The role of vitamin E in T-cell differentiation and the decrease of cellular immunity with aging

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Abstract : Spontaneously hypertensive rats (SHR) as a model for aging were used in this experiment and fed a regular (50 IU/Kg diet) or high vitamin E (500IU/Kg diet) diet for 6weeks. At 12weeks old, they were killed and assayed. Although proliferation of thymic lymphocytes was significantly decreased in SHR fed the regular diet compared to Wistar Kyoto rats (WKY) fed the same diet, high vitamin E diet enhanced proliferation of thymic lymphocytes in SHR to almost the levels in WKY fed the regular diet. In addition, the expressions of both CD4 and CD8 antigens on $CD4^+CD8^+$ T cells, immature T cells existing in thymic cortex, were also decreased in SHR, and significantly improved by high vitamin E diet. These results suggest that high vitamin E diet enhances thymic lymphocyte proliferation through increased T-cell differentiation in thymus. Then, the effect of vitamin E on T-cell differentiation in thymus was investigated by using male Fisher rats. 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 500IU/Kg diet) for 7weeks. Although the percentages of $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells in thymocytes were significantly greater in the high vitamin E group, the percentage of $CD4^+CD8^-$ T cells inversely decreased in the vitamin E-free group compared to the regular group. We have tried to investigate the mechanism of the increased T-cell differentiation in thymus of rats fed the high vitamin E diet through cytokine production, and thymic epithelial cell (TEC) and macrophage functions. We have found that vitamin E enhances T-cell differentiation through the increase of not macrophage but TEC function in thymus, which is associated with the increased binding capacity of TEC to immature T cells via increased expression of adhesion molecule, ICAM-1. These results suggest that vitamin E is a potent nutrient for promoting health in the aged via the improvement of cellular immunity decreased with aging. J. Med. Invest. 45: 1-8, 1998

Key words **:** *vitamin E, thymic epithelial cells (TEC), adhesion molecule, ICAM-1, rats*

INTRODUCTION

It is estimated that by the 21st century, one fourth of Japan's population will be older than 65 years of age. Accompanying this increased percentage of elderly people will be an increased incidence of geriatric disorders and infectious diseases associated with the less of cellular immunity that occurs during aging. If this decrease of cellular immunity can be diminished, it may improve the health of older persons and prolong life expectancy. Some reports have described beneficial effects of vitamin E supplementation on cellular immune functions in aged mice (1). This finding may be related to the decrease in the serum vitamin E level during aging and the actions of vitamin E as an antioxidant and immunostimulator.

We initiated an investigation of the effect of vitamin

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E supplementation on cellular immune functions with aging and tried to establish a good experimental model. A good model in mice, the senescence-accelerated mouse (SAM) (2), had been already established. However, a good model for aging had not been established in rats. Spontaneously hypertensive rats (SHR) were derived from Wistar Kyoto rats (WKY) by Okamoto and Aoki (3) and are now used widely as an animal model for the study of human essential hypertension. Because SHR exhibit an accelerated decrease in, and abnormalities of, cellular immune functions with aging. Takeichi et al. (4, 5) have proposed that SHR are a good model for the study of not only human essential hypertension but also the mechanism of aging.

We examined effects of vitamin E supplementation on cellular immunity during aging in SHR. We found that vitamin E levels in serum and thymus decreased significantly more during aging in SHR than in WKY (6). Takeichi et al (7) reported that the early decrease of cellular immunity in SHR was associated with thymic dysfunction following increased production of natural thymocytotoxic autoantibody (NTA). Based on these two studies, we specu-lated that the increased production of NTA in thymus might be associated with the decrease of vitamin E level in thymus and that vitamin E may play an important role in T cell differentiation in thymus. This paper describes not only the role of vitamin E in T-cell differentiation, but also the protective action of vitamin E against injuries to the thymus and bone marrow after X-ray irradiation.

Effect of Vitamin E Supplementation on the Decrease of Cellular Immunity in SHR with Aging

In SHR, mitogensis and natural killer cell (NK) activity of splenocytes declined remarkably early in life, whereas alveolar macrophage (AM) showed greater phagocytic activity compared with levels in WKY (6). Furthermore, vitamin E supplementation (500IU/Kg diet) prevented the decrease in proliferation of thymocytes or splenocytes observed in SHR after phytohemagglutinin (PHA) and concanavalin A (Con A) stimulation. This recovery in SHR fed the high vitamin E to the level in WKY fed the control diet (50IU/kg diet) was closely related to the increasing vitamin E levels in plasma and thymus, which were almost equal to those of WKY fed the control diet.

Many reports have shown that vitamin E deficiency impairs both humoral and cellular immunities. Bendich (8) reported that vitamin E deficiency caused impaired T-cell function and moderate impairment of B cell function. Vitamin E deficiency also decreased the mitogenic response of T cells, which was reversed after vitamin E repletion (9). From these reports, it is suggested that decreased cellular immune function in SHR is evoked by the decrease of vitamin E level in plasma or thymus.

Vitamin E deficiency causes increased production of prostaglandin E_2 (PGE_2), which depresses T cell functions by increasing the cellular cyclic adenosine monophosphate (cAMP) level (10). We also found that restoration of vitamin E levels in both thymus and spleen in SHR to the levels of WKY by feeding the high vitamin E diet improved T-cell mitogenesis in thymocytes and splenocytes (6). NK activity of splenocytes in SHR was not affected by the high vitamin E diet and was significantly lower than that of WKY. However, phagocytic activity of AM against opsonized sheep red blood cells (SRBC) was significantly higher in SHR than in WKY.

As it is known that macrophages are nonspecifically activated in athymic, nude mice (11), AM function in SHR, which show decreased function of T cells, also may be nonspecifically activated. This activation may represent compensation for the defect in the T-cell-mediated immune system of SHR with aging.

Several possible explanations for decreased vitamin E levels in serum and some immune tissues of SHR have been considered. First, because levels of vitamin E in serum, thymus, and spleen of SHR increased when the rats consumed the high vitamin E diet, the decreased vitamin E level in SHR does not appear to be due to malabsorption of vitamin E. Second, the corn oil used in this experiment contains a considerable quantity of polyunsaturated fatty acids, which also may further promote the wastage of vitamin E.

Although many studies show decreased T-cell function in vitamin E deficiency (12), macrophage functions appear to be well-maintained. We have found that vitamin E deficiency causes enhancement rather than depression of AM function (13). The difference between T-cell and AM functions in vitamin E deficiency may result from differences in the sensitivity of the two systems PGE_2, which increases in concentration in vitamin E-deficient animals (10) and suppresses cellular immune functions. To data, several reports have shown that high vitamin E diets enhance lymphocyte functions (1, 8, 14). We also have found that high vitamin E diets enhance mitogenesis of splenic lymphocytes and phagocytosis of AM against opsonized SRBC, which was closely associated with the decreased production of PGE_2 following the inkate of high vitamin E diet. As mentioned previously, both vitamin E-deficeint and high vitamin E diets induced higher levels of phagocytic activity of AM in rats. However,

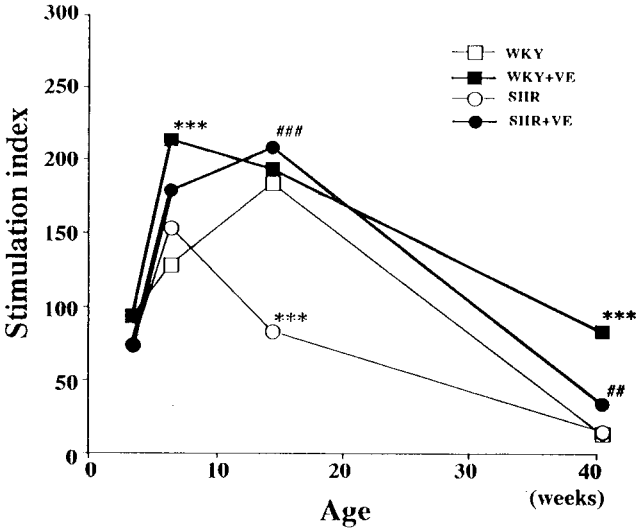

Fig.1. Proliferation of thymocytes with Con A in WKY and SHR fed control or high vitamin E diet for 40weeks. Thymocytes with Con A(5μg/ml) were plated in 96-well microtiter plates, incubated at 37℃ in a 5% CO_2 incubator for 72hr, and then pulsed with [$_3$H] thymidine. After 20hr, the cells were harvested and their radioactivity was determined with a liquid scintillation counter. Data were presented as the stimulation index, which was calculated by assigning a value of 1 to the radioactivity of thymocytes cultured with medium and comparing this to the radioactivity of thymocytes from each group treated with Con A. Values are the mean ± SD from 5 rats, Significantly different from WKY fed the control diet (***P<0.001). Significantly different from SHR fed the control diet (##P<0.01, ###P<0.001).

the mechanisms by which each diet enhanced phagocytic activity of AM against opsonized SRBC appeear to be different.

In addition, the decrease in T-cell functions in SHR is also associated with increased production of natural thymocytotoxic autoantibody (NTA) in SHR with aging (7). We also have found that NTA titer in serum of SHR steadily increased with aging, and that vitamin E supplementation suppresses this increase (15). This change resembles that of proliferation of thymocytes with Con A with aging in SHR (Fig. 1). The increase of NTA in SHR may be relevant to the impairment of T-cell differentiation and maturation in thymus of SHR. In other words, the decrease of NTA titer in the serum of SHR fed a high vitamin E diet may be, in part, associated with vitamin E-induced restoration of cellular immune functions that decrease with aging in SHR. These results suggest that vitamin E-induced restoration of cellular immune functions that deteriorate with aging in SHR is related to normalization of T-cell differ-entiation in thymus of SHR.

Effects of Vitamin E Deficiency and Supplementation on T-cell Differentiation in Thymus

Although many reports have shown substantial effects of vitamin E on immune functions, few have described the effects of vitamin E on differentiation of T cells in thymus. From the results of experiments with SHR (6, 8), we have speculated that vitamin E is an important factor in T-cell differentiation in thymus. To test this hypothesis, 6-week-old F344 rats were separated into three groups and fed diets containing various levels of α-tocopheryl acetate:0 (vitamin E-free), 50 (regular), and 500 (high vitamin E) IU/kg diet for 7 weeks.

The number of thymocytes in rats fed the vitamin E-free diet was significantly lower than in those fed the regular diet. As T-cell development occurs in young rats mainly in the thymus (16), the decreased number of thymocytes in rats fed the vitamin E-free diet appears to be due to a decrease in the number of T cells in thymocytes. Because T cells in thymocytes come from bone marrow and differentiate in thymus, the decreased number of T cells in thymocytes in the vitamin E-free group may result from decreased proliferation of stem cells in bone marrow. However, vitamin E deficiency did not affect proliferation of bone marrow cells [unpublished data].

Another possible explanation for the decreased number

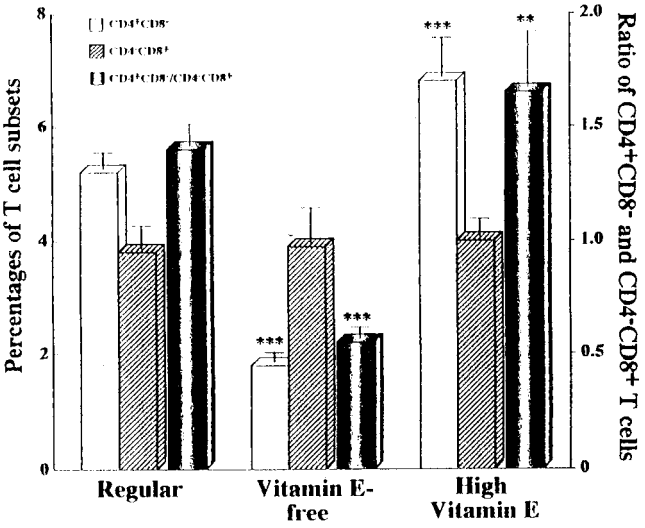

Fig.2. Percentages of $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells, and the ratio of $CD\ 4^+CD\ 8^-$ and $CD\ 4^-CD\ 8^+$ T cells in thymocytes of rats fed the regular, vitamin E-free and high vitamin E diets. Thymocytes were isolated from rats fed regular, vitamin E-free and high vitamin E diets for 7weeks and stained with both fluorescein isothiocyanate (FITC)-conjugated anti-rat CD mAb and phycoerythrin (PE)-conjugated anti-rat CD8 mAb. After they were fixed in 0.1% paraformaldehyde, they were analyzed with a FACScan flow cytometer and Consort 30 software program. Values are the mean ± SD for 10 rats per group and indicate the percentage of cells in the population per 10^4 thymic lymphocytes. Significantly different from the regular group (**P<0.01, ***P<0.001).

of thymocytes in vitamin E-deficient rats is that vitamin E deficiency may induce a defect of T-cell differentiation and/or maturation in thymus. In the present study, rats fed the vitamin E-free diet showed not only a decreased number of thymocytes but also a significant decrease of the percentage of $CD4^+CD8^-$ (helper/inducer) T cells in thymocytes compared with rats fed the regular diet (Fig. 2). This suggests that vitamin E deficiency decreases proliferation of $CD4^+CD8^-$ T cells in thymic medulla. In general, both the variety 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 interactions with macrophages (17). Most T cells (>95%), coming from bone marrow and present in the thymic cortex will be eliminated (by a process called apoptosis), and the remaining cells will continue to proliferate in thymic medulla (18).

Interleukin-2 (IL-2), which is produced by activated T cells, is needed for the proliferation of T cells (19). Thymocytes from rats fed the vitamin E-free diet produced significantly less IL-2 than those from rats receiving vitamin E. In fact, their IL-2 activity could not be detected in the biological assay using the growth of IL-2-dependent cytotoxic lymphoid line-2 (CTLL-2) cells. Vitamin E deficiency could cause the marked decrease of IL-2 production in thymocytes because 1) macrophage function may be depressed by vitamin E deficiency and 2) the capacity of T cells producing IL-2 may be lowered by vitamin E deficiency. Because in our previous study vitamin E deficiency did not cause a decrease of alveolar macrophage function but actually increased phagocytic activity against opsonized SRBC (13), vitamin E deficiency at least does not appear to decrease macrophage function.

In rats fed the high vitamin E diet, the number of thymocytes was not altered, but the percentages of both $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells in their thymocytes increased significantly (Fig.2). Further, IL-2 production by the thymocytes was significantly greater than that in rats fed the regular diet.

We have previously noted that AM can be greatly activated by high vitamin E diets (20). Therefore, it is also conceivable that an increase of macrophage function as APC in thymus may bring about the increased production of IL-2. In addition, the marked increases of $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells in thymocytes may be directly related to the increased production of IL-2 by thymocytes in thymic medulla of rats fed the high vitamin E diet.

Suppression of immune functions by vitamin E deficiency and enhancement by supplementation of vitamin E have been attributed to differences in PGE_2 synthesis from fatty acids in phospholipids in cell membranes. Vitamin E

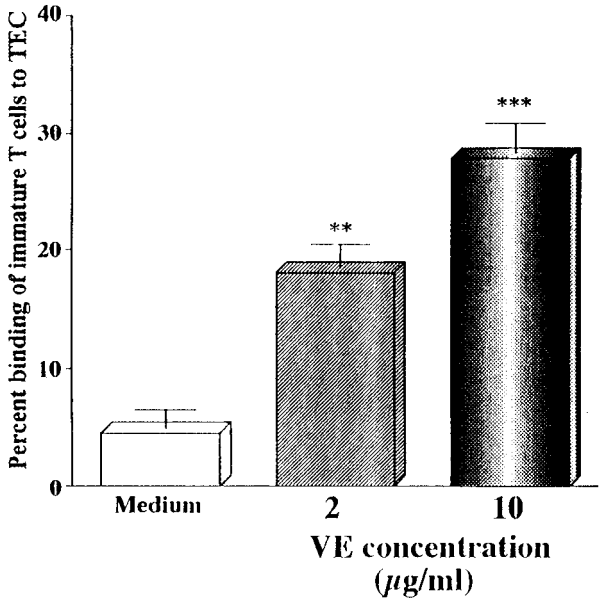

Fig.3. In vitro effect of vitamin E on the binding of immature T cells ($CD4^+CD8^-$) to thymic epithelial cells (TEC). Immature thymic lymphocytes were isolated by discontinuous Percoll gradient centrifugation from thymocytes of rats fed the regular diet. Isolated immature lymphocytes were labeled by in vitro incubation with Na_2 (^{51}Cr) O_4 for 1hr. IT45-R1, a thymic epithelial cell line established from rat thymus cells, were incubated with vitamin E (2 or 10μg/ml) for 24hr, then cultured with ^{51}Cr-labeled immature T cells. After 4hr, they were washed vigorously and then destroyed by adding 1 N NaOH. The radioactivity of remaining immature thymic lymphocytes was measured with a gamma counter. Binding capacity of TEC to immature thymic lymphocytes was calculated by comparing the radioactivity of the experimental group with the total radioactivity of immature thymic lymphocytes. Values are the mean±SEM. Significantly different from the culture with medium (**P<0.01, ***P<0.001).

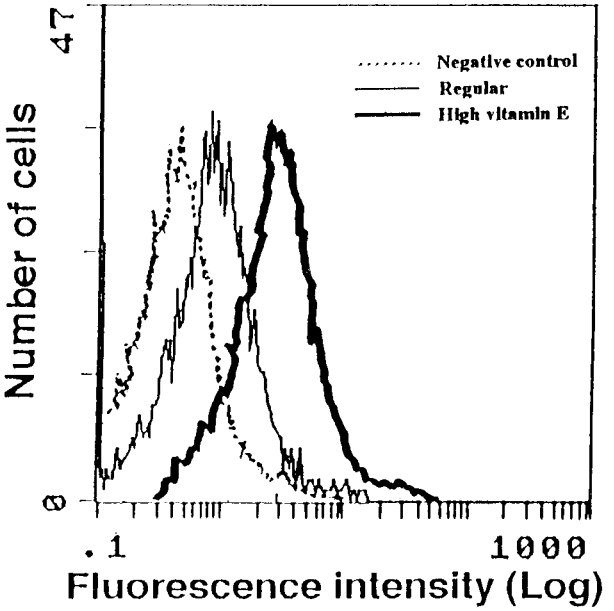

Fig.4. Effect of vitamin E on the expression of ICAM-1 on immature ($CD4^+CD8^+$) T cells. The expression of adhesion molecule, ICAM-1, was analyzed by flow cytometry using FITC-conjugated antibody of ICAM-1.

protects phospholipids in cell membranes from peroxidation through inhibition of phospholipase A_2 activity (21). This inhibition decreases production of arachidonic acid from membrane phospholipids, thereby decreasing production of PGE_2. Because PGE_2 exerts an inhibitory effect on cellular immune functions by increasing the cAMP concentration in immune cells (10), vitamin E is thought to modulate immune functions through its effect on PGE_2 synthesis.

In addition, PGE_2 has an inhibitory effect on IL-2 production by activated T cells (22). A marked decrease of IL2 production following consumption of the vitamin E-free diet and an increase after consumption of the high vitamin E diet may be closely related to the degree of PGE_2 synthesis. In fact, we have observed an increase in PGE_2 production in vitamin E deficiency and a decrease after high vitamin E supplementation. These changes in PGE_2 production by thymocytes may affect production of IL-2 by T cells in thymocytes and result in a decreased percentage of $CD4^+CD8^-$ T cells in thymocytes of the vitamin E-free group and an increased proportion in those of the high vitamin E group. As addition of indomethacin, an inhibitor of PGE_2 synthesis, to the thymocyte cultures stimulated IL-2 production by thymocytes of rats fed the vitamin E-free diet, the increased production of PGE_2 in thymocytes appears to be related to the decreased production of IL-2.

Thymic epithelial cells (TEC) play an important role in the differentiation of T cells (23). We have examined effects of vitamin E supplementation on TEC function. The ability of TEC isolated from thymocytes of rats fed the high vitamin E diet to bind to immature T cells was greater than that of TEC from rats fed the regular diet (Fig. 3). Anderson *et al.* (24) have shown that the contact of immature T cells to TEC is more important than soluble factors from TEC in the positive selection of $CD4^+CD8^+$ T cells. We also have found that the supernate of a TEC culture does not affect the differentiation of T cells *in vitro*. This suggests that vitamin E enhances T-cell differentiation through increased binding of immature T cells to TEC in thymic cortex. Although it is not known why vitamin E increases the binding of immature T cells to TEC, vitamin E may enhance the expression of adhesion molecules. In fact, we have found that vitamin E supplementation or *in vitro* addition of vitamin E to the culture medium enhances the expression of intercellular adhesion molecule-1 (ICAM-1) in TEC (Fig.4). However, *in vitro* incubation with macrophages isolated from rats fed the high vitamin E diet did not induce a significant changes in T-cell subsets in immature T cells (25). These results suggest that vitamin E enhances T-cell differentiation through not macrophage function (negative selection) but the increased binding capacity of TEC to immature T cells via increased expression of ICAM-1 (positive selection).

Nagel *et al.* (26) have reported that the percentage of $CD4^-CD8^+$ (suppressor/killer) T cells is significantly lower in elderly subjects than in younger subjects. In our experiment, the percentage of $CD\ 4^-CD\ 8^+$ T cells in thymocytes was significantly greater in the high vitamin E group than in the regular group. This suggests that vitamin E is able to prevent or delay the decrease of cellular immune functions associated with aging. Meydani et al (27) found that vitamin E supplementation improves immune responsiveness in healthy elderly subjects;this responsiveness is mediated by a decrease in levels of PGE_2 and/or other lipid perox-idation products. We found that vitamin E is important for enhancing and/or maintaining T-cell differentiation in thymus and in preventing decline of cellular immune functions with aging.

Table 1. Effect of X-ray irradiation on the percentages of T cell subsets in thymocytes of rats fed control or high vitamin E diet

Days after X-ray irradiation	$CD\ 4^-\ CD\ 8^-$		$CD\ 4^-\ CD\ 8^+$		$CD\ 4^+CD\ 8^-$		$CD\ 4^+CD\ 8^+$	
	Cont.	Vit. E	Cont.	Vit. E	Cont.	Vit. E	Cont.	Vit. E
Pre	2.8±0.4[a]	2.9±0.5	4.3±0.3	4.6±0.2	8.6±0.4	8.4±0.3	84.3±3.1	84.1±4.2
2	12.5±1.0***	8.7±0.9***	13.6±1.2***	13.8±1.0***	32.1±1.8***	34.6±2.0***	41.8±2.8***	42.9±3.1***
5	21.6±1.5***	7.4±0.5***	14.5±1.5***	5.2±0.4*	29.6±2.2***	14.2±1.0***	34.3±2.3***	73.2±4.2*
9	2.4±0.2	1.6±0.1**	2.6±0.1***	2.4±0.2***	10.3±0.8**	7.7±0.4*	89.7±3.6	88.3±3.3

[a]Data shows are means ± SD of 4 rats and indicate the percentage of cells in the population per 10,000 thymic lymphocytes. *P< 0.05, **P<0.01,***P<0.001 (vs Pre).

Effect of Vitamin E Supplementation on the Decrease of Cellular Immunity after X-Ray Irradiation

X-ray irradiation induces oxidative damage in the body (28). In particular, high doses of X-rays are toxic to rapidly dividing cells such as bone marrow cells (BMC) (29). Bone marrow is the source of precursor cells, called stem cells, for major blood cells : erythrocytes, leukocytes, and platelets. Depression of bone marrow function after X-ray irradiation may result in varying degrees of leukopenia, decreased activity of the host immune system, increased infection, and a higher incidence of cancer (30). Konings and Drijver (31) reported that vitamin E deficiency decreased the LD_{50} dose of X-ray irradiation in mice. Sakamoto and Sakka (32) also found that a high vitamin E diet increased the LD_{50} dose in X-ray irradiated mice. In addition, Konings *et al.* (33) demonstrated that vitamin E supplementation protected against lipid peroxidation induced by radiation. As mentioned previously, we found that vitamin E supplementation enhances the differentiation of T cells in thymus and transiently restores the early decrease of T-cell function in SHR.

We also examined effects of vitamin E supplementation and vitamin E deficiency on the loss of cellular immunity after X-ray irradiation. As shown in Table 1, vitamin E supplementation induced an early recovery from thymic injury following X-ray irradiation. The percentage of $CD4^+CD8^+$ T cells in thymocytes was markedly decreased in all groups on day 2 ; the extent of the decrease depended on the level of vitamin E in the diet. The proportion was restored to the level before X-ray irradiation in the high vitamin E group by day 5 (34). In addition, by day 9, vitamin E supplementation restored the proliferation of thymic lymphocytes in response to PHA or Con A to the level before X-ray irradiation. However, as production of IL-2 from thymocytes showed a marked decrease even on day 9 after X-ray irradiation in the high vitamin E group, the beneficial effect of vitamin E supplementation on the proliferation of thymic lymphocytes does not appear to be due to the increased production of T-cell growth factor, Il-2, but rather appears to be associated with the early recovery of the normal proportions of T-cell subsets in thymocytes.

Some trials have evaluated the protective effects of vitamin E on the side effects induced by radiotherapy for bone marrow transplantation and the treatment for tumors in brain (35). Our study also suggests that vitamin E supplementation is effective for patients receiving bone marrow transplantation or having unresectable tumors from the viewpoints of both enhancing T cell differentiation in thymus and inducing the prompt recovery of the loss of cellular immunity induced by radiation.

CONCLUSION

A role for vitamin E in T-cell differentiation in thymus has been demonstrated in studies in which vitamin E supplementation reduced the decline in cellular immune functions associated with aging. The spontaneously hypertensive rat (SHR) is a satisfactory model animal, not only for human essential hypertension, but also for aging. Because the vitamin E status of the SHR declined during aging, the marked decrease observed in cellular immune functions was associated with decreases of vitamin E levels in plasma and immune organs such as thymus and spleen. Vitamin E supplementation temporally reversed the decrease in proliferation of thymocytes from SHR in response to Con A. Evaluation of the effects of vitamin E deficiency and vitamin E supplementationm on T-cell differentiation in thymus revealed that the degree of T-cell differentiation depended on vitamin E levels in thymus. That is, decreased vitamin E levels in thymus induced a lower percentage of $CD4^+CD8^-$ T cells whereas increased levels induced higher percentages of both $CD4^+CD8^-$ and $CD4^+CD8^+$ T cells.

With regard to the mechanism of the effect of vitamin E, we initially observed an increased production of IL-2 and decreased production of PGE_2 by thymocytes from rats fed the high vitamin E diet, and subsequently found that the binding capacity of TEC for immature T cells increased. This may indicate increased expression of ICAM-1. It appears that vitamin E has an ability to enhance T-cell differentiation in both thymic cortex and medulla. However, the effects of vitamin E on both negative and positive selections by macrophages existing in the border between thymic cortex and medulla must be clarified.

Because vitamin E enhances T-cell differentiation in thymus, vitamin E supplementation may induce early growth and increased differentiation of transplanted bone marrow cells in patients with leukemia, aplastic aemia and tumors. This may result in more rapid recovery of patients undergoing bone marrow transplantations and shorter hospital stays.

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