Effect of Vitamin E on Contact Sensitization Responses 
Induced by 2,4-Dinitrochlorobenzene in Mice

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Summary The effect of vitamin E on the contact sensitization responses induced in mice by 2,4-dinitrochlorobenzene (DNCB) was studied. Mice were fed a vitamin E-adequate or a vitamin E-deficient diet for 5 weeks. The amounts of thiobarbituric acid-reactive substances in the spleens and draining auricular lymph nodes of mice were decreased by dietary vitamin E. Dietary vitamin E prevented lipid peroxidation in the spleens and lymph nodes of mice. Contact sensitization develops in two phases, induction (sensitization) and elicitation. Following sensitization to DNCB on ears, draining lymph node responses, i.e., lymph node weight, total lymph node cell number and in vitro lymph node cell proliferation as assessed by [3H]methyl thymidine incorporation, were examined. These responses, activated by DNCB, were lower in the mice fed a vitamin E-deficient diet as compared with those of the mice fed a vitamin E-adequate diet. In the elicitation phase, lymphocytes from sensitized mice respond to the antigen and blastogenate in vitro. The blastogenesis of spleen lymphocytes in the DNCB-sensitized mice was decreased by vitamin E deficiency, which was enhanced by exogenously adding vitamin E. It was found that vitamin E deficiency decreases the contact sensitization responses to DNCB in mice, but responses were restored by exogenous vitamin E. In conclusion, vitamin E may participate in the lymphocyte responses to contact allergens through scavenging reactive oxygen species.

Key Words vitamin E, 2,4-dinitrochlorobenzene, contact sensitization, lymphocyte blastogenesis

Vitamin E plays an important role in protecting the cellular membrane structures against damage from reactive oxygen species and reactive products of lipid peroxidation (1–4). In addition, it has been shown that vitamin E stimulates

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mitogenic responses of lymphocytes (5-14) and antibody production (15). It has been suggested that vitamin E modulates immune responses. However, there is little information about the effect of vitamin E on lymphocyte activity to contact allergens.

It is known that contact hypersensitivity develops in two phases, induction and elicitation (16). Following the first encounter with a skin sensitizing chemical, hapten-specific lymph node cell activation is induced at the lymph node draining site of the exposure (induction phase). When the lymphocytes from the sensitized mice contact the antigen again, they respond to the chemical and proliferate in vitro (elicitation phase) (17).

In this study, the effect of vitamin E on lymphocyte responses during the contact sensitization of mice was examined. Mice were fed a vitamin E-adequate diet or a vitamin E-deficient diet, and the amount of thiobarbituric acid-reactive substances was measured as an index of lipid peroxidation (18). Mice were sensitized by 2,4-dinitrochlorobenzene (DNCB), a potent sensitizer used for many years to induce contact sensitization (19). The draining auricular lymph node activation responses of mice were measured using a method of local lymph node assay, to examine responses during the induction phase following sensitization on ears (20). To assess the lymphocyte reaction during elicitation of sensitization, the in vitro blastogenesis of spleen lymphocytes was measured using mice sensitized on flank with a subsequent challenge on ears.

MATERIALS AND METHODS

Chemicals. dl-α-Tocopherol and 2,4-dinitrobenzenesulfonic acid sodium salt (DNBS) were obtained from Tokyo Kasei Organic Chemicals (Tokyo, Japan). DNCB and concanavallin A (Con A) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Thiobarbituric acid (TBA) was purchased from Nacalai Tesque (Kyoto, Japan). Freund’s complete adjuvant (FCA) was purchased from Difco Laboratories (MI, USA). Fetal bovine serum (FBS) was obtained from Life Technologies (NY, USA).

Animals. Female BALB/c strain mice, 3-weeks-old, were obtained from Japan SLC (Shizuoka, Japan). Mice were fed a normal solid diet with adequate vitamin E content (F-2 type, 5 mg% vitamin E; Funabashi Farm, Chiba, Japan) or a vitamin E-deficient diet (AIN-93M Composition, 0 mg% vitamin E; Oriental Yeast, Tokyo, Japan) for 5 weeks.

TBA test. The TBA test was performed according to a method previously described (18). Mice were sacrificed, and their spleens and auricular lymph nodes were quickly removed. The tissues were washed well with cold Hanks’ balanced salt solution (HBSS) and lyophilized. The lyophilized tissues were homogenized in a cold 1.15% KCl solution at a concentration of 30%. Protein content was determined according to the method of Lowry et al (21) using bovine serum albumin as the reference standard. In a test tube, 0.70 mL of water containing 0.10-0.20 mL homogenate, 50 μL 0.8% butylated hydroxytoluene solution in acetic acid, 0.20 mL
8.1% sodium dodecyl sulfate solution, 1.50 mL 20% acetic acid solution adjusted to pH 3.5 with 10 mol/L NaOH, 0.10 mL 40 mmol/L ferric chloride, and 1.50 mL 0.8% TBA solution were added in this order. The mixture was kept at exactly 5°C for 60 min and then heated at 100°C for 60 min. After cooling, the mixture was extracted with 1.0 mL water and 5.0 mL n-butanol: pyridine (15:1, v/v). The mixture was centrifuged at 3,000 rpm for 10 min, and absorbance of the organic phases was measured at 532 nm. The absorbance of the blank reaction mixture was subtracted. The amount of red pigment was determined by the absorbance and molecular extinction coefficient of 1:2 malonaldehyde:TBA adduct, 156,000.

Local lymph node assay. Local lymph node assay was performed according to a method previously described (19) with slight modification (22). Groups of mice (n = 3) were treated on both ears by painting 25 μL of a DNCB solution in acetone: olive oil (4:1) (AOO) at the indicated concentration or the vehicle alone (control) daily for 3 consecutive days. On the fourth day, draining auricular lymph nodes were excised, pooled for each experimental group and weighed. Lymph node cell suspensions were prepared by mechanical disaggregation through sterile 200-mesh gauze. The cells were transferred through nylon mesh into a 15 mL centrifuge tube, and the tube was centrifuged at 1,200 rpm for 5 min. The lymph node cell suspensions were washed once with HBSS and total lymph node cell number was determined using an automated cell counter. The lymph node cells were resuspended in RPMI-1640 culture medium supplemented with 25 mmol/L HEPES, 100 units/mL penicillin, 100 μg/mL streptomycin and 10% FBS (FBS-RPMI) at a concentration of 5 × 10⁶ cells/mL. The lymph node cell suspensions (200 μL) were seeded into 96-well culture plates (4 wells per group) and cultured with 18.5 kBq [³H]methyl thymidine (³HTdR) at 37°C for 24 h in a humidified atmosphere of 5% CO₂ in air. Culturing was terminated by a semiautomatic cell harvester, and ³HTdR incorporation was determined for each experimental group. No statistical analysis was done in this study, but the difference of the lymph node activity obtained from each group is meaningful, as has been described (16).

Blastogenesis of spleen lymphocytes. The effect of exogeneously added vitamin E on the blastogenesis of lymphocytes from non-sensitized mice was examined as follows. Spleen lymphocyte suspensions from untreated mice fed a vitamin E-adequate diet were prepared by mechanical disaggregation of spleen through sterile 200-mesh gauze. The cells were treated with 0.83% ammonium chloride solution for lysing red blood cells, and the lymphocytes were washed twice in HBSS and resuspended in FBS-RPMI. The lymphocyte suspensions (200 μL) were seeded into a 96-well plate at a concentration of 2.5 × 10⁶ cells/mL (4 wells per group). For the in vitro test, dl-α-tocopherol was first dissolved in ethanol at a concentration of 20 mg/mL, and then diluted with FBS-RPMI. Ethanol at the final concentration of 0.0005–0.5% in the culture well did not affect the cell activity. Twenty microliters of a solution of dl-α-tocopherol and/or Con A in FBS-RPMI at the indicated final concentration was added. Control wells received an equal volume of the medium alone. The lymphocyte suspensions were cultured at 37°C for 72 h in a humidified
atmosphere of 5% CO₂ in air. The cultures were pulsed with 18.5 kBq ³HTdR for 6 h before harvesting with a semiautomatic cell harvester. ³HTdR incorporation was determined by a liquid scintillation counter.

The effect of dietary vitamin E and exogenously added vitamin E on the blastogenesis of spleen lymphocytes from the DNCB-sensitized mice was examined as follows. Mice were sensitized according to the method described by Gad et al. (23), with small modification. Groups of mice (n = 4), fed a vitamin E-adequate or a vitamin E-deficient diet, received 50 μL of 1% DNCB in FCA emulsion by intradermal injection into the shaved flank. On the fifth day after induction, the mice were challenged on ears by painting 20 μL of 1% DNCB in AOO to confirm positive increase in ear thickness. On the seventh day after the challenge, spleen lymphocytes were obtained as described above. To the lymphocyte suspensions (200 μL) at a concentration of 2.5 × 10⁶ cells/mL (4 wells per group), a 20 μL solution of DNBS in FBS-RPMI and/or dl-α-tocopherol in FBS-RPMI (each final concentration at 100 μg/mL) was added. Control wells received an equal volume of the medium alone. The mixture was then cultured at 37°C for 72 h. The cultures were pulsed with ³HTdR for 6 h, and ³HTdR incorporation was determined.

Statistical analysis. Data were analyzed by two-way ANOVA to test for the effects of dietary vitamin E, and exogenously added vitamin E and DNCB. Differences associated with p values of <0.05 were regarded as statistically different by Fisher's least-significant difference test.

RESULTS

Lipid peroxidation in tissues of mice fed vitamin E-adequate and vitamin E-deficient diets

Because it has been shown that vitamin E levels of the livers and spleens of rats fed a vitamin E-deficient diet for 5 weeks were remarkably lower than those fed a vitamin E-adequate diet (24), mice fed a vitamin E-adequate diet or vitamin E-deficient diet for 5 weeks were used in this study. The amount of thiobarbituric acid-reactive substances (TBARS) was expressed by the amount of red pigment obtained in the TBA assay. The mean TBARS ± SD value of spleens from 3 mice fed a vitamin E-adequate diet was 0.18 ± 0.02 nmol/mg protein, whereas that from 3 mice fed a vitamin E-deficient diet was 0.37 ± 0.12 nmol/mg protein. Vitamin E deficiency caused a significant increase in the amount of TBARS in the spleens (p < 0.05). The amount of TBARS in pooled auricular lymph nodes from 3 mice fed a vitamin E-adequate diet was 0.35 nmol/mg protein, whereas that from 3 mice fed a vitamin E-deficient diet was 0.43 nmol/mg protein. Hence, in the experiments reported here, dietary vitamin E significantly prevented lipid peroxidation in the spleens and auricular lymph nodes of mice.

Effect of vitamin E on local lymph node activation induced by DNCB

The effect of vitamin E on the in vivo responses provoked during the induction
Table 1. Effect of vitamin E on draining auricular lymph node responses induced by painting DNCB on ears.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lymph node weight (mg)</th>
<th>Lymph node cell number ($\times 10^6$)</th>
<th>Lymph node cell proliferation $^3$HTdR incorporation (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E-adequate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29.4</td>
<td>16.81</td>
<td>4,700</td>
</tr>
<tr>
<td>DNCB</td>
<td>57.4</td>
<td>66.40</td>
<td>33,486</td>
</tr>
<tr>
<td>Vitamin E-deficient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.8</td>
<td>12.96</td>
<td>3,296</td>
</tr>
<tr>
<td>DNCB</td>
<td>51.1</td>
<td>56.59</td>
<td>27,321</td>
</tr>
</tbody>
</table>

Groups of mice ($n=3$) were fed a vitamin E-adequate or vitamin E-deficient diet for 5 weeks. The mice received 25$\mu$L of a solution of 1% DNCB in AOO or the control vehicle alone on ears daily for 3 consecutive days. On the fourth day after induction, draining auricular lymph nodes were excised, pooled for each experimental group. Lymph node weight, lymph node cell number and $^3$HTdR incorporation into the cells (an average of 4 culture wells) were measured as described in Materials and Methods.

Effect of vitamin E on mouse spleen lymphocyte blastogenesis

The effect of vitamin E on the blastogenesis of spleen lymphocytes from non-sensitized mice was studied. Spleen lymphocytes obtained from mice fed a vitamin E-adequate diet were incubated with $^3$HTdR in the presence of 0.1–100$\mu$g/mL $dl\text{-}a$-tocopherol. Exogeneously added vitamin E alone did not show any significant blastogenesis responses of spleen lymphocytes. At first, spleen lymphocytes were incubated with 0, 5 or 10$\mu$g/mL Con A. Maximum mitogenic response was obtained at a concentration of 5$\mu$g/mL Con A, but the response was not enhanced by the addition of $dl\text{-}a$-tocopherol (data not shown). Then, spleen lymphocytes were cultured with 0.25–1$\mu$g/mL Con A and 0–100$\mu$g/mL...
Fig. 1. Effect of *dl*-α-tocopherol on Con A-induced blastogenesis of spleen lymphocytes from mice fed a vitamin E-adequate diet. Spleen lymphocytes (5 × 10⁵ cells/well) were cultured with various concentrations of Con A in the presence of *dl*-α-tocopherol at the indicated concentration for 72 h. The cells were pulsed for 6 h with ³HdR, and ³HdR incorporation was measured.

dl-α-tocopherol. Mitogenic responses of spleen lymphocytes induced by 1 μg/mL Con A were increased in the presence of *dl*-α-tocopherol at concentrations between 0.1 and 100 μg/mL (Fig. 1, closed circles). Mitogenic responses of the cells by 0.5 μg/mL Con A were also enhanced in the presence of *dl*-α-tocopherol at these concentrations (Fig. 1, open circles). The stimulation effect of *dl*-α-tocopherol was maximal at 10 μg/mL. The results obtained here were consistent with earlier observations showing that vitamin E enhances lymphocyte mitogenic responses (5, 6, 8, 9, 11–13).

The effect of vitamin E on the blastogenesis of spleen lymphocytes from DNCB-sensitized mice was studied. Mice fed a vitamin E-adequate or vitamin E-deficient diet received DNCB on the shaved flank. On the fifth day after induction, the mice were challenged on their ears with DNCB to confirm positive increase in ear thickness. Before challenging, there was no difference in ear thickness between the mice fed the vitamin E-adequate and vitamin E-deficient diets. Increase in ear thickness after challenging the mice fed a vitamin E-deficient diet was 34% lower than that of the mice fed a vitamin E-adequate diet (data not shown).

On the seventh day after challenging, spleen lymphocyte suspensions were obtained. The blastogenesis of spleen lymphocytes obtained from control mice fed the vitamin E-adequate diet was a little higher than that from the control mice fed a vitamin E-deficient diet (Table 2, control). In vitro addition of 10 μg/mL *dl*-α-tocopherol to the lymphocyte cultures caused a slight increase in blastogenesis
Table 2. Effect of \textit{dl-\alpha-}tocopherol on blastogenesis of spleen lymphocytes from mice fed a vitamin E-adequate or a vitamin E-deficient diet and sensitized with DNCB.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>( ^{3} \text{HTdR incorporation (dpm)} )</th>
<th>Vitamin E-adequate</th>
<th>Vitamin E-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>671 ± 79</td>
<td>582 ± 196</td>
</tr>
<tr>
<td>\textit{dl-\alpha-}Tocopherol</td>
<td></td>
<td>2,225 ± 458</td>
<td>2,106 ± 446</td>
</tr>
<tr>
<td>DNBS</td>
<td></td>
<td>18,915 ± 382</td>
<td>12,313 ± 3,380(^b)</td>
</tr>
<tr>
<td>DNBS + \textit{dl-\alpha-}tocopherol</td>
<td></td>
<td>22,822 ± 1,639(^a)</td>
<td>18,291 ± 1,907(^{a,b})</td>
</tr>
</tbody>
</table>

Groups of mice (\( n=4 \)) were fed a vitamin E-adequate or vitamin E-deficient diet for 5 weeks. The mice received 1% DNCB in FCA emulsion by intradermal injection on the shaved flank. On the fifth day after induction, the mice were challenged on ears with 1% DNCB in AOO. On the seventh day after challenging, spleen lymphocytes were obtained. The lymphocyte suspensions were cultured with 100 \( \mu \text{g/mL} \) DNBS and/or 10 \( \mu \text{g/mL} \) \textit{dl-\alpha-}tocopherol for 72 h. Control wells received an equal volume of the medium alone. The cells were pulsed for 6 h with \( ^{3} \text{HTdR} \), and \( ^{3} \text{HTdR} \) incorporation was measured. Each value is the mean \( ^{3} \text{HTdR} \) incorporation ± SD.

\(^a\) DNBS plus \textit{dl-\alpha-}tocopherol groups showed significant increase as compared to DNBS groups (\( p<0.05 \)).

\(^b\) Vitamin E-deficient groups showed significant decrease as compared to vitamin E-adequate groups (stimulation with DNBS or DNBS plus \textit{dl-\alpha-}tocopherol, \( p<0.05 \)).

(Table 2, \textit{dl-\alpha-}tocopherol). In these cases, there was no significant difference between the sensitized mice fed the vitamin E-adequate and vitamin E-deficient diets. When spleen lymphocytes were cultured with water-soluble DNBS (100 \( \mu \text{g/mL} \)), whose antigenic determinant group is common to that of DNCB, the blastogenesis of spleen cells from both groups of mice increased extensively (Table 2, DNBS).

The blastogenesis of spleen lymphocytes caused by DNBS in both groups of mice was significantly increased when spleen lymphocytes were cultured in the presence of \textit{dl-\alpha-}tocopherol (Table 2, DNBS + \textit{dl-\alpha-}tocopherol). The extent of increase from mice fed the vitamin E-adequate diet was significantly greater than that from mice fed the vitamin E-deficient diet. These results indicate that vitamin E enhanced in vitro blastogenesis of spleen lymphocytes in DNCB-induced sensitization.

DISCUSSION

The effect of vitamin E on contact sensitization responses induced by DNCB in mice was studied. It was found that vitamin E deficiency decreased lymphocyte activation induced by DNCB. Dietary vitamin E is important to cause lymphocyte responses to DNCB in mice. Exogenous vitamin E enhanced the in vitro blastogenesis of mouse spleen lymphocytes in DNCB-induced sensitization. There is much literature reporting the stimulatory effect of vitamin E on various responses...
of lymphocytes to mitogen (5–15). However, to our knowledge, this is the first demonstration of the effect of vitamin E on sensitization response to contact allergens.

There was a significant correlation between plasma vitamin E concentrations and immune responses in rats (11). The degree of vitamin E content in the tissues of animals may vary with the diet, species and feeding time. Although the determination of the level of vitamin E content in blood is important to assess the condition of vitamin E deficiency, it is difficult to collect blood from mice. The measurement of TBARS values of tissues is a possible indication of the degree of lipid peroxidation (25). Therefore, we measured the amounts of TBARS of tissues, and assessed the condition of vitamin E deficiency. Kikugawa et al reported that vitamin E levels of blood, livers and spleens of rats fed a vitamin E-deficient diet for 5 weeks were remarkably lower than those fed a vitamin E-adequate diet (24). In this study, the amounts of TBARS in the spleens and lymph nodes of mice fed a vitamin E-deficient diet showed a significant increase in comparison to those of mice fed a vitamin E-adequate diet. Consequently, dietary vitamin E prevented lipid peroxidation in the spleens and auricular lymph nodes of the mice. The mice fed a vitamin E-deficient diet for 5 weeks were thought to be in vitamin E-deficient condition.

Skin sensitizing chemicals induce lymph node cell activation in the draining site of the exposure (16, 20). In vivo lymph node responses provoked during the induction of contact sensitization in mice were investigated using a local lymph node assay (20, 22). Weight of draining auricular lymph nodes, lymph node cell number in the lymph nodes and 3HTdR incorporation into the cells of control or DNBCB-treated mice fed a vitamin E-adequate diet were higher than those of control mice fed a vitamin E-deficient diet (Table 1). Vitamin E deficiency decreased the auricular lymph nodes responses. The lipid peroxidation of lymph nodes by vitamin E deficiency decreased lymph node responses in the mice. It is likely that reactive oxygen species were key species to the lymphocyte responses. This result suggests that vitamin E may be important to maintain immune reactivity in contact sensitization to chemicals.

Cell culturing conditions are conducive for the production of reactive oxygen intermediates. The oxygen tension is higher than that in lymphoid organs and the oxidative products of cultured cells are not usually removed during the culturing periods. Peroxidation of lymphocyte membrane significantly depressed the in vitro response to mitogen (26). 2-Mercaptoethanol (2-ME) is usually added to the culture medium for in vitro lymphocyte proliferation assay, as a synthetic antioxidant agent to enhance the (cellular) response (27, 28). This enhancement was mediated by the enhanced availability of glutathione to prevent cell membrane lipid peroxidation (29). Oonishi et al found that, in the presence of 2-ME, there was no enhancement of proliferation by α-tocopherol (30). Because 2-ME might mask the effect of dl-α-tocopherol, we did not add 2-ME to the culture medium.

The lymphocytes from sensitized animals proliferate following in vitro in-
cubation of the antigen. The extent of increase of blastogenesis in the spleen lymphocytes of mice fed a vitamin E-deficient diet was significantly lower than that of mice fed a vitamin E-adequate diet (Table 2). This demonstrates that vitamin E deficiency decreases the blastogenesis in the elicitation of contact sensitization. Macrophages included in splenocytes have important roles in blastogenesis and generate reactive oxygen when stimulated. It was reported that the vitamin E content of macrophages was significantly reduced following exposure to oxidative stress (31). Harris et al reported that vitamin E-deficient rats impaired macrophage and neutrophil functions by decreased protection of autooxidation damage (32). Active oxygen production may be a trigger of lymphocyte proliferation, but lipid peroxidation of cell membranes decreases cell activity. Fountain et al suggested that vitamin E played a role in stabilizing the fluidity of the lymphocyte membrane by preventing the autooxidation of the unsaturated acyl chain of phospholipids of the lymphocyte membrane during blastogenesis (33). It was reported that lipid peroxidation and vitamin E deficiency impaired the depolarization of mitochondrial membrane potential and mass loss of the organelles of spleen lymphocytes from rats (34, 35). Lehmann and McGill found morphological change in the mitochondria of reticulocytes and lymphocytes from vitamin E-deficient rats by electron microscope study (36). These results lead to the assumption that vitamin E is an essential constituent of all the membrane found in cells including the plasma, mitochondria and nuclear membranes, and that vitamin E deficiency would reduce the energy-yielding reactions. This suggests that the antioxidant property of vitamin E indirectly affects the sensitization responses. In the animals fed a vitamin E-deficient diet, macrophage membrane receptors of the Ia antigen were decreased (37), interleukin production was lowered (38) and immunosuppressive prostaglandins production was decreased (39). These also may have contributed to the suppression of contact sensitization responses in the mice fed the vitamin E-deficient diet.

The in vitro addition of dl-a-tocopherol enhanced the proliferation of spleen lymphocytes (Fig. 1 and Table 2). The responses of spleen lymphocytes induced by 1 and 0.5 μg/mL Con A were increased in the presence of dl-a-tocopherol (Fig. 1). The concentrations of Con A were sub-optimal concentrations that cause mild spleen lymphocyte mitogenesis. The stimulative effect of dl-a-tocopherol was maximal at 10 μg/mL, and enhancement was decreased at higher concentrations of dl-a-tocopherol (Fig. 1). Roy et al reported that lower concentrations of α-tocopherol enhanced the proliferation of murine spleen lymphocytes in response to sub-optimal concentrations of Con A, but they inhibited the proliferation to a supra-optimal level of Con A (40). It is also possible that the optimum response to Con A was shifted by an interaction between tocopherol and Con A (40). Higher concentrations of Con A are cytotoxic, and the toxicity may be potentiated at higher levels of tocopherol.

The blastogenesis of spleen lymphocytes induced by DNBS in DNBC-sensitized mice was significantly increased when cultured in the presence of dl-a-tocopherol.
(Table 2). The stimulating effect of vitamin E on blastogenesis in response to antigen is similar to the mitogenic responses to Con A. Hoffeld showed that the enhancement of lipopolysaccharide-stimulated proliferation by various chemicals that block various steps in the lipid peroxidation process, such as 2-ME and vitamin E, was mediated by the enhanced availability of reduced glutathione in the culture medium. This supports the hypothesis that the enhancement of cellular responses by vitamin E is mediated by its lipid antioxidant activity. Meydani et al reported that high levels of dietary vitamin E decreased the level of immunosuppressive prostaglandins in spleen cells and enhanced lymphocyte functions \((39, 41)\). Furthermore, dietary vitamin E could modulate cytokine production by splenocytes and thymocytes \((42)\). Cytokines, such as interleukin-1 and 2, interferone and tumor necrosis factor, play crucial roles in immune responses. Oonishi et al found that treatment with vitamin E could increase mitogenic response by the enhanced production of interleukin 1 from macrophages \((30)\). Brohee and Neve showed that a dietary high dose of vitamin E treatment induced a small decrease in T cell, especially CD4+ cells (T-helper cells), in blood from young adult mice, and the number of reactive specific lymphocyte subsets affected the change in immune reactivity \((43)\). These results suggest that the stimulation of spleen lymphocyte proliferation by vitamin E was mediated by action as an immunomodulator rather than an antioxidant. However, it is not clear whether a dietary high dose of vitamin E would enhance contact sensitization responses. We need to compare the reactivity of lymph nodes among the mice fed a vitamin E-adequate diet, vitamin E-deficient diet or vitamin E-excessive diet following the application of contact allergens.

In summary, in the mice fed a vitamin E-deficient diet, there was a decrease in lymph node responses induced by DNCB in contact sensitization. It is likely that reactive oxygen species were key species to the lymphocyte responses, and the lymphocyte responses were restored by dietary vitamin E. The in vitro addition of \(dl\-\alpha\)-tocopherol stimulated lymphocyte proliferation in mice in response to contact allergen. Vitamin E is important to maintain immune reactivity in contact sensitization to chemicals.

REFERENCES


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