By eleven wk of age, only 90% of mice were surviving in the 269-IU vitamin E groups while 100% of the mice were surviving in the 694-IU vitamin E groups. At thirteen wks, most of the mice in the 269-IU vitamin E groups had enlarged lymph nodes while there were no visible signs of enlarged lymph nodes in the FO-694-IU vitamin E diet group suggesting ω-3 fatty acids containing 694-IU vitamin E levels of vita-

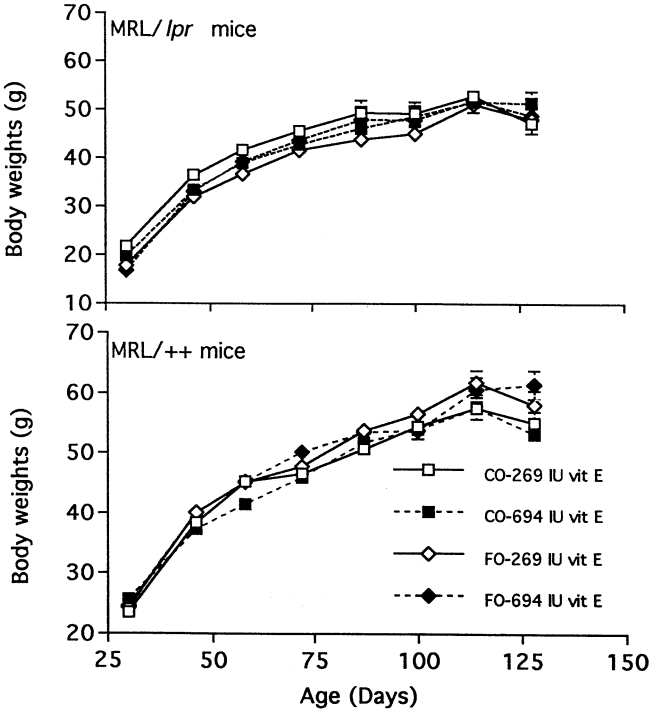

Fig. 1. Effects of dietary lipids and vitamin E on body weights of MRL/*lpr* and MRL/++ mice. Values are Mean ± SEM, n = 10 mice/group. CO: Corn oil; FO: Fish oil; vitamin E-269 IU and vitamin E-694 IU refer to vitamin E levels/kg diet.

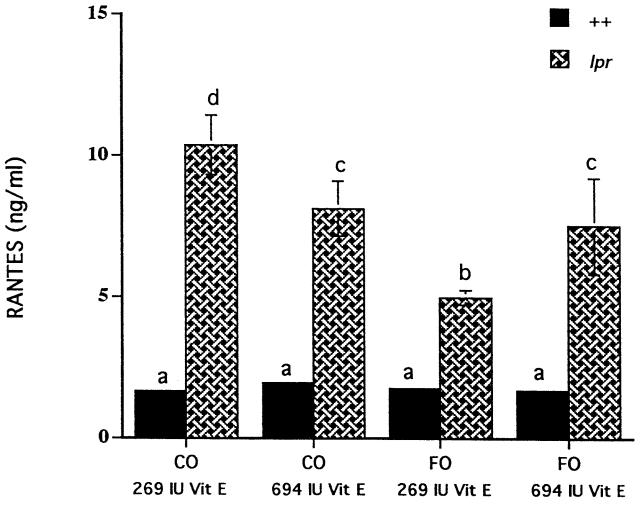

Fig. 2. Effects of dietary lipids and vitamin E on production of RANTES by spleen cells cultured in the presence of PHA in MRL/*lpr* and MRL/++ mice. Values are Mean ± SEM, n = 10 mice/group. Means with different superscripts are significantly different at $p < 0.05$ as revealed by FISHER's PLSD test; CO: Corn oil; FO: Fish oil; vitamin E-269 IU and vitamin E-694 IU refer to vitamin E levels/kg diet, RANTES: Regulated upon Activation, Normal T cell Expressed and Secreted.

min E may offer protection against enlargement of lymph nodes in the MRL/*lpr* mouse model.

3.2. Effects of dietary lipids and vitamin E on chemokines in the PHA stimulated spleen cell culture supernatants cells in MRL/lpr and MRL/++ mice

3.2.1. RANTES levels

Production of RANTES by PHA-stimulated spleen cells was significantly higher in MRL/*lpr* compared to MRL/++ mice ($p < 0.0001$). The effects of dietary lipids and vitamin E on RANTES production by spleen cells are presented in Fig. 2. The type of oil consumed did not affect production of RANTES by spleen cells of MRL/++ mice, but it did influence the levels of this chemokine in MRL/*lpr* mice (MRL/*lpr* mice develop spontaneous lymphadenopathy and other immunological abnormalities). The FO based diets significantly lowered the RANTES production by spleen cells of autoimmune prone-MRL/*lpr* mice compared to the CO fed mice ($p < 0.0001$). The levels of vitamin E did not affect RANTES production in both MRL/*lpr* and MRL/++ mice. There was a significant two-way interaction between oil and vitamin E ($p = 0.02$) suggesting differential effects of ω-3 fatty acids containing FO on RANTES production in the two types of mice. The FO decreased the production of RANTES in MRL/*lpr* mice but not in the MRL/++ mice. A highly significant interaction was observed between and oil and type of mice ($p = 0.003$), suggesting FO had selective effects depending on the strain of mice. A significant three-way interaction between oil, type of mice and vitamin E levels ($p = 0.008$) on the production of RANTES by spleen cells was also observed suggesting differential effects of oil and vitamin E on the RANTES production by

Table 3
Effects of dietary lipids and vitamin E levels on enlargement of lymph nodes and survival in MRL/*pr* mice

Age (Wks)	OIL	269 IU-Vitamin E				694 IU-Vitamin E			
		0	+	++	Survival*	0	+	++	Survival*
9 wks	CO	5	5	–	10	9	1	–	10
	FO	8	1	1	10	10	–	–	10
11 wks	CO	1	5	4	9	1	5	4	10
	CO	7	–	2	9	9	1	–	10
13 wks	CO	–	2	7	9	–	6	2	8
	CO	7	1	2	10	9	–	–	9

* Survival is reported for 10 mice/group; Scores are arbitrarily assigned to evaluate incidence and size of enlarged lymph nodes in mice as follow: 0: no enlarged lymph nodes (LN), +: 2-3 LNs, ++: several enlarged LNs.

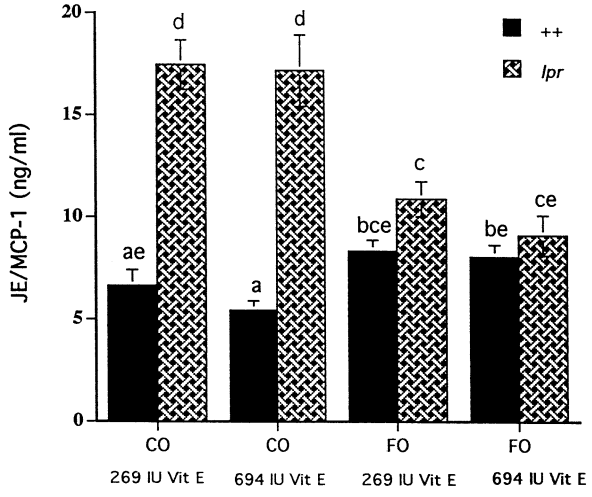

Fig. 3. Effects of dietary lipids and vitamin E on production of MCP-1 by spleen cells cultured in the presence of PHA in MRL/*lpr* and MRL/++ mice. Values are Mean ± SEM, n = 10 mice/group. Means with different superscripts are significantly different at $p < 0.05$ as revealed by FISHER's PLSD test; CO: Corn oil; FO: Fish oil; vitamin E-269 IU and vitamin E-694 IU refer to vitamin E levels/kg diet. MCP-1: Monocyte Chemotactic Protein-1.

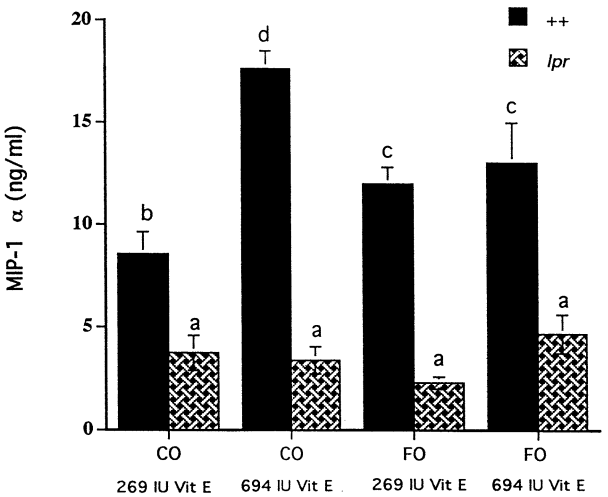

Fig. 4. Effects of dietary lipids and vitamin E on production of MIP-1α by spleen cells cultured in the presence of PHA in MRL/*lpr* and MRL/++ mice. Values are Mean ± SEM, n = 10 mice/group. Means with different superscripts are significantly different at $p < 0.05$ as revealed by FISHER's PLSD test; CO: Corn oil; FO: Fish oil; vitamin E-269 IU and vitamin E-694 IU refer to vitamin E levels/kg diet. MIP-1α: Macrophage Inflammatory Protein-1α.

spleen cells in the autoimmune-prone MRL/*lpr* and control MRL/++ mice.

3.2.2. JE/MCP-1 levels

The production of pro-inflammatory JE/MCP-1 by splenocytes in response to PHA was significantly higher in the autoimmune-prone MRL/*lpr* than in the congenic control MRL/++ mice ($p < 0.0001$) (Fig. 3). In MRL/*lpr* mice fed the FO diets, the JE/MCP-1 production by spleen cells was significantly lower compared to the CO diets fed groups ($p < 0.0001$). In the groups of mice fed the CO-based diets, significant differences in JE/MCP-1 production between the MRL/*lpr* and MRL/++ mice were observed. There was no significant difference in the JE/MCP-1 production between the MRL/*lpr* and MRL/++ mice fed the FO diets suggesting FO may normalize the RANTES production by spleen cells to the levels observed in control MRL/++ mice. The level of vitamin E in the diets had no significant effect on the JE/MCP-1 production by spleen cells of both autoimmune-prone MRL/*lpr* and MRL/++ congenic control mice. A highly significant two-way interaction occurred between type of oil and type of mice ($p < 0.0001$) on the production of MCP-1 by spleen cells suggesting differential effects of FO on pro-inflammatory MCP-1 production by spleen cells in the autoimmune-prone MRL/*lpr* and congenic control MRL/++ mice.

3.2.3. MIP-1α levels

The effects of oils, vitamin E, and type of mice on the levels of MIP-1α production by spleen cells are presented in Fig. 4. The MIP-1α production by spleen cells was higher in MRL/++ mice than in autoimmune-prone MRL/*lpr* mice ($p < 0.0001$). The type of oil had no significant effect on MIP-1α production by spleen cells of the autoimmune-prone MRL/*lpr* and control MRL/++ mice. Vitamin E levels significantly affected the production of MIP-1α by spleen cells of autoimmune-prone MRL/*lpr* and control MRL/++ mice. The spleen cells of mice fed diets containing 694 IU vitamin E produced significantly higher levels of MIP-1α than the groups of mice fed 269 IU vitamin E ($p < 0.0001$). A significant two-way interaction between vitamin E and type of mice ($p = 0.02$) suggesting differential effect of vitamin E levels on the two types of mice. A significant three-way interaction between oil, type of mice and vitamin E levels ($p = 0.0003$) on the production of MIP-1α by spleen cells were observed suggesting vitamin E levels had no effect on production of MIP-1α by spleen cells of MRL/*lpr* mice while high levels of vitamin E increased MIP-1α levels in CO-fed MRL/++ mice though not in the FO-fed mice.

4. Discussion

RA and JA are common chronic inflammatory joint diseases where the pathogenesis is influenced by the number of leukocytes within the inflamed joints. The ω-3 lipids are reported to have beneficial effects in delaying specific autoimmune diseases. The effects of dietary lipids (ω-6 and ω-3) and vitamin E on the levels of splenic chemokines (RANTES, JE/MCP-1 and MIP-1α) in MRL/*lpr* and MRL/++ mice were examined in this study. The data suggest that RANTES and MCP-1 production by PHA activated spleen cells was significantly higher in MRL/*lpr*

mice compared to MRL/++ mice and ω-3 fatty acids-containing FO-based diets significantly decreased the levels of these two chemokines. Higher levels of vitamin E (694 IU vitamin E/kg diet) had no effect on RANTES and MCP-1 production by spleen cells of the MRL mice while it increased MIP-1 α levels.

The possible mechanisms of action of ω-3 lipids have been suggested to be through altering cell membrane composition by changing signaling pathways, which is crucial for the regulation of the immune response [15], vascular integrity [16] and cell adhesion [17], or by reducing the levels of specific pro-inflammatory cytokines [18,19]. Elevated levels of MCP-1 in serum have been reported in patients with systemic JA, especially at current systemic features [20]. As a potent monocyte chemoattractant, MCP-1 significantly increased in both the synovial fluid and serum of patients with RA [21]. A study in the mouse model has suggested the benefit of an antagonist of MCP-1 in preventing the onset of arthritis [22]. MCP-1 is produced by various cells, including monocytes, T and B lymphocytes, endothelial cells and synovial cells. JE/MCP-1 is expressed by macrophages [23], mast cells, endothelial cells [24]. In human myelomonocytic leukemia cell lines, MCP-1 production was enhanced by IL-3, IL-6, IFN-γ, GM-CSF, M-CSF, and TNF-α [25]. Cytokines such as TNF-α, IL-1, IFN-γ and PDGF can induce the expression of mouse JE/MCP-1 [26,27].

In the present study, significantly higher production of JE/MCP-1 by PHA-activated spleen cells was observed in MRL/*lpr* mice compared to MRL/++ mice. MCP-1 is one of the most potent monocyte chemoattractant that plays a critical role in the development of arthritis [28]. The antagonist of MCP-1 can inhibit arthritis in the MRL/*lpr* mouse model [22] suggesting that lower MCP-1 levels may be beneficial in the treatment of arthritis. Data from the present study suggest that JE/MCP-1 levels were modulated by the FO-based diets. The JE/MCP-1 production by spleen cells was significantly lower in autoimmune-prone MRL/*lpr* mice fed the FO diets, compared to MRL/*lpr* mice fed the CO diets. Expression of MCP-1 is reduced by dietary ω-3 fatty acids [29]. In the present study, vitamin E levels did not affect JE/MCP-1 production by spleen cells in MRL mice.

RANTES may be involved in the progression of inflammatory arthritis. Elevated levels of RANTES have been found in both the synovial fluid [30] and serum of patients with RA [31]. Anti-RANTES antibody is reported to neutralize the chemotactic activity of peripheral blood monocytes in the joints of RA patients suggesting a role for RANTES in recruiting more monocytes to the inflamed joints [30]. The results from our present study demonstrated that the production of RANTES by spleen cells in MRL/*lpr* mice can be modulated by the types of dietary lipids. MRL/*lpr* mice fed the FO based diets had significantly lower RANTES production by spleen cells than the MRL/*lpr* fed the CO based diets. Feeding FO to mice results in suppressed T cell proliferation [31]. Lower levels of RANTES in MRL/*lpr* mice fed the FO diets may be the results of this mechanism. RANTES binding to chemokine receptor CCR5 induces phosphorylation of four distinct serine residues on the CCR5 carboxy terminus by a G-protein coupled receptor kinase (GRK)-mediated mechanism [32].

As inflammatory mediators, elevated levels of MIP-1 chemokines occur in chronic inflammatory disease. The chemotactic property of MIP-1 chemokines may be one of the potential mechanisms for disease progression in inflammatory joint disease. MIP-1α and MIP-1β preferentially attract $CD8^+$ and $CD4^+$ T cells, respectively [33]. Adhesion of T cells to VCAM-1 may be the major effect of MIP-1β in mobilizing lymphocytes [34]. MIP-1α and MIP-1β play a role on lymphocyte chemoattractants in RA [35] and are mainly produced by macrophages and other cell types, including B and T lymphocytes. The expression of MIP-1α and MIP-1β was shown to increase in mononuclear cells isolated from synovial fluid of RA patients [36]. MIP-1α levels were significantly elevated in both the synovial fluid and serum of patients with RA [37].

There are no reports on the effects of FO and vitamin E on chemokines levels in arthritis or autoimmune disease. To our knowledge this is the first study reporting the effects of marine oils and vitamin E on chemokine levels in autoimmune -prone MRL/*lpr* mice. RA patients on FO supplementation have been able to lower or discontinue their use of NSAIDs [38,39]. Daily supplementation with 2.6 g of ω-3 fatty acids resulted in significant clinical improvement [40]. In addition, it is recommended that patients should consume dietary supplements containing 3–6 g ω-3 fatty acids daily for ≥12 wks [41].

Similar to ω-3 fatty acids, vitamin E is also thought to modulate the immune response by altering PGE production [42]. The requirement for vitamin E increases with high levels of either ω-6 or ω-3 PUFAs, and it has been suggested that the consumption of ω-3 fatty acids reduce vitamin E concentrations in the blood and tissues than ω-6 fatty acids [43,44]. In addition to the regular anti-inflammatory treatment, vitamin E supplementation (1.2 g/day) provides an analgesic effect in RA patients [45].

MIP-1α is mainly produced by macrophages and it is chemotactic for macrophages, T cells and PMNs. An important role for MIP-1α in mediating type II collagen induced arthritis in a murine model has been suggested [46]. Data from the present study indicate that there are significantly higher levels of MIP-1α production by spleen cells in MRL/++ mice compared to MRL/*lpr* mice. Vitamin E significantly affected the levels of MIP-1α. Significantly higher MIP-1α production by spleen cells was found in mice that were fed the diets containing higher levels of vitamin E (694 IU vitamin E/kg diet), but not the mice that were fed the diets containing low levels of vitamin E. Vitamin E has been also reported to exert anti-inflammatory action through the inhibition of the arachidonic acid pathway. The studies from animals and humans have suggested

that vitamin E may have beneficial effects on the clinical symptoms of arthritis. Fish oil and vitamin E may provide a specific effect on MIP-1α levels in MRL/*lpr* and MRL/++ mice.

The beneficial effects of FO on chronic conditions are most consistently observed when vitamin E is also present in the diet [8,9,47]. Unfavorable results have been observed when FO was fed along with inadequate levels of vitamin E [44] of cell membranes. Earlier studies on autoimmune mice fed FO have indicated that 75 IU of vitamin E is inadequate to restore loss of plasma membrane vitamin E and 500 IU was sufficient to replenish both serum and hepatic vitamin E levels to reduce free radical generation 9450. By increasing vitamin E levels, peroxidation of hepatic microsomes could be prevented [44]. Our earlier studies on NZB/NZWF1 autoimmune-prone mice have indicated that ω3 lipids containing adequate levels of vitamin E delay the onset of autoimmune disease ion these mice through enhancing the activities and expression of antioxidant enzymes [44]. The MRL/*lpr* autoimmune-prone mice used in the present study have a weak antioxidant defense system compared to their congenic control MRL/++ mice [49]. The mRNA expression for SOD, GSHPX and the activities for these enzymes were lower in the livers and kidneys of these mice and free radical generation by liver and kidney were higher in these mice [49]. Generally due to imbalances in antioxidant defense system, cytokines and lipid mediators in autoimmune mice these mice can tolerate a higher level of ω3 lipids and vitamin E in their diet [18,19].

In summary, the data obtained from this study provide evidence that in a mouse model for RA higher levels of specific chemokines by spleen cells were produced and ω-3 lipids and vitamin E interventions modulate the levels of specific chemokines. This action may result in ameliorating the clinical symptoms of the disease. The major findings from this study indicate that ω-3 lipids-containing FO diets may be beneficial in decreasing the levels of specific chemokines. Further research is required to understand the role of ω-3 lipids on the expression of chemokine receptors and anti-chemokine therapy in autoimmunity. Research is also required to elucidate the effects of ω-3 lipids and antioxidants on localization and expression of chemokines and their receptors.

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