Vitamin E Is an Important Factor in T Cell Differentiation in Thymus of F344 Rats

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Summary The effect of vitamin E (dl-a-tocopheryl acetate) on T cell differentiation in thymus of F344 rats was examined in this study. The rats were divided into three groups: vitamin E-free, regular and high vitamin E groups and fed a diet containing various levels of vitamin E (0, 50, and 585 mg/kg diet) for 7 weeks. The number of thymocytes was significantly lower in the vitamin E-free group relative to the regular group. Although the proportions of both CD4+CD8− and CD4−CD8+ T cells in thymocytes were significantly greater in the high vitamin E group, the proportion of CD4+CD8− T cells inversely decreased in vitamin E-free group compared to that of the regular group. The ratio of CD4+CD8−/CD4−CD8+ T cells increased in the high vitamin E group (p<0.01) and significantly decreased in the vitamin E-free group (p<0.001) compared to that of the regular group. Although the marked changes of T cell subsets were not seen in peripheral blood lymphocytes (PBL), the ratio of CD4+CD8−/CD4−CD8+ T cells was significantly lower in the vitamin E-free group and significantly greater in the high vitamin E group compared to that of the regular group. Production of interleukin (IL) 2 by thymocytes following the stimulation with Con A for 48 h increased about threefold in the high vitamin E group compared to the regular group. Conversely, thymocytes from rats fed the vitamin E-free diet showed a significant decrease of IL2 production compared to that of the regular group. Prostaglandin E2 (PGE2) production from thymocytes was significantly lower in the high vitamin E group compared to that of the regular group, whereas thymocytes of rats fed the vitamin E-free diet showed a significant increase of PGE2 production compared to that of rats fed the regular diet. Furthermore, in vitro addition of indomethacin provided a restoration of IL2 production from thymocytes of rats fed the vitamin E-free diet to the level of rats fed the regular diet. These results suggest that vitamin E plays an important role in T cell differentiation in

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thymus, which may be related to the action of vitamin E as antioxidant.

**Key Words** vitamin E, T cell differentiation, thymus, interleukin 2, prostaglandin E2, rats

Vitamin E acts as an important antioxidant in cellular membranes and its main function is to protect the unsaturated bonds of cellular membrane phospholipids against free radical attack (1). In addition, it has been shown that vitamin E also modulates immune responses. Tengerdy et al. have reported that dietary supplementation with vitamin E leads to enhanced humoral immune responses and increases resistance to bacterial infection in mice and chickens (2). Tanaka et al. have found that dietary supplementation of vitamin E induces the enhancement of helper T cell activity in mice (3). We have also reported that high intakes of vitamin E increases the functions of splenic lymphocytes and alveolar macrophages (4). Conversely, Sheffy and Schultz have found that lymphoproliferative responses with mitogens were depressed in cells from vitamin E-deficient dogs (5). Langweiler et al. have reported that sera from vitamin E-deficient dogs contain a factor capable of greatly suppressing mitogen-induced blastogenesis (6). We have also found that production of macrophage-activating factor from splenic lymphocytes stimulated with concanavalin A (Con A) is much less in rats fed the vitamin E-deficient diet (7). Although there have been many reports showing the great effect of vitamin E on immune functions, there have been few reports concerning the effects of vitamin E on the differentiation of T cells in thymus.

The purpose of this study is to demonstrate the effects of vitamin E on the differentiation of T cells in thymus by monitoring the proportions of CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells with two-color flow cytometry and to evaluate the possible association between the effect of vitamin E on T cell differentiation in thymus and the action of vitamin E as an antioxidant.

**EXPERIMENTAL METHODS**

*Animals and diets.* In the present study, specific-pathogen-free, inbred F344 male rats, 6 weeks old, weighing 100 g, were purchased from Japan SLC, Inc. (Shizuoka) and divided into three groups: vitamin E-free, regular, and high vitamin E groups. Each group consisted of 10 rats. They were fed a diet containing various levels of vitamin E (0, 50, and 585 mg/kg diet) for 7 weeks (Table 1). Food and water were given in free access. Body weight and food intake were measured daily. The animals were killed under anesthesia with sodium pentobarbital (5 mg/100 g body weight, Abbott Laboratories, North Chicago, IL) after consumption of experimental diets for 7 weeks. The thymus of each animal was removed aseptically and used for the assays. In this paper, the representative data from three experiments are shown in each figure.

*Thymocyte suspension.* Thymus was minced with scissors and passed through
**Table 1. Composition of the basal diet.**

| Ingredient               | Concentration (g/100 g) |
|--------------------------|-------------------------|
| Vitamin-free casein¹     | 20                      |
| Sucrose                  | 10                      |
| Cornstarch               | 57                      |
| Stripped corn oil²       | 8                       |
| Mineral mixture³         | 4                       |
| Vitamin mixture⁴         | 1                       |

¹Oriental Yeast, Tokyo, Japan. ²Eisai Pharmaceutical Co., Tokyo, Japan. ³The mineral mixture had the following composition (mg/100 g): K, 420; P, 990; Na, 250; Mg, 74.9; Fe, 27.0; Zn, 5.1; Mn, 2.2; Cu, 0.57; I, 0.46. All the materials used in this experiment were gifts of Eisai Pharmaceutical Co., Tokyo, Japan, and were analytical grade. ⁴The vitamin mixture had the following composition (mg/100 g): thiamine, 2.4; riboflavin, 8.0; pyridoxine, 1.6; cyanocobalamin, 0.001; ascorbic acid, 60.0; menadione, 10.4; biotin, 0.04; folic acid, 0.4; Ca-pantothenate, 10.0; p-aminobenzoic acid, 10.0; myo-inositol, 12.0; niacin, 12.0; choline-chloride, 400.0. The following vitamins were also added, in mg/kg diet: retinyl acetate, 0.03; cholecalciferol, 0.005. Regular and high vitamin E diets were prepared by adding 50 or 585 mg of dl-a-tocopherol acetate per kilogram diet, respectively. All the vitamins were also gifts of Eisai Pharmaceutical Co., Tokyo, Japan.

a stainless steel sieve in RPMI 1640 culture medium (Gibco Laboratories, Grand Island, NY). The cells were washed twice with cold RPMI 1640 and used for the following assays.

**Preparation of peripheral blood lymphocytes (PBL).** PBL were isolated from heparinized peripheral blood by using Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation and counted microscopically.

**Proliferation of thymocytes and PBL.** Thymocytes and PBL isolated from each animal were adjusted to $1 \times 10^7$ and $2 \times 10^6$ cells/ml in RPMI 1640 culture medium supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethansulfonic acid (HEPES, Sigma Chemical Co., St. Louis, MO), 5 μM 2-mercaptoethanol (2-ME, Sigma), and 5% heat-inactivated fetal bovine serum (FBS, Gibco Laboratories, Grand Island, NY), respectively. Thymocytes and PBL with or without mitogens such as phytohemagglutinin (PHA, 10 μg/ml, Sigma) and concanavalin A (Con A, 5 μg/ml, Sigma), were plated in 96-well microtiter plates, incubated at 37°C in humidified incubator with 5% CO₂ and 95% air for 72 h, and then pulsed with $[^3H]$thymidine (specific activity 25 μCi/mmol, New England Nuclear, Boston, MA). After 18 h, they were harvested by an automated sample harvester (Flow Laboratories, Rockville, MD). The radioactivity was determined by a liquid scintillation counter (LSC-703, Aloka Corp., Tokyo). The data are indicated as counts per minutes (cpm).

**Flow cytometry analysis.** Thymocytes and PBL isolated from each animal
were stained with both fluorescein isothiocyanate (FITC)-conjugated anti-rat CD4 monoclonal antibody (mAb) (W3/25, Serotec Ltd., Oxford, UK) and phycoerythrin (PE)-conjugated anti-rat CD8 mAb (OX8, Serotec Ltd., Oxford, UK) for two-color staining. Stained cells were fixed in 0.1% paraformaldehyde in saline and analyzed with a FACScan flow cytometer and Consort 30 software program (Becton Dickinson, Co., Mountain View, CA) after excluding dead cells by using forward and side light scatters.

**IL2 assay.** Thymocytes (1×10^7 cells/ml) isolated from each animal were mixed with alveolar macrophages (1×10^5 cells) harvested from the same animal as antigen-presenting cells (APC), and they were cultured with Con A (5.0μg/ml) in RPMI 1640 culture medium supplemented with 100mM sodium pyruvate (Sigma), 10% heat-inactivated FBS, and 5μM 2-ME for 48 h. Then, IL2 activity in the culture medium was measured by the proliferation of IL2-dependent CTLL-2 cells (8). Five thousand CTLL cells in a volume of 100μl were added to each well and the plates were incubated at 37°C for 24 h. Then 1μCi of [3H]thymidine was added to each well and further incubated for 24 h. The incorporation of [3H] thymidine into DNA of CTLL cells was measured by a liquid scintillation counter (LSC-703, Aloka Corp., Tokyo). IL2 activity in the culture medium was expressed as units/ml in comparison with the activity of murine recombinant IL2 (Genzyme, Boson, MA).

**Prostaglandin E2 production from thymocytes.** Thymocytes (1×10^7 cells/ml) were incubated with lipopolysaccharide (LPS; 10μg/ml) in 5% incubator at 37°C for 48 h. Then, the supernatants of the thymocyte cultures were harvested and used for the PGE2 assay. PGE2 concentration in the supernatant was determined by prostaglandin E2 enzyme immunoassay (EIA) system (Amersham International plc, Amersham, UK).

**Vitamin E concentrations in plasma and thymus.** The concentrations of α-tocopherol in plasma and the supernatants of thymic homogenates, which were prepared by 10ml of RPMI 1640 medium, were determined by HPLC (9).

**Statistical analysis.** Results are presented as M±SEM (n=10). Differences between the regular and two experimental groups were analyzed by using Student's two-tailed t-test. The difference associated with p value <0.05 was regarded as statistically significant.

RESULTS

**Vitamin E concentrations in plasma and thymus**

The concentrations of vitamin E in plasma and thymus were dependent on the contents of vitamin E in the diets. The concentrations of vitamin E in plasma and thymus were significantly lower in the vitamin E-free group (p<0.01) and significantly higher in the high vitamin E group (p<0.001) compared to that of the regular group (Fig. 1A and B).
Fig. 1. \(\alpha\)-Tocopheryl concentrations in plasma (A) and thymus (B) of rats fed regular (50 mg/kg), vitamin E-free (0 mg/kg) and high vitamin E diet (585 mg/kg) for 7 weeks. Values are M±SEM for 10 rats per group. Significantly different from the regular group: ** \( p<0.01 \), *** \( p<0.001 \).

**Food intake, and body and thymic weights**

Daily food intakes of the regular and two experimental groups were not significantly different throughout the experiment, and the daily weight gain of each group was also not significantly different. There were not significant differences in the final body weights among the three groups. There were also not significant differences in the thymic weights between the regular and two experimental groups (Table 2).

**Numbers of thymocytes and peripheral blood lymphocytes (PBL)**

As shown in Table 2, the number of thymocytes in rats fed the vitamin E-free diet was significantly lower than in rats fed the regular diet. The number of peripheral blood lymphocytes (PBL) was also significantly lower in the vitamin E-free group compared to the regular group.

Table 2. Thymic weight and numbers of thymocytes and peripheral blood lymphocytes (PBL) of rats fed regular, vitamin E-free, and high vitamin E diets for 7 weeks.\(^1\)

| Groups     | Thymus (g/100 g BW) | Thymocytes \((\times 10^7/0.1 \text{ g thymus})\) | PBL \((\times 10^6/\text{ml blood})\) |
|------------|---------------------|-----------------------------------------------|----------------------------------|
| Regular    | 0.13±0.01           | 2.21±0.16                                     | 2.63±0.26                       |
| VE-free    | 0.12±0.01           | 1.77±0.11\(^2\)                               | 2.76±0.05                       |
| High VE    | 0.14±0.02           | 1.96±0.13                                     | 3.30±0.49                       |

\(^1\)Values are M±SEM \((n=10)\). \(^2\)Significantly different from the regular group, \( p<0.01 \).
diet was significantly lower in comparison with that of the regular group. In contrast to the number of thymocytes, no significant difference in the number of PBL was found between the regular and two experimental groups.

**Proportions of CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells in thymocytes and PBL**

The proportions of CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells in thymocytes were significantly greater in the high vitamin E group compared to those of the regular group (Fig. 2A). Further, the ratio of CD4⁺CD8⁻/CD4⁻CD8⁺ T cells was also significantly higher in the high vitamin E group than that of the regular group. In contrast, thymocytes from rats fed the vitamin E-free diet showed a significant decrease in the proportion of CD4⁺CD8⁻ T cells, which resulted in a significant decrease of CD4⁺CD8⁻/CD4⁻CD8⁺ T cell ratio. In PBL, there were no significant changes in the proportions of CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells as shown in thymocytes from both the vitamin E-free and the high vitamin E groups (Fig. 2B). However, the ratio of CD4⁺CD8⁻/CD4⁻CD8⁺ T cells in PBL was significantly greater in the high vitamin E group and significantly lower in the vitamin E-free group compared to that of the regular group.

**Proliferations of thymocytes and PBL with PHA and Con A**

In the high vitamin E group, proliferations of thymocytes with PHA and Con

![Graph A](image)

**Fig. 2.** Proportions of CD4⁺CD8⁻ (■) and CD4⁻CD8⁺ (▲) T cells, and the ratio of CD4⁺CD8⁻/CD4⁻CD8⁺ T cells (△) in thymocytes (A) and PBL (B) of rats fed regular, vitamin E-free and high vitamin E diets for 7 weeks. Values are M±SEM for 10 rats per group. Significantly different from the regular group: *p<0.05, **p<0.01, ***p<0.001.

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Effects of vitamin E-free and high vitamin E diets on responses of rat thymocytes (A) and PBL (B) to mitogens. After rat thymocytes and PBL were isolated from rats fed regular, vitamin E-free, and high vitamin E diets, they were cultured for 4 days with phytohemagglutinin (PHA) and concanavalin A (B). At 17 to 24 h before the termination of the culture, 50 μl of [3H]thymidine (20 μCi/ml) was added to each well. Each value is the M±SEM of 10 rats. Significantly different from the regular group: * p<0.05, ** p<0.01, *** p<0.001.

A were similar to those of the regular group as shown in Fig. 3A. In contrast, thymocytes of rats fed the vitamin E-free diet showed significantly decreased responses to PHA and Con A (p<0.01 and p<0.001). In the proliferation of PBL with PHA and Con A, the high vitamin E group showed significant increases compared to those of the regular group. The proliferations of PBL from rats fed the vitamin E-free diet were significantly lower compared to those of the regular group (Fig. 3B).

**Interleukin (IL) 2 production by thymocytes**

Although IL2 production was increased about threefold in thymocytes of rats fed the high vitamin E diet compared to that of the regular group, the thymocytes from rats fed the vitamin E-free diet did not show any production of IL2 following *in vitro* stimulation with Con A for 48 h (Fig. 4).
Fig. 4. Interleukin 2 production by thymocytes of rats fed regular, vitamin E-free, and high vitamin E diets for 7 weeks. IL2 activity was measured in the supernatants of thymocytes cultured with concanavalin A (Con A) for 48 h and assessed in CTLL-2 cell growth assay as described in MATERIALS AND METHODS. Values are M±SEM of 10 rats. Significantly different from the regular group: ***p<0.001; ND, not detected.

Prostaglandin E2 (PGE2) production by thymocytes.

PGE2 produced from thymocytes of rats fed the regular diet was 212±38 pg/ml. In the thymocytes of the high vitamin E group, the production of PGE2 was significantly lower compared to that of the regular group (Fig. 5). In contrast, PGE2 production from thymocytes of rats fed the vitamin E-free diet was significantly higher than that of the regular group.

In vitro effect of indomethacin on IL2 production from thymocytes

To investigate the effect of PGE2 on IL2 production, thymocytes were cultured with 10μM of indomethacin, an inhibitor of prostaglandin synthesis, for 48 h and then IL2 activity in their supernatants was measured by CTLL-2 cell growth assay. As shown in Fig. 6, the addition of indomethacin to the thymocyte cultures could
enhance the production of IL2 from the thymocytes of rats fed the regular and vitamin E-free diets. However, the production of IL2 from thymocytes of the high vitamin E group was not influenced by the addition of indomethacin.

DISCUSSION

Rats fed the vitamin E-free and the high vitamin E diets for 7 weeks showed no changes in body and thymic weights compared to those of rats fed the vitamin E-free diet. Eskew et al. have found that rats fed diet deficient in both vitamin E and selenium decreased their food intakes and began to lose weight by the fourth week. However, they did not notice any changes of food intake and weight gain in rats fed vitamin E-deficient diet (10), which was in agreement with our results in this study. There are relatively few studies on the effects of vitamin E on thymic cellularity and functions. In the present study the number of thymocytes was significantly lower in rats fed the vitamin E-free diet compared to that of rats fed the regular diet as shown in Table 2. Since T cell development mostly takes place in the thymus (11), the decreased number of thymocytes in rats fed the vitamin E-free diet appears to be due to the decrease of T cell number in thymocytes. Because T cells in thymocytes come from bone marrow and differentiate in thymus, the decreased number of T cells in thymocytes seen in the vitamin E-free group may be relevant to the decreased proliferation of stem cells in bone marrow. However, vitamin E deficiency did not have any effects on the proliferation of bone marrow cells (unpublished data). Thus, a possible explanation for the decreased number of thymocytes in vitamin E-deficient rats is that vitamin E deficiency may induce the deficit of T cell differentiation and/or maturation in thymus. In the present study rats fed the vitamin E-free diet showed not only the decreased number of thymocy-
tes but also a significant decrease of the proportion of CD4<sup>+</sup>CD8<sup>-</sup> (helper/inducer) T cells in thymocytes compared to that of rats fed the regular diet. This result suggests that vitamin E deficiency may cause the decreased proliferation of CD4<sup>+</sup>CD8<sup>-</sup> T cells in thymic medulla. In general, the repertoire of T cell antigen receptors and the capacity to distinguish self from non-self develop during the differentiation and/or maturation of T cells in thymus through the interaction with antigen-presenting cells (APC) (12). Most of T cells (>95%), coming from bone marrow and existing in thymic cortex, are going to eliminate, which is called as apoptosis and the remaining cells further proliferate in thymic medulla (13). Vitamin E deficiency may cause the defect of T cell proliferation in thymic medulla rather than the defect of the repertoire of T cell antigen receptors in thymic cortex.

IL2, which is produced by activated T cells, is needed for the proliferation of T cells (14). As shown in Fig. 4, the production of IL2 from thymocytes was significantly lower in rats fed the vitamin E-free diet and their IL2 activity could not be detected in the assay used in this experiment. The reasons why the vitamin E deficiency causes the marked decrease of IL2 production in thymocytes are follows: that is, 1) macrophage function as APC may be depressed in vitamin E deficiency and 2) the capacity of T cells producing IL2 may be lowered in vitamin E deficiency. As our previous study has shown that vitamin E deficiency causes not the decrease of alveolar macrophage function, but the increase of their phagocytic activity against opsonized sheep red blood cells (8), at least vitamin E deficiency does not appear to decrease the functions of macrophages. As shown in Fig. 2A, the proportion of CD4<sup>+</sup>CD8<sup>-</sup> T cells in thymocytes was significantly lower in the vitamin E-free group compared to that of rats fed the regular diet. Since IL2 is produced by mature T cells (CD4<sup>+</sup>CD8<sup>-</sup> and CD4<sup>-</sup>CD8<sup>+</sup>) (15), the decreased production of IL2 by thymocytes in the vitamin E-free group may be explained by the decreased capacity of T cells producing IL2, which is closely related to the decreased proportion of T cell subset (CD4<sup>+</sup>CD8<sup>-</sup>). Further, this result also appears to be related to the decreased proliferation of thymocytes and PBL with PHA and Con A in rats fed the vitamin E-free diet as shown in Fig. 3A and B.

Conversely, rats fed the high vitamin E diet showed no change in thymocyte number, but the proportions of CD4<sup>+</sup>CD8<sup>-</sup> and CD4<sup>-</sup>CD8<sup>+</sup> T cells in their thymocytes were significantly increased (Fig. 2A). Further, the production of IL2 by thymocytes was also significantly greater in rats fed the high vitamin E diet compared to that of rats fed the regular diet. As we have previously found that alveolar macrophages can be greatly activated by high vitamin E diets (4), the increase of macrophage function as APC in thymus also ensues, which may bring the increased production of IL2 by thymocytes. In addition, the marked increases of CD4<sup>+</sup>CD8<sup>-</sup> and CD4<sup>-</sup>CD8<sup>+</sup> T cells in thymocytes may be directly related to the higher production of IL2 in thymocytes of rats fed the high vitamin E diet. In PBL there were no significant changes in the proportions of T cell subsets as shown in thymocytes of the vitamin E-free and the high vitamin E groups. However, the ratio of CD4<sup>+</sup>CD8<sup>-</sup>/CD4<sup>-</sup>CD8<sup>+</sup> T cells was significantly greater in the high
vitamin E group and significantly lower in the vitamin E-free group compared to that of rats fed the regular diet, which may explain enhanced or depressed proliferations of PBL with mitogens such as PHA and Con A as shown in Fig. 3B.

Up to now, the mechanism by which vitamin E suppresses or enhances immune functions described previously in most of papers has been explained by the degree of prostaglandin E\(_2\) (PGE\(_2\)) synthesis from phospholipids in cellular membrane. Vitamin E suppresses the peroxidation of phospholipids in cellular membrane via the inhibition of phospholipase A\(_2\) activity (16). It results in the decreased production of arachidonic acid from membrane phospholipids, which induces the decreased production of PGE\(_2\). Since it is known that PGE\(_2\) has an inhibitory effect on cellular immune functions via the increase of cAMP concentration in immune cells (17), it is considered that vitamin E modulates immune functions via PGE\(_2\) synthesis. Furthermore, it is known that PGE\(_2\) also has the inhibitory effect on IL2 production by T cells (18). A marked increase or significant decrease of IL2 production following the intake of high vitamin E or vitamin E-free diet as shown in Fig. 4 may be also closely related to the degree of PGE\(_2\) synthesis. In fact, our results are coincident with it and have shown the increase of PGE\(_2\) production in vitamin E deficiency and the decrease of PGE\(_2\) production in the high vitamin E group as reported previously (19,20). These changes of PGE\(_2\) production from thymocytes may affect the production of IL2 by T cells in thymocytes and result in decreased or increased proportions of CD4\(^+\)CD8\(^-\) T cells in thymocytes of the vitamin E-free and the high vitamin E group, respectively. Since the in vitro addition of indomethacin, an inhibitor of PGE\(_2\) synthesis, to the thymocyte cultures had the stimulatory effect on IL2 production from thymocytes of rats fed the vitamin E-free diet (Fig. 6), the higher production of PGE\(_2\) in thymocytes of rats fed the vitamin E-free diet appears to be relevant to the decreased production of IL2. In summary, the effects of vitamin E on T cell differentiation and/or maturation in thymus are closely related to the antioxidative action of vitamin E, which primarily causes the decreased production of PGE\(_2\) in the high vitamin E group and the increased production of PGE\(_2\) in the vitamin E-free group from cell membrane phospholipids, with resultant increased or decreased production of IL2 by thymocytes, respectively.

There are many theories concerning the mechanism of aging. One of them is that the accumulation of lipid peroxides is associated with aging (21). As it is known that vitamin E inhibits lipid peroxidation (1), vitamin E is called the vitamin for preventing the aging. Nagel et al. have reported that the proportion of CD4\(^-\)CD8\(^+\) (suppressor/killer) T cells is significantly lower in the aged group in comparison to the young group (22). In this experiment, the proportion of CD4\(^+\)CD8\(^-\) T cells in thymocytes was significantly greater in the high vitamin E group compared to that of the regular group as shown in Fig. 2A. Furthermore, the ratio of CD4\(^+\) CD8\(^-\)/CD4\(^+\) CD8\(^+\) T cells in PBL was significantly higher in the high vitamin E group. These results may indicate a possibility that vitamin E has an ability to prevent or delay the decrease of cellular immune functions with aging. In fact,
Meydani et al. have also found that vitamin E supplementation improves immune responsiveness in healthy elderly subjects, which is mediated by a decrease in PGE₂ and/or other lipid peroxidation products (23). Corwin and Shloss (24) found that although vitamin E did stimulate the response of BALB/c spleen cells to lipopolysaccharide (LPS), the response was absent in athymic nude mice. Based on those results, they have suggested that vitamin E is an important thymic factor to produce mature helper T cells. The present findings have confirmed the above observations that vitamin E is an important nutrient to enhance and/or maintain T cell differentiation in thymus and to prevent the decrease of cellular immune functions with aging.

This is the first report showing that vitamin E plays an important role in differentiation and maturation of T cells in thymus. Since it is known that thymic epithelial cells are also closely related to T cell differentiation and maturation in thymus (25), further work is in progress to elucidate the thorough mechanism of vitamin E on T cell differentiation and maturation in thymus.

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