Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects[1-4]

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-α-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) E_2, 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)* PGE_2 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 PGE_2 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), hydroxeicosatetraenoic acid (HETE), and leukotriene (LT) (13, 14). PGE_2 was shown to suppress lymphocyte proliferation and lymphokine synthesis (15–17). Other oxidative metabolites of activated macrophages, such as H_2O_2, was also shown to suppress lymphocyte proliferation (18, 19). Increased PGE_2 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 PGE_2 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-

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 $20 \pm 1.5\%$ ($\bar{x} \pm$ SD) protein, $31.5 \pm 2.8\%$ fat, with a ratio of polyunsaturated to saturated fatty acids (P:S) of 0.41 ± 0.09, and $49 \pm 2\%$ 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 PGE_2 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 PGE_2 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-α-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 PGE_2 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), tricophyton (metagrophytes), and proteus (mirabilis). 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 trypan blue exclusion method. Cells were then suspended at appropriate concentrations for measurement of mitogenic lymphocyte proliferation, IL-2, IL-1, and PGE_2 formation.

Lymphocyte proliferation was measured by [^{3}H]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 1×10^9 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% CO_2 and 95% humidity. Four hours before termination of incubation, 18.5 GBq of [^{3}H]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 (1×10^9/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

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 [^{3}H]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 5×10^9 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 (1×10^9 cells/L in the first 16 subjects or 5×10^9 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% CO_2, humidified incubator. Cell-free supernatant was saved at -70 °C for PGE_2 analysis by radioimmunoassay as described by Hwang et al (35) and McCosh et al (36). PGE_2 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 PGE_2 was greater for larger PGE_2 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 (1×10^7) 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 1
Age, sex, and weight of subjects*

Group	Age	Weight	
		Before treatment	After treatment
	y	*kg*	
Placebo			
Male ($n = 4$)	62 ± 1	77.6 ± 8.4	76.3 ± 8.4
Female ($n = 10$)	73 ± 3	67.7 ± 2.9	67.3 ± 2.9
Vitamin E supplemented			
Male ($n = 5$)	69 ± 3	82.8 ± 4.5	82.5 ± 5.4
Female ($n = 13$)	71 ± 2	66.7 ± 3.3	65.8 ± 3.4

* $\bar{x} \pm$ 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 ($p < 0.008$) and γ-tocopherol concentrations decreased threefold (0.028 ± 0.005 before vs 0.009 ± 0.002 nmol/10^7 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/10^7 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, ie, 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) α-tocopherol concentration in elderly subjects*

Group	Plasma α-tocopherol		PBMC α-tocopherol	
	Before	After	Before	After
	μmol/L		nmol	
Placebo	26.2 ± 1.7 (n = 14)	23.9 ± 1.4 (n = 14)	0.14 ± 0.04 (n = 6)	0.19 ± 0.03 (n = 6)
Vitamin E supplemented	25.6 ± 1.4 (n = 17)	70.9 ± 6.3† (n = 17)	0.12 ± 0.02 (n = 5)	0.39 ± 0.05‡ (n = 5)

* $\bar{x}$ ± SEM.
†‡ Significantly different from before-treatment values: †$p < 0.0001$, ‡$p < 0.001$.

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

Group	Before treatment	After treatment
	ccpm	
Placebo (n = 14)	24 478 ± 2 729	21 954 ± 2 908
Vitamin E supplemented (n = 18)	20 551 ± 1 927	23 770 ± 2 991†

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

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

sis). 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 α-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 PGE$_2$ 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 PGE$_2$ 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-α-tocopheryl acetate/d for 30 d significantly improved DTH, an in vivo mea-

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

Group	Cumulative index	
	Before treatment	After treatment
	mm	
Placebo (n = 13)	16.5 ± 2.2	16.9 ± 2.1
Vitamin E supplemented (n = 16)	14.2 ± 2.9	18.9 ± 3.5†

* $\bar{x}$ ± 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*

Group	Before treatment	After treatment
	kU/L	
Placebo (n = 14)	31.8 ± 8.3	37.5 ± 12.5
Vitamin E supplemented (n = 18)	35.6 ± 9.1	49.6 ± 12.6†

* $\bar{x}$ ± SEM.
† Significantly different from before-treatment values, $p < 0.05$ (paired Student's t test).

TABLE 6
Effect of vitamin E supplementation on PGE_2 formation by PBMCs of elderly subjects*

Group	Control cultures		PHA-stimulated cultures	
	Before treatment	After treatment	Before treatment	After treatment
	In pmol/L			
Placebo (n = 14)	7.3 ± 1.6	6.6 ± 1.7	8.3 ± 1.6	8.4 ± 1.6
Vitamin E supplemented (n = 18)	7.8 ± 1.6	7.7 ± 1.6	9.1 ± 1.6	8.5 ± 1.5†

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

TABLE 7
Effect of vitamin E supplementation on plasma lipid peroxide concentration in elderly subjects*

Group	Before treatment	After treatment
	μmol/L	
Placebo (n = 14)	2.26 ± 0.76	2.20 ± 0.55
Vitamin E supplemented (n = 18)	2.76 ± 0.67	1.20 ± 0.60†

* $\bar{x}$ ± SEM.
† Significantly different from before-treatment values, $p < 0.001$ (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
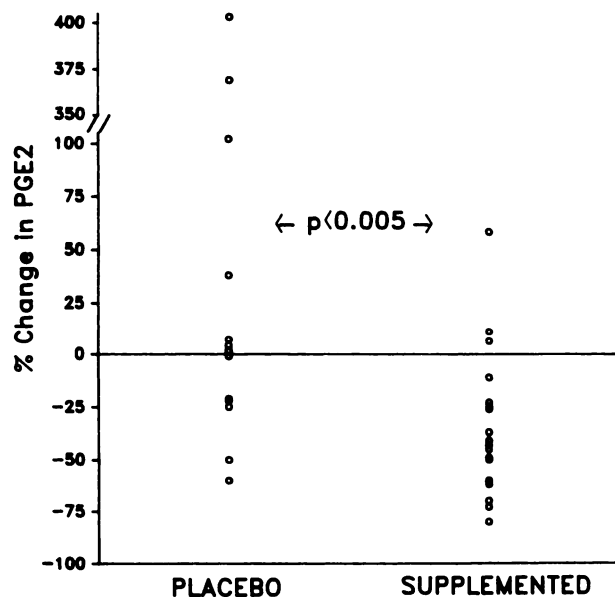
type of test. The variability in response could be partly due to differences in plasma or PBMC concentration of tocopherol after supplementation. The greatest relative change after vitamin E supplementation was observed in IL-2 production followed by cumulative DTH score and mitogenic response to Con 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 PGE_2 production and/or decreases in other lipid-peroxidation products. PGE_2 suppresses lymphocyte proliferation and IL-2 production. Increased PGE_2 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 PGE_2 (49). Of particular interest is our observation that PBMCs from healthy elderly subjects synthesize significantly more PGE_2 than do those of young subjects (unpublished observations, 1990). In this study we observed a significant reduction in PGE_2 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.

PGE_2 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 lipoxygenase products (51, 52) including LT B_4, 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 ($\sim$30 times

FIG 1. Effect of vitamin E supplementation on PHA-stimulated PGE_2 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 PGE_2 on immune function, it is plausable that the immunostimulatory effect of vitamin E is due to a reduction in PGE_2 synthesis and a concommitant 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 y. 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. ⓔ

References

1. Siskind GW. Immunological aspects of aging: an overview. In: Schimke RT, ed. Biological mechanism in aging. Bethesda, MD: US Department of Health and Human Services, 1980:455–67.
2. Kay MMB. An overview of immune aging. Mech Ageing Dev 1978;9:39–59.
3. Hallgren HM, Buckley CE, Gilbersten VA, Yunis EJ. Lymphocyte phytohemagglutinin responsiveness, immunoglobulins and auto-antibodies in aging humans. J Immunol 1973;4:1101–11.
4. Buckley CG, Buckley EG, Dorsey FC. Longitudinal changes in serum immunoglobulin levels in older humans. Fed Proc 1974;33: 2036–9.
5. Makinodan T. Cellular basis of immunologic aging. In: Schimke RT, ed. Biological mechanisms in aging. Bethesda, MD: US Department of Health and Human Services, 1981:488–500.
6. Roberts-Thompson IC, Yvonschaiyud V, Whittingham S. Aging, immune response and mortality. Lancet 1974;2:368–70.
7. Goodwin JS, Searles RP, Tung KSK. Immunological responses of a healthy elderly population. Clin Exp Immunol 1982;48:403–10.
8. Thoman ML, Weigle WO. Lymphokines and aging: interleukin-2 production and activity in aged animals. J Immunol 1981;127: 2101–6.
9. Miller RA, Stutman O. Decline in aging mice of anti-TNP cytotoxic T cell response attributable to loss of lyt-2⁻ IL-2 producting helper cell function. Eur J Immunol 1981;11:751–6.
10. Chang MP, Makinodan T, Peterson WJ, Strehler BL. Role of T cells and adherent cells in age-related decline in murine interleukin 2 production. J Immunol 1982;129:2426–30.
11. Gillis S, Kozak R, Durante M, Menkler MK. Immunological studies of aging. Decreased production of and response to T cell growth factor by lymphocytes from aged humans. J Clin Invest 1982;67: 937–42.
12. Unanue ER. Cooperation between mononuclear phagocytes and lymphocytes in immunity. N Engl J Med 1980;303:977–85.
13. Humes JL, Bonney RJ, Pebes L, et al. Macrophage synthesize and release prostaglandins in response to inflammatory response. Nature 1977;269:149–50.
14. Bonney RJ, Opas EE, Humes JL. Lipoxygenase pathway of macrophages. Fed Proc 1985;44:2933–6.
15. Goodwin JS, Messner RP, Peake GT. Prostaglandin suppression of mitogen stimulated leukocytes in culture. J Clin Invest 1974;54: 368–78.
16. Webb DR, Rogers TJ, Nowowiejski I. Endogenous prostaglandin synthesis and the control of lymphocyte function. Proc NY Acad Sci 1980;332:260–70.
17. Gordon D, Bray MA, Morley J. Control of lymphokine secretion by prostaglandins. Nature 1976;29:401–2.
18. Metzger Z, Hoffeld JT, Oppenheim JJ. Macrophage mediated suppression I. Evidence of participation of both hydrogen peroxide and prostaglandins in suppression of murine lymphocyte proliferation. J Immunol 1980;124:983–8.
19. Zoschke DC, Messner RP. Suppression of human lymphocyte mitogenesis mediated by phagocyte-released reactive oxygen species; comparative activities in normal and in chronic granulomatous disease. Clin Immunol Immunopathol 1984;32:29–40.
20. Rosenstein MM, Strauser HR. Macrophage induced T cell mitogen suppression with age. J Reticuloendothel Soc 1980;27:159–66.
21. Meydani SN, Meydani M, Verdon CP, Shapiro AC, Blumberg JB, Hayes KC. Vitamin E supplementation suppresses prostaglandin E₂ synthesis and enhances the immune response of aged mice. Mech Ageing Dev 1986;34:191–201.
22. Tengerdy RP, Mathias MM, Nockels CF. Effect of vitamin E on immunity and disease resistance. In: Prasad A, ed. Vitamins, nutrition and cancer. Basel, Switzerland: Karger, 1986:123–33.
23. Harman D. The free-radical theory of aging. In: Pryor WA, ed. Free radicals in biology. Vol 5. New York: Academic Press, 1982: 255–73.
24. Horvarth PM, Ip C. Synergistic effect of vitamin E and selenium in the chemoprevention of mammary carcinogenesis in rats. Cancer Res 1983;43:5335–41.
25. Menkes MS, Comstock GW, Vuilleumier JP, Helsing KJ, Rider AA, Brookmezer R. Serum beta-carotene, vitamins A and E, selenium, and the risk of lung cancer. N Engl J Med 1986;315:1250–4.
26. Knekt P, Aromaa A, Maatela J, et al. Serum vitamin E and risk

of cancer among Finnish men during a 10-year follow-up. Am J Epidemiol 1988;127:28–41.

27. Meydani SN, Cathcart CA, Hopkins RE, Meydani M, Hayes KC, Blumberg JB. Antioxidants in experimental amyloidosis of young and old mice. In: Glenner GG, Asserman EP, Benditt E, Calkins E, Cohen AS, Zucker-Franklin D, eds. Fourth International Symposium of Amyloidosis. New York: Plenum Press, 1986:683–92.

28. Harman D. Free radical theory of aging: beneficial effect of antioxidants on the life span of male NZB mice; role of free radical reaction in the deterioration of immune system with age and in pathogenesis of systemic lupus erythematosus. Age 1980;3:64–73.

29. Chevance M, Brubacher G, Herbeth B, et al. Immunological and nutritional status among the elderly. In: De Wick AL, ed. Lymphoid cell functions in aging. Paris: Eurage, 1984:231–7.

30. Chevance M, Brubacher G, Herbeth B, et al. Immunological and nutritional status among the elderly. In: Chandra RK, ed. Nutrition, immunity, and illness in the elderly. New York: Pergamon Press, 1985:137–42.

31. Boyum AI. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968;97(suppl 21):77–89.

32. Gillis S, Fern MM, Ou W, Smith KA. T-cell growth factor: parameters of production and a quantitative micro-assay for activity. J Immunol 1978;120:2027–32.

33. Dinarello CA, Lonnemann G, Maxwell R, Shaldon S. Ultrafiltration reject human interleukin-1-inducing substances derived from bacterial cultures. J Clin Microbiol 1987;25:1233–8.

34. Lisi PJ, Chu CW, Koch GA, Endres S, Lonnemann G, Dinarello CA. Development and use of radioimmunoassay for interleukin-1B. Lymphokine Res 1987;6:229–44.

35. Hwang DH, Mathias MM, Dupont J, Meyer DL. Linoleate enrichment of diet and prostaglandin metabolism in rats. J Nutr 1986;105:995–1002.

36. McCosh EJ, Meyer DL, Dupont J. Radioimmunoassay of prostaglandin E_1, E_2, $F_{2\alpha}$ in unextracted plasma, serum and myocardium. Prostaglandins 1976;12:472–86.

37. Meydani SN, Yogeeswaran G, Liu S, Baskar S, Meydani M. Fish oil and tocopherol induced changes in natural killer cell mediated cytotoxicity and PGE_2 synthesis in young and old mice. J Nutr 1988;118:1245–52.

38. Bieri JG, Tollinery TJ, Catigani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr 1979;32:2143–9.

39. Meydani SN, Shapiro AC, Meydani M, McCauley J, Blumberg JB. Effect of age and dietary fat (fish oil, corn oil, and coconut oil) on tocopherol status of C57BL/6Nia mice. Lipids 1987;22:345–50.

40. Handelman GJ, Epstein WL, Machlin LJ, Van Kuijk JGM, Dratz EA. Biopsy method for human adipose with vitamin E and lipid measurements. Lipids 1988;23:598–604.

41. Yagi K. Assay for serum lipid peroxide level and its clinical significance. In: Yagi K, ed. Lipid peroxides in biology and medicine. New York: Academic Press, 1982:223–42.

42. Wintrobe MM, Lee GR, Boggs DR, et al. Principles of hematology

examination. In: Wintrobe MM, ed. Clinical hematology. Philadelphia: Lea and Febiger, 1981:21–5.

43. Buckley CE III. Delayed hypersensitivity skin testing. In: Rose NR, Fiedman H, Fahey JL, ed. Manual of clinical laboratory immunology. 3rd ed. Washington, DC: American Society for Microbiology, 1980:260–73.

44. Miller RA. The cell biology of aging: immunological models. J Gerontol Biol Sci 1989;44:B4–8.

45. Nagel JE, Chopra RK, Chrest FJ, et al. Decreased proliferation, interleukin 2 synthesis and interleukin 2 receptor expression are accompanied by decreased mRNA expression in phytohemagglutinin-stimulated cells from elderly donors. J Clin Invest 1988;81:1096–101.

46. Inamiza T, Chang MP, Makinodan T. Influence of age on the production and regulation of interleukin-1 in mice. Immunology 1985;55:447–55.

47. Becker MJ, Farkas R, Schneider M, Drucker A, Klajman A. Cell mediated cytotoxicity in humans: age-related decline as measured by xenogeneic assay. Immunol Immunopathol 1979;14:204–10.

48. Cobleigh MA, Braun DP, Harris JE. Age-dependent changes in human peripheral blood B cells and T cell subsets: correlation with mitogen responsiveness. Clin Immunol Immunopathol 1980;15:162–73.

49. Goodwin JS, Messner RP. Sensitivity of lymphocytes to prostaglandin E_2 increased in subjects over age 70. J Clin Invest 1980;332:260–70.

50. Knudsen PJ, Dinarello CA, Storm TB. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. J Immunol 1986;31:89–94.

51. Goetzel EJ. Vitamin E modulates the lipoxygenation of arachidonic acid in leukocytes. Nature 1981;288:183–5.

52. Meydani SN, Stocking LM, Shapiro AC, Meydani M, Blumberg JB. Fish oil and tocopherol induced changes in ex-vivo synthesis of spleen and lung leukotriene B_4 in mice. Ann NY Acad Sci 1988;524:395–8.

53. Rola-Plaszczynski M, Lemaire I. Leukotrienes augment interleukin 1 production by human monocytes. J Immunol 1985;135:3958–61.

54. Duchateau J, Delepesse G, Vrijens R, Collet H. Beneficial effects of oral zinc supplementation on the immune responsiveness of old people. Am J Med 1981;70:1001–4.

55. Bogden JD, Oleske JM, Lavenhar MA, et al. Zinc and immunocompetence in elderly people: effects of zinc supplementation for 3 months. Am J Clin Nutr 1988;48:655–63.

56. Trickler D, Shklar G. Prevention by vitamin E of experimental oral carcinogenesis. JNCI 1987;78:1615–9.

57. Fletcher JD, Koch GA, Endres E, et al. Anergy and the severly ill surgical patient. Aust NZ J Surg 1986;56(2):17–20.

58. Harman D, Miller RW. Effect of vitamin E on the immune response to influenza virus vaccine and incidence of infectious disease in man. Age 1986;9:21–3.

59. Bendich A, Garbiel E. Dietary vitamin E requirement for optimum immune response in the rat. J Nutr 1986;116:675–81.