ELSEVIER

Nutrition 21 (2005) 940–948

NUTRITION

www.elsevier.com/locate/nut

Basic nutritional investigation

Opposite effects of low and high dose supplementation of vitamin E on survival of MRL/*lpr* mice

Chia-Chien Hsieh, Ph.D., and Bi-Fong Lin, Ph.D.*

Department of Biochemical Science and Technology, Institute of Microbiology and Biochemistry, College of Life Science, National Taiwan University, Taipei, Taiwan, Republic of China

Manuscript received July 13, 2004; accepted November 30, 2004.

Abstract

Objective: The purpose of this study is to investigate the effects of vitamin E supplementation on the autoimmune disease course in MRL/lymphoproliferative mice.

Methods: Three-month-old MRL/lymphoproliferative *lpr* female mice were fed an AIN-76 diet containing 50 mg/kg (control), 250 mg/kg (E5), 375 mg/kg (E7.5), or 500 mg/kg (E10) all-rac-α-tocopheryl acetate. Eight mice per group were killed for analysis after two months of experimental diets, and the rest of the mice were followed up to observe their proteinuria levels and life span.

Results: The data suggest that the life span of the E5 group was longer than the E10 group. Though α-tocopherol content in the plasma, liver, and kidneys increased in the mice fed the diet supplemented with vitamin E, the thiobarbituric acid reactive substance values in the liver and kidneys among these groups were not significantly different. IgM anti-ds-DNA and anticardiolipin antibodies were significantly higher in the E10 group than in those of the other groups. Phytohemagglutinin-stimulated interleukin (IL)-2 secretion was significantly lower, but concanavalinA-stimulated IL-4 and IL-10 production was significantly higher in the E10 group compared with the control group. The in vitro study also showed decreased IL-2 secretion and messenger RNA expression in phytohemagglutinin-stimulated splenocytes cultured in medium supplemented with high doses of vitamin E, but increased IL-2 with low doses of vitamin E.

Conclusions: Our data suggest that low and high dose supplementation of vitamin E has the opposite effect on the survival of MRL/*lpr* mice. The inhibitory effect of Th1 from high vitamin E content may not be beneficial for those suffering from Th2 prone autoimmune diseases, such as systemic lupus erythematosus.

Keywords: Vitamin E; MRL/*lpr* mice; Life span; Autoantibody; IL-2

Introduction

MRL/*lpr* mice, homozygous for the lymphoproliferative (*lpr*) mutation, develop spontaneous systemic lupus erythematosus (SLE)-like autoimmune disease [1,2]. This is characterized by the production of a rheumatoid factor, several autoantibodies, immune complex-mediated glomerulonephritis, vasculitis, arthritis, and a greater than 50% mortality by 5 to 6 mo of age [3]. Double negative ($CD4^-$ $CD8^-$) T-cells in *lpr* mutant mice are distinct from other T-cells in their low production of interleukin-2 (IL-2) in response to mitogen stimulation or TCR/CD3 engagement. Low levels of IL-2 production in *lpr* double negative T-cells are due to the increased instability of messenger RNA (mRNA) and the reduced activation of the IL-2 gene promoter [4]. Cytokine imbalances may play a significant role in the acceleration of lupus like autoimmune disease [5]. Patients with SLE have elevated serum levels of Th2 cytokines, such as IL-4, IL-6, and IL-10, but decreased production of Th1 cytokines, including IL-2 and gamma interferon (IFN-γ). The shifting of Th1 to Th2 immune responses leads to B-cells hyperactivity and the production of pathogenic autoantibodies [6]. Subsequently, an age-related decline has been observed in the production of IL-2 and

* Corresponding author. Tel.: +886-2/2363-0231 Ext. 2473; fax: +886-2/2362-1301.

E-mail address: bifong@ntu.edu.tw. (B.-F. Lin)

0899-9007/05/$ – see front matter
doi:10.1016/j.nut.2004.11.021

Table 1
Composition of experimental diets

Ingredient (g/kg diet)*	Control	E5	E7.5	E10
Soybean oil	50	50	50	50
Casein	200	200	200	200
Methionine	3	3	3	3
Corn starch	325	325	325	325
Sucrose	325	325	325	325
Cellulose	50	50	50	50
Choline	2	2	2	2
AIN-76 mineral mixture	35	35	35	35
AIN-76 vitamin mixture†	10	10	10	10
all-rac-α-tocopheryl acetate (mg/kg)	50	250	375	500
Energy (kJ/kg)	16 170	16 170	16 170	16 170
Unsaturated fatty acid‡	42	42	42	42

* Sources of ingredients: casein, ICN (Aurora, Ohio); cornstarch (Seoul, Korea); sucrose Taiwan sugar Co. (Taipei, Taiwan); cellulose, J. Betten-Maier & Söhne; Rosenberg (Holzmuhle, Germany); soybean oil, President Co. (Tainan, Taiwan); methionine and choline (Sigma); AIN-76 mineral mixture and AIN-76 vitamin mixture, ICN Pharmaceuticals (Aurora, Ohio).

† American Institute of Nutrition (1977). The diets were added vitamin mixture without vitamin E and then supplemented with different content of vitamin E, respectively.

‡ Unsaturated fatty acid contains monounsaturated fatty acid and polyunsaturated fatty acid.

IFN-γ, whereas the levels of IL-4, IL-10, IL-1, and tumor necrosis factor-alpha (TNF-α) have been observed to progressively increase with age [7].

Reactive oxygen species have been implicated as a cause of tissue damage [8,9] and may play a role in the inflammatory process of autoimmune disease. Vitamin E is the most effective chain-breaking, peroxyl radical scavenger that protects biologic membranes against lipid peroxidation [10,11]. In addition to its antioxidant properties, vitamin E supplementation has a variety of effects on the immune response system, including the enhancement of humoral and cellular immune reactions, subsequent regulation of cytokine balance and the immune system, T-cell differentiation, proliferative responses of the lymphocytes to mitogens, and the reduction of anti-inflammatory reactions [12,13]. On the other hand, the novel mechanisms of the antiatherosclerotic and anticarcinogenic properties involve modulation of cellular signaling, transcriptional regulation, and induction of apoptosis [14,15].

Some studies have reported that serum from SLE patients has a lower α-tocopherol concentration, superoxide dismutase, glutathione peroxidase, and plasma levels of malonedialdehyde levels are significantly higher than in controls [16,17]. MRL/*lpr* mice have lower mRNA expression of antioxidant enzymes and weak superoxide dismutase and glutathione peroxidase enzyme activities. Such mice also have a higher level of C20:4 (*n*-6) in their liver and kidneys, which may increase membrane peroxidation, contributing to development of autoimmune renal disease [18].

Though vitamin E supplementation has numerous beneficial effects on the host immune system [12–15], few studies concerning the effects of vitamin E on autoimmunity have been reported. Therefore, the aim of this study is to investigate antioxidant vitamin E on the course of the disease in MRL/*lpr* mice by observing autoantibody and cytokine production, and IL-2 mRNA expression.

Material and methods

Experimental animals and diets

Female MRL/*lpr* mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The animal room was maintained on a 12 h light-dark cycle with constant temperature (25 ± 2°C) and humidity. Each group included 22 mice. Three-month-old MRL/*lpr* mice were fed one of four experimental diets containing the following: all-rac-α-tocopheryl acetate 50 mg/kg (control), 250 mg/kg (E5), 375 mg/kg (E7.5), or 500 mg/kg (E10). The composition of the experimental diets is summarized in Table 1. Eight mice in each group were killed at five mo of age after being fed an experimental diet for two mo. The rest of the mice were maintained on the diets to determine autoantibody levels. Follow-up proteinuria and life span were determined to confirm pathologic changes.

Determination of proteinuria and life span

Proteinuria was measured in a semiquantitative manner, using tetrabromophenol paper (Yeongdong, Pharmaceutical Co., Seoul, Korea) on fresh urine samples. This colorimetric assay, which is relatively specific for albumin, was graded from 1+ to 4+, and the approximate protein concentrations were as follows: 1+: 0.3 g/L; 2+: 1 g/L; 3+: 10 g/L; 4+: greater than 10 g/L. High-grade proteinuria was defined as higher than 2+ (1 g/L). Mice were followed up for life span and proteinuria every week.

Determination of α-tocopherol

The α-tocopherol content in tissue homogenates were extracted as follows: 500 μL samples were pipetted into glass tubes, followed by 1% pyrogallol ethanol (1 mL) and a methanol solution of tocopheryl acetate as an internal standard. Then 5 mL *n*-hexane was added, and the mixture was shaken for 3 min. The samples were centrifuged at 1500 rpm for 5 min, and 4 mL of the upper layer was transferred to a new tube and dried to remove the solvent. The samples were then redissolved in 200 μL of methanol and analyzed by reverse-phase high performance liquid chromatography. The column was endcapped with RT-C18 (5 μm) (Merck, Darmstadt, Germany). Detection after separation was performed using an ultraviolet detector (Jasco, 821-FP Intelligent Spectrofluorometer, Tokyo, Japan). To calculate vitamin E concentrations, the peak area was determined following the α-tocopherol standard curve ($\epsilon = 3260^{-1}M^{-1}$ cm, 292 nm in absolute ethanol).

Determination of thiobarbituric acid-reactive substances (TBARS)

Oxidized tissue lipids were measured by tissue homogenate malondialdehyde production. One milliliter tissue homogenate and 1 mL 10% trichloroacetic acid were mixed and then centrifuged at 1500 rpm for 10 min. One mL of the upper layer was collected to 1 mL 0.4% 2-thiobarbituric acid, and 0.1 mL 0.2% butylated hydroxy toluene was added and mixed. The mixture was incubated at 50°C for 1 h. After cooling, 2 mL of isobutanol was added, and the mixture was mixed in a vortex until clear, and then was centrifuged. The sample supernatant was measured at excitation 515 nm and emission 550 nm. Yields of malondialdehyde were calculated using 1,1,3,3,-tetra-methoxy propane as a standard.

Assay of anti-double-stranded DNA (dsDNA) and anticardiolipin antibodies

Mice were bled retro-orbitally every month to determine the circulating autoantibody levels. The anti-dsDNA and anticardiolipin antibodies in the serum of MRL/*lpr* mice were determined by enzyme-linked immunosorbent assay (ELISA), according to Lin et al. [19,20]. The anti-dsDNA and anticardiolipin antibody results were expressed as ELISA units, which were calculated as the absorbance at 415 nm using a positive control of monoclonal antibody (mAb) specific for IgG and IgM. The mAb 10F10A3 and mAb 6H7B5 from the laboratory were prepared for use as positive controls for anti-dsDNA IgG and IgM assays, and mAb 9D12A2 and mAb 9C1A4 for anticardiolipin IgG and IgM assays, respectively.

Cell culture and determination of cytokines

The spleens were removed and single cells suspension were isolated by lysing erythrocytes with ammonia chloride-*tris* buffer and washed three times with Hank's balanced saline solution before use. Spleen cells at a concentration of 5×10^6 cells/mL were placed in 24-well plates in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% TCM (mouse serum replacement, Celox Corp., Hopkins, MN). Mitogens (phytohemagglutinin [PHA] 20 mg/L; concanavalinA, 10 mg/L, Sigma) were utilized as a stimulus for cell cultures. Suspensions were cultured at 37°C in a 5% CO_2 humidified incubator for 24 h. The collected supernatants were assayed for levels of cytokines by sandwich ELISA, as reported by Lin et al. [19]. The assay antibodies were purchased from PharMingen (San Diego, CA). The data were calculated according to the cytokine standard curve.

Reverse transcriptase polymerase chain reaction (RT-PCR): Quantitation of IL-2 mRNA

Total cellular RNA from the spleen was extracted using an RNeasy Mini kit (Qiagen, GmbH, Germany). The RNA was reverse transcripted to complementary DNA through RT-PCR, using a Pro STAR high fidelity RT-PCR system kit (Stratagene, La Jolla, CA). The cycles were as follows: 15 min at 37°C, 1 min at 95°C for RT formation, 30 s at 95°C for denaturation and 30 s at 57°C for annealing, and 2 min at 68°C for elongation. The samples underwent 30 cycles of amplified products and then 10 min at 68°C for complete reaction. The primers had the following sequence for $\acute{5}$ and $\acute{3}$ primers, respectively: IL-2, 5′ primer, 5′-TTCAAGCTCCACTTCAAGCTCTACAGCGGAAG-3′; and 3′ primer, 5′-GACAGAAGGCTATCCATCTCCT CAGAAAGTCC-3′ β-actin, 5′ primer, 5′-GTGGGC CGCTCTAGGCACCAA-3′; and 3′ primer, 5′-CTCTT TGATGTCACGCACGATTTC-3′. PCR products were visualized after electrophoresis on 2% agarose gels by staining with ethidium bromide. The IL-2 products were present at 413 base pair (bp), and β-actin was present at 540 bp. The results from electrophoresis were quantified by the Microcomputer Imaging Device (MCID-M4 ver 3.0, Imaging Research Inc., St. Catharines, Ontario, Canada).

Statistical analysis and survival analysis

Data were expressed as mean ± SEM for each group. Significance was determined by analysis of Duncan's Multiple Range test using the SAS software program (SAS/STAT version 6; SAS Institute, Cary, NC). Survival analysis was compared between two different curves using the Cox proportional hazards regression test (STATA version 6.0; Stata Corp., College Station, TX). The difference was considered significant at a *P* value less than or equal to 0.05.

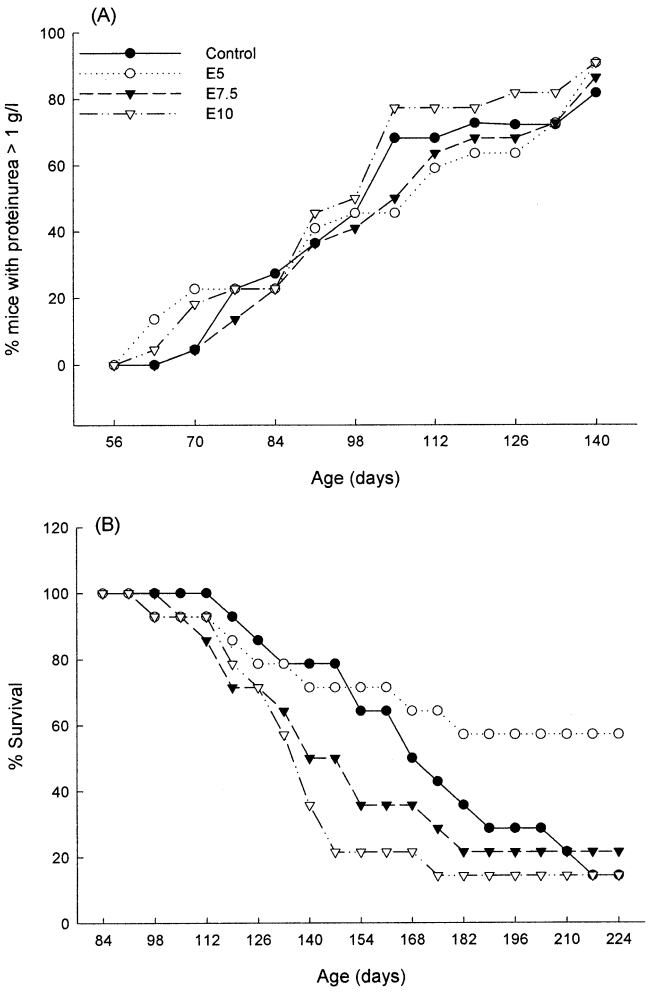

Fig. 1. (A) proteinuria and (B) survival curve of MRL/*lpr* mice fed on diets containing 50, 250, 375, or 500 mg all-rac-α-tocopheryl acetate/kg diet. Mice with proteinuria were defined as higher than 1 g/L protein in urine, $n = 22$ for each group. Life span data were analyzed by the Cox proportional hazards regression test, $P = 0.05$; $n = 14$ for each group.

Results

There was no significant difference in body weight gain and food intake in mice consuming the four diets containing different amounts of vitamin E in this study (data not shown). The mice began to have proteinuria greater than 1 g/L at day 70. Half of the mice developed proteinuria by day 98, and 90% by day 140 (Fig. 1A). The mortality of the control group reached 50% at day 168 (Fig. 1B). At this time, the E5 group had a higher survival than the other groups. The life span of the E5 group was significantly longer (213 ± 76 days) than the E10 group (157 ± 49 days) ($P = 0.05$). The life span of the control group was 177 ± 45 days, and that of the E7. 5 group was 162 ± 57 days. Low dose supplementation (E5 group) improved survival, whereas high dose supplementation (E10 group) decreased survival rates compared with the low dose group.

Plasma α-tocopherol concentrations were determined at 5 mo of age (Table 2). The level in the E10 group was significantly higher than the other groups ($P < 0.05$). Liver and kidney α-tocopherol levels significantly increased with vitamin E supplementation and reached the plateau in the E7.5 group, suggesting that a higher dose of vitamin E in the E10 diet may not retain higher α-tocopherol in tissue than the E7.5 diet. The kidney TBARS values determined by the concentration of malondialdehyde, a product of lipid peroxidation, showed the same tendency. The liver TBARS values were not significantly different among these groups. Although the antioxidant vitamin E supplement in the diets was reflected in different tissues, it did not significantly decrease the TBARS level.

To investigate the effect of vitamin E supplement on autoantibody production, data are expressed as the ratio of serum antibody titers after diet treatment to serum antibody titers before treatment (Table 3). The anti-dsDNA and anticardiolipin antibodies tended to increase with age. The levels of IgM anti-dsDNA ($P < 0.05$) and anticardiolipin ($P < 0.001$) antibodies were increased in mice fed the E10 diet compared with other groups.

Cytokine profiles of splenocytes, including IL-2, IFN-γ, IL-4, and IL-10, were determined. As shown in Table 4, Th1 type cytokines IL-2 secreted from splenocytes with or without PHA stimulation were significantly lower in mice of the E10 group compared with the control group ($P < 0.05$). The levels of PHA-stimulated IFN-γ production were

Table 2
α-tocopherol levels in liver, kidney and plasma and tbars level in liver and kidney of MRL/*lpr* mice fed diets containing different amounts of vitamin E*

	Control	E5	E7.5	E10
α-tocopherol (μg/g tissue)				
Liver	20.1 ± 1.6^{c}	45.1 ± 2.3^{b}	76.7 ± 4.2^{a}	75.1 ± 4.3^{a}
Kidney	15.0 ± 0.9^{c}	19.3 ± 1.9^{b}	23.5 ± 1.1^{a}	19.4 ± 0.6^{ab}
Plasma (μg/ml)	5.4 ± 0.4^{c}	7.3 ± 0.5^{b}	8.9 ± 0.6^{b}	16.7 ± 2.0^{a}
TBARS contain (nmol/g tissue)				
Liver	3.7 ± 0.6	4.0 ± 0.4	3.1 ± 0.5	4.5 ± 0.6
Kidney	13.9 ± 2.3	12.7 ± 2.6	7.8 ± 1.4	8.6 ± 1.1

$^{a, b, c}$Means values within a row with unlike superscript letters were significantly different ($P < 0.05$) as determined by the Duncan's Multiple Range test.

* The diets were as follows: control: all-rac-α-tocopheryl acetate 50 mg/kg; E5: 250 mg/kg; E7.5: 375 mg/kg; E10: 500 mg/kg. Values are means ± SEM for seven to eight mice per group.

Table 3
Serum anti-dsDNA and cardiolipin autoantibodies content of female MRL/*lpr* mice fed diets containing different amounts of vitamin E*

		Control	E5	E7.5	E10
Anti-dsDNA autoantibodies (ELISA unit)†					
IgG	3.5 mo	2.62 ± 0.48	2.37 ± 0.48	1.99 ± 0.61	2.62 ± 0.36
	4.5 mo	3.24 ± 1.01	2.85 ± 0.62	5.55 ± 1.36	3.97 ± 0.70
IgM	3.5 mo	3.46 ± 0.75^{b}	3.23 ± 0.53^{b}	4.24 ± 0.72^{ab}	6.26 ± 1.30^{a}
	4.5 mo	3.79 ± 0.89^{b}	2.83 ± 0.63^{b}	3.44 ± 0.90^{b}	8.20 ± 3.16^{a}
Anti-cardiolipin autoantibodies (ELISA unit)					
IgG	3.5 mo	2.08 ± 0.30	2.31 ± 0.69	1.33 ± 0.28	1.20 ± 0.48
	4.5 mo	1.72 ± 0.31	2.29 ± 0.43	1.51 ± 0.23	1.58 ± 0.36
IgM	3.5 mo	0.92 ± 0.12^{b}	0.87 ± 0.11^{b}	0.99 ± 0.13^{b}	2.00 ± 0.37^{a}
	4.5 mo	1.11 ± 0.13^{b}	1.79 ± 0.21^{b}	1.93 ± 0.25^{b}	5.72 ± 1.21^{a}

$^{a, b}$Means values within a row with unlike superscript letters were significantly different ($P < 0.05$) as determined by the Duncan's Multiple Range test. dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay.

* The diets were as follows: control: all-rac-α-tocopheryl acetate 50 mg/kg; E5: 250 mg/kg; E7.5: 375 mg/kg; E10: 500 mg/kg. Values are means ± SEM for seven to eight mice per group.

† Data are expressed as the ratio of serum antibody titer after diet treatment (3.5, 4.5 months) to serum antibody titer before treatment (2.5 months).

not significantly different among the four groups. In contrast, Th2 type cytokines IL-4 and IL-10 secreted by concanavalinA-stimulated splenocytes were significantly higher in the E10 group compared with the control group ($P = 0.0332$ and $P = 0.0211$ for IL-4 and IL-10, respectively) (Table 5). The results suggest that high doses of vitamin E in the E10 diet could decrease IL-2 and increase IL-4 and IL-10 levels.

To investigate whether high doses of vitamin E inhibit IL-2 production via suppression of IL-2 gene expression,

Table 4
IL-2 and IFN-γ produced by splenocytes in female MRL/*lpr* mice fed diets containing different amounts of vitamin E*

	Control	E5	E7.5	E10
IL-2 (unit/10^6 cell)				
Spontaneous†	2.57 ± 0.58^{a}	0.64 ± 0.24^{b}	2.11 ± 0.54^{a}	0.24 ± 0.08^{b}
PHA‡	5.13 ± 1.17^{a}	3.20 ± 0.73^{ab}	3.21 ± 0.73^{ab}	2.30 ± 0.30^{b}
IFN-γ (unit/10^6 cell)				
Spontaneous	ND^{c}	0.48 ± 0.04^{a}	0.21 ± 0.09^{b}	ND^{c}
PHA	2.01 ± 0.48	2.26 ± 0.50	1.00 ± 0.30	2.10 ± 0.78

$^{a, b, c}$Means values within a row with unlike superscript letters were significantly different ($P < 0.05$) as determined by Duncan's Multiple Range test. IFN-γ, interferon-gamma; IL-2, interleuken-2; ND, not detectable; RPMI, Roswell Park Memorial Institute; TCM, mouse serum replacement.

* The diets were as follows: control: all-rac-α-tocopheryl acetate 50 mg/kg; E5: 250 mg/kg; E7.5: 375 mg/kg; E10: 500 mg/kg. Values are means ± SEM for seven to eight mice per group.

† Splenocytes at a concentration of 5×10^6 cells/mL in RPMI-1640 medium supplemented with 10% TCM were incubated for 24 h. Spontaneous: cells were incubated without stimulation.

‡ PHA: 20 mg/L of phytohemagglutinin was added for stimulation of splenocytes.

Table 5
IL-4 and IL-10 produced by mitogen stimulated splenocytes in female MRL/*lpr* mice fed diets containing different amounts of vitamin E*

	Control	E5	E7.5	E10
IL-4 (pg/10^8 cell)				
Spontaneous†	1.29 ± 0.93^{b}	0.41 ± 0.27^{b}	3.92 ± 2.07^{b}	10.9 ± 0.82^{a}
ConA‡	0.88 ± 0.58^{c}	5.71 ± 2.41^{bc}	9.29 ± 2.11^{ab}	11.5 ± 1.39^{a}
IL-10 (pg/10^6 cell)				
Spontaneous	3.47 ± 1.71	3.74 ± 2.74	3.56 ± 1.64	ND
ConA	1.09 ± 1.02^{b}	0.20 ± 0.18^{b}	15.4 ± 8.66^{ab}	23.5 ± 6.74^{a}

$^{a, b, c}$Means values within a row with unlike superscript letters were significantly different ($P < 0.05$) as determined by Duncan's Multiple Range test. conA, concanavalin A; IL, interleuken; RPMI, Roswell Park Memorial Institute; TCM, mouse serum replacement.

* The diets were as follows: control: all-rac-α-tocopheryl acetate 50 mg/kg, E5: 250 mg/kg, E7.5: 375 mg/kg, E10: 500 mg/kg. Values are means ± SEM for seven to eight mice per group.

† Splenocytes at a concentration of 5×10^6 cells/mL in RPMI-1640 medium supplemented with 10% TCM were incubated for 24 h. Spontaneous: cells were incubated without stimulation.

‡ Stimulation with the ConA 5 mg/L for IL-4 secretion, and ConA 10 mg/L for IL-10 secretion.

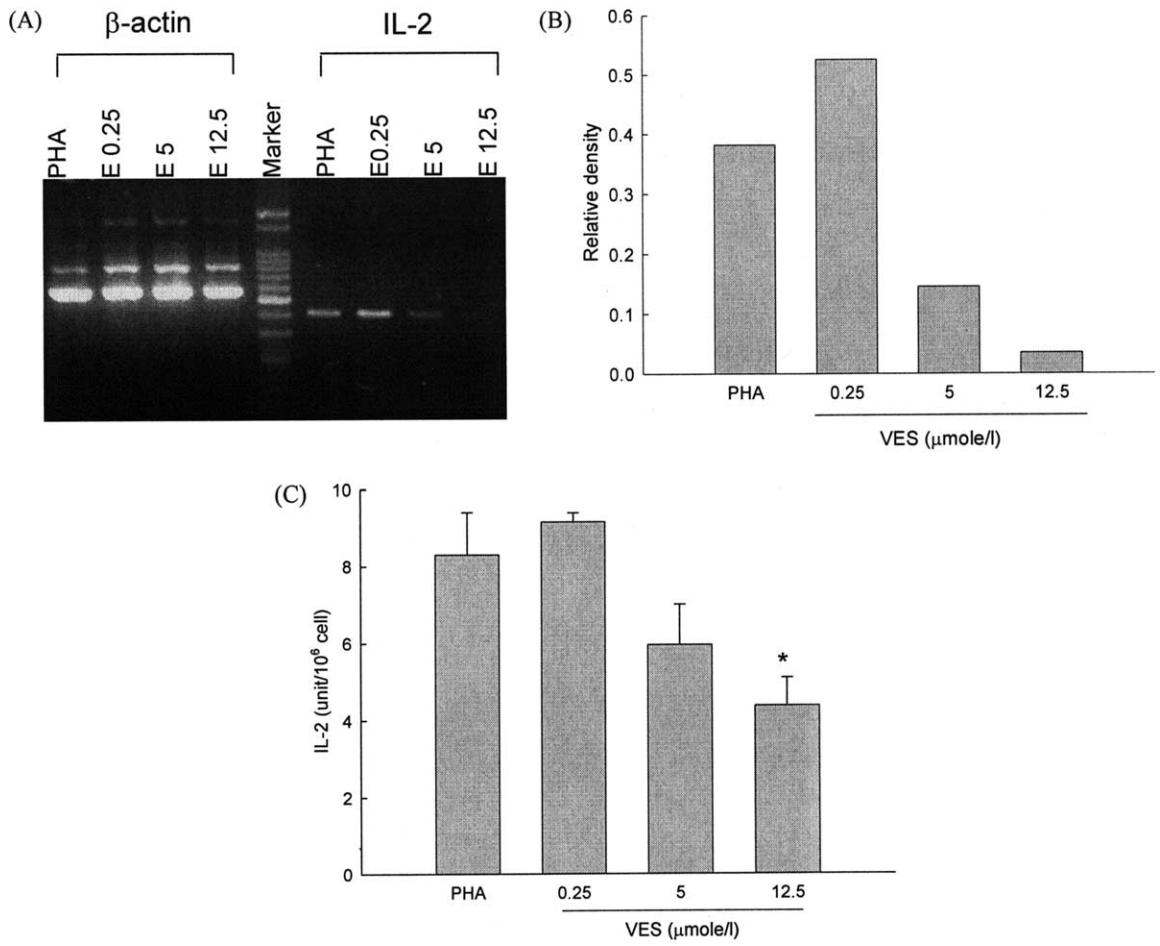

Fig. 2. (A) IL-2 mRNA expression, (B) IL-2 mRNA relative density quantified by comparing with the IL-2 dose-dependent curve, and (C) IL-2 secretion by PHA-stimulated spleen cells from 16-wk-old MRL/*lpr* mice ($n = 3$, mean ± SEM). *Significantly different from the PHA stimulation ($P < 0.05$) was analyzed by Student's t test. The spleen cells were treated with 0.25, 5, or 12.5 μmol/L α-tocopheryl succinate (VES) and analyzed by semiquantitative RT-PCR. Total RNA was isolated after 17 h activation and PCR products were visualized after electrophoresis. The data are representative of two independent experiments. IL-2, interleukin-2; mRNA, messenger RNA; PHA, phytohemagglutinin; RT-PCR, reverse transcription polymerase chain reaction; VES, vitamin E succinate.

we determined the levels of IL-2 secretion and mRNA in PHA stimulated cells in the presence of various concentrations of vitamin E (Fig. 2). Splenocytes from 16-wk-old MRL/*lpr* mice were treated with α-tocopheryl succinate (vitamin E succinate [VES]) at doses of 0.25, 5.0, or 12.5 μmol/L for 17 h. Figure 2B shows the relative density of the IL-2 mRNA expression using β-actin as an internal control. The mRNA IL-2 was 37% higher when treated with 0.25 μmol/L low dose VES. However, levels of IL-2 transcripts were diminished about 62% and 91% when treated with 5 and 12.5 μmol/L of VES, respectively. The IL-2 levels from supernatant also showed the same tendency with vitamin E treatment (Fig. 2C), suggesting that high doses of vitamin E significantly decrease IL-2 secretion at both mRNA levels and cytokine production. Therefore, the opposite effect of low and high doses of vitamin E on IL-2 production may be due to the regulation of gene expression.

Discussion

It has been implicated that oxidative stress may cause tissue damage and may play a role in the inflammatory process of autoimmune disease [8,9]. In this study, the mice fed a low dose vitamin E supplement had a significantly prolonged life span, suggesting that low doses of vitamin E may protect in the later stages of autoimmune processes. However, high doses of vitamin E supplementation did not exert an antioxidant effect on autoimmune-prone mice. Although the vitamin E concentration of tissues was elevated through vitamin E supplementation, the TBARS levels were not significantly decreased. Therefore, high doses of vitamin E may affect autoimmune progression through mechanisms other than antioxidant effects.

In SLE, studies suggest that the anticardiolipin antibodies, in conjunction with elevated levels of anti-dsDNA, are highly specific for glomerulonephritis in patients with

lupus [21]. A previous study showed that the production of IgG anticardiolipin antibodies increased in the NZB/W F1 autoimmune-prone female mice fed on a high-fat diet at an earlier age, and thus shortened the life span [20]. The IgG anticardiolipin antibodies were also higher in mice fed an oxidized frying oil diet, which may be due to the oxidative breakdown of membrane phospholipid by a high oxidized frying oil diet [22]. Besides, vitamin E has been shown to enhance antibody production in several animal studies [23]. Cooney et al. [24] demonstrated a positive association between plasma total tocopherols and antibody levels, especially antioxidized DNA autoantibody levels. This present study also showed significantly higher anti-dsDNA and anticardiolipin IgM production in MRL/*lpr* mice fed a high vitamin E diet, which may play a role in accelerating SLE development.

In addition, autoimmunity and aging may lead to cytokine imbalance. MRL/*lpr* mice have been characterized with higher populations of double negative T-cells which can only produce extremely low levels of IL-2, suggesting adequate levels of IL-2 are necessary for the alleviation of autoimmune disease severity [4,25]. In our study of the IL-2 secretion from splenocytes both in vivo and in vitro, high doses of vitamin E significantly decreased IL-2 secretion at the mRNA level and cytokine production. On the contrary, IL-4 is a key cytokine directing differentiation and development of naive Th cells into Th2 cells [26]. The IL-10 inhibits Th1 responses and is a potent cofactor for B-cell survival, proliferation, differentiation, and Ig secretion, which play important roles in the pathogenesis of SLE [27]. Higher IL-4 and IL-10 secretions from concanavalinA-stimulated splenocytes were consistent with higher autoantibody production in mice fed on the E10 diet in our study. These data suggest that a diet with high vitamin E decreased IL-2 and increased IL-4 and IL-10 production, thereby promoting autoimmune disease development in autoimmune-prone mice.

There are still limited studies showing the effect of vitamin E supplementation on IL-2 secretion. The positive effects are usually observed in subjects under stress conditions such as aging, malnutrition, disease, infection, and immunization [28–32]. Either 100 mg dL-α-tocopheryl acetate supplementation for three months in elderly subjects, or 400 IU/d for six or eight weeks in healthy volunteers have been undertaken with no significant beneficial effects on the immune response, though higher plasma α-tocopherol level was also detected in the vitamin E group [33,34]. Moreover, dietary vitamin E has been reported to negatively influence IL-2 secretion from mitogen-stimulated peripheral blood lymphocytes in a free-living elderly population [35]. Therefore, the beneficial effects of vitamin E supplementation on immune function, which may depend on physiologic needs, still need to be further investigated.

The effect of vitamin E supplementation on autoimmunity is worthy of investigation since oxidative stress may contribute to its etiology. An early study showed that 0.4 mg/d of vitamin E supplementation eliminated autoimmune deterioration in MRL/*lpr* mice when compared with a vitamin E deficient group [36]. The daily vitamin E intake in their study was lower than that in our E5 group. The results suggest that low doses of vitamin E supplementation from an early stage could prevent autoimmune disease development. Although Venkatraman and Chu [37] reported that high vitamin E supplementation (500 mg/kg diet) had higher IL-2 production by spleen cells compared with lower doses (75 mg/kg diet) in MRL/*lpr* mice, the higher oil content (10%) used in their diet must be noted. Higher oil concentration may increase oxidative stress and expend some vitamin E. Therefore, the effect of vitamin E at the 500 mg/kg diet containing 10% corn oil in their study may not be the same with the diet containing 5% soybean oil used in our experiment.

Therefore, the dose of vitamin E may exert different effects and needs to be further clarified. Yu et al. [38] also indicated that enhanced cellular proliferation of mouse T-cell line (EL-4 cells) treated with 0.1 μg/mL VES via increased IL-2 production, whereas cellular proliferation was inhibited with high doses of 10 to 20 μg/mL VES. High concentrations of vitamin E may inhibit cell proliferation by interacting with cytosolic protein kinase C, thus preventing its membrane translocation, activation, and decreasing IL-2 production [39]. From our IL-2 mRNA results, a low dose of 0.25 μmol/L VES increased IL-2 mRNA expression, but high doses of 5 or 12.5 μmol/L decreased IL-2 mRNA expression, also suggesting that low doses and high doses of vitamin E exert opposite effects on the IL-2 production of immune cells.

The mechanisms of high concentrations of vitamin E on IL-2 production are now under investigation in our lab. Some studies have revealed that vitamin E plays roles beyond that of an antioxidant. More and more studies are suggesting that vitamin E may directly, or by modulating the intracellular redox status, downregulate the activation of NF-κB [40]. NF-κB is essential for the development of a Th1-type response and is the pivotal regulator of proinflammatory gene expression [41]. It has been documented that NF-κB is one of the transcription factors for the IL-2 promoter [42]. Thus, the opposite effect of high doses of vitamin E to decrease IL-2 expression can be explained. Recently, high doses of α-tocopherol (250 to 1000 μM) were also shown to induce peroxisome proliferator-activated receptor-gamma (PPAR-γ) expression in a dose-dependent fashion in hepatocytes [43]. The activation of PPAR-γ is also capable of inhibiting the expression of IL-2 by activated T-cells. On the contrary, IL-4 can induce the upregulation of expression of PPAR-γ in T cells [44]. Therefore, whether low and high dose vitamin E exerts different effects on IL-2 expression through the pathways of NF-κB or PPAR-γ is currently under study. Nevertheless, the evidence of decreased IL-2 through gene expression, but increased IL-4 secretion in our study, suggests that the imbalance of cytokines may be the possible

mechanism of action of the high doses of vitamin E supplementation.

In, conclusion, low dose of vitamin E improved survival, whereas high dose of vitamin E decreased the survival of MRL/*lpr* mice compared with lower dose. Low- and high-dose supplementations of vitamin E have opposite effects on the survival of MRL/*lpr* mice. High concentration of vitamin E inhibits IL-2 secretion and stimulates IL-4 and IL-10 production, and therefore, may not be beneficial for Th2 prone autoimmune diseases such as systemic lupus erythematosus.

This research was supported by a grant, NSC 89-2320-B-002-164, from the National Science Council of the Republic of China.

References

[1] Watanabe-Fukunage R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 1992;356: 314–7.

[2] Kotzin BL. Systemic lupus erythematosus. Cell 1996;85:303–6.

[3] Cohen PL, Eisenberg RA. Lpr and gld: single gene models of systemic autoimmunity and lymphoproliferative disease. Annu Rev Immunol 1991;9:243–69.

[4] Liang HE, Hsueh YP, Wu CC, Hsu SC, Han SH, Lai MZ. Atypical signaling defects prevent IL-2 gene expression in lpr/lpr CD4-CD8- cells. J Biomed Sci 1998;5:297–304.

[5] Takahaski S, Fossati L, Iwamoto M, Merino R, Motta R, Kobayakawa T, et al. Imbalance towards Th1 predominance is associated with acceleration of lupus-like autoimmune syndrome in MRL mice. J Clin Invest 1996;97:1597–604.

[6] Akahoshi M, Nakashima H, Tanaka Y, Kohsaka T, Nagano S, Ohgami E, et al. Th1/ Th2 balance of peripheral T helper cells in systemic lupus erythematosus. Arthritis Rheum 1999;42:1644–8.

[7] Segal R, Bermas BL, Dayan M, Kalush F, Shearer GM, Mozes E. Kinetics of cytokine production in experimental systemic lupus erythematosus. J Immunol 1997;158:3009–16.

[8] Blount S, Griffiths H, Emery P, Lunec J. Reactive oxygen species modify human DNA, eliciting a more discriminating antigen for the diagnosis of systemic lupus erythematosus. Clin Exp Immunol 1990; 81:384–9.

[9] Ruiz-Torres P, Lucio J, González-Rubio M, Rodríguez-Puyol M, Rodríguez-Puyol D. Oxidant antioxidant balance in isolated glomeruli and cultured mesangial cells. Free Radic Biol Med 1997;22:49–56.

[10] Packer L. Protective role of vitamin E in biological systems. Am J Clin Nutr 1991;53:1050S–5S

[11] Rock CL, Jacob RA, Bowen PE. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. J Am Diet Assoc 1996;96:693–702.

[12] Meydani SN, Beharka AA. Recent developments in vitamin E and immune response. Nutr Rev 1996;56:S49–S58.

[13] Lee CYJ, Wan JMF. Vitamin E supplementation improves cell-mediated immunity and oxidative stress of men and women. J Nutr 2000;130:2932–7.

[14] Brigelius-Flohè R, Kelly FJ, Salonen JT, Neuzil J, Zingg JM, Azzi A. The European perspective on vitamin E: current knowledge and future research. Am J Clin Nutr 2002;76:703–16.

[15] Rimbach G, Minihane AM, Majewicz J, Fischer A, Pallauf J, Virgli F, et al. Regulation of cell signaling by vitamin E. Proc Nutr Soc 2002;61:415–25.

[16] Comstock GW, Burke AE, Hoffman SC, Helzlsouer KJ, Bendich A, Masi AT, et al. Serum concentrations of α-tocopherol, β-carotene, and retinol preceding the diagnosis of rheumatoid arthritis and systemic lupus erythematosus. Ann Rheum Dis 1997;56:323–5.

[17] Bae SC, Kim SJ, Sung MK. Impaired antioxidant status and decreased dietary intake of antioxidants in patients with systemic lupus erythematosus. Rheumatol Int 2002;22:238–43.

[18] Venkatraman JT, Chandrasekar B, Kim JD, Fernandes G. Genotype effects on the antioxidant enzymes activity and mRNA expression in liver and kidney tissues of autoimmune-prone MRL/MpJ-lpr/lpr mice. Biochem Biophys Acta 1994;1213:167–75.

[19] Lin BF, Huang CJ, Chiang BL, Jeng SJ. Dietary fat influences Ia antigen expression, cytokines and prostaglandin E2 production of immune cells in autoimmune-prone NZB×NZW F1 mice. Br J Nutr 1996;75:711–22.

[20] Lin BF, Jeng SJ, Chiang BL, Huang CC. Dietary fat affects lipids and anti-cardiolipin antibody levels in autoimmune-prone NZB/W F1 mice. Br J Nutr 1997;77:657–69.

[21] Loizou S, Samarkos M, Norsworthy PJ, Cazabon JK, Walport MJ, Davies KA. Significance of anticardiolipin and anti-β_2-glycoprotein I antibodies in lupus nephritis. Rheumatology 2000;39:962–8.

[22] Lin BF, Jeng SJ. Effects of dietary frying oil on lipids and anti-cardiolipin antibody levels in autoimmune-prone NZB/W F1 mice. Nutr Res 1997;17:1047.

[23] Finch JM, Turner RJ. Effects of selenium and vitamin E on the immune responses of domestic animals. Res Veter Sci 1996;60:97–106.

[24] Cooney RV, Maskarinec G, Franke AA, Okinaka L, Karkoszka J, Wilkens LR, et al. Association of tocopherols with circulating autoantibody levels against an oxidized DNA nucleoside in humans. Free Radic Biol Med 2001;31:460–8.

[25] Wu WM, Suen JL, Lin BF, Chiang BL. Tamoxifen alleviates disease severity and decreases double negative T cells in autoimmune MRL-lpr/lpr mice. Immunology 2000;100:110–8.

[26] Li-Weber M, Giaisi M, Treiber MK, Krammer PH. Vitamin E inhibits IL-4 gene expression in peripheral blood T cells. Eur J Immunol 2002;32:2401–8.

[27] Beebe AM, Cua DJ, Malefyt RW. The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). Cytokine Growth Factor Rev 2002;13:403–12.

[28] Meydani SN, Barklund MP, Liu S, Meydani M, Miller RA, Cannon JG, et al. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. Am J Clin Nutr 1990;52:557–63.

[29] Wang Y, Huang DS, Wood S, Watson RR. Modulation of immune function and cytokine production by various levels of vitamin E supplementation during murine AIDS. Immunopharmacology 1995; 29:225–33.

[30] Moriguchi S, Maekawa K, Okamura M, Oonishi K, Kishino Y. Vitamin E prevents the decrease of cellular immune function with aging in spontaneously hypertensive rats. Nutr Res 1995;15:401.

[31] Han SN, Wu D, Ha WK, Beharka A, Smith DE, Bender BS, et al. Vitamin E supplementation increases T helper 1 cytokine production in old mice infected with influenza virus. Immunology 2000;100: 487–93.

[32] Pallast EG, Schouten EG, De Waart FG, Fonk HC, Doekes G, Blomberg BM. Effect of 50- and 100-mg vitamin E supplements on cellular immune function in noninstitutionalized elderly person. Am J Clin Nutr 1999;69:1273–81.

[33] De Waart FG, Portengen L, Doekes G, Verwaal CJ, Kok FJ. Effect of 3 months vitamin E supplementation on indices of the cellular and humoral immune response in elderly subjects. Br J Nutr 1997;78: 761–74.

[34] Kaul N, Devaraj S, Grundy SM, Jialal I. Failure to demonstrate a major anti-inflammatory effect with alpha tocopherol supplementation (400 IU/day) in normal subjects. Am J Cardiol 2001;87:1320–3.

[35] Payette H, Rola-Pleszczynski M, Ghadirian P. Nutrition factors in relation to cellular and regulatory immune variables in a free-living elderly population. Am J Clin Nutr 1990;52:927–32.

[36] Weimann BJ, Hermann D. Inhibition of autoimmune deterioration in MRL/lpr mice by vitamin E. Inter J Vit Nutr Res 1999;69:255–61.

[37] Venkatraman JT, Chu WC. Effects of dietary ω3 and ω6 lipids and vitamin E on proliferative response, lymphoid cell subsets, production of cytokines by spleen cells, and splenic protein levels for cytokines and oncogenes in MRL/MpJ-lpr/lpr mice. J Nutr Biochem 1999;10:582–97.

[38] Yu W, Sanders BG, Kline K. Modulation of murine EL-4 thymic lymphoma cell proliferation and cytokine production by vitamin E succinate. Nutr Cancer 1996;25:137–49.

[39] Boscoboinik D, Szewczyk A, Hensey C, Azzi A. Inhibition of cell proliferation by α-tocopherol. J Biol Chem 1991;266:6188–94.

[40] Rimbach G, Minihane AM, Majewicz J, Fischer A, Pallauf J, Virgli F, et al. Regulation of cell signaling by vitamin E. Proc Nutr Soc 2002;61:415–25.

[41] Liand Q, Verma IM. NF-κB regulation in the immune system. Nature Rev Immunol 2002;2:725–34.

[42] Aringer M. T lymphocyte activation—an inside overview. Acta Med Austriaca 2002;1:7–13.

[43] Davies GF, Mcfie PJ, Khandelwal RL, Roesler WJ. Unique ability of troglitazone to up-regulate peroxisome proliferator-activated receptor-γ expression in hepatocytes. J Pharmacol Exp Ther 2002; 300:72–7.

[44] Daynes RA, Jones DC. Emerging roles of PPARs in inflammation and immunity. Nat Rev Immunol 2002;2:748–59.