Basic nutritional investigation

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

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Abstract

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

Methods: Three-month-old MRL/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. © 2005 Elsevier Inc. All rights reserved.

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 (CD4̶ CD8̶) 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

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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 malondialdehyde 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].

Table 1

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

* Sources of ingredients: casein, ICN (Aurora, Ohio); cornstarch (Seoul, Korea); sucrose Taiwan sugar Co. (Taipei, Taiwan); cellulose, J. Betten-Maier & Söhne; Rosenberg (Holzmühle, 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.

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.
were then redissolved in 200 μL samples to a new tube and dried to remove the solvent. The samples were 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 (ε = 3260−1 M−1 cm, 292 nm in absolute ethanol).

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 (ε = 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% CO₂ 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 RNasey Mini kit (Qiagen, GmbH, Germany). The RNA was reverse transcribed 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 5 and 3 primers, respectively: IL-2, 5′ primer, 5′-TTCAAGCTCCACTTCAAGCTACGGAAG-3′; and 3′ primer, 5′-GACAGAAGGCTATCCATCTCCTCAGAAAGTCC-3′; β-actin, 5′ primer, 5′-GTGGGC CGCTCTAGGCACAA-3′; and 3′ primer, 5′-CTCTTG ATGTCACGCACGATTTC-3′. PCR products were visualized after electrophoresis on 2% agarose gels with 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.
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 $\alpha$-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 $\alpha$-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 $\alpha$-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>
<thead>
<tr>
<th>Table 2</th>
<th>$\alpha$-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*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>$\alpha$-tocopherol (μg/g tissue)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>20.1 ± 1.6$^c$</td>
</tr>
<tr>
<td>Kidney</td>
<td>15.0 ± 0.9$^a$</td>
</tr>
<tr>
<td>Plasma (μg/ml)</td>
<td>5.4 ± 0.4$^a$</td>
</tr>
<tr>
<td>TBARS contain (nmol/g tissue)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.9 ± 2.3</td>
</tr>
</tbody>
</table>

$a, b, c$ 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-$\alpha$-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.
for seven to eight mice per group.

IL-10 (pg/10^6 cell)

...higher in the E10 group compared with the control group...contrast, Th2 type cytokines IL-4 and IL-10 secreted by concanavalinA-stimulated splenocytes were significantly...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,

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.

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>E5</th>
<th>E7.5</th>
<th>E10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA autoantibodies (ELISA unit)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG 3.5 mo</td>
<td>2.62 ± 0.48</td>
<td>2.37 ± 0.48</td>
<td>1.99 ± 0.61</td>
<td>2.62 ± 0.36</td>
</tr>
<tr>
<td>4.5 mo</td>
<td>3.24 ± 1.01</td>
<td>2.85 ± 0.62</td>
<td>5.55 ± 1.36</td>
<td>3.97 ± 0.70</td>
</tr>
</tbody>
</table>
| IgM 3.5 mo    | 3.46 ± 0.75b | 3.23 ± 0.53b | 4.24 ± 0.72ab | 6.26 ± 1.30*
| 4.5 mo        | 3.79 ± 0.89b | 2.83 ± 0.63b | 3.44 ± 0.90b | 8.20 ± 3.16a |

Anti-cardiolipin autoantibodies (ELISA unit)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>E5</th>
<th>E7.5</th>
<th>E10</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG 3.5 mo</td>
<td>2.08 ± 0.30</td>
<td>2.31 ± 0.69</td>
<td>1.33 ± 0.28</td>
<td>1.20 ± 0.48</td>
</tr>
<tr>
<td>4.5 mo</td>
<td>1.72 ± 0.31</td>
<td>2.29 ± 0.43</td>
<td>1.51 ± 0.23</td>
<td>1.58 ± 0.36</td>
</tr>
</tbody>
</table>
| IgM 3.5 mo    | 0.92 ± 0.12b | 0.87 ± 0.11b | 0.99 ± 0.13b | 2.00 ± 0.37*
| 4.5 mo        | 1.11 ± 0.13b | 1.79 ± 0.21b | 1.93 ± 0.25b | 5.72 ± 1.21a |

* † 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.

1 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).

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

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

* † 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, interleukin-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.

1 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.

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*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>E5</th>
<th>E7.5</th>
<th>E10</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4 (pg/10^6 cell) Spontaneous†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|                | 1.29 ± 0.93b | 0.41 ± 0.27b | 3.92 ± 2.07b | 10.9 ± 0.82*
| ConA†          | 0.88 ± 0.58a | 5.71 ± 2.41b | 9.29 ± 2.11b | 11.5 ± 1.39*
| IL-10 (pg/10^6 cell) Spontaneous† |         |      |      |      |
|                | 3.47 ± 1.71 | 3.74 ± 2.74 | 3.56 ± 1.64 | ND |
| ConA†          | 1.09 ± 1.02b | 0.20 ± 0.18b | 15.4 ± 8.66b | 23.5 ± 6.74* |

* † 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.

1 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.

1 Stimulation with the ConA 5 mg/L for IL-4 secretion, and ConA 10 mg/L for IL-10 secretion.
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 antichondroilip 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 antichondroilip 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 antioxidant DNA autoantibody levels. This present study also showed significantly higher anti-dsDNA and antichondroilip 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 decreased 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.

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References


