Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects

Simin Nikbin Meydani, M Patrice Barklund, Sandra Liu, Mohsen Meydani, Richard A Miller, Joseph G Cannon, Frank D Morrow, Ross Rocklin, and Jeffrey B Blumberg

ABSTRACT The effect of vitamin E supplementation on the immune response of healthy older adults was studied in a double-blind, placebo-controlled trial. Subjects (n = 32) resided in a metabolic research unit and received placebo or vitamin E (800 mg dl-a-tocopheryl acetate) for 30 d. Alpha-tocopherol content of plasma and peripheral blood mononuclear cells (PBMCs), delayed-type hypersensitivity skin test (DTH), mitogen-stimulated lymphocyte proliferation, as well as interleukin (IL)-1, IL-2, prostaglandin (PG) E₂, and serum lipid peroxides were evaluated before and after treatment. In the vitamin E-supplemented group 1) α-tocopherol content was significantly higher (p < 0.0001) in plasma and PBMCs, 2) cumulative diameter and number of positive antigen responses in DTH response were elevated (p < 0.05), 3) IL-2 production and mitogenic response to optimal doses of concanavalin A were increased (p < 0.05), and 4) PG E₂ synthesis by PBMCs (p < 0.005) and plasma lipid peroxides (p < 0.001) were reduced. Short-term vitamin E supplementation improves immune responsiveness in healthy elderly individuals; this effect appears to be mediated by a decrease in PG E₂ and/or other lipid-peroxidation products. Am J Clin Nutr 1990;52:557–63.

KEY WORDS Vitamin E, immune response, aging, prostaglandin

Introduction

Considerable evidence indicates that aging is associated with altered regulation of the immune system (1). Age-related functional changes have been well characterized for both humoral and cell-mediated immune responses (2–4). Although all cell types of the immune system show age-related changes, the major alterations occur in the T cells (5).

In vivo, T-cell–dependent cell-mediated functions, such as delayed type hypersensitivity skin test (DTH) (6, 7), graft vs host reaction (2), and resistance to challenge with syngeneic and allogeneic tumors and parasites (5), are depressed with age. In vitro the proliferative response of human and rodent lymphocytes to phytohemagglutinin (PHA) and concanavalin A (Con A) become depressed with age (2). Several groups showed that antigen- and mitogen-stimulated interleukin (IL)-2 production declines with age and contributes to the T-cell–mediated defects observed with aging (8–11). Cooperation between monocytes and lymphocytes is essential in antigen recognition, lymphocyte differentiation and eventual antibody production, and development of the effector state of cellular immunity, ie, the DTH phase (12). In addition to presenting antigen, macrophages synthesize IL-1 which induces the production of IL-2 by the activated T cells. Macrophages have a high concentration of arachidonic acid in their membrane phospholipids. Upon stimulation, macrophages release up to 50% of their arachidonic acid content in the form of oxygenated metabolites, eg, prostaglandin (PG), hydroxicosatetraenoic acid (HETE), and leukotriene (LT) (13, 14). PG E₂ was shown to suppress lymphocyte proliferation and lymphokine synthesis (15–17). Other oxidative metabolites of activated macrophages, such as H₂O₂, was also shown to suppress lymphocyte proliferation (18, 19). Increased PG E₂ production by macrophages from aged rats (20) and mice (21) was reported.

Vitamin E was shown to decrease PG production in immune cells (21, 22) and enhance cell-mediated immunity in young (22) and old (21) animals. A significant decrease of PG E₂ synthesis in spleen in old C57Bl/6J mice was associated with an enhanced DTH, in vitro spleen mitogenic responses to Con A but not to PHA, and IL-2 formation (21). One of the biologic changes associated with aging is an increase in free radical formation with subsequent damage to cellular processes. Several studies investigated the free radical theory of aging and the role of antioxidants, including vitamin E, on the life expectancy of rodents (23). Vitamin E supplementation was shown to be protective against age-associated diseases such as cancer (24–26) and amyloidosis (27). An increase in the average life span of short-lived autoimmune-prone NZB/NZW mice receiving vitamin E supplements was reported by Harman (28). Furthermore, a community-based survey by Chevance et al (29, 30) showed a positive correlation between plasma vitamin E con-


1 From the USDA Human Nutrition Research Center on Aging at Tufts University; the Department of Pathology, Boston University School of Medicine; and the Department of Medicine, New England Medical Center Hospitals and Tufts University School of Medicine, Boston.


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4 Address reprint requests to SN Meydani, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111. Received August 21, 1989. Accepted for publication November 8, 1989.
centrations and DTH responses and a negative correlation between plasma vitamin E and the number of recent infections. Therefore, we investigated the effect of vitamin E supplementation on in vivo and in vitro indices of cell-mediated immune response in healthy elderly subjects.

Subjects and methods

Study subjects and experimental design

Thirty-two healthy men and women, ≥ 60 y of age, with no known medical illness and receiving no prescription medication, were recruited from the Boston area. Those using vitamin supplements and/or any nonsteroidal antiinflammatory drugs were excluded. All subjects passed a complete physical examination and had normal weight-for-height. Blood and urine samples from each subject were obtained for complete blood and differential counts, clinical chemistry profile, and routine urinalysis before subjects were admitted. The study was approved by the Tufts University/New England Medical Center Human Investigation Review Committee (HIRC). All volunteers signed a HIRC-approved written consent form.

Volunteers were randomly assigned to a placebo or vitamin E-supplemented group. All subjects resided and consumed their meals in the Metabolic Research Unit of the USDA Human Nutrition Research Center on Aging at Tufts University. A 3-d-cycle menu consisting of foods typical to the American diet and adequate in all nutrients was served throughout the study. The basal 3-d menu contained 20 ± 1.5% (X ± SD) protein, 31.5 ± 2.8% fat, with a ratio of polysaturated to saturated fatty acids (P:S) of 0.41 ± 0.09, and 49 ± 2% carbohydrate. The amounts of fat and protein, the P:S, and all other dietary components except for carbohydrates were kept constant for all subjects. The total calorie content provided by the basal 3-d menu (1768 ± 46 kcal/d) was adjusted by changing the carbohydrate content to meet each subject's calorie requirement and to maintain the subject's weight.

Subjects were weighed weekly and their vital signs were monitored daily. Subjects were advised to continue their normal activity and to avoid excessive sun exposure. This precaution was taken because membrane lipid peroxidation and PGE2 formation have been implicated as major components of ultraviolet-induced skin injury.

During the first 2 wk of the study all subjects received a placebo capsule with breakfast and with dinner. On days 8, 10, and 12, 40 mL fasting blood and 24-h urine samples were obtained for different in vitro immunologic tests and PGE2 analyses as baseline or presupplementation values. On day 12, 30 mL additional blood was collected for biochemical measures. Subjects were then administered a DTH. Upon completion of the 48-h evaluation of the skin test, the placebo group continued consuming two placebo capsules containing soybean oil whereas the vitamin E group consumed two vitamin E capsules containing 400 mg dl-alpha-tocopheryl acetate in soybean oil (Hoffmann-LaRoche, Inc, Nutley, NJ) daily for 30 d. At the completion of the test period three fasting blood and 24-h urine samples were collected every other day for postsupplementation analyses (except for the first 10 subjects for whom one blood sample was collected 15 d after supplementation and the remaining two samples were collected 30 and 32 d after supplementation. The amount of blood withdrawn was similar to that collected at baseline.

The study was conducted in a double-blind fashion with the codes broken only after all data collection had been completed. The average of three baseline and three postsupplementation values for immunologic tests and PGE2 were used in analyses.

Procedures

DTH was assessed with multitest CMI (Merieux Institute, Inc, Miami), a single-use, disposable applicator of acrylic resin with eight heads loaded with a glycerine control and the following seven recall antigens: tetanus toxoid, diphtheria toxoid, streptococcus (group C), mycobacterium tuberculosis, candida (albicans), trichophytan (metagrophytes), and protein (mira-

bils). The diameter of positive reaction was measured 24 and 48 h after administration of the test. The antigen score was calculated as the total number of positive antigens and the cumulative score was calculated as the total diameter of induration of all the positive reactions. According to the manufacturer's instructions, an induration of ≥ 2 mm was considered positive. If a positive reaction to the glycerine control was observed, the diameter of its induration was subtracted from all the other positive reactions. The test was administered by the same nurse before and after supplementation for each subject and the diameter of induration was measured by the same person before and after supplementation.

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood according to the procedure of Boyum (31). PBMCs were removed from the interface and washed twice in RPMI 1640 supplemented with 100 mg/L penicillin, 100 g/L streptomycin, 2 mmol/L-glutamine/L, and 25 mmol HEPES/L (Gibco, Grand Island, NY). Cells were resuspended in medium and counted under a light microscope. Cell viability was assessed by using the trypsin blue exclusion method. Cells were then suspended at appropriate concentrations for measurement of mitogenic lymphocyte proliferation, IL-2, IL-1, and PGE2 formation.

Lymphocyte proliferation was measured by [3H]thymidine incorporation after stimulation with T-cell and B-cell mitogens. Dilutions of mitogens between 1 and 100 mg/L for PHA (PHA-P, Difco, Detroit) and Con A (Sigma, St Louis) and 0.015% to 0.15% for Staphylococcus aureus Cowan I (SAC; Zysorbin, Zymed, San Francisco) were prepared in RPMI 1640 with 100 mL fetal bovine serum (FBS)/L and optimal dilution for each mitogen was determined. One hundred microliters of each mitogen was plated in triplicate into 96-well, flat-bottomed microtiter plates (Becton Dickinson, Oxnard, CA). PBMC were suspended at 1 × 10^6 cells/L in RPMI 1640; 100 μL of the cell suspension was plated with and without mitogens and incubated for 72 h at 37 °C in an atmosphere of 5% CO2 and 95% humidity. Four hours before termination of incubation, 18.5 GBq of [3H]thymidine (specific activity 247.9 GBq/mol, New England Nuclear, Boston) in 20 μL was added to each well. Cells were harvested onto glass microtiter filter paper by use of a cell harvester (PHD, Cambridge, MA). Filter disks were placed in minivials and counted in a liquid-scintillation counter (Beckman Instruments, Palo Alto, CA). The results are reported as corrected counts per minute (ccpm), the average cpm of mitogen-stimulated cultures minus the average cpm of cultures without mitogens.

Cells (1 × 10^6/L) in RPMI with 100 mL FBS/L were cultured in 24-well flat-bottomed plates (Becton Dickinson) with Con A (10 mg/L for 48 h). Cell-free supernatant was stored at -70 °C for later analysis of IL-2. IL-2 activity was measured with a
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microassay method described by Gillis et al. (32). Recombinant human IL-2 (Genzyme Corp, Boston) was used as standard. One unit per milliliter is defined as the amount of recombinant IL-2 that causes a half maximal incorporation of [3H]thymidine in 5 × 10^3 cytotoxic T-cell line (CTLL) cells in culture. IL-2 activity was calculated by using probit analysis (32). CTLL cells were a gift from S Gillis of Immunex (Seattle).

Human PBMCs were suspended at 5 × 10^3 cells/L in minimal essential medium (MEM) (supplemented per liter with 2 mmol glutamine, 100 kU penicillin, 100 mg streptomycin, and 20 mL heat-inactivated AB serum) and subjected to ultrafiltration to remove endotoxins (33). This cell suspension (0.5 mL) was cultured in 0.5 mL MEM or 0.5 mL of 60 µg/L endotoxin (lipopolysaccharide Escherichia coli 1335, Sigma) for 24 h. Cell-free supernatant was stored at −70 °C for measurement of IL-1 concentration by radioimmunoassay (34).

Human PBMC (1 × 10^5 cells/L in the first 16 subjects or 5 × 10^5 cells/L for the remainder of the subjects) were cultured in 24-well, flat-bottomed plates (Becton Dickinson) in the presence or absence of PHA (10 mg/L for the first 16 subjects and 2.5 mg/L for the remainder of the subjects) and 50 mL FBS/L for 48 h in a 37 °C, 5% CO₂, humidified incubator. Cell-free supernatant was saved at −70 °C for PGE₂ analysis by radioimmunoassay as described by Hwang et al (35) and McCosh et al (36). PGE₂ antibody was a gift from J Dupont of Iowa State University and M Mathias of Colorado State University. The antibody cross-reactivity and specificity were described (37). Because variability in PGE₂ was greater for larger PGE₂ values, the data was log transformed.

Serum immunoglobulin G (IgG), IgM, and IgA were measured by an immunoturbidimetric method by using a centrifugal analyzer (Cobas Fara, Roche Diagnostic System, Montclair, NJ) and antibodies (Atlantic Antibodies, Inc, Scarborough, ME).

PBMCs (1 × 10^5) and plasma samples were saved under nitrogen at −70 °C for tocopherol analysis. Plasma was analyzed by a modified high-pressure liquid chromatography (HPLC) method of Bieri et al (38) as previously described (39). Tocopherol in PBMCs was analyzed by adapting the method described by Handelman et al (40).

Plasma thiobarbituric reactive substances (TBARS) were measured by the method of Yagi (41). The procedure measures malondialdehyde and other aldehydes and end products of lipid peroxidation that can react with thiobarbituric acid forming a red chromophore.

Complete blood count was obtained with a hematology analyzer (Baker 9000, Serono-Baker Instrument Inc, Allentown, PA) (42) and white cell differential was assessed by microscopic examination of blood smears after Wright-Giemsa staining. In addition, urine and blood samples were used to evaluate changes in nutrition and general-health status during the study period by standard methods.

**Statistical analysis**

Data were analyzed by paired Student’s t test (for variables with normal distribution) or paired Wilcoxon signed-rank test (for variables not having normal distribution). Chi-square analysis was used to detect differences between groups in percent of subjects showing a change. Normality was determined with Wilk-Shapiro W statistics by use of Clinfo (Clinfo BBN Research System, Cambridge, MA).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Age, sex, and weight of subjects*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight</td>
</tr>
<tr>
<td></td>
<td>Before treatment</td>
</tr>
<tr>
<td>Group</td>
<td>Age</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
</tr>
<tr>
<td>Male (n = 4)</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>Female (n = 10)</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>Vitamin E supplemented</td>
<td></td>
</tr>
<tr>
<td>Male (n = 5)</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Female (n = 13)</td>
<td>71 ± 2</td>
</tr>
</tbody>
</table>

* x ± SEM.

**Results**

Table 1 shows the weight, age, and sex distribution in each group. There was no difference in average weight between the two groups before supplementation. All subjects maintained body weight throughout the study regardless of treatment. Subjects in both groups had normal values for plasma total protein, albumin, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, lactic dehydrogenase, and urinary creatinine. Supplementation with vitamin E did not change these variables (data not shown).

Blood cell counts (erythrocyte, hemoglobin, hematocrit, platelet count) in placebo and vitamin E-supplemented groups were within normal ranges and did not change during the study (data not shown). White-blood-cell and differential counts of the subjects were within normal ranges and did not change significantly during the study period in either group (data not shown).

The plasma α-tocopherol concentrations before supplementation were similar in placebo and vitamin E-supplemented groups (Table 2). Although no change in plasma α-tocopherol concentration was observed in placebo group, the concentrations in vitamin E-supplemented group increased threefold (p < 0.0001). Except for a small but significant increase in plasma zinc concentration, no changes were observed in the plasma concentration of other nutrients (fat- and water-soluble vitamins, minerals, and trace elements; data not shown).

No significant difference was observed in PBMC α-tocopherol concentrations between the two groups before supplementation (Table 2), and the concentrations did not change in the placebo group. In the vitamin E-supplemented group α-tocopherol concentrations rose threefold (p < 0.0008) and γ-tocopherol concentrations decreased threefold (0.028 ± 0.005 before vs 0.009 ± 0.002 nmol/10^10 cells after supplementation, p < 0.008). γ-tocopherol concentrations did not change in the placebo group (0.035 ± 0.009 before vs 0.049 ± 0.014 nmol/10^10 cells after supplementation).

Positive DTH responses (an induration ≥ 2 mm) were recorded at both 24 and 48 h and the highest value was recorded for each antigen. However, it was suggested that the DTH reactions occur ≥ 48 h after an antigenic challenge (43). Therefore, the analyses were calculated both ways, i.e., with 48-h values and with the highest value of the 24- and 48-h recordings. The
TABLE 2
Effect of vitamin E supplementation on plasma and peripheral blood mononuclear cell (PBMC) \( \alpha \)-tocopherol concentration in elderly subjects*  

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma ( \alpha )-tocopherol</th>
<th>PBMC ( \alpha )-tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu \text{mol/L} )</td>
<td>( \text{nmol} )</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Placebo</td>
<td>26.2 ± 1.7</td>
<td>23.9 ± 1.4</td>
</tr>
<tr>
<td>(( n = 14 ))</td>
<td>(( n = 14 ))</td>
<td>(( n = 6 ))</td>
</tr>
<tr>
<td>Vitamin E supplemented</td>
<td>25.6 ± 1.4</td>
<td>70.9 ± 6.3†</td>
</tr>
<tr>
<td>(( n = 17 ))</td>
<td>(( n = 17 ))</td>
<td>(( n = 5 ))</td>
</tr>
</tbody>
</table>

* \( \bar{x} \pm \text{SEM} \).
†† Significantly different from before-treatment values: \( t < 0.0001 \), \( t_p < 0.001 \).

statistical significance was the same regardless of which values were analyzed. The cumulative score significantly increased in vitamin E–treated group whereas no change in cumulative score was observed in the placebo group (Table 3). Seventy-one percent of the subjects in the vitamin E–supplemented group had an increase in their cumulative score, with a mean increase of 52.8 ± 20.0% in the vitamin E–supplemented group, compared with a 3.7 ± 3.7% increase in the placebo group. Percent change in the supplemented group is significantly higher than that in the placebo group at \( p = 0.04 \). The antigen score (total number of positive responses) was also significantly increased in the vitamin E–supplemented group (2.5 ± 0.38 before vs 3.1 ± 0.48 after, \( p < 0.05 \)) whereas no change was noted in the placebo group (3.2 ± 0.45 before vs 3.3 ± 0.42 after placebo).

Table 4 shows the mitogenic response to optimal concentrations (10 mg/L) of Con A. A significant increase in Con A–stimulated mitogenic response was observed in the vitamin E–supplemented group. No significant change in response to Con A was observed in the placebo group. No significant change was observed in response to PHA or SAC in either placebo or vitamin E–supplemented group (data not shown).

IL-2 formation in response to Con A was significantly increased in the vitamin E–supplemented group (\( p < 0.05 \)) (Table 5). In contrast, no significant change in IL-2 production was observed in the placebo group. Although 29% of subjects in the placebo group showed an increase in IL-2 formation, 64% of the subjects in the vitamin E–supplemented group had an increase in IL-2 concentration (\( p < 0.0002 \) by chi-square analysis). The percent change in IL-2 concentration in the vitamin E–supplemented group (67 ± 24%) was significantly (\( p < 0.025 \)) higher than that in the placebo group (−7 ± 20%). Furthermore, in the vitamin E–supplemented group, a positive correlation (\( r = 0.50, p = 0.056 \)) was noted between changes in IL-2 concentration and changes in plasma \( \alpha \)-tocopherol concentration. No significant change in endotoxin-stimulated IL-1 production was observed in either group (data not shown).

No significant change was observed in serum immunoglobulin concentrations in either the placebo or the vitamin E–supplemented group (data not shown).

Table 6 shows PGE2 production by the placebo and vitamin E–supplemented groups before and after supplementation. There was no significant change in unstimulated cultures in either group. However, in the vitamin E–supplemented subjects but not in the placebo group, a highly significant decrease was observed in PHA-stimulated PGE2 formation (Table 6). The percent decrease in the vitamin E group was significantly (\( p < 0.005 \)) higher than that of the placebo group (Fig 1).

A highly significant decrease in plasma lipid peroxides was observed in the vitamin E–supplemented group with no significant change in placebo group (Table 7).

**Discussion**

This study represents the first double-blind, placebo-controlled trial of the effect of vitamin E supplementation on the immune response of healthy elderly individuals. Supplementation of healthy elderly individuals with 800 mg dl-\( \alpha \)-tocopheryl acetate/d for 30 d significantly improved DTH, an in vivo mea-

TABLE 4
Effect of vitamin E supplementation on Con A–induced lymphocyte proliferation by PBMCs from elderly subjects*  

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (( n = 14 ))</td>
<td>24 478 ± 2 729</td>
<td>21 954 ± 2 908</td>
</tr>
<tr>
<td>Vitamin E supplemented (( n = 18 ))</td>
<td>20 551 ± 1 927</td>
<td>23 770 ± 2 991†</td>
</tr>
</tbody>
</table>

* \( \bar{x} \pm \text{SEM} \).
† Significantly different from before-treatment values, \( p < 0.05 \) (paired Wilcoxon signed-rank test).

TABLE 3
Effect of vitamin E supplementation on DTH in elderly subjects*  

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (( n = 13 ))</td>
<td>16.5 ± 2.2</td>
<td>16.9 ± 2.1</td>
</tr>
<tr>
<td>Vitamin E supplemented (( n = 16 ))</td>
<td>14.2 ± 2.9</td>
<td>18.9 ± 3.5†</td>
</tr>
</tbody>
</table>

* \( \bar{x} \pm \text{SEM} \).
† Significantly different from before-treatment values \( p < 0.05 \) (paired Wilcoxon signed-rank test).

TABLE 5
Effect of vitamin E supplementation on Con A–induced IL-2 production by PBMCs from elderly subjects*  

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (( n = 14 ))</td>
<td>31.8 ± 8.3</td>
<td>37.5 ± 12.5</td>
</tr>
<tr>
<td>Vitamin E supplemented (( n = 18 ))</td>
<td>35.6 ± 9.1</td>
<td>49.6 ± 12.6†</td>
</tr>
</tbody>
</table>

* \( \bar{x} \pm \text{SEM} \).
† Significantly different from before-treatment values, \( p < 0.05 \) (paired Student's \( t \) test).
sure of cell-mediated immunity, and enhanced the in vitro mitogenic response to the T-cell mitogen Con A but not to PHA or the B-cell mitogen SAC. Furthermore, a significant increase in IL-2 formation in response to Con A was observed after vitamin E supplementation. The increase in IL-2 concentration was positively correlated with changes in vitamin concentration. No effect on IL-1 formation was noted. Decreases in DTH, mitogenesis to Con A and PHA, and IL-2 production are well documented in both senescent rodents and older adults (44–46) whereas changes in B-cell response (47) and IL-1 production are equivocal (10, 46–48). The results of this clinical trial closely parallel our earlier studies in aged mice where vitamin E supplementation significantly improved DTH, mitogenic response to Con A (but not to PHA), and IL-2 formation (21).

It is interesting that the majority but not all of the vitamin E–supplemented subjects showed an enhancement of immune response. However, the responders showed improvement in all indices. The quantitative change of the immune indices was variable in the responders depending on the individual and the type of test. The variability in response could be partly due to differences in plasma or PBMC concentration of tocopherol after supplementation. The greatest relative change after vitamin E supplementation was observed in IL-2 production followed by cumulative DTH score and mitogenic response to Con A. Changes in IL-2 were directly correlated with changes in plasma vitamin E concentration. The lack of a larger average change in Con A and PHA responses might be due to the greater variability inherent in these assays. In addition to large interindividual variation, substantial intraindividual differences were also observed in mitogen-stimulated lymphocyte-proliferation tests. All in vitro culture assays were performed in the presence of FBS, a poor source of tocopherol. This medium may diminish the effect of vitamin E during the longer culture periods used in lymphocyte-proliferation assays relative to shorter culture periods utilized for the IL-2 assay. In both our previous animal experiment (21) and the present study, vitamin E supplementation improved Con A- but not PHA-induced lymphocyte proliferation. This implies specificity of the vitamin E effect because these mitogens stimulate different T-cell populations.

The immunostimulatory effect of vitamin E might be mediated by decreases in PGE₂ production and/or decreases in other lipid-peroxidation products. PGE₂ suppresses lymphocyte proliferation and IL-2 production. Increased PGE₂ production and lipid peroxidation have been found in aged animals (21, 23). Lymphocytes from elderly individuals are also more sensitive to the inhibitory effect of PGE₂ (49). Of particular interest is our observation that PBMCs from healthy elderly subjects synthesize significantly more PGE₂ than do those of young subjects (unpublished observations, 1990). In this study we observed a significant reduction in PGE₂ production by PBMCs and in plasma lipid peroxides (TBAR) of elderly subjects supplemented with vitamin E. Because malonaldehyde is produced as a by-product of arachidonic acid metabolism, the relative contribution to the reduction in total plasma TBAR by decreases in arachidonic acid metabolism and decreases in the formation of other lipid peroxides is not clear. PGE₂ was shown to decrease IL-1 production by monocytes (50). In this study no significant increase in IL-1 production was observed. However, vitamin E can inhibit the synthesis of lipoxigenase products (51, 52) including LT B₄, which was shown to enhance endotoxin-stimulated IL-1 production (53).

Except for a small but statistically significant increase in plasma zinc concentration, the status of other nutrients was unaffected by vitamin E supplementation. Although supplementation of elderly subjects with 440 mg zinc/d (~30 times

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**TABLE 6**

<table>
<thead>
<tr>
<th>Placebo ($n=14$)</th>
<th>Vitamin E supplemented ($n=18$)</th>
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<tbody>
<tr>
<td>Before treatment</td>
<td>7.3 ± 1.6</td>
</tr>
<tr>
<td>After treatment</td>
<td>6.6 ± 1.7</td>
</tr>
<tr>
<td>8.3 ± 1.6</td>
<td>7.7 ± 1.6</td>
</tr>
<tr>
<td>8.4 ± 1.6</td>
<td>9.1 ± 1.6</td>
</tr>
</tbody>
</table>

* $\bar{x} \pm$ SEM.
† Significantly different from before-treatment values, $p < 0.0004$ (paired Student’s t test).

**TABLE 7**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo ($n=14$)</td>
<td>2.26 ± 0.76</td>
<td>2.20 ± 0.55</td>
</tr>
<tr>
<td>Vitamin E supplemented ($n=18$)</td>
<td>2.76 ± 0.67</td>
<td>1.20 ± 0.60†</td>
</tr>
</tbody>
</table>

* $\bar{x} \pm$ SEM.† Significantly different from before-treatment values, $p < 0.001$ (paired Student’s t test).

**FIG 1.** Effect of vitamin E supplementation on PHA-stimulated PGE₂ production by PBMCs of elderly subjects. Each circle denotes one subject.
the recommended dietary allowance) was shown to improve DTH response (54), the increase in plasma zinc concentration in our subjects was minor (~5%) compared with the threefold increase in plasma and PBMC α-tocopherol concentrations. Furthermore, a change in plasma zinc concentration does not indicate a proportionate change in the tissue concentration of the mineral (55). A recent double-blind, placebo-controlled study by Bogden et al (55) showed no significant effect of zinc supplementation on immune response of elderly subjects.

In conclusion, our data indicate that short-term supplementation of most healthy elderly subjects treated with 800 mg dl-α-tocopheryl acetate significantly improves several indices of cell-mediated immunity. In view of the known effects of PGE₂ on immune function, it is plausible that the immunostimulatory effect of vitamin E is due to a reduction in PGE₂ synthesis and a concomitant increase in IL-2 production. Epidemiologic studies indicate a lower incidence of infectious disease in elderly subjects with high plasma tocopherol concentrations (30). Population groups maintaining high plasma tocopherol concentrations were also noted to possess a lower incidence of cancer (25, 56). Improved DTH response in hospitalized patients was shown to decrease sepsis and mortality (57). However, Harman and Miller (58) were not able to show a difference in antibody development against influenza virus vaccine or the incidence of infectious disease in elderly patients from a chronic-care facility supplemented with 200 or 400 mg tocopherol/d for 1 year. Unfortunately, data on the health and nutrition status, medication use, antibody concentrations, and other pertinent indices were not reported so it is difficult to interpret their findings.

It was demonstrated in young rodents that a higher than standard intake of tocopherol is necessary to promote optimal immune responsiveness (59). Although our study suggests that many elderly individuals might benefit from a supplementary intake of vitamin E, such public health recommendations can only be considered after longer-term studies with lower amounts of tocopherol are completed. This point will be especially important in determining if the immunostimulatory effect observed is due to pharmacologic or physiologic effect of vitamin E. Nevertheless, it is encouraging to note that a single nutrient supplement can enhance immune responsiveness in healthy elderly subjects consuming the recommended amounts of all nutrients. This is especially significant because dietary intervention represents the most practical approach for delaying or reversing the rate of decline of immune function with age.

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