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Vitamin E-Enhanced IL-2 Production in Old Mice: Naive But Not Memory T Cells Show Increased Cell Division Cycling and IL-2-Producing Capacity[1] FREE

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Vitamin E-Enhanced IL-2 Production in Old Mice: Naive But Not Memory T Cells Show Increased Cell Division Cycling and IL-2-Producing Capacity[1]

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Aging is associated with reduced T cell function, as demonstrated by decreased T cell proliferation and IL-2 production. These changes respond to supplemental vitamin E both in animals and humans, in part by the reduction of T cell suppressive PGE_2, the production of which by macrophages is increased with age. To evaluate whether vitamin E has a direct PGE_2-independent effect on T cell responses, T cells purified from the spleens of young and old mice were preincubated with vitamin E or vehicle control. Activation-induced cell division of T cells from old mice was lower than that by young, and the production of IL-2 following 48-h activation was less by T cells from old mice. There was an age-related decline in both the number of IL-2^+ T cells and the amount of IL-2 produced per cell. Despite decreased IL-2 protein at 48 h, the expression of IL-2 mRNA at 6 h and IL-2 protein production at 6 and 16 h was greater by T cells from old mice compared with that of young. Age-related decline in cell division and IL-2 production at 48 h was only observed within the naive T cell subpopulation. Vitamin E increased both cell-dividing and IL-2-producing capacity of naive T cells from old mice, with no effect on memory T cells. These data indicate that naive T cells exhibit the greatest age-related defect and show for the first time that supplemental vitamin E has direct immunoenhancing effect on naive T cells from old mice. ***The Journal of Immunology,*** **2001, 167: 3809–3817.**

Age-dependent deterioration of the immune system is believed to contribute to higher incidence of morbidity and mortality from infection, neoplastic, and possibly autoimmune diseases. Age-related changes in the T cell compartment of the immune system have been reported both in humans and in experimental animal models. One of the hallmarks of age-related changes is a shift toward greater proportions of Ag-experienced memory T cells with fewer T cells of the virgin or naive phenotype (1–3). Naive T cells have different response kinetics to Ag challenge than memory T cells, with Ag-experienced memory T cells responding faster and to a lower Ag dose than naive T cells (4). However, recent evidence indicates that naive, Ag-inexperienced T cells also show an age-related functional decline (5, 6). Using a transgenic model with naive T cells specific for pigeon cytochrome *c* (PCC),[3] naive PCC-specific T cells from old mice were shown to have reduced ability to proliferate and produce IL-2 (5). Furthermore, they showed intracellular defects in assembling an effective immunologic membrane synapse (6). These studies provide strong evidence that intrinsic age-related changes in naive T cells contribute to the age-related decline in the response to new or infrequently encountered Ag.

The significance of IL-2 for cellular immunity is evident by its role in mediating clonal expansion of activated T cells (7). The induction of IL-2 production by activated T cells results in marked up-regulation of high-affinity IL-2R on T cells and, thus, responsiveness to IL-2 and progression through the cell cycle, as well as differentiation of naive T cells into effectors. Therefore, it has been suggested that alteration in IL-2 production with age significantly contributes to the age-dependent dysregulation of cellular immunity (8).

Some of the age-related changes in T cell function may involve free radical damage to critical cell components and other age-related changes accumulated over the life span of the organism. In T cells, the intracellular balance between oxidizing and reducing conditions is closely linked to the cellular proliferative capacity. Production of reactive oxygen species (ROS) by activated macrophages during an inflammatory response as well as cellular depletion of reduced glutathione (GSH), a major cellular reducing agent, has been shown to depress T cell responses (9–11). The effect of ROS on T cell function has been found to be different between naive and memory T cells. Lohmiller et al. (12) reported that memory T cells had higher levels of GSH compared with naive T cells. Exposure to ROS had a greater suppressive effect on functional measures of naive T cells compared with that of memory T cells.

Vitamin E is the most biologically active fat-soluble antioxidant capable of protecting unsaturated fatty acids in cellular membranes from per-oxidation and thus contributing to membrane stability (13). Both human clinical trials and animal studies have shown a beneficial effect of supplemental vitamin E on immune function in the aged. Meydani et al. (14) reported that when old mice were fed 500 ppm of vitamin E, macrophage production of the T cell-suppressive lipid mediator PGE_2 was lowered. In addition, measures of cell-mediated immune function, such as delayed-type hypersensitivity (DTH) response, in vitro T cell proliferation and IL-2 production in response to the T cell mitogen Con A were increased

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[3] Abbreviations used in this paper: PCC, pigeon cytochrome *c*; ROS, reactive oxygen species; GSH, reduced glutathione; DTH, delayed-type hypersensitivity; HSD, honestly significant difference.

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compared with those fed 30 ppm (an adequate dietary level) of vitamin E. A dietary supplement of 800 mg of vitamin E for 30 days to men and woman over 60 years of age, increased DTH, mitogenic response to Con A, and the production of IL-2 (15). A subsequent randomized double-blind, placebo-controlled trial by Meydani et al. (16) found that vitamin E supplementation improved DTH response in a group of healthy elderly subjects. In addition, Ab titer against T cell-dependent hepatitis B vaccine was significantly increased in subjects receiving supplemental vitamin E. Previous studies from our laboratory show that vitamin E exerts its effect in part by reducing macrophage PGE_2 production (14, 17). However, preliminary results also indicated a direct effect of vitamin E on T cells independent of its effect on macrophage PGE_2 production. Therefore, the purpose of the present study was first to determine whether vitamin E can enhance T cell function in old mice, independently of its enhancing effects mediated through the reduction of macrophage PGE_2 production, and, second, to determine the mechanism of the direct enhancing effect of vitamin E on T cell function of old mice.

Materials and Methods

Animals

Young (6 mo) and old (26 mo) male C57BL/6 mice fed autoclaved Purina (Ralston, St. Louis, MO) mouse chow and water ad libitum were housed in filtered cages maintained at a constant temperature (23°C) with a 12-h light-dark cycle. Mice were euthanized via CO_2 asphyxiation and spleens were aseptically removed and placed in sterile, endotoxin-free RPMI 1640 (BioWhittaker, Walkersville, MD) medium supplemented with 5% heat-inactivated FBS, 25 mM HEPES, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Life Technologies, Grand Island, NY; complete RPMI). All handling and animal conditions were approved by the Animal Care and Use Committee of the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Mice exhibiting tumors, splenomegaly, grossly visible skin lesions, or significant pathology were excluded from the study.

Purification of $CD3^+$ spleen cells

Single-cell suspensions were obtained by disrupting spleens between two sterile frosted glass slides. RBCs were lysed using a hemolytic ammonium chloride-based Gey's solution. Splenocytes depleted of RBCs were washed and resuspended in degassed buffer consisting of PBS, 2 mM EDTA, and 0.5% BSA (Sigma, St. Louis, MO). The cell suspension was then incubated with a mixture of mAb (anti-Mac-1, anti-MHC class II, and anti-NK) directly conjugated to immunomagnetic beads (Miltenyi Biotec, Auburn, CA). Following a 15-min incubation at 4°C, cells were washed and T cells negatively selected using a magnetic-activated cell sorter separation column (Miltenyi Biotec) equipped with a flow restrictor. To assess T cell enrichment, aliquots of unseparated and negatively selected cells were stained with PE-conjugated anti-CD3 and FITC-conjugated anti-CD19 (both from BD PharMingen, San Diego, CA) or FITC-conjugated anti-Mac-1 (Caltag Laboratories, Burlingame, CA) mAb. Cytofluorographic analysis was performed on a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA) using standard acquisition and analysis software.

T cell supplementation with vitamin E

A stock solution of natural vitamin E was prepared by dissolving RRR-α-tocopherol (Henkel, Gulph Mills, PA) in absolute ethanol to a final concentration of 37 mg/ml. To optimize cellular uptake, a solution of vitamin E was then mixed in FBS at 1 mg/ml and incubated in a 37°C water bath for 1 h in the dark with intermittent vortexing. Purified T cells, adjusted to a concentration of 2×10^6 cells/ml, were preincubated in complete RPMI supplemented with vitamin E (46 μM) in a 37°C and 5% CO_2 incubator for 4 h. This level of vitamin E represents the average plasma α-tocopherol levels of humans taking a daily vitamin E supplement of 200 IU (18). T cells at the same concentration were also suspended in complete RPMI with 0.06% ethanol as vehicle control. Vitamin E-loaded T cells were washed twice in serum-free PBS before continuing. To compare the uptake of vitamin E by T cells from young and old mice, 5×10^6 T cells incubated with or without vitamin E as above were analyzed by HPLC with electrochemical detection as described by Wu et al. (19).

Proliferation assay

For the assessment of T cell proliferative capacity, 96-well round-bottom cell culture plates (Nunc, Roskilde, Denmark) were coated with 50 μl of anti-CD3ϵ mAb (hamster anti-mouse, clone 145-2C11; anti-CD3i; BD PharMingen) at 5 μg/ml in sterile PBS. Plates were incubated at 37°C for 90 min and then washed twice with PBS before cells were plated. In addition to anti-CD3i, cells were also activated with soluble anti-CD28 mAb (hamster anti-mouse, clone 37.51; BD PharMingen) at 2 μg/ml (anti-CD3i/CD28). Cells were plated in triplicate for each treatment at 2×10^5 cells/well for 64 h, pulsed with 0.5 μCi of [^{3}H]TdR (New England Nuclear, Boston, MA) in a 20-μl volume of RPMI, and then incubated for an additional 8 h. The cells were harvested onto glass filter paper and proliferation was quantified as the amount of [^{3}H]TdR incorporated into DNA, as determined by liquid scintillation counting (Beckman Coulter, Fullerton, CA). Data are expressed as absolute counts per minute.

Secreted and intracellular IL-2 determination

Purified T cells were plated at 1×10^6 cells/well of a 24-well tissue culture plate following preincubation with vitamin E or vehicle control. The cells were then subjected to various stimulation times with anti-CD3i/CD28 or for initial experiments with anti-CD3i only. Cell-free supernatants were collected and stored at −70°C for later analysis. Secreted IL-2 concentration was determined by an ELISA according to the manufacturer's instructions (BD PharMingen) using reference standard curves prepared with known amounts of mouse rIL-2 (BD PharMingen). For intracellular staining of IL-2, monensin (Sigma) at 2 μM was added to each well for the last 5 h of a 16-h activation period. The cells were collected and incubated with purified anti-CD16/CD32 mAb (Fc block; BD PharMingen) for 5 min and then with PE-conjugated anti-Thy1.2 mAb (Caltag Laboratories) in staining buffer containing 0.1% NaN_3 (Sigma) and 1% FBS in PBS for 30 min at 4°C. All staining procedures were performed at 4°C in the dark. The cells were then washed with staining buffer and fixed in 2% formaldehyde for 20 min. Fixed cells were washed again in staining buffer, followed by an incubation with allophycocyanin-conjugated anti-IL-2 mAb (BD PharMingen) or allophycocyanin-conjugated anti-IgG2b (BD PharMingen) isotype control in permeabilization buffer containing 0.1% NaN_3, 0.1% saponin (Sigma), and 1% FBS in PBS for 30 min. For the detection of intracellular IL-2 in naive and memory T cells separately, a final concentration of 2 μM monensin was added to each well for the last 10 h of a 48-h activation period. The cells were harvested and incubated with Fc block as before and then with PE-conjugated anti-phagocytic gp1 (anti-CD44) mAb (BD PharMingen). Intracellular staining of IL-2 was performed as described above. The cells were then washed in permeabilization buffer and resuspended in staining buffer for immediate analysis on a FACSCalibur flow cytometer (BD Biosciences) as previously described.

PGE_2 production

Purified T cells were plated at 4×10^5 cells/well of a 96-well tissue culture plate following preincubation with vitamin E or vehicle control. After 48 h of stimulation with anti-CD3i or anti-CD3i/CD28, the plates were centrifuged and cell-free supernatants collected and stored at −70°C for later analysis. PGE_2 was analyzed by RIA as described by Wu et al. (20). To rule out any possible involvement of PGE_2, T cells from young and old mice were also activated and plated with the cyclooxygenase inhibitor indomethacin (Sigma) at 4 μg/ml, and then assayed for proliferation and IL-2 production by ELISA as described above.

RNA preparation and RNase protection assay

RNA was obtained from 1×10^7 T cells/sample following activation for 0, 6, 16, and 26 h as described above using a guanidinium thiocyanate-phenol-chloroform-based extraction procedure according to manufacturer's instructions (Ambion, Austin, TX). Following ethanol precipitation, the RNA pellet was reconstituted in 10–30 μl of RNase-free water. Total RNA purity and quantity were determined by measuring absorbance at 260 and 280 nm on a UV spectrophotometer (Shimadzu, Columbia, MD). For relative quantitation of IL-2 mRNA transcripts, an RNase protection assay was conducted. Briefly, the ^{32}P-labeled antisense single-stranded IL-2 and L32 RNA probes were synthesized by using an in vitro transcription kit (Ambion) with a T7 RNA polymerize-directed synthesis. Linearized single-stranded 204-nt mIL-2 and 141-nt mL32 DNA fragments were used as templates (BD PharMingen) with [α-^{32}P]UTP (800 Ci/mmol; New England Nuclear) as the labeled nucleotide. The transcribed probes were purified by extraction with phenol-chloroform and precipitated with ammonium acetate and ethanol. Before hybridization, RNA samples were evaporated in a SpeedVac concentrator. The overnight hybridization and digestion reaction were conducted using 8 μg of total sample RNA per

5.75×10^5 cpm labeled probe and a combination of RNase T1 and A (Ambion), respectively. For the separation of protected dsRNA fragments, the denatured samples were electrophoresed in standard 5% acrylamide/8 M urea gels. A set of RNA markers (Ambion) were transcribed as described above and run in parallel with samples. Gels were exposed to BioMax MS film (Kodak, Rochester, NY) at −70°C and autoradiographs were analyzed by densitometry (Alpha Innotech, San Leandro, CA).

Cell division analysis of naive and memory T cells

For cell division analysis, purified T cells were incubated with 0.2 μM of the cell tracing compound CFSE (Molecular Probes, Eugene, OR) for 7 min in PBS at room temperature. Staining was stopped with an equal volume of FBS and the cells were washed twice in complete RPMI containing 5% FBS. CFSE-loaded T cells were then activated for 48 h. Upon harvesting, cells were stained with PE-conjugated anti-CD44 mAb or a PE-conjugated IgG2b isotype control Ab (both from BD PharMingen) as described above. In addition, CFSE and CD44 fluorescence was measured in resting T cells. The expression pattern of the CD44 Ag and cell division analysis was determined on a FACSCalibur flow cytometer (BD Biosciences). For the proportion of naive and memory cells going through cell division cycles during the 48-h culture period, each two-dimensional dot plot region representing zero, one, and two cell division cycles was evaluated for low and high expression patterns of the CD44 Ag as described above. The zero cell division cycle histogram peaks and dot plot regions were identified by the peak CFSE fluorescence of unactivated resting T cells.

Statistics

Data were analyzed by a two-way ANOVA with significant differences evaluated by a Tukey's honestly significant difference (HSD) post hoc procedure using Systat 9 statistical software (Systat, Evanston, IL). Data are reported as mean ± SEM. Significance was set at $p < 0.05$.

Results

Uptake of α-tocopherol by T cells from young and old mice

T cells were purified from the spleens of young and old C57BL/6 mice. Cytofluorographic analysis demonstrated that ≥94% of the enriched cell population expressed CD3, an Ag that defines mature T cells (data not shown). Cells staining positive for macrophages (Mac-1 Ag) or B cells (CD19 Ag) represented <1% of the total flow-through population. In vitro supplementation of T cells with 46 μM vitamin E for 4 h increased cellular vitamin E content in T cells from young and old mice as determined by HPLC analysis ($p < 0.001$; data not shown). There was no significant difference in the ability of T cells from young and old mice to take up vitamin E under the conditions of this experiment. Therefore, any functional differences due to vitamin E cannot be contributed to differential uptake of the vitamin by T cells from young and old mice.

Effect of vitamin E on the age-dependent decline in T cell proliferation and IL-2 production

Proliferative capacity of purified T cells from young and old mice was compared using immobilized anti-CD3 mAb only (anti-CD3i) or in a combination with soluble anti-CD28 mAb (anti-CD3i/CD28). This mimics T cell activation by providing a primary signal via the TCR-CD3 complex and a secondary signal through the CD28 T cell coreceptor. The proliferative capacity of T cells from old mice in response to anti-CD3i or anti-CD3i/CD28 was significantly lower ($p < 0.001$) when compared with T cells from young mice (Fig. 1). The addition of anti-CD28 mAb did not increase proliferation of T cells from young or old mice above what was observed when anti-CD3i was used alone. Anti-CD28 mAb activation alone did not induce proliferation above resting T cells. When T cells were supplemented with vitamin E-enriched medium, anti-CD3i- and anti-CD3i/CD28-induced proliferative capacity of T cells from old mice was significantly enhanced (>4-fold increase; $p < 0.05$), whereas young T cells did not show a significant increase. The positive effect of vitamin E on the proliferative response of T cells in a mixed splenocyte culture originating from old mice has previously been reported (17). This was, in part, due to vitamin E-induced reduction in T cell-suppressive lipid mediator PGE_2, the production of which by macrophages is increased with age. Purified T cells were used to exclude macrophages, a major cellular source of PGE_2. To rule out any possible role of PGE_2 originating from T cells or contaminating macrophages, we evaluated whether purified T cells originating from young and old mice could be stimulated to produce PGE_2. No PGE_2 was detected in any of the samples collected from activated T cells of young or old mice (data not shown). Furthermore, T cell activation in the presence of the cyclooxygenase inhibitor, indomethacin, did not alter the vitamin E-induced effects in old T cells (data not shown). Thus, the enhancing effect of vitamin E on the proliferation of purified T cells is not mediated through PGE_2.

IL-2 regulates the clonal expansion of T cells upon activation. The concentration of IL-2 in the supernatants of young and old T cells activated for 48 h was measured by an ELISA (Fig. 2). T cells

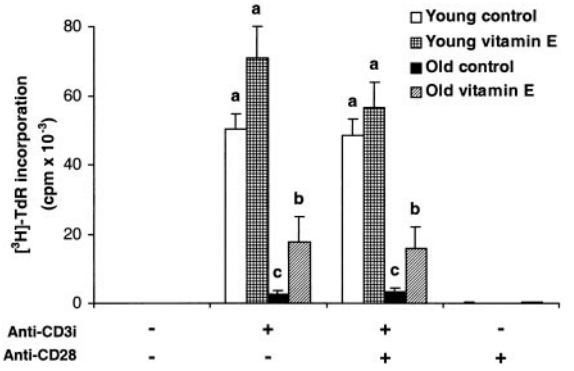

FIGURE 1. Effect of age and vitamin E on the proliferation of T cells. T cells from young and old mice ($n = 5$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) or vehicle control for 4 h and activated with immobilized anti-CD3 alone or in combination with soluble anti-CD28 mAb for 72 h. Cells were pulsed with 0.5 μCi of [^{3}H]TdR for the last 8 h of culture and [^{3}H]TdR uptake was determined by liquid scintillation counting. Bars with different letters within each group are significantly different ($p < 0.05$) by a two-way ANOVA followed by Tukey's HSD post hoc procedure.

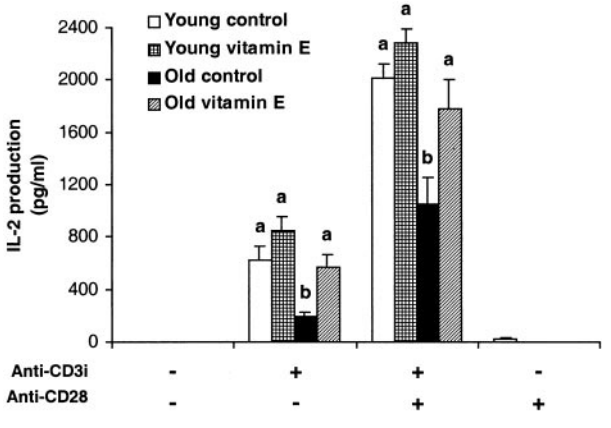

FIGURE 2. Effect of age and vitamin E on IL-2 production by T cells. T cells from young and old mice ($n = 5$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) or vehicle control for 4 h and activated with immobilized anti-CD3 alone or in combination with soluble anti-CD28 mAb for 48 h. Supernatants were collected and IL-2 protein was assayed by an ELISA. Bars with different letters within each group are significantly different ($p < 0.05$) by a two-way ANOVA followed by Tukey's HSD post hoc procedure.

from old mice produced significantly less IL-2 compared with T cells from young mice, both when activated with anti-CD3i ($p < 0.01$) or with anti-CD3i/CD28 ($p < 0.01$). To evaluate the effect of vitamin E on the ability of T cells from young and old mice to produce IL-2, T cells supplemented with vitamin E were activated with anti-CD3i or anti-CD3i/CD28. Preincubation with vitamin E significantly increased both anti-CD3i- and anti-CD3i/CD28-induced IL-2 secretion by activated T cells from old mice ($p < 0.05$). However, activation-induced secretion of IL-2 by young T cells was not increased by vitamin E supplementation. Vitamin E restored IL-2 secretion by T cells from old mice to a level that was not significantly different from that observed by T cells from young mice. As was observed with proliferation, indomethacin did not have an effect on IL-2 production or the ability of vitamin E to increase IL-2 production by old T cells (data not shown).

To rule out the possibility that the difference in IL-2 secretion was due to a difference in proliferative capacity and thus a difference in cell numbers, we analyzed T cell cultures from young and old mice by intracellular staining of IL-2 and flow cytometry. Flow cytometric analysis of anti-CD3i/CD28-induced intracellular accumulation of IL-2 revealed that significantly fewer T cells from old mice were IL-2^+ when compared with young mice ($p < 0.01$; Fig. 3*A*). In addition, IL-2-specific fluorescence of IL-2^+ T cells from old mice was less ($p < 0.05$) compared with that of young mice, indicating lower activation-induced intracellular accumulation of IL-2 per T cell from old mice (Fig. 3*B*). Supplementing T cells from old mice with vitamin E resulted in an increased number of activation-induced IL-2^+ cells ($p < 0.01$; Fig. 3*A*) as well as an increased level of IL-2 accumulated per cell ($p < 0.05$; Fig. 3*B*). No effect of vitamin E on T cells from young mice was observed. Thus, for T cells originating from old mice, supplementation with vitamin E increased the number of IL-2^+ T cells as well as intracellular IL-2 protein levels, resulting in higher total secreted IL-2 comparable to levels observed for T cells originating from young mice.

IL-2 mRNA expression is higher in T cells from old mice

One major regulatory site for IL-2 production is at the transcriptional level. Therefore, we next investigated whether the effect of age and vitamin E on IL-2 was at a pre- or a posttranscriptional level. A time course study for IL-2 mRNA expression showed that maximum IL-2 mRNA was obtained following 6 h of activation for T cells from both young and old mice (data not shown). T cells from additional young and old mice were next activated with anti-CD3i/28 for 6 h, and total RNA was extracted and analyzed for levels of IL-2 mRNA transcripts. Despite lower IL-2 protein levels at 48 h, old T cells had significantly higher IL-2 mRNA expression levels at 6 h compared with that of young T cells ($p < 0.05$; Fig. 4). Preincubation with vitamin E did not have an effect on IL-2 mRNA expression in young or old T cells. This indicated that the age-related defect in IL-2 production and the effect of vitamin E are posttranscriptional events. Alternatively, T cells originating from old mice may have responded faster to activation than young T cells, and vitamin E could have mediated its effect on a slow responding T cell subpopulation. This prompted us to determine whether the lower IL-2 protein levels in T cells from old mice at 48 h and its reversal by vitamin E would also be observed at earlier time points.

Time course for the effect of age and vitamin E on IL-2 production

To better characterize the effect of age and vitamin E on anti-CD3i/CD28-induced IL-2 secretion, supernatants were collected at five different time points of activation: 0, 6, 16, 26, and 48 h. As previously observed, at 48 h T cell cultures from young mice had produced significantly greater amounts of IL-2 protein compared with those of the old mice ($p < 0.05$; Fig. 5). In agreement with IL-2 mRNA expression, at earlier time points of 6 and 16 h, T cells from old mice produced more IL-2 protein compared with T cells from young mice ($p < 0.05$). At 26 h, IL-2 protein secretion by T cells from young and old mice was comparable. Supplementation with vitamin E increased the IL-2 protein level by old T cells only at 48 h ($p < 0.05$), the time point at which IL-2 secreted by old T cells was lower than that by young T cells. There was no indication of an effect of vitamin E at any of the earlier time points. As was previously seen, the secretion of IL-2 at 48 h by old T cells preincubated with vitamin E was not significantly different from that of young controls at the same time point. These results demonstrate that T cells from young and old mice have a different time course pattern for IL-2 secretion. Furthermore, they suggest that the lower overall IL-2 production and proliferation by T cells from old mice was due to the inability of an IL-2-producing T cell

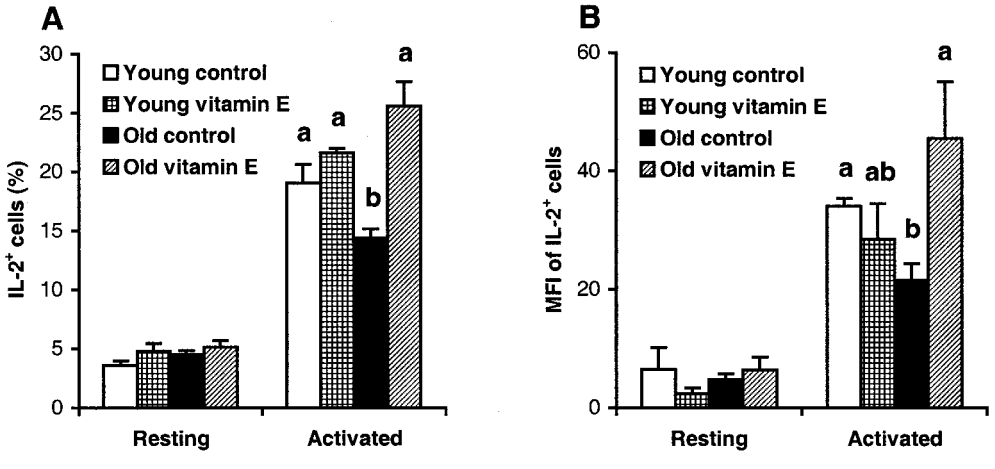

FIGURE 3. Effect of vitamin E on intracellular IL-2 levels in T cells from young and old mice. Purified T cells ($n = 5$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) for 4 h and activated with immobilized anti-CD3 and soluble anti-CD28 mAb for 18 h. T cells were treated with monensin, an inhibitor of IL-2 secretion, permeabilized, and stained with fluorochrome-conjugated anti-IL-2 and anti-Thy1.2 mAbs. Cell fluorescence was measured on a flow cytometer. *A*, The proportion of IL-2^+ T cells and *B*, the linearized mean fluorescence intensity (MFI) of IL-2^+ T cells. Bars with different letters within each panel are significantly different ($p < 0.05$) by a two-way ANOVA followed by Tukey's HSD post hoc procedure.

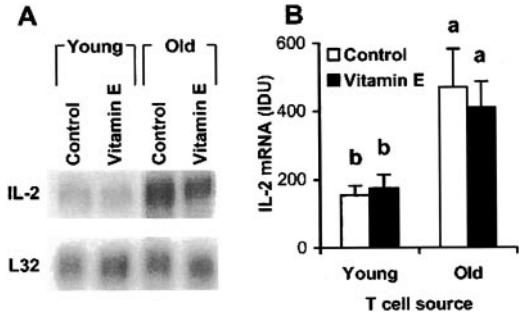

FIGURE 4. Effect of age and vitamin E on activation-induced IL-2 mRNA transcripts. Purified T cells ($n = 5$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) for 4 h and activated with immobilized anti-CD3 and soluble anti-CD28 mAb for 6 h. Relative amounts of IL-2 mRNA transcripts were determined by extracting RNA followed by an RNase protection assay using an in vitro-transcribed ^{32}P-labeled antisense ssIL-2 and L32 housekeeping RNA probes. Autoradiographs were analyzed by densitometry. *A*, One representative autoradiograph of five and *B*, mean integrated density units (IDU) ± SEM. Bars with different letters are significantly different ($p < 0.05$) by a two-way ANOVA followed by Tukey's HSD post hoc procedure.

population in old mice to maintain proliferation and IL-2 production. In addition, these results suggest that vitamin E increased IL-2 production by preserving the proliferative and/or IL-2-producing ability of this particular T cell population from the old mice.

CD44 expression and cell division analysis

A major change which occurs in the T cell compartment of the immune system with age is a gradual decrease in the proportion of naive T cells with a corresponding increase in the proportion of Ag-experienced memory T cells (2, 3). Naive T cells have different response kinetics to Ag challenge than memory T cells, with Ag-experienced memory cells responding faster and to a lower Ag dose than naive T cells do (4). This led us to speculate that the higher production of IL-2 and higher IL-2 mRNA expression at an earlier time point from old mice was due to a higher proportion of memory cells among old T cells. On the other hand, the lower IL-2 production at a later time point reflected either the inability of memory T cells from old mice to maintain IL-2 production or naive T cells from old mice to initiate proliferation and IL-2 production. We hypothesized that vitamin E increases IL-2 production by maintaining the function of memory T cells or by initiating cell division of naive T cells from old mice. Thus, we investigated the expression patterns of CD44, a T cell surface marker expressed at high levels on memory T cells (CD44^{high}) and at low levels on naive T cells (CD44^{low}).

As previously reported by others (1, 2), resting T cells from old mice were predominantly memory T cells of the CD44^{high} phenotype, whereas T cells from young mice were predominantly naive T cells of the CD44^{low} phenotype ($p < 0.05$; Fig. 6). Vitamin E did not have an effect on the percent resting naive or memory T cells. To investigate the progression of naive and memory T cells through cell division cycles separately, cells were labeled with CFSE cell-tracing reagent before activation and then stained for CD44 expression following 48 h of activation. Compared with naive T cells from young mice, naive T cells from old mice were less able to progress through one and two cell division cycles ($p < 0.05$; Figs. 7 and 8). Vitamin E increased the ability of naive T cells from old mice to progress through one as well as two cell division cycles ($p < 0.05$). This effect of vitamin E was not observed for memory T cells from old mice. This confirmed that it is the naive but not the memory T cell population from the old mice that fails to divide when activated. In addition, higher production of IL-2 at an early time point is a reflection of the memory-rich population of T cells from old mice, which responds with faster kinetics. However, at a later time point, naive T cells from old mice failed to respond, thus resulting in lower overall IL-2 production by old T cells. Furthermore, vitamin E mediates its effect by increasing the ability of naive T cells to respond to stimuli by proliferating and producing IL-2.

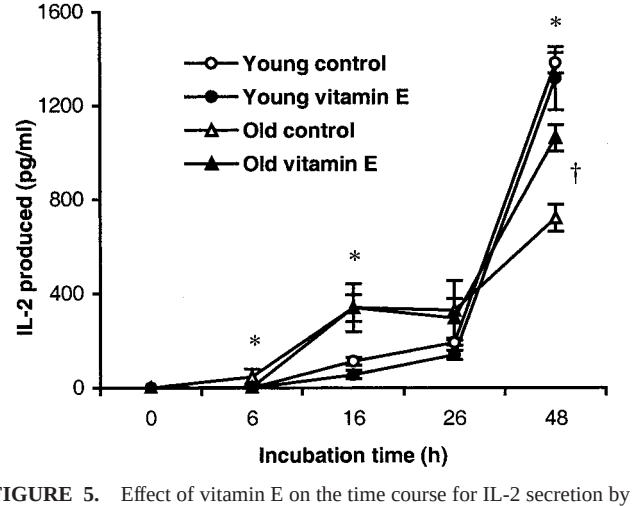

FIGURE 5. Effect of vitamin E on the time course for IL-2 secretion by T cells from young and old mice. Purified T cells ($n = 5$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) for 4 h and activated with immobilized anti-CD3 and soluble anti-CD28 mAb for the times indicated. Cell supernatants were harvested and assayed for IL-2 by an ELISA. *, Significant difference between young and old ($p < 0.05$) by a two-way ANOVA followed by Tukey's post hoc procedure. †, Significant difference between old control and old vitamin E supplemented ($p < 0.05$) by a two-way ANOVA followed by Tukey's HSD post hoc procedure.

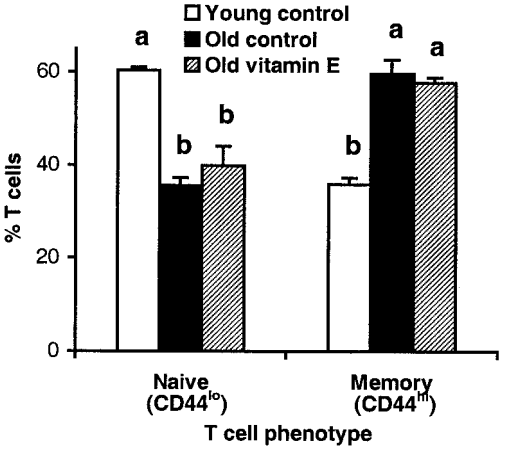

FIGURE 6. Effect of age and vitamin E on CD44 expression patterns of unactivated T cells from young and old mice. Purified unactivated T cells ($n = 3$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) for 4 h and then stained with fluorochrome-conjugated anti-CD44 and anti-Thy1.2 mAbs. T cell expression of CD44 was analyzed on a flow cytometer. T cells were divided into naive and memory phenotypes based on low or high expression of the CD44 Ag, respectively. The mean ± SEM of three independent experiments for young control, old control, and old supplemented with vitamin E are shown. Bars with different letters are significantly different ($p < 0.01$) within each phenotype by an ANOVA followed by Tukey's HSD post hoc procedure.

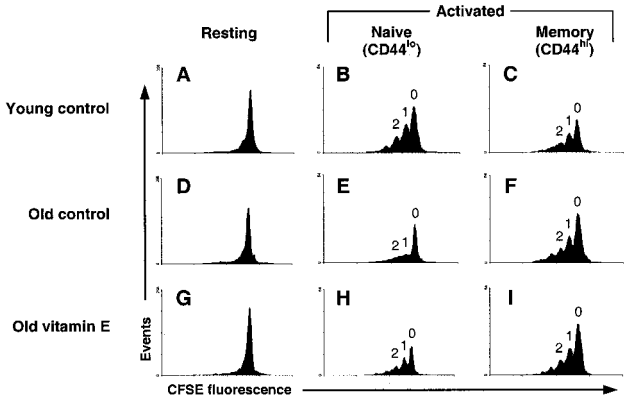

FIGURE 7. Effect of age and vitamin E on the progression of T cells through cell cycle division. Purified T cells were preincubated with 46 μM RRR-α-tocopherol (vitamin E) for 4 h, labeled with CFSE, and activated with immobilized anti-CD3 and soluble anti-CD28 mAb for 48 h. Cells were harvested, stained for CD44 expression, and analyzed on a flow cytometer. One representative histogram for each young control (*A–C*), old control (*D–F*), and old preincubated with vitamin E (*G–I*) are shown. Cell cycle division patterns are shown for unactivated T cells (*A, D,* and *G*), activated naive ($CD44^{low}$) T cells (*B, E,* and *H*), and activated memory ($CD44^{high}$) T cells (*C, F,* and *I*). Peaks representing cell division cycles 0, 1, and 2 are also indicated.

Effect of age and vitamin E on intracellular IL-2 levels in naive and memory T cells

To further confirm that the age-related defect in IL-2 production was due to a change in the proliferative capability of naive T cells and that the effect of vitamin E on IL-2 production was specifically mediated through this population, T cells were activated for 48 h and then stained for CD44 expression and for intracellular IL-2. This allowed us to determine IL-2 production by naive and memory T cells separately. In agreement with the data obtained for cell proliferation, naive T cells from old mice failed to produce IL-2 at the same level as young naive T cells. Both the proportion of $IL\text{-}2^+$ T cells (Fig. 9*A*) and the amount of IL-2 produced per cell (Fig. 9*B*) was less for naive T cells from old compared with that of young ($p < 0.05$). Vitamin E increased both the proportion of $IL\text{-}2^+$ naive T cells ($p < 0.05$) and the amount of IL-2 produced per naive T cell ($p < 0.05$). More memory T cells from old mice were $IL\text{-}2^+$ ($p < 0.05$), but there was no age difference in the amount of IL-2 produced per memory T cell. Vitamin E did not have an effect on IL-2 production by memory T cells from young or old mice. Thus, it is the naive T cell population that exhibits the greatest age-related defect in IL-2 production, and supplemental vitamin E is able to specifically enhance the functional properties of this population of T cells.

Discussion

In this study, we sought to investigate the effect of vitamin E, a potent lipid-soluble antioxidant, on age-related T cell functional defects using purified T cells free from the influence of immunosuppressive macrophage PGE_2. Cell-free supernatants from purified T cells stimulated to produce PGE_2 did not show detectable PGE_2 levels. In addition, the use of a cyclooxygenase inhibitor, indomethacin, did not alter the proliferative response or IL-2 production of T cells from young or old mice, indicating that in the absence of macrophages, PGE_2 does not contribute to age-associated T cell functional defects. To investigate the effect of vitamin E on proliferative response and IL-2 production independently of its effect on macrophage PGE_2 production, we supplemented purified T cells from young and old mice with vitamin E by an in vitro method. In agreement with previous studies, over a 48-h activation period, T cells from old mice had less proliferative and IL-2-producing capacity than T cells from young mice. Vitamin E supplementation increased both proliferation and total IL-2 production by T cells from old mice, with no effect on young T cells.

The age-related shift in the proportions of T cells from mainly those that have not encountered Ag (naive) to those that have (memory) is a major change that influences T cell-mediated immunity in the aged (2, 3). This, in part, results from decreased output of positively selected immature naive T cells from the aging thymus. Naive T cells have different response kinetics to Ag challenge than memory T cells, with Ag-experienced memory cells responding faster and to a lower Ag dose than naive T cells (4, 21). In addition, the requirements for cell activation have been shown to be different between naive and memory T cells, with naive T cells more dependent on costimulatory signals provided by APCs through the CD28 Ag expressed on T cells (21). Therefore, the anti-CD3i/CD28 stimulation protocol used in the present study should have not compromised naive T cell activation.

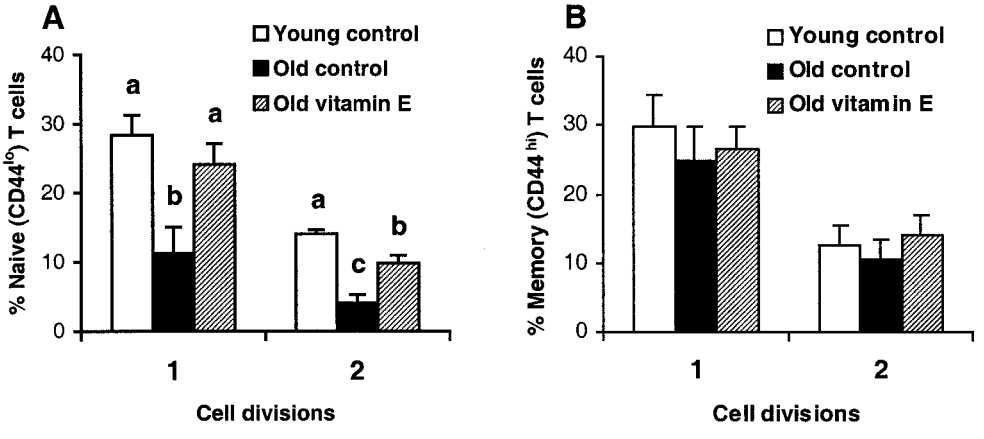

FIGURE 8. Effect of age and vitamin E on the expression patterns of CD44 at one and two cell division cycles. Purified T cells ($n = 3$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) for 4 h, labeled with CFSE (0.2 μM), and activated with immobilized anti-CD3 and soluble anti-CD28 mAb for 48 h. Cells were harvested, stained for CD44 expression, and analyzed on a flow cytometer. Each two-dimensional dot plot region representing zero, one, and two cell division cycles was evaluated for low ($CD44^{low}$) and high ($CD44^{high}$) expression patterns of the CD44 Ag. *A,* Naive ($CD44^{low}$) T cells and *B,* Memory ($CD44^{high}$) T cells. Bars with different letters within each group are significantly different ($p < 0.05$) by an ANOVA followed by Tukey's HSD post hoc procedure.

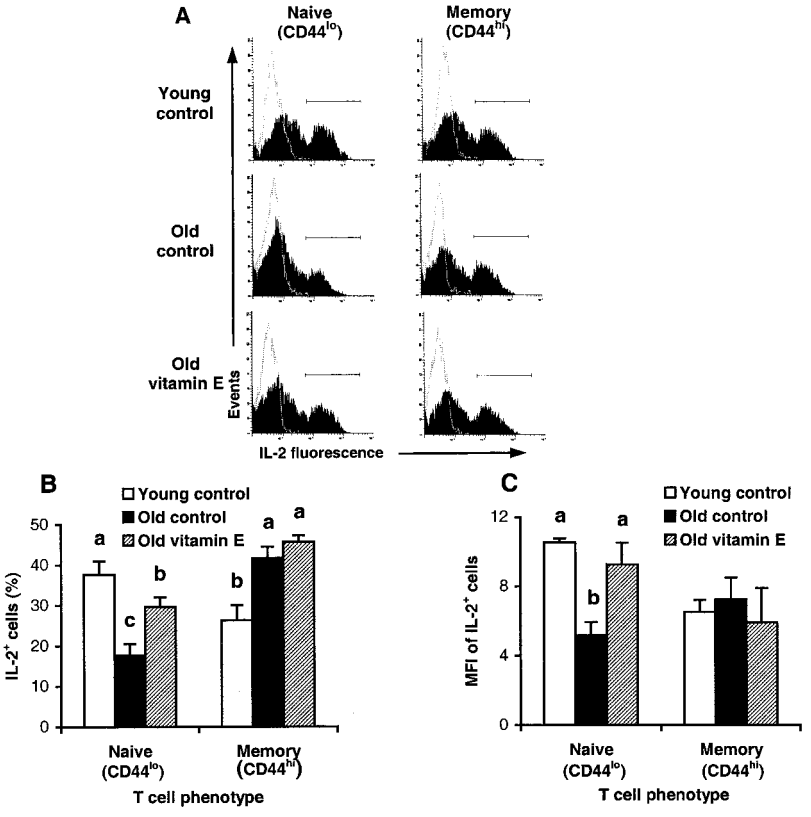

FIGURE 9. Effect of age and vitamin E on intracellular IL-2 by naive and memory T cell subsets. Purified T cells ($n = 5$) were preincubated with 46 μM RRR-α-tocopherol (vitamin E) for 4 h and activated with immobilized anti-CD3 and soluble anti-CD28 mAb for 48 h. Cells were treated with monensin, an inhibitor of IL-2 secretion, for the last 10 h of activation. Harvested cells were stained with fluorochrome-conjugated anti-CD44 mAb, permeabilized, and stained with fluorochrome-conjugated anti-IL-2. T cells were divided into naive and memory phenotypes based on low or high expression of the CD44 Ag, respectively. Cell fluorescence was measured on a flow cytometer. *A,* One representative histogram for naive ($CD44^{low}$, *left*) and memory ($CD44^{high}$, *right*) T cells from each of the three groups: young control (*top*), old control (*middle*), and old vitamin E supplemented (*bottom*). *B,* Relative proportion (mean ± SEM) of $IL\text{-}2^+$ T cells and *C,* linearized mean fluorescence intensity (MFI) of $IL\text{-}2^+$ T cells. Bars with different letters within each phenotype are significantly different ($p < 0.05$) by an ANOVA followed by Tukey's HSD post hoc procedure.

The age-related decrease in T cell function has been reported for both the memory (1, 22, 23) as well as the naive (5, 6, 24) T cell subpopulations. The critical role IL-2 plays in the T cell response is reflected in its ability to up-regulate high-affinity IL-2R by T cells and, therefore, responsiveness to IL-2 (7). IL-2 is also critical for T cells to progress through the cell cycle and for the differentiation of naive T cells into effectors. The age-related defect in IL-2 production is an important contributing factor to the decline in T cell function with age (8). Reduced output of IL-2 by naive T cells may indeed influence the generation of functional memory T cells, as responsive but IL-2-negative naive T cells have been shown to become unresponsive memory T cells (25). Using transgenic mice with naive T cells specific for PCC, Linton et al. (5) reported that naive Ag-inexperienced transgene-positive T cells isolated from old mice had lower proliferative and IL-2-producing capacity when compared with naive transgene-positive T cells from young mice. This was supported by Garcia and Miller (6), who found that naive PCC-specific T cells from old mice had at least two age-related functional defects in the early stages of T cell activation: they were defective in translocating signaling proteins to the cell membrane and forming an immunologic synapse, and those which did form a synapse had less nuclear translocation of NFAT, a transcription factor critical for IL-2 production.

We used the anti-phagocytic gp1 Ag (the murine equivalent of CD44) and flow cytometry to separate naive and memory T cell subsets based on low and high expression of this Ag, respectively (26). The age-related defect in activation induced IL-2 production and proliferation at 48 h was mainly observed in naive ($CD44^{low}$) T cells when anti-CD3i/CD28 mAb was used, an activation protocol known to be particularly effective for stimulating naive T cells (21). We hypothesized that vitamin E increased the total IL-2 production by old T cells by either preserving the proliferation of naive T cells and/or by boosting memory T cell function. When young and old T cells supplemented with vitamin E were examined for their ability to divide in response to activation over a 48-h period, vitamin E significantly increased the ability of naive T cells from old mice to progress through one as well as two cell division cycles. This effect of vitamin E was not observed for memory T cells from old mice. Furthermore, vitamin E supplementation increased IL-2 production by naive T cells from old mice, while there was no effect on IL-2 production by memory T cells.

During the inflammatory response, lymphocytes are exposed to increased levels of intra- and extracellular ROS. This initial increase in ROS has an important effect on a subsequent proliferative response as well as on other cell functions. As Harman (27) first proposed in 1956, oxygen-derived free radicals cause damage to cells, which leads to age-associated impairments in functions at organ and cellular levels. This is supported by studies indicating that aging is associated with increased free radical burden with resulting damage to cellular proteins and lipid components (28, 29). The exposure of T cells to oxidative stress results in decreased IL-2 production, as a consequence of reduced intracellular calcium response, and altered activity of transcription factors required for IL-2 transcription (30, 31). These alterations are similar to changes which have been reported to occur in aged T cells (31–33). There is ample evidence demonstrating that ROS and oxidative stress play an integral role in T cell activation (11, 30, 34–37). The expression of the TCR/CD3ζ chain was reported to decrease in response to H_2O_2 or diamide, which resulted in the suppression of the Ag-specific T cell response (11). Treatment with *N*-acetylcysteine, a glutathione precursor known to increase cellular GSH, prevented this effect. Other signaling components may respond to oxidative stress due to the presence of redox-sensitive amino acid residues (35). Thus, oxidative stress may have various effects on T cell function by influencing multiple signaling components. Another factor that may contribute to the age-associated loss of T cell response is an increase in T cell apoptosis with age. This increase

has been reported for both TNF-α and activation-induced apoptosis (38, 39). Decreased anti-CD3-induced apoptosis of T cells from old mice has also been reported (40). Antioxidants may decrease apoptotic events in various cell systems, particularly when apoptosis is induced by oxidative stress. However, a recent study showed that vitamin E and catalase failed, while GSH did prevent apoptosis when human peripheral T cells were induced via Fas- and CD2-mediated signals (41). This suggests that an antiapoptotic effect of vitamin E is not likely.

The age-associated decline in T cell response can be partially reversed by dietary (14–16, 42) or in vitro (10, 17, 19, 43) antioxidant supplementation. Dietary vitamin E supplementation was found to stimulate T helper cell activity in a dose-dependent manner in mice immunized with a hapten-carrier conjugate (44). Sakai and Moriguchi (45) described increased proliferative response and IL-2 production of T cells from old rats in response to Con A when they were fed a high vitamin E diet for 12 mo. It has been reported from work in our laboratory that mitogen-activated macrophages and mixed splenocytes from old mice produce more PGE_2 than corresponding cells from young mice (17, 46). The age-related decline in some of the T cell immune parameters such as proliferative response and IL-2 production has been contributed to the age-associated increase in macrophage PGE_2 production resulting from increased cyclooxygenase 2 activity in the old mice (15, 46). The effect of supplemental vitamin E on lowering macrophage PGE_2 production and the resulting enhancement of cell-mediated immunity have previously been described both for mice and for human subjects (15–17, 46). However, other effects of supplemental vitamin E on T cell immunity that may not involve PGE_2 have been suggested (17).

In the present study, we demonstrate that vitamin E can also enhance the function of T cells from old mice directly, independent from its macrophage PGE_2-lowering effect. The effect of vitamin E is mediated through increasing IL-2 production by naive T cells from old mice. This leads to greater cell dividing capacity by naive T cells without an effect on the memory T cell population. The effect of age on the naive T cell population and the preferential effect of vitamin E on naive but not memory T cells from old mice may be due to an underlying difference in the susceptibility of naive and memory T cells to oxidative stress-induced damage. This is supported by a recent report that memory T cells from old mice are more resistant to oxidative injury than naive T cells (12).

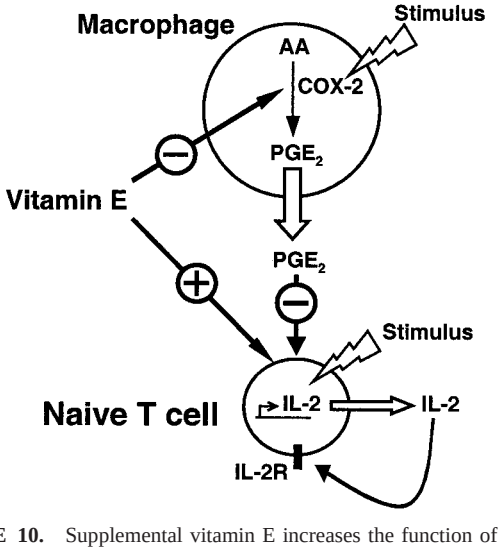

FIGURE 10. Supplemental vitamin E increases the function of T cells from the aged by at least two different mechanisms. As previously shown, vitamin E reduces the age-related increase in the production of T cell-suppressive PGE_2 by macrophages. In addition, we show here a direct PGE_2-independent effect of vitamin E on the function of naive T cells in the aged.

In summary, as reported by others, we show here that aging is characterized by decreased T cell proliferation and IL-2 production. We also show that vitamin E increases the proliferative capacity of purified T cells from old mice. This effect of vitamin E is mediated through increasing the production of IL-2, the major auto- and paracrine growth factor of activated T cells. Furthermore, T cells from old mice responded faster to activation than T cells from young mice, with greater IL-2 protein and mRNA expression at an early time point. At a later time point though, young T cells proliferated more vigorously and secreted more IL-2 than old T cells. The faster response of old T cells was in part due to the higher proportion of fast responding memory T cells in old mice. However, it was the naive T cell subpopulation that showed the age-associated defect in progressing through cell division and producing IL-2, and thus failed to match the overall response of the young T cells in IL-2 production at a later time point. Furthermore, our data demonstrate that vitamin E significantly increased proliferation and IL-2 production of T cells from old mice. The effect of vitamin E was mediated through increasing the cell dividing- and IL-2-producing capacity of the naive T cell subpopulation. Thus, in addition to its inhibition of PGE_2 production by macrophages, vitamin E increases the function of T cells from old mice through a direct effect on T cells (Fig. 10). This enhancing effect is mediated through increased IL-2 production and cell dividing-capacity of naive T cells. This is the first demonstration of a nutrient having a direct enhancing effect on naive T cells, a population of T cells that exhibits the greatest age-related defect. These findings will have important implications for developing strategies to reverse age-associated defects in T cell-mediated immune function. Further studies are needed to elucidate the mechanisms by which vitamin E specifically enhances the function of naive T cells in old mice.

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References

1. Lerner, A., T. Yamada, and R. A. Miller. 1989. Pgp-1^{high} T lymphocytes accumulate with age in mice and respond poorly to concanavalin A. *Eur. J. Immunol. 19:977.*
2. Ernst, D. N., M. V. Hobbs, B. E. Torbett, A. L. Glasebrook, M. A. Rehse, K. Bottomly, K. Hayakawa, R. R. Hardy, and W. O. Weigle. 1990. Differences in the expression profiles of CD45RB, Pgp-1 and 3G11 membrane antigens and in the patterns of lymphokine secretion by splenic $CD4^+$ cells from young and aged mice. *J. Immunol. 145:1295.*
3. Miller, R. A. 1996. The aging immune system: primer and prospectus. *Science 273:70.*
4. Rogers, P. R., C. Dubey, and S. L. Swain. 2000. Qualitative changes accompany memory T cell generation: faster, more effective responses at lower doses of antigen. *J. Immunol. 164:2338.*
5. Linton, P.-J., L. Haynes, N. R. Klinman, and S. L. Swain. 1996. Antigen-independent changes in naive CD4 T cells with aging. *J. Exp. Med. 184:1891.*
6. Garcia, G. G., and R. A. Miller. 2001. Single-cell analyses reveal two defects in peptide-specific activation of naive T cells from aged mice. *J. Immunol. 166: 3151.*
7. Katzen, D., E. Chu, C. Terhorst, D. Y. Leung, M. Gesner, and R. A. Miller. 1985. Mechanisms of human T cell response to mitogens: IL-2 induces IL-2 receptor expression and proliferation but not IL-2 synthesis in PHA-stimulated T cells. *J. Immunol. 135:1840.*
8. Thoman, M. L., and W. O. Weigle. 1981. Lymphokines and aging: interleukin-2 production and activity in aged animals. *J. Immunol. 127:2102.*
9. Metzger, Z., J. T. Hoffeld, and J. J. Oppenheim. 1980. Macrophage-mediated suppression I. Evidence for participation of both hydrogen peroxide and prostaglandin in suppression of murine lymphocyte proliferation. *J. Immunol. 124:983.*

10. Wu, D., S. N. Meydani, J. Sastre, M. Hayek, and M. Meydani. 1994. In vitro glutathione supplementation enhances interleukin-2 production and mitogenic response of peripheral blood mononuclear cells from young and old subjects. *J. Nutr. 124:655.*
11. Otsuji, M., Y. Kimura, T. Aoe, Y. Okamoto, and T. Saito. 1996. Oxidative stress by tumor-derived macrophages suppresses the expression of CD3ζ chain of T-cell receptor complex and antigen-specific T-cell responses. *Proc. Natl. Acad. Sci. USA 93:13119.*
12. Lohmiller, J. J., K. M. Roellich, A. Toledano, P. S. Rabinovitch, N. S. Wolf, and A. Grossmann. 1996. Aged murine T-lymphocytes are more resistant to oxidative damage due to the predominance of the cells possessing the memory phenotype. *J. Gerontol. Biol. Sci. 51A*:B132.
13. Machlin, L. J., and A. Bendich. 1987. Free radical damage: antioxidant defenses. *FASEB J. 1:441.*
14. Meydani, S. N., M. Meydani, C. P. Verdon, A. C. Shapiro, J. B. Blumberg, and K. C. Hayes. 1986. Vitamin E supplementation suppresses prostaglandin E_2 synthesis and enhances the immune response of aged mice. *Mech. Ageing Dev. 34:191.*
15. Meydani, S. N., M. P. Barklund, S. Liu, M. Meydani, R. A. Miller, J. G. Cannon, F. D. Morrow, R. Rocklin, and J. B. Blumberg. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am. J. Clin. Nutr. 52:557.*
16. Meydani, S. N., M. Meydani, J. B. Blumberg, L. S. Leka, G. Siber, R. Loszewski, C. Thompson, M. C. Pedrosa, R. D. Diamond, and B. D. Stollar. 1997. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. *J. Am. Med. Assoc. 277:1380.*
17. Beharka, A. A., D. Wu, S. N. Han, and S. N. Meydani. 1997. Macrophage prostaglandin production contributes to the age-associated decrease in T cell function which is reversed by the dietary antioxidant vitamin E. *Mech. Ageing Dev. 93:59.*
18. Meydani, S. N., M. Meydani, J. B. Blumberg, L. S. Leka, M. Pedrosa, R. Diamond, and E. J. Schaefer. 1998. Assessment of the safety of supplementation with different amounts of vitamin E in healthy older adults. *Am. J. Clin. Nutr. 68:311.*
19. Wu, D., M. Meydani, A. A. Beharka, M. Serafini, K. R. Martin, and S. N. Meydani. 2000. In vitro supplementation with different tocopherol homologues can affect the function of immune cells in old mice. *Free Radical Biol. Med. 28:643.*
20. Wu, D., C. Mura, A. A. Beharka, S. N. Han, K. E. Paulson, D. Hwang, and S. N. Meydani. 1998. Age-associated increase in PGE_2 synthesis and COX activity in murine macrophages is reversed by vitamin E. *Am. J. Physiol. 275:C661.*
21. Dubey, C., M. Croft, and L. Swain. 1996. Naive and effector CD4 T cells differ in their requirements for T cell receptor versus costimulatory signals. *J. Immunol. 157:3280.*
22. Philosophe, B., and R. A. Miller. 1990. Diminished calcium signal generation in subsets of T lymphocytes that predominate in old mice. *J. Gerontol. Biol. Sci. 45:B87.*
23. Hobbs, M. V., W. O. Weigle, D. J. Noonan, B. E. Torbett, R. J. McEvilly, R. J. Koch, G. J. Cardenas, and D. N. Ernst. 1993. Patterns of cytokine gene expression by $CD4^+$ T cells from young and old mice. *J. Immunol. 150:3602.*
24. Beckman, I., K. Shepherd, F. Firgaira, and M. Ahern. 1995. Age-related defects in CD2 receptor-induced activation in human T-cell subsets. *Immunology 86:533.*
25. Saparov, A., F. H. Wagner, R. Zheng, J. R. Oliver, H. Maeda, R. D. Hockett, and C. T. Weaver. 1999. Interleukin-2 expression by a subpopulation of primary T cells is linked to enhanced memory/effector function. *Immunity 11:271.*
26. Budd, R. C., J. C. Cerottini, C. Horvath, C. Bron, T. Pedrazzini, R. C. Howe, and H. R. MacDonald. 1987. Distinction of virgin and memory T lymphocytes: stable acquisition of the Pgp-1 glycoprotein concomitant with antigenic stimulation. *J. Immunol. 138:3120.*
27. Harman, D. 1956. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol. 11:298.*
28. Stadtman, E. R. 1992. Protein oxidation and aging. *Science 257:1220.*
29. Ames, B. N., M. K. Shigenaga, and T. M. Hagen. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA 90:7915.*
30. Flescher, E., J. A. Ledbetter, G. L. Schieven, N. Vela-Roch, D. Fossum, H. Dang, N. Ogawa, and N. Talal. 1994. Longitudinal exposure of human T lymphocytes to weak oxidative stress suppresses transmembrane and nuclear signal transduction. *J. Immunol. 153:4880.*
31. Pahlavani, M. A., and M. D. Harris. 1998. Effect of in vitro generation of oxygen free radicals on T cell function in young and old rats. *Free Radical Biol. Med. 25:903.*
32. Grossmann, A., J. A. Ledbetter, and P. S. Rabinovitch. 1990. Aging-related deficiency in intracellular calcium response to anti-CD3 or concanavalin A in murine T-cell subsets. *J. Gerontol. Biol. Sci. 45:B81.*
33. Pahlavani, M. A., M. D. Harris, and A. Richardson. 1995. The age-related decline in the induction of IL-2 transcription is correlated to changes in the transcription factor NFAT. *Cell. Immunol. 165:89.*
34. Los, M., H. Schenk, K. Hexel, P. A. Baeuerle, W. Dröge, and K. Schulze-Osthoff. 1995. IL-2 gene expression and NF-κB activation through CD28 requires reactive oxygen production by 5-lipoxygenase. *EMBO J. 14:3731.*
35. Lander, H. M., D. P. Hajjar, B. L. Hempstead, U. A. Mirza, B. T. Chait, S. Campbell, and L. A. Quilliam. 1997. A molecular redox switch on $p21^{ras}$: structural basis for the nitric oxide-$p21^{ras}$ interaction. *J. Biol. Chem. 272:4323.*
36. Schieven, G. L., J. M. Kirihara, D. E. Myers, J. A. Ledbetter, and F. M. Uckun. 1993. Reactive oxygen intermediates activate NFκB in a tyrosine kinase-dependent mechanism and in combination with vanadate activate the $p56^{lck}$ and $p59^{fyn}$ tyrosine kinases in lymphocytes. *Blood 82:1212.*
37. Schieven, G. L., R. S. Mittler, S. G. Nadler, J. M. Kirihara, J. B. Bolen, S. B. Kanner, and J. A. Ledbetter. 1994. ZAP-70 tyrosine kinase, CD45, and T cell receptor involvement in UV- and H_2O_2-induced T cell signal transduction. *J. Biol. Chem. 269:20718.*
38. Aggarwal, S., S. Gollapudi, and S. Gupta. 1999. Increased TNF-α-induced apoptosis in lymphocytes from aged humans: changes in TNF-α receptor expression and activation of caspases. *J. Immunol. 162:2154.*
39. Herndon, F. J., H.-C. Hsu, and J. D. Mountz. 1997. Increased apoptosis of $CD45RO^-$ T cells with aging. *Mech. Ageing Dev. 94:123.*
40. Spaulding, C. C., R. L. Walford, and R. B. Effros. 1997. The accumulation of non-replicative, non-functional, senescent T cells with age is avoided in calorically restricted mice by an enhancement of T cell apoptosis. *Mech. Ageing Dev. 93:25.*
41. Déas, O., C. Dumont, B. Mollereau, D. Métivier, C. Pasquier, G. Bernard-Pomier, F. Hirsch, B. Charpentier, and A. Senik. 1997. Thiol-mediated inhibition of Fas and CD2 apoptotic signaling in activated human peripheral T cells. *Int. Immunol. 9:117.*
42. Furukawa, T., S. N. Meydani, and J. B. Blumberg. 1987. Reversal of age-associated decline in immune responsiveness by dietary glutathione supplementation in mice. *Mech. Ageing Dev. 38:107.*
43. Franklin, R. A., Y. M. Li, S. Arkins, and K. W. Kelley. 1990. Glutathione augments in vitro proliferative responses of lymphocytes to concanavalin A to a greater degree in old than in young rats. *J. Nutr. 120:1710.*
44. Tanaka, J., H. Fujiwara, and M. Torisu. 1979. Vitamin E and immune response. I. Enhancement of helper T cell activity by dietary supplementation of vitamin E in mice. *Immunology 38:727.*
45. Sakai, S., and S. Moriguchi. 1997. Long-term feeding of high vitamin E diet improves the decreased mitogen response of rat splenic lymphocytes with aging. *J. Nutr. Sci. Vitaminol. 43:113.*
46. Hayek, M. G., S. N. Meydani, M. Meydani, and J. B. Blumberg. 1994. Age differences in eicosanoid production of mouse splenocytes: effects on mitogen-induced T-cell proliferation. *J. Gerontol. Biol. Sci. 49:B197.*