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Vitamin E ingestion improves several immune functions in elderly men and women

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Abstract

The effects of diet supplementation with the antioxidant vitamin E (200 mg daily) on several blood neutrophil, lymphocyte and natural killer cell functions have been investigated in healthy elderly men and women before supplementation, after 3 months of supplementation and 6 months after the end of supplementation (post-supplementation). In parallel, samples of healthy adult men and women were used as age controls. In elderly men and women, an impairment of immune functions was observed in comparison with the respective adult controls and the intake of vitamin E resulted in a significant enhancement of immune parameters in both elderly men and women, bringing their values close to those in the adults. These effects were not found in post-supplementation samples in several but not in all functions. The present findings suggest that supplementation with vitamin E can produce an improvement of immune functions and therefore of health in aged people.

Keywords: *Vitamin E, ageing, lymphocytes, neutrophils, men, women*

Introduction

Ageing is associated with a decline in cell and tissue functions, including those of the immune system, both in humans and in many other mammalian species [1–4]. The immunosenescence seems to contribute significantly to morbidity and mortality in the elderly through the age-related increase of cancer and especially of infectious diseases [5]. Thus, it is presently accepted that immune function is related not only with health but with longevity as well [4,6,7] and individuals who live longer in good health, such as centenarians or very old animals, are equipped with optimal immune cell defense mechanisms [8].

Free radicals, because of their high potential to damage biological systems, have been proposed as contributing factors to ageing [9–11]. However, since the reactive oxygen species (ROS) have a pivotal role in the regulation of many cellular processes [12], an adequate oxidant–antioxidant balance is very important for maintaining cell functions. This balance is critical in the cells of the immune system, since these cells synthesize ROS as key agents for their functions [13] and they are particularly sensitive to oxidative stress because of the high content of polyunsaturated fatty acids in their plasma membranes [14]. Moreover, the integrity and function of lipids, proteins and nucleic acids as well as the control of signal transduction of gene expression in immune cells are very dependent on the maintenance of the oxidant–antioxidant balance [14–16]. Therefore, it is not surprising that immune cells usually contain higher

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concentrations of antioxidant compounds than other cells [17].

With ageing, an imbalance between oxidant production and antioxidant levels appears in favour of the former with resulting oxidative stress [11,18]. Antioxidants, such as vitamins E and C, β-carotene and others, have been proposed as reagents able to retard, reverse or prevent the oxidative damage and therefore the general physiological impairment associated with ageing [11] and in particular immunosenescence [3,4,19–21].

Vitamin E is the most important lipid-soluble antioxidant present in body tissues and is considered the first line of defense against lipid peroxidation [22]. Vitamin E deficiency in aged humans is relatively scarce in the Western countries [23] and for this reason, there is little work on deficiency of this vitamin and immune function. The few studies performed on this subject show that the vitamin E deficiency seems to be linked to impaired cell-mediated immunity [24]. In addition, previous studies have shown that vitamin E supplementation improves T-cell mediated function in the elderly [20,25–28] and that the optimal dose is 200 IU/day [25]. *In vitro* and *in vivo* animal studies by us and others indicate that vitamin E can also improve the function of other cells of the immune system [29–33]. No information, however, is available on the effect of vitamin E supplementation on a relevant function of lymphocytes such as the chemotaxis or the effects of this supplementation on the function of neutrophils or NK cells in the elderly. Thus, we conducted a study to determine the effect of vitamin E supplementation in elderly healthy men and women at 200 mg/day during a short period of time (3 months) on several immune functions, as well as the time of prevalence of these effects. The study was carried out on the three most representative immune cells in human peripheral blood, namely lymphocytes, phagocytic cells, i.e. neutrophils, and NK cells and on several important functions that have been shown to change with age [3,7,16]. In lymphocytes, the adherence to endothelium, the mobility directed to the infectious focus by a chemoattractant gradient (chemotaxis), the proliferative response to mitogens and the IL-2 production have been analysed. In neutrophils we have studied the different steps of their phagocytic process, such as adherence to endothelium, chemotaxis, ingestion of foreign agents and their destruction with the help of oxygen free radicals, starting with superoxide anion. In NK cells the cytotoxic activity against tumoral cells was analysed.

Materials and methods

Subjects

A group of 33 elderly women and men (mean age $\pm$ SD: 70.4 ± 5.1 years old) and 30 adult women and men (29.7 ± 4.9 years old) who volunteered were used for this study. All elderly subjects were selected according to the immunogerontological 'SENIEUR' protocol [34]. All subjects showed good health, as defined by normal findings in physical, haematological and biochemical screening tests, they had normal body weight and physical examination, they did not take any medication for at least 2 months before the start of the study, did not smoke and gave informed written consent. Exclusion criteria were abnormal laboratory values, malignancies, inflammation and infection influencing the immune system. The participating women and men were not hospitalized during the course of the investigation. They resided in their homes and consumed a physician-supervised balanced Mediterranean diet that was not very rich in poly-unsaturated fatty acids, since the main cooking fat was olive oil. There was no change in the diet throughout the study for any of the subjects. The study protocol was carried out in agreement with the Declaration of Helsinki (1989) and was approved by the Ethics Committee of the La Paz Hospital.

Vitamin E supplementation

Elderly subjects (18 women and 15 men) received a daily supplement of 200 mg of dl-alpha-tocopherol (Alcala Farma) for 3 months. This dose was chosen on the basis of previous work from our laboratory [27] and especially of the work of Meydani et al. [25] in which that dose of vitamin E was the most effective at improving T-cell mediated function.

Peripheral venous blood samples from elderly subjects were drawn by vein puncture from 9–10 am, in tubes with EDTA (for determination of immune cell functions), before (BT), after 3 months of supplementation (T) and 6 months after the end of supplementation, without intake of vitamin E (post-supplementation, PT). At each time point five men and five women, healthy adults, were studied and the 15 men and 15 women used as controls.

Separation of blood neutrophils and lymphocytes

Cells were obtained from EDTA samples by centrifugation at 300 g for 30 min in a density gradient (1.114) using monopoly resolving medium (Flow Laboratories, McLean, VA). Differential migration during centrifugation resulted in the formation of two halos and a red blood cell pellet. The superior halo consisted of mononuclear lymphocytes and monocytes and the inferior halo of polymorphonuclear neutrophils. The cells were harvested, washed twice in Hank's medium for neutrophils or RPMI medium (Gibco, Burlington, Ontario, Canada) for lymphocytes, counted and adjusted to 5×10^5 neutrophils/ml medium and 1×10^6 lymphocytes/ml medium. Cell viability was checked by the trypan blue exclusion test. Viable cells were over 98%.

Assays of neutrophil functions

The adherence capacity of neutrophils was measured following the method described by McGregor et al. [35]. This method mimics *in vitro* the adherence of neutrophils to the vascular endothelium and it has been followed for evaluating adherence in other studies [36]. Briefly, 1 mL blood (diluted 1:1 with Hank's medium) was placed in a Pasteur pipette in which 50 mg of nylon fibre was packed to a height of 1.25 cm. After 10 min, the effluent had drained by gravity. The percentage of adherence or adherence index (AI) was calculated as follows:

$$\mathrm{AI} = 100 - \frac{(\text{neutrophils per mL of effluent samples}}{\text{neutrophils per mL of original samples})} \times 100.$$

The induced mobility or chemotaxis was evaluated by a modification [36,37] of the original technique described by Boyden [38], which measures the mobility capacity of neutrophils towards an infectious focus. Aliquots of 0.3 ml of the neutrophil suspension (10^6 neutrophils/ml) were deposited in the upper compartment of a Boyden chamber separated by a filter of nitrocellulose (Millipore, Mildford, MA) of 3 μm pore diameter. Fmet-phe-leu (Sigma, St. Louis, MO), a chemoattractant agent for neutrophils, was put in the lower compartment at 10^{-8} M to induce chemotaxis. After 3 h of incubation at 37°C and 5% CO_2, the filter was fixed (methanol 50%) and stained (Diff-Quick pack; Dade, Düdingen, Switzerland). The chemotactic index (ChI), representing the total number of neutrophils counted by optical microscopy (immersion objective) on one-third of the lower face of the filters, was calculated.

The phagocytosis assay was carried out following the method previously described [36] for ingestion of inert particles (latex beads). Aliquots of 200 μL of neutrophil suspension were incubated on migration inhibition factor (MIF) plates (Sterilin, Teddington, UK) for 30 min and the adherent monolayer was washed with PBS (phosphate buffer saline) at 37°C, and 20 μL latex beads (1.09 μm diluted to 1% PBS, Sigma) were added. After 30 min of incubation, the plates were washed, fixed (methanol 50%) and stained with the Diff-Quick pack and the number of particles ingested by 100 neutrophils was determined by optical microscopy (immersion objective) as phagocytosis index (PhI).

Superoxide anion production, the first response in the respiratory burst, which starts the destruction of ingested micro-organisms, was evaluated by its capacity to reduce nitroblue tetrazolium (NBT). The assay was carried out following the method described previously [36]. Aliquots of 250 μL of neutrophil suspension were mixed with 250 μL of NBT (1 mg/mL in PBS, Sigma) and 20 μL latex bead (1.09 μm diluted to 1% PBS, Sigma) suspension (stimulated samples) and 20 μL of PBS (non-stimulated samples). After 60 min of incubation, the reaction was stopped with 0.5 N HCl, the samples were centrifuged, the supernatants discarded and the reduced NBT extracted with dioxane (Sigma). Supernatant absorbance at 525 nm was determined in a spectrophotometer using dioxane as a blank control. The data obtained were expressed as nmol NBT reduced by 10^6 neutrophils by extracting in a standard curve of NBT reduced with 1,4-dithioerythritol (Roche, Basel, Switzerland).

Assays of lymphocyte function

Lymphocyte adherence and chemotaxis methods were similar to the above described in neutrophils, previously carried out with lymphocytes [39].

The lymphoproliferation assay was performed by a standard method, previously used by us [27,37,40]. The suspensions of mononuclear leukocytes were adjusted to 10^6 lymphocytes/mL of RPMI (Gibco) supplemented with gentamicin (1 mg/mL, Gibco) and 10% foetal bovine serum (FBS) (Gibco), previously inactivated by heat (30 min at 56°C). Aliquots of 200 μL were dispensed in plates of 96 wells (Costar, Cambridge, MA) and 20 μL of phytohemagglutinin (PHA, Flow) to 20 mg/L was used as mitogen; 20 μL of PBS were added to controls. After 48 h of incubation, 0.5 μCi/well ^{3}H-thymidine (Dupont, Boston, MA) was added, followed by another 24 h of incubation. The cells were harvested in a semiautomatic harvester and thymidine uptake was measured in a beta counter (LKB, Upsala, Sweden) for 1 min. The results were expressed as ^{3}H-thymidine uptake (cpm), both in basal and PHA stimulated cells.

The natural killer activity was measured following an enzymatic colourimetric assay for cytolysis measurements of target cells (Cytotox 96 TM Promega, Boerhinger Ingelheim, Ingelheim, Germany) based on the determination of LDH using tetrazolium salts. This method has been demonstrated to provide identical values (within experimental error) to those obtained by parallel ^{51}Cr release assays in murine cells [32]. Cells K-562 from a human lymphoma were used as targets in the assay. These cells were maintained in complete medium (RPMI-1640 plus 10% FBS), being checked and counted periodically. Target cells were seeded in 96-well U-bottom culture plates at 10^4 cells/well in RPMI medium without phenol red. Effector cells (lymphocytes) were added at 10^5 cells/well. The effector/target rate used, 10/1, was found by us to be responsible for similar results to those obtained in a previous work with radioactive techniques [32]. The plates were centrifuged at 250 g for 4 min to facilitate cell-to-cell contacts and then they were incubated for 4 h. After incubation, LDH activity was measured by addition of the enzyme substrate and absorbance recording at 490 nm. Four

kinds of control measurements were performed: a target spontaneous release, a target maximum release, an effector spontaneous release and a volume correction control to adjust the volume change caused by the addition of lysis solution to the maximum release control wells. To determine the percentage of target cells killed, the following equation was used:

$$\% \text{ lysis} = ((E - ES - TS)/(M - TS)) \times 100$$

where E is the mean of absorbance in the presence of effector cells, ES the mean of absorbance of effector cells incubated alone and M the mean of maximum absorbance after incubating target cells with lysis solution.

Interleukin-2 release assay

The concentration of interleukin-2 (IL-2) was determined on supernatants of lymphocyte cultures in the presence of ConA following a method previously described by us [40]. After 48 h of incubation with ConA (1 mg/ml), the supernatants were collected and frozen at -20°C until assay. IL-2 was measured using an ELISA kit (R & D Systems, Minneapolis, MN).

Statistical study

Data are expressed as the mean $\pm$ SD of the values corresponding to subjects, being each value the mean of duplicate assays (two samples from the same blood). The data were evaluated statistically by the one-way analysis of variance (ANOVA) for paired observations, used to evaluate vitamin E supplementation in the aged groups, followed by the Scheffe's F post-hoc procedure. The two-way ANOVA test for unpaired observations was used for age and gender groups, followed by the Scheffe's F-test. The normality of the samples was confirmed by the Kolmogorov-Smirnov test, $p < 0.05$ being the minimum level of significance.

Results

The results of the neutrophil activities expressed in the phagocytic process are shown in Table I. Regarding adherence capacity of PMN neutrophils, the aged groups before supplementation (BT) showed higher values ($p < 0.001$) of adherence indexes (AI) than adult controls. After vitamin supplementation (T), the values of AI were decreased with respect to the corresponding BT values in the female and male groups ($p < 0.01$), showing similar values to those of cells from the adults. After 6 months without vitamin E ingestion (PT) the values of AI remained lower than those of BS ($p < 0.05$) in both men and women. The chemotaxis indexes (CI) of neutrophils of elderly women and men, before supplementation (BT), were lower than those of the adult groups ($p < 0.001$). After supplementation (T), these indexes showed significantly higher values ($p < 0.05$) than those found in BT, although these values were still lower ($p < 0.05$ in women and $p < 0.001$ in men) than those of adults. In the PT groups the CI brought the values near those of the BT. The phagocytosis indexes (PI), lower in neutrophils from elderly women than in those from adult women ($p < 0.05$), increased after supplementation with vitamin E ($p < 0.01$ in women and $p < 0.001$ in men). In the PT groups the values decreased ($p < 0.01$ in women and $p < 0.05$ in men) with respect to the values after supplementation. The values of superoxide anion production in stimulated and non-stimulated neutrophils in the aged BT groups were significantly higher ($p < 0.01$ in women and $p < 0.001$ in men) than in the adult groups. After supplementation (T) there was a significant decrease

Table I. Several functions of neutrophils from elderly men and women before (BT) and after (T) a daily supplementation of 200 mg vitamin E for 3 months, as well as after 6 months post-supplementation (PT).

	Women				Men			
		Elderly ($n=18$)				Elderly ($n=15$)		
Functions	Adult ($n=15$)	BT	T	PT	Adult ($n=15$)	BT	T	PT
Adherence (AI)	41 ± 10	67 ± 10^{c}	39 ± 15**	52 ± 8*^{a}	50 ± 8	63 ± 5^{c}	47 ± 12**	48 ± 10*
Chemotaxis (CI)	627 ± 139	357 ± 80^{c}	486 ± 125*^{a}	410 ± 102^{b}	691 ± 87	427 ± 68^{c}	512 ± 72*^{c}	377 ± 64^{c}
Ingestion (PI)	176 ± 40	141 ± 32^{a}	220 ± 46**	151 ± 43^{++}	132 ± 30	139 ± 26	264 ± 37***^{c}	203 ± 46*$^{b+}$
O_2^- levels (nmol/10^6 cells)								
NS	36 ± 16	57 ± 18^{b}	32 ± 13*	50 ± 15^{+}	39 ± 17	91 ± 19^{c}	26 ± 3***	60 ± 15*$^{++}$
S	51 ± 11	77 ± 17^{b}	48 ± 19**	74 ± 14^{+}	57 ± s20	116 ± 16^{c}	47 ± 13***	70 ± 12**$^{+}$

The data are expressed as the mean ± SD of the values. *$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$ with respect to the corresponding BT values. $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ with respect to the corresponding adult value. $^{+}p < 0.05$ and $^{++}p < 0.01$ with respect to the corresponding S values. AI (adherence index) = 100 − (neutrophils per mL of effluent samples/neutrophils per mL of original samples) × 100. CI (chemotaxis index) was the number of neutrophils in the lower face of the filter. PI (phagocytosis index) was the number of the latex beads ingested per 100 neutrophils. NS (non-stimulated), S (stimulated samples with latex bead suspension).

($p < 0.05$ or $p < 0.01$, for non-stimulated and stimulated samples, respectively, in women and $p < 0.001$ in men) in superoxide production, with similar values to those found in adults. In the PT groups the values were increased ($p < 0.05$ in women and $p < 0.05$ or $p < 0.01$ in men for stimulated and non-stimulated samples, respectively) with respect to those after supplementation (T). However, in men the superoxide levels remained lower than in BT groups ($p < 0.01$ in stimulated samples and $p < 0.05$ in non-stimulated samples).

With respect to the lymphocyte functions studied, the results of adherence (AI) and chemotaxis (CI) are shown in Figure 1. The AI of lymphocytes from elderly subjects before supplementation (BT) were higher than those from adults ($p < 0.05$ in women and $p < 0.01$ in men). After vitamin supplementation (T), the values of AI were decreased with respect to the corresponding BT values in women and men ($p < 0.01$), showing similar values to those in cells from adults. In PT the values of AI, in both men and women, were similar to those found before

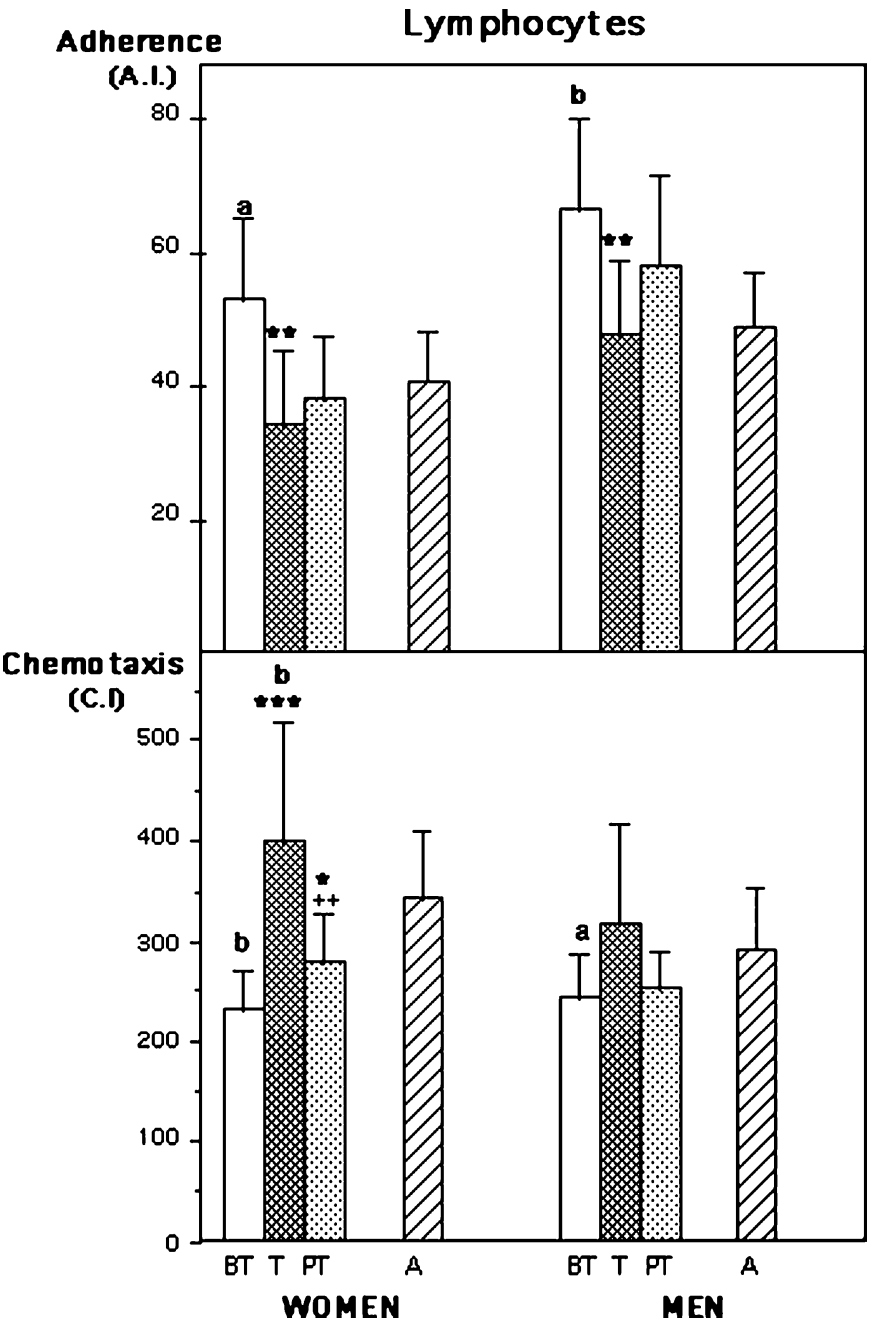

Figure 1. Adherence capacity (up) and chemotaxis capacity (down) of lymphocytes from elderly subjects before (BT), after 3 months of vitamin E supplementation (T) and 6 months after the end of supplementation without vitamin E intake (PT). Each column represents the mean $\pm$SD of the values corresponding to 18 women or 15 men, each value being the mean of duplicate assays. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ with respect to the corresponding BT values. $^{a}p < 0.05$ and $^{b}p < 0.01$ with respect to the corresponding adult (A) values (15 women and 15 men). $^{++}p < 0.01$ with respect to the corresponding T-values.

supplementation (BT). The chemotaxis of lymphocytes was lower in elderly men ($p < 0.05$) and women ($p < 0.01$) than in the adults. With vitamin E supplementation this function increased in cells from women ($p < 0.001$), decreasing after 6 months without supplementation ($p < 0.01$), although preserving values higher than those before supplementation ($p < 0.05$).

The lymphoproliferative capacity in response to PHA, the IL-2 release and the NK activity are shown in Figure 2. These parameters were lower in cells from elderly men and women than in adults ($p < 0.001$ in proliferation and IL-2 and $p < 0.05$ and $p < 0.01$ in NK of women and men, respectively). After the supplementation with vitamin E (T) all these functions were stimulated ($p < 0.001$ in proliferation and $p < 0.01$ in IL-2 and NK), showing similar or higher values ($p < 0.01$ in proliferation of lymphocytes from women) than in adults. After 6 months without supplementation of vitamin E (PT) the values decreased, being similar to those found for BT, with the exception of NK activity in cells from men, which maintain values higher ($p < 0.05$) than BT.

Discussion

The present work shows that the intake of 200 mg/day of vitamin E supplementation in elderly men and women during 3 months results in a significant improvement of several functions of lymphocytes, neutrophils and NK cells, which are those that suffer an impairment with age [3,4,16]. These results expand and confirm the previous data about the enhanced T-cell-mediated immunity in healthy elderly human subjects by vitamin E supplementation [19,25,27,28].

The oxidation theory of ageing has been supported by the increased vitality and life-span of mice administered antioxidants [41]. Moreover, in unpublished work from our laboratory, we have also found that mice with an antioxidant-supplemented diet show a significant increase in their life-span. Other experimental evidence supports this fact, showing that antioxidants such as vitamin E may prevent or delay the oxidative stress and the physiological impairment associated with ageing [11]. Since the immune system is a health marker and longevity predictor [4,6,7] and since its age-related impairment [1–4,42] may be a consequence of oxidative stress [4,18], diet supplementation with antioxidants has been investigated as a way to prevent or even reverse that age-related immune dysfunction [3,4,21], thus increasing health and therefore life span.

In the present work, a study of several functions of the three main immune cells, lymphocytes, phagocytes (neutrophils) and NK cells, has been carried out in healthy elderly men and women in comparison with those cells from adult subjects. It is known that the changes in the immune cells with ageing are specially related with T lymphocyte activity, which is decreased as regards proliferative response to antigens or mitogens, one of the central events implicated in the development of the immune response, and in its IL-2 production. In agreement with previous results from us and other authors [3,4,27] the lymphoproliferative response to the mitogen PHA and the IL-2 production by T lymphocytes were found to be decreased in the present study in both elderly men and women with respect to adults. Other lymphocyte functions such as chemotaxis were also decreased in elderly subjects in agreement with previous results [3,4]. However, as regards functions of the non-specific immune response there is no agreement on the effects of age [43,44]. Thus, some authors have found a diminished activity of certain functions of phagocytes and NK cells, while other studies have established that these and other functions do not decline or are stimulated with age [3,4,16,43]. Although there are contradictory results concerning changes in natural killer (NK) cell activity with ageing, most previous research shows a decrease [3,4,32], similar to the results of the present work. Other functions such as chemotaxis and ingestion of phagocytes were also decreased in elderly subjects as in previous studies [3,4,16]. However, adherence and superoxide anion levels were increased with age. Adherence of lymphocytes or phagocytes is the first event in the immune and inflammatory response and it is a function that precedes the migration (i.e. chemotaxis) of immune cells. Leukocyte adherence increases in an oxidative situation such as ageing or endotoxic shock, because free radicals stimulate the expression of adherence molecules [4,16,27]. With respect to the age-related changes in the levels of superoxide anion there are contradictory data. In peritoneal leukocytes from mice the intracellular levels of superoxide decrease whereas the extracellular levels increase with age [16]. Previous results have shown an increase in the levels of superoxide anion in neutrophils from elderly women (data in the process of being published) in agreement with the results of the present work.

Vitamin E is widely recognized as a major lipid-soluble antioxidant present in the biological membrane, although this vitamin scavenges also ROS in the body aqueous compartments, protecting cells against oxidative stress damage. Experimental studies have provided evidence for a role of vitamin E in protecting the immune function in young animals using supplementation with higher than usual levels of this vitamin [29]. These results are more evident in elderly subjects in which the beneficial effect of dietary vitamin E supplementation has been clearly shown [20,25,28]. Further, the modulating effects of vitamin E found in the present study, bringing the immune values close to those of the adults, occurs after a short period of ingestion (3 months) and with

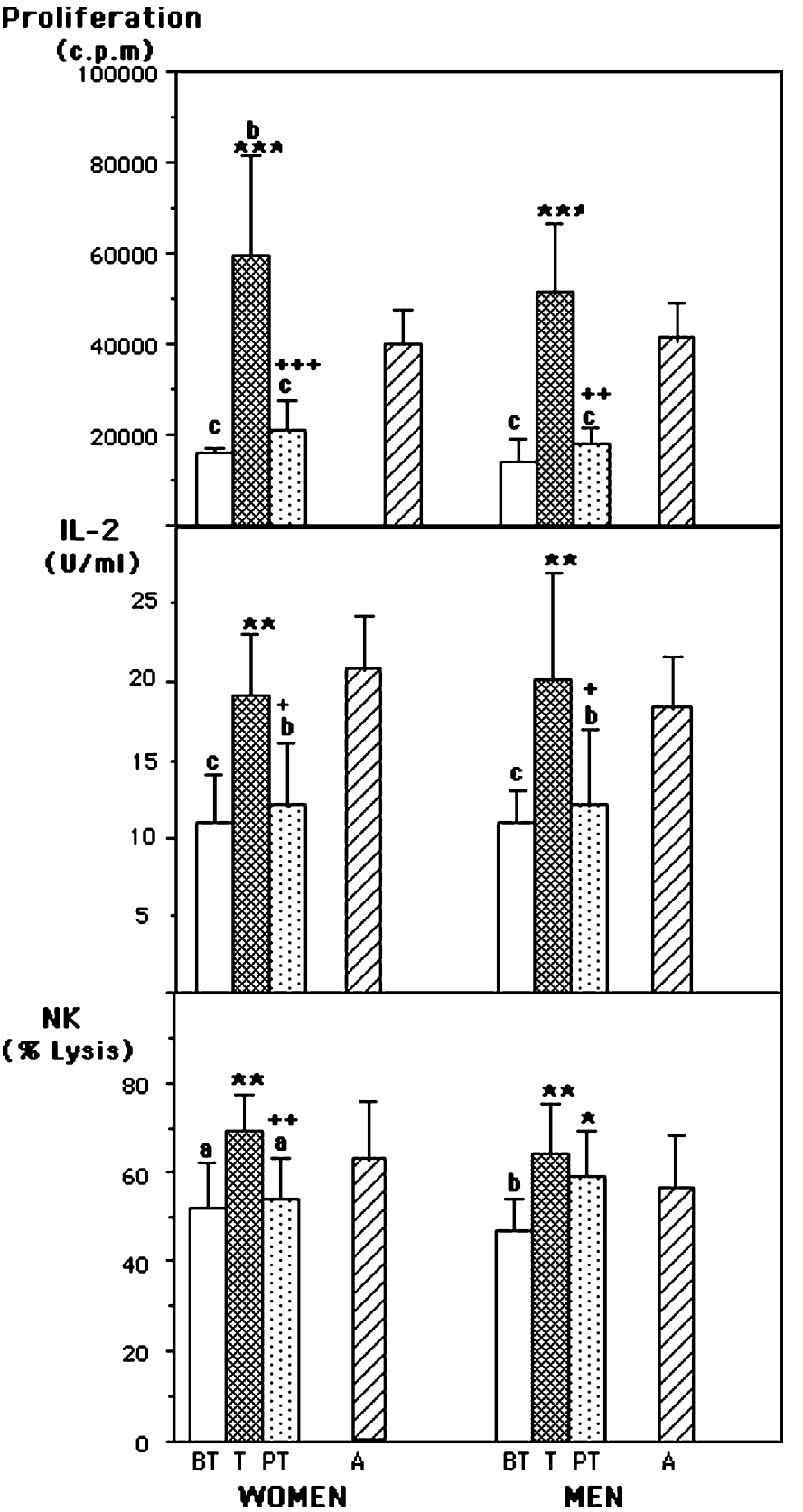

Figure 2. Proliferation, IL-2 production and NK activity of lymphocytes from elderly subjects before (BT), after 3 months of vitamin E supplementation (T) and 6 months after the end of supplementation without vitamin E intake (PT). Each column represents the mean ± SD of the values corresponding to 18 women or 15 men, each value being the mean of duplicate assays. $^{*}p < 0.05$ $^{**}p < 0.01$ and $^{***}p < 0.001$ with respect to the corresponding BT values. $^{a}p < 0.05$, $^{b}p < 0.01$ and $^{c}p < 0.001$ with respect to the corresponding adult (A) values (15 women and 15 men). $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ with respect to the corresponding T-values.

a dose of 200 mg/day. Some of these effects have been observed previously, especially in relation with lymphocyte proliferation in response to mitogen and IL-2 production [25,28] as well as adherence capacity [27]. However, other effects of vitamin E observed in the present study have been described for the first time, such as the effects on neutrophil functions, NK activity and lymphocyte chemotaxis. In another study, our group has also shown that vitamin C, together with the same amount of vitamin E used in the present study, improves several immune functions (in lymphocytes and neutrophils) in old women bringing their values close to those of adults [4,37]. In the present study only the vitamin E supplementation was able to produce similar results.

The increase with ageing in the adherence capacity was lowered after ingestion of vitamin E, which has been previously observed by us [27]. Similarly, vitamin E ingestion decreased superoxide production bringing its values near those of adults. The ingestion of other antioxidants appears to slow down the increase in superoxide production by phagocytes that usually occurs with age [21], an effect that may be beneficial since it prevents an excessive oxidative stress. Other functions such as chemotaxis and phagocytic capacities, which decrease with ageing, are increased after vitamin E intake. This means that these cells are activated regarding their ability to reach and ingest bacterial and other foreign bodies, i.e. to improve their defense function.

The most widely studied effects of vitamin E supplementation on immune functions analysed in the present study have been on T-cell proliferation to mitogens and the IL-2 production, functions that decrease in elderly humans. This fact seems to reflect a progressively decreasing proportion of functional T-cells rather than a uniform decline in function of all cells, which could be due to the excessive apoptosis of those lymphocytes [45]. Vitamin E increases the T-cell proliferation and IL-2 production of naive T-cells (the subpopulation of lymphocytes that decreased more with ageing) in old mice, with no effect on memory T-cells [20,46]. This effect seems to be mediated by the increase of the percentage of old CD4+ T-cells capable of forming an effective immune synapse [47]. In addition, the presence of multiple intracellular signalling deficiencies as well as changes on the expression of genes associated with signal transduction, transcriptional regulation and apoptosis pathways in T-cells could be the cause of the impaired proliferative response of T-cells with ageing and vitamin E has a significant effect on the expression of these genes associated with the cell cycle and Th1/Th2 balance in old cells [48]. Because there are data supporting the idea that immune function in ageing is similar to that in inflammatory conditions [4] and that antioxidants also have anti-inflammatory effects, they may act in this way on immune function [49]. Thus, it has been found that vitamin E acts reducing prostaglandin production by phagocytes, which contributes to the age-associated decrease in T-cell proliferation [20,28]. Moreover, vitamin E does not exert an indiscriminate stimulating effect on the immune system against disturbances like those caused by ageing. Instead, this antioxidant shows an immunoregulatory effect, increasing or depressing immune functions depending on the particular function and cell state, as observed previously [27]. Since the changes shown in the immune parameters studied bring the values closer to those of adult subjects, we can hypothesize that the daily intake of 200 mg of vitamin E by aged people can improve their immune response at the level not only of lymphocyte and NK functions, but the neutrophil activities as well. Since those immune function parameters have been suggested to be markers of health and longevity [4], vitamin E supplementation could be useful to improve the quality of life and functional longevity of elderly subjects.

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