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Nutrient Regulation of the Immune Response: The Case of Vitamin E

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The influence of nutrition on immune function was recognized in the early 1800s by Menkel when he described the thymic atrophy associated with severe malnutrition. Discovery of vitamins in the early 1900s was followed by reports on their contribution to host defenses, including immune responses.¹ These early observations were continued in several laboratories by the use of more advanced immunologic techniques. Vitamin E is perhaps the most studied nutrient related to the immune response. Evidence accumulated over the years and in many species indicates that vitamin E is an essential nutrient for the normal function of the immune system. Furthermore, results from several studies suggest that beneficial effects of certain nutrients, such as vitamin E on reducing disease risk, might be through their effects on the immune response. In this chapter, the regulatory roles of nutrients on immune function and their clinical significance will be discussed using vitamin E as an example.

Overview of the Immune System and Immunologic Methods

The immune system is a complex system involving various cells and lymphoid organs. Lymphoid organs can be classified as central (or primary) lymphoid organs—where lymphocytes arise and mature—and peripheral (or secondary) lymphoid organs—where mature lymphocytes respond to foreign antigens. Central lymphoid organs include bone marrow (where B and T lymphocytes originate and B lymphocytes mature) and the thymus (where T lymphocytes mature). Peripheral lymphoid organs include lymph nodes, the spleen, gut-associated lymphoid tissues such as tonsils, Peyer patches,

adenoids, and the appendix. The immune system comprises different types of cells, including lymphocytes, granulocytes, and monocytes.

Lymphocytes (B and T lymphocytes and natural killer [NK] cells) are the major players of the immune response. B lymphocytes, when activated, differentiate into plasma cells that secrete antibodies. T lymphocytes can be subdivided into cytotoxic T lymphocytes (CTLs), which are recognized by surface protein marker CD8, and T helper cells, which are recognized by protein marker CD4. CTLs kill cells infected with viruses and tumor cells. T helper cells can be further divided into T helper 1 (Th1) and T helper 2 (Th2) cells according to their cytokine production. Interferon (IFN)- γ is the signature cytokine of Th1 cells, and Th1 cells mainly promote cellular immunity and activate macrophages. Th2 cells produce interleukin-4 (IL-4), IL-5, and IL-10 and promote humoral immune responses by stimulating B-cell growth and differentiation. NK cells (also called large granular lymphocytes) can lyse tumor cells and virus-infected cells without overt antigenic stimulation. Granulocytes containing abundant cytoplasmic granules are classified into three types according to the staining characteristics of the predominant granules: neutrophils, basophils, and eosinophils. Monocytes circulate in the blood and differentiate into macrophages once they have migrated to tissues. Macrophages play an important role in the innate immune response as phagocytes and in the inflammatory response by producing soluble molecules such as cytokine, prostaglandins, and nitric oxide.

Immune responses are classified as innate and specific (or acquired) immune responses. The innate immune response provided by phagocytes—macrophages and neu-

trophils—and NK cells is a first line of defense against many common microorganisms. Phagocytes engulf and digest many microorganisms to combat a wide range of bacteria. In innate immune responses, there is no discrimination among foreign substances (lack of specificity) and prior exposure does not enhance subsequent exposure (lack of memory). Specific immune response is mediated by lymphocytes and provides long-term protection against specific antigens. Specific immune responses are classified into two types based on the components of the immune system that mediate the responses: humoral immunity is mediated by antibodies produced by plasma cells (effector cells of B lymphocytes) and cell-mediated immunity is mediated by T lymphocytes. In humoral immune responses, antibodies eliminate extracellular microbes; in cell-mediated immune responses, T lymphocytes activate macrophages to kill intracellular microbes or CTLs are activated to destroy viral-infected or tumor cells.

Various techniques are used to measure immune responses and to understand their underlying mechanisms. Immune response can be determined *in vitro* using specific immune cells by evaluating the different cytokines produced, the ability of immune cells to proliferate in response to antigens or mitogens, their ability to kill target cells, and their ability to help other immune cells. Immune status is evaluated *in vivo* by determining protection or resistance against challenge with infections, antibody response after vaccination, delayed type hypersensitivity (DTH) reactions, and phenotypic enumeration of lymphocyte subpopulations.

The lymphocyte proliferation assay is the most commonly used technique for evaluating cell-mediated immunity. Although this assay can provide information re-

lated to overall immunologic competence, it provides little information about the functional capabilities of the responding cells. In this assay, polyclonal mitogens (e.g., phytohemagglutinin [PHA], concanavalin A [ConA], pokeweed mitogen, lipopolysaccharide [LPS]), anti-CD3, or direct triggering of intracellular activation pathways with phorbol ester and calcium ionophore are used to initiate the growth response; proliferating lymphocytes are then quantitated by measuring incorporation of [³H]thymidine. These stimuli induce many or all lymphocytes of a given type to proliferate, and therefore the response of a heterogeneous population of cells is measured.

Determination of types and amounts of cytokines produced can provide information about the potential effector mechanisms of T cells (functional capabilities) and inflammatory responses of the host over the course of a disease. For example, the quantity of IL-2 synthesized is an important determinant of the magnitude of the T-cell-dependent immune response. Cytokines are hormone-like soluble proteins produced by different cell types that are involved in growth and differentiation of lymphocytes, communication between cells, and regulation of immune and inflammatory responses. Many cytokines have been discovered; those often used in studying the effects of nutrition on immune responses are briefly described in Table 1. Levels of cytokines can be determined by bioassay, radioimmunoassay, or enzyme-linked immunosorbent assay.

The effector function of CTLs can be determined by the ⁵¹Cr release assay, which measures the ability of CTLs to kill target cells radioactively labeled with sodium chromate. CTLs kill target cells on the basis of cell-surface antigen recognition. The development and activity of

Table 1. Functions of Selected Cytokines Commonly Measured in Nutritional Immunology

Cytokine	Cell Source	Functions
IL-2	T cells	T-cell growth
IFN- γ	Th1 cells, CTLs, NK cells	Activation of macrophages and NK cells Antiviral activity B-cell differentiation
IL-4	Th2 cells	Production of IgE by B cells Inhibition of macrophage activation Growth and differentiation of Th2 cells
IL-1 α or IL-1 β	Macrophages, epithelial and endothelial cells	Mediation of local inflammation Induction of acute phase response
TNF- α	Macrophages, T cells, NK cells	Activation of macrophages and induction of nitric oxide production Induction of acute phase response
IL-6	Macrophages, T cells, vascular endothelial cells, fibroblasts, etc.	T- and B-cell growth and differentiation Induction of acute phase response

IL, interleukin; Th1, T helper 1; IFN, interferon; CTL, cytotoxic T lymphocyte; NK, natural killer; IgE, immunoglobulin E; Th2, T helper 2; TNF, tumor necrosis factor.

CTLs against specific pathogens can be detected by the use of appropriate target cells.

The fluorescence-activated cell sorter is a powerful tool for defining and enumerating the lymphocyte population. Each lymphocyte can be identified with the use of fluorescent dyes. Size and granularity of lymphocytes, changes in lymphocyte population, developmental stages of lymphocytes, cellular production of cytokines, and cell cycle status can be determined by fluorescence-activated cell sorter analysis.

The DTH response has been widely used to assess cell-mediated immunity *in vivo*. When small amounts of antigens are injected into subcutaneous tissue, a T-cell-mediated local inflammatory reaction occurs over 24 to 72 hours after the challenge, resulting in local skin swelling, erythema, and induration. The intensity of the DTH reaction is determined by measuring the diameter of the induration area and the number of positive responses to different antigens. In animal models, antigens are injected subcutaneously into the footpad of a previously challenged animal and the extent of footpad swelling is measured with a caliper.

B lymphocytes contribute to acquired immunity by secreting antibodies, and the *in vivo* response of B cells is often determined by analyzing specific antibody production after immunization. Antibody response to most antigens requires help from antigen-specific T cells. However, some antigens, such as bacterial polysaccharides, can directly induce B cells to produce antibody in the absence of help from T cells. Therefore, depending on the type of antigen used—T-cell dependent or independent—analysis of antibody production can also provide information on the functional capabilities of T cells.

Nutrient Regulation of the Immune Response

Nutritional deficiency leads to increased susceptibility to infection because of the effect on immune function. In humans, malnutrition usually includes deficiencies of several nutrients and rarely deficiency of a single nutrient. However, the effect of individual nutrient deficiencies on immune function has been reported with the use of animal models and clinical studies. Nutrients reported to affect immune function include zinc, iron, selenium, copper, magnesium, folic acid, β -carotene, and vitamins A, B₆, B₁₂, C, D, and E. The effects of zinc, iron, selenium, copper, and vitamins A, B₆, C, D, and E on immune function have been extensively studied. Detailed review articles are available on the effects of zinc,² iron,³ selenium,⁴ copper,⁵ β -carotene,⁶ vitamin B₆,^{7,8} vitamin C,^{9,10} and vitamin D,¹¹ and several books include in-depth information on individual nutrients and immune function.^{12,13} Effects of deficiencies of some individual nutrients on immune functions are summarized in Table 2.

Vitamin E Deficiency Impairs Immune Response

Studies with different species of experimental animals indicate that vitamin E deficiency impairs both humoral and cell-mediated immune functions. Immunologic changes observed in animals with vitamin E deficiency include lower antibody response to sheep red blood cells (SRBCs),^{14,15} depressed lymphocyte response to mitogens,¹⁶ and impaired chemotaxis in response to bacterial culture filtrate.¹⁷

Tengerdy et al.¹⁴ showed that vitamin-E-deficient mice had fewer plaque-forming cells and a lower hemagglutination titer in response to SRBC injection than did mice fed a diet containing vitamin E at 50 to 60 mg/kg diet. The addition of vitamin E (2035 mg/kg diet) to the diet significantly increased the response to SRBCs. A lower response to SRBCs in vitamin-E-deficient mice was reportedly due to impaired accessory cell function.¹⁵ Macrophages from vitamin-E-deficient mice expressed less Ia antigen and acted as suppressor cells. The effect of vitamin E deficiency on cell-mediated immune response was demonstrated in experiments in which lymphocyte proliferation in response to T-cell mitogens (ConA and PHA) was shown to be depressed in vitamin-E-deficient rats.¹⁶ F344 rats fed a vitamin-E-deficient diet for 7 weeks had significantly fewer thymocytes, lower mitogenic response to PHA and ConA, and lower IL-2 production.¹⁸ Vitamin E deficiency also affected phagocyte function and bactericidal activity. Polymorphonuclear cells from peritoneal exudates of vitamin-E-deficient rats showed lower chemotactic and ingestive activities.¹⁷ Furthermore, vitamin E deficiency augmented the adverse effect of prolonged low-level exposure to ozone on pulmonary bactericidal capacity.¹⁹ Recently, vitamin E deficiency was shown to increase the virulence of the otherwise amyocarditic strain of coxsackievirus.²⁰ This increase in virulence was shown to be due to specific nucleotide changes in the viral genome in the vitamin-E-deficient host.

In contrast to the well-documented need for vitamin E in the maintenance of immune function in animals, limited studies have evaluated the effect of vitamin E deficiency in humans. The immune response is seldom mentioned when manifestations of vitamin E in humans are described, mainly because in humans, except for premature low-birth-weight infants, a primary severe deficiency of vitamin E rarely occurs. However, a secondary deficiency of vitamin E does occur in subjects with certain diseases such as primary biliary cirrhosis,²¹ chronic cholestasis,²² cystic fibrosis,²³ and intestinal fat malabsorption.²⁴ Unfortunately, when Horwitt's group^{25,26} conducted the most extensive study of the effect of vitamin E deficiency in humans, a comprehensive evaluation of immune response was not performed. Furthermore, most studies of vitamin E deficiency secondary to other causes have focused on red blood cell hemolysis and neurologic

Table 2. Nutrient Deficiency and Immune Function

Nutrient Deficiency	Effects of Deficiency on Immune Function	Possible Mechanism	References
Protein-energy malnutrition	Thymic atrophy ↓ DTH ↓ No. of rosette-forming T lymphocytes ↓ NK activity	Unavailability of essential nutrients; altered metabolism	Cunningham-Rundles, 1982 ⁹³
Vitamin B ₆ deficiency	↓ Antibody response ↓ DTH ↓ IL-1 β , IL-2, IL-2 receptor ↓ NK activity ↓ Lymphocyte proliferation	Effects on rate of production of 1-carbon units and capabilities to synthesize nucleic acids and proteins; serine-hydroxymethyl transferase is B ₆ -dependent enzyme	Chandra and Sudhakaran, 1990 ⁷ ; Trakatellis et al., 1997 ⁸
Copper deficiency	↓ DTH ↓ Lymphocyte proliferation ↓ T-cell–dependent production of antibody ↓ IL-2 production ↓ IL-2 mRNA Neutropenia	Effects on copper-dependent cellular antioxidant enzymes that affect transcriptional factors sensitive to redox status	Failla and Hopkins, 1998 ⁵
Iron deficiency	↓ Lymphoid cell development ↓ NK activity ↓ IL-1 production ↓ DTH ↓ Bactericidal capacity	Effects on enzymes such as mitochondrial aconitase and ribonucleotide reductase of which iron is a cofactor; effects on production of reactive oxygen species	Dallman, 1987 ³
Selenium deficiency	↓ IgG, IgM titers ↓ Antibody production ↓ Neutrophil chemotaxis ↑ Virulence of coxsackievirus	Antioxidant	McKenzie et al., 1998 ⁴ ; Turner and Finch, 1991 ⁹⁴ ; Spallholz et al., 1990 ⁹⁵
Zinc deficiency	Thymic activity (↓ Thymulin activity) Depletion of developing B cells in marrow ↓ T- and B-cell proliferation ↓ DTH ↓ NK activity ↓ IL-2, IFN γ production Impaired chemotactic response	Effects on activity of enzymes involved in replication and transcription, binding of NF- κ B to DNA	Fraker et al., 2000; Shankar and Prasad, 1998 ⁹⁶ ; Fraker and King, 2004 ⁹⁷

DTH, delayed-type hypersensitivity; IFN, interferon; Ig, immunoglobulin; IL, interleukin; NF, nuclear factor; NK, natural killer.

symptoms of vitamin E deficiency with no attention to immunologic changes. As a result, erythrocyte hemolysis and neurologic function are the most often used markers of vitamin E deficiency.^{22,26}

A negative response to DTH skin response, low mitogenic response to PHA and ConA, low IL-2 production, and polyneuropathy were observed in a patient with severe vitamin E deficiency secondary to an intestinal malabsorptive disorder.²⁴ After vitamin E supplementation by intramuscular injection of vitamin E, mitogenic response and IL-2 production increased significantly and DTH response to three of the antigens became positive (Table

3). In a case report by Adachi et al.,²⁷ decreased NK activity was observed in a 16-month-old boy with Shwachman syndrome associated with severe vitamin E deficiency. Oral supplementation of 100 mg/d vitamin E for 8 weeks normalized the NK activity, which decreased again when vitamin E supplementation was discontinued for 16 weeks. A lower mitogenic response to pokeweed mitogen and ConA was observed in children with low serum vitamin E levels (<10th percentile) compared with those with higher vitamin E levels (>90th percentile).²⁸ Although the results obtained from human studies are limited, when put together with extensive and reproduci-

Table 3. Vitamin E Deficiency and the Immune Response in Humans (Adapted from Kowdley KV, Mason JB, Meydani SN, Cornwall S, Grand RJ. Vitamin E deficiency and impaired cellular immunity related to intestinal fat malabsorption. *Gastroenterology*. 1992;102:2139–2142).

Parameter	Date		
	July 7	July 14	November 14
Plasma α -tocopherol (μ mol/L)	9.3	21	27
Mitogenic response (cpm)			
PHA	846	35,520	54,215
ConA	338	13,125	29,828
IL-2 (U/mL)			
PHA	2.6	114.9	ND
ConA	<1	11.2	ND
DTH (No. of positive responses/total mm induration)	ND	0/0	3/11.5

ConA, concanavalin A; DTH; delayed-type hypersensitivity; IL, interleukin; ND, not determined; PHA, phytohemagglutinin.

ble data from different animal species, they strongly indicate that impairment of immune function should be included among the biologic effects of vitamin E deficiency.

Vitamin E Supplementation Enhances Immune Function

Dietary supplementation of vitamin E above the recommended levels has been shown to enhance both cell-mediated and humoral immune responses in various species of animals (Table 4). One of the most commonly used *in vitro* indices of cell-mediated immune response is the proliferative response of lymphocytes to mitogens such as ConA and PHA. Higher lymphocyte proliferation in response to ConA, PHA, or LPS stimulation was observed in rodents fed diets supplemented with vitamin E ranging from 50 to 2500 mg/kg diet.^{29–32} Meydani et al.³⁰ also showed that old mice fed a vitamin-E-supplemented diet (500 mg/kg diet vs. a control diet with 30 mg/kg) for 6 weeks had significantly higher DTH response and IL-2 production and lower immunosuppressive prostaglandin E₂ (PGE₂) production. In a short-term study, young F344 rats were fed diets containing vitamin E at 50 (control group), 100, 250, 500, or 2500 mg/kg diet for 10 days.³¹ Lymphocyte responses to PHA, ConA, and LPS, phagocytic activity of alveolar macrophages, and splenic NK activity were measured. Significantly higher lymphocyte responses to ConA were observed in rats fed diets containing >100 mg vitamin E/kg diet compared with the control group. The maximal proliferative response to ConA was observed in rats fed the diet with 500 mg vitamin E/kg diet. The response to PHA was not significantly different among groups. Splenic NK activity and phagocytosis of opsonized SRBCs by alveolar macrophages were higher in animals fed >250 and >100 mg vitamin E/kg diet, respec-

tively. Bendich et al.²⁹ investigated the requirement of vitamin E for several different parameters in spontaneously hypertensive rats (SHR/Ncr1BR). The dietary requirement for optimal lymphocyte response to mitogens was >50 mg/kg diet, which was higher than the requirement for prevention of erythrocyte hemolysis (50 mg/kg), prevention of myopathy (15 mg/kg), or normal growth and spleen-body weight ratio (7.5 mg/kg). *In vitro* supplementation of vitamin E also influences the mitogenic response of lymphocytes. Beharka et al.³³ showed that *in vitro* addition of vitamin E increased ConA-stimulated cell proliferation when macrophages from old mice were co-cultured with purified T cells from either old or young mice or when macrophages from young mice were co-cultured with purified T cells from old mice. IL-2 production was also increased with vitamin E supplementation in cocultures of macrophages from old mice and purified T cells from either old or young mice.

Vitamin E supplementation enhances the humoral response. Tanaka et al.³⁴ showed that vitamin E enhances antibody response to immunization with a hapten-carrier conjugate when mice have been primed with the same carrier. A shift from immunoglobulin M (IgM) to IgG was also reported. Vitamin E can also act as an adjuvant. When added to inactivated and emulsified vaccines, replacing 20% or 30% of mineral oil, vitamin E can induce more rapid and higher antibody response than control vaccines in chicks.³⁵

The immunostimulatory effect of vitamin E supplementation was shown to be transferred to the offspring. Chicks fed diets supplemented with vitamin E had significantly higher tetrahydrofuran-stimulated bursal lymphocyte proliferation and higher ConA- and phorbol 12-myristate 13 acetate-stimulated splenic lymphocyte proliferation than did control chicks.³⁶

Table 4. Vitamin E Supplementation and Immune Responses in Animals

Species	Dosage and Duration	Results	Reference
Young rats (N = 6/group)	50 or 200 mg/kg diet for 8–10 weeks	↑ Lymphocyte proliferation (ConA, LPS)	Bendich et al., 1986 ²⁹
Old mice (N = 10/group)	500 mg/kg diet for 6 weeks	↑ Lymphocyte proliferation (ConA, LPS) ↑ DTH response ↑ IL-2 production ↓ PGE ₂ production	Meydani et al., 1986 ³⁰
Young and old mice (N = 5/group)	500 IU for 9 weeks	↑ Lymphocyte proliferation (ConA) in young ↔ Lymphocyte proliferation (ConA) in old ↑ IFN-γ in young under restraint stress ↔ IFN-γ in old under restraint stress	Wakikawa et al., 1999 ⁹⁸
Young rats (N = 10/group)	50, 100, 250, 500, or 2500 mg/kg diet for 7 days	↑ Lymphocyte proliferation (>100 mg/kg diet, ConA) (>250 mg/kg diet, LPS) ↑ NK activity (>250 mg/kg diet)	Moriguchi et al., 1990 ³¹
Old rats (N = 5/ group)	585 mg/kg diet for 12 months	↑ Lymphocyte proliferation (ConA, PHA) ↑ IL-2 production	Sakai and Moriguchi, 1997 ³²
Young calves (N = 8/group)	125, 250, or 500 IU/d for 24 weeks	↑ Lymphocyte proliferation (PHA, ConA, pokeweed mitogen) ↑ Antibovine herpesvirus antibody titer to booster in 125–IU/d group	Reddy et al., 1987 ⁹⁹
Young mice (N = 8/group)	200 mg/kg diet for 6–12 weeks	↑ Antibody response ↑ Helper T-cell activity	Tanaka et al., 1979 ³⁴
Young pigs (N = 10/group)	100,000 IU/ton for 10 weeks	↑ Antibody response to <i>Escherichia coli</i>	Ellis and Vorhies, 1976 ¹⁰⁰
Mice (N = 10/ group)	500 mg/kg diet for 6 months	↓ IL-6 and PGE ₂ (unstimulated) production by macrophages ↓ nitric oxide production (LPS) by macrophages	Beharka et al., ⁴⁴

ConA, concanavalin A; DTH, delayed-type hypersensitivity; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PGE₂, prostaglandin E₂; PHA, phytohemagglutinin.

Over the past 15 years, several double-blind, placebo-controlled trials investigated the effects of vitamin E supplementation on immune functions (Table 5). These studies suggest that vitamin E supplementation is more effective in improving the immune response in elderly subjects, who have a dysregulated immune response, compared with that in young subjects.

Does Vitamin E Enhance Immune Response in the Aged?

Age-associated dysregulation of immune function has been clearly demonstrated in both animals and humans. Immunologic changes observed with aging include a decrease in lymphocyte proliferation, DTH response, and IL-2 production; a shift toward Th2 response; an increase in T cells expressing memory phenotype; a decrease in

antibody response to vaccination; and an increase in the production of suppressive factors such as PGE₂.^{37,38} T cells are considered to be the immune cells most vulnerable to the deleterious effects of aging. The impairment of T-cell functions—including a decline in T-cell proliferation and IL-2 production—is related to an increase in memory cells and to changes in signal transduction pathways such as defects in immediate calcium mobilization and protein phosphorylation after contact with an activating stimulus. Increased production of immunosuppressive PGE₂ by macrophages has also been shown to contribute to the age-associated decline of T-cell function.^{37,39}

Vitamin E supplementation has an immunostimulatory effect in aged mice,³⁰ rats,³² and humans.^{40–42} In an early study by Meydani et al.,⁴⁰ 32 healthy elderly men and women >60 years were supplemented daily with either placebo or 800 mg *dl*- α -tocopheryl acetate for 30

Table 5. Vitamin E Supplementation and Immune Responses in Humans

Subjects	Age	Amount and Duration of Supplementation*	Effects	Reference
Adults and teenagers (N = 18)	25–30; 13–18	300 mg/d for 3 weeks	↓ Lymphocyte proliferation ↔ DTH ↓ Bactericidal activity	Prasad, 1980 ⁴⁷
Adults (N = 31) and premature infants (N = 10)	24–31	600 mg/d for 3 months† 40 mg/kg body weight for 8–14 days	↓ Chemiluminescence	Okano et al., 1991 ⁵³
Cigarette smokers (N = 60)	33 + 4	900 IU/d for 6 weeks	↓ Chemiluminescence	Richards et al., 1990 ⁵²
Sedentary young and elderly (N = 21)	22–29; 55–74	800 IU/d for 48 days	↓ IL-6 secretion ↓ Exercise-enhanced IL-1β secretion	Cannon et al., 1991 ⁴⁵
Adults (N = 26)	25–35	233 mg/d for 28 days‡	↑ Lymphocyte proliferation ↑ Total T cells, CD4 T cells ↓ Plasma malondialdehyde ↓ Urinary 8-OHDG	Lee and Wan, 2000 ⁴³
Institutionalized elderly	63–93	200 mg/d for 4 months	↑ Total serum protein; α-2 and β-2 globulin fractions	Ziemiński et al., 1986 ⁴⁸
Institutionalized adults and elderly (N = 103)	24–104	200 or 400 mg /d for 6 months	↔ Antibody development to influenza virus	Harman and Miller, 1986 ⁶²
Elderly (N = 32)	≥60	800 mg/d for 30 days	↑ Lymphocyte proliferation ↑ DTH ↑ IL-2 production ↓ PGE ₂ production	Meydani et al., 1990 ⁴⁰
Elderly (N = 88)	≥65	60, 200, or 800 mg/d for 235 days‡	↑ DTH and antibody titer to hepatitis B with 200 and 800 mg	Meydani et al., 1997 ⁴¹
Elderly (N = 74)	≥65	100 mg/d for 3 months	↔ Lymphocyte proliferation ↔ IgG, IgA levels	De Waart et al., 1997 ⁴⁶
Elderly (N = 161)	65–80	50 or 100 mg/d for 6 months	↑ No. of positive DTH responses with 100 mg ↑ Diameter of induration of DTH response in a subgroup with 100 mg ↔ IL-2 production	Pallast et al., 1999 ⁴²
Hypertriglyceridemic (N = 12) and normolipidemic (N = 8) adults	49.5 + 9.6 55.8 + 12.1	600 IU/d for 6 weeks†	↓ Superoxide production ↓ TNF-α, IL-1β, IL-8 production	van Tits et al., 2000 ¹⁰¹

*Supplemented with *dl*-α-tocopheryl acetate unless indicated.

†Supplemented with RRR-α-tocopherol.

‡Supplemented with *dl*-α-tocopherol.

DTH, delayed-type hypersensitivity; 8-OHDG, 8-hydroxydeoxyguanosine; Ig, immunoglobulin; IL, interleukin; PGE₂, prostaglandin E₂; TNF, tumor necrosis factor.

days. Vitamin E supplementation was associated with increased DTH response, proliferative response to ConA, and IL-2 production. Decreased PHA-stimulated PGE₂ production by peripheral blood mononuclear cells and decreased plasma lipid peroxide concentration were observed with vitamin E supplementation (Figure 1). In a more recent study, the effect of 4.5 months of vitamin E supplementation on in vivo indices of immune function was investigated in healthy elderly men and women >65 years; 88 subjects were supplemented daily with placebo or 60, 200, or 800 mg *dl*- α -tocopherol.⁴¹ All three vitamin-E-supplemented groups showed a significant increase in DTH response compared with baseline. When DTH was expressed as median percentage change, subjects in the 200 mg/d group had a 65% increase, significantly greater ($P = 0.04$) than that of the placebo group (17%). Although the median percentage changes in the 60 and 800 mg/d groups (41% and 49%, respectively) were similar to the change in the 200 mg/d group (65%), these changes were not statistically different from that of the placebo group. A significant increase in antibody titer to hepatitis B was observed in the 200 and 800 mg/d groups. The 200 mg/d group also had a significant increase in antibody titer to tetanus vaccine. Lee and Wan⁴³ reported a significant increase in proliferative response to PHA or LPS and a significant decrease in plasma malondialdehyde and urinary DNA adduct 8-hydroxy-2'-deoxyguanosine after short-term supplementation with vitamin E (233 mg *dl*- α -tocopherol/d for 28 days) in Chinese adults. In addition to its effect on cell-mediated immunity, vitamin E may have anti-inflammatory effects; it has been shown to reduce production of proinflammatory mediators such as IL-1, IL-6, PGE₂, and nitric oxide in animals⁴⁴ and humans.⁴⁵ Long-term supplementation of vitamin E (55 mg/kg diet for 6 months) decreased the production of unstimulated IL-6 and LPS-stimulated

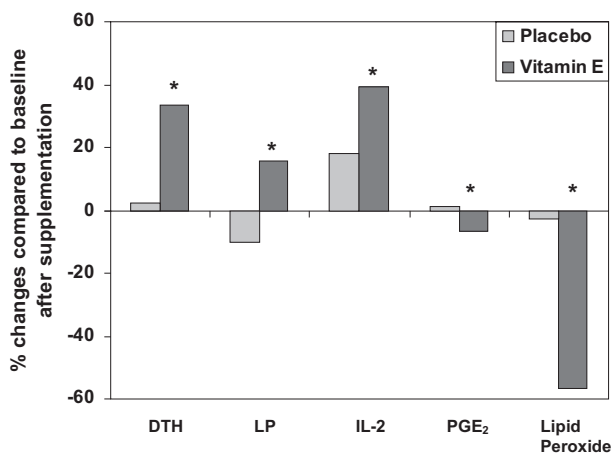


Figure 1. Effect of vitamin E supplementation (800 mg/d for 30 days) on immune responses of healthy older adults. *Significant changes from baseline at $P < 0.05$. Abbreviations: DTH, delayed-type hypersensitivity response; LP, lymphocyte proliferation; IL-2, interleukin-2; PGE₂, prostaglandin E₂. Data adapted from Meydani SN, Barklund MP, Liu S, et al. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am J Clin Nutr.* 1990;52:557–563.

nitric oxide by peritoneal macrophages in mice.⁴⁴ In humans, supplementation with 800 mg vitamin E for 60 days prevented exercise-induced elevation of IL-1 and significantly decreased the production of IL-6.⁴⁵

De Waart et al.⁴⁶ observed no significant changes in mitogenic response to ConA and PHA; IgG and IgA levels against *Penicillium*; and IgG4 levels against egg, milk, and wheat proteins after 3 months of supplementation with vitamin E at 100 mg/d. The lower dose of vitamin E and the use of previously frozen lymphocytes for determination of mitogenic response and elevation of antibody levels without previous specific vaccination may have contributed to the discrepancy observed between the results from De Waart et al.⁴⁶ and Meydani et al.⁴¹ Prasad⁴⁷ measured mitogenic response to PHA, DTH response to PHA, and bactericidal activity against *Escherichia coli* of leukocytes in 13 young adults (25–30 years) and 5 young boys (13–18 years) after 3 weeks of supplementation with vitamin E at 300 mg/d. Bactericidal activity and mitogenic response were measured in the young adults, and DTH was measured in the young boys. DTH was tested by measuring induration after intradermal injection of PHA. Bactericidal activity and mitogenic response decreased after 3 weeks of supplementation, and there was no significant difference in DTH response after supplementation. However, only a few subjects were used and no placebo group was included. Pallast et al.⁴² supplemented healthy elderly subjects 65 to 80 years of age with 50 or 100 mg vitamin E for 6 months. Subjects in the vitamin-E-supplemented group showed a significant increase in DTH (induration diameter and number of positive reactions) compared with their own baseline values. Only the change in the number of positive DTH reactions tended to be larger in the 100-mg-supplemented group than the placebo group ($P = 0.06$). A significantly greater improvement in cumulative DTH score and number of positive DTH reactions was observed in a subgroup of subjects who received 100 mg vitamin E and had low baseline DTH reactivity (\leq positive DTH reactions). There was no significant difference in PHA-stimulated IL-2 production in the vitamin-E-treated groups compared with the placebo group, and IFN- γ production tended to be lower in groups receiving vitamin E. Significant increases in total serum protein and α -2 and β -globulin fractions were observed in older subjects supplemented with vitamin E at 2000 mg/d for 4 months.⁴⁸

Differences in results among these human studies may reflect the difference in age of subjects, doses of supplementation (resulting in varied levels of changes in plasma vitamin E levels) (Table 6, Figure 2), and methodology, as well as subjects' vitamin E status at baseline. Mean plasma vitamin E level changes after supplementation with 19.3 μ mol/L, an increase from baseline level of 17.9 μ mol/L by 300 mg/d supplementation of young subjects in the study by Prasad⁴⁷; 16.7 μ mol/L, an increase from the baseline level of 33.0 μ mol/L by 100-mg/d supplementation in the study by De Waart et al.⁴⁶; and 10.1 and 15.8 μ mol/L, an increase from baseline level of 28.8 and 31.1 μ mol/L by 50

Table 6. Changes in Plasma Vitamin E Levels and DTH Responses

Vitamin E Dose (mg/d)	Change in Blood Vitamin E Levels ($\mu\text{mol/L}$)	Vitamin E Levels After Supplementation ($\mu\text{mol/L}$)	Change in Diameter of Induration (mm)	Reference
0	-1.2	23.3	2.0	Meydani et al., 1997 ⁴¹
50	10.1	38.9	4.6	Pallast et al., 1999 ⁴²
60	11.2	38.4	5.0	Meydani et al., 1997 ⁴¹
100	15.8	46.9	6.0	Pallast et al., 1999 ⁴²
200	25.4	51.0	10.0	Meydani et al., 1997 ⁴¹
800	45.7	71.5	11.0	Meydani et al., 1997 ⁴¹

and 100 mg/d supplementation, respectively, in the study by Pallast et al.⁴² On the other hand, in the studies by Meydani et al.,^{40,41} plasma vitamin E levels increased from 25.6 to 70.9 $\mu\text{mol/L}$ with 800 mg/d supplementation for 30 days, and serum vitamin E levels increased from 25.6 and 25.8 $\mu\text{mol/L}$ to 51.0 and 71.5 $\mu\text{mol/L}$ with 200 and 800 mg/d supplementation, respectively, for 4.5 months. Considering the results from the study by Meydani et al.,⁴¹ in which subjects in the upper tertile of serum vitamin E concentration ($>48.4 \mu\text{mol/L}$) after supplementation had higher antibody response to hepatitis B and higher DTH responses than those in the lower tertile of serum vitamin E, the amount of increase in vitamin E level achieved in the studies by others^{42,46,47} might not have been adequate to observe a highly significant effect. It is also noteworthy that Lee and Wan⁴³ observed a significant increase in cell-mediated immune response with a 13.4- $\mu\text{mol/L}$ increase in plasma vitamin E level, a level of increase comparable to

those observed by others,^{42,46} with 100 mg supplementation. However, De Waart et al.⁴⁶ observed no significant change, and Pallast et al.⁴² observed significant improvement only in a subgroup of subjects with low baseline vitamin E status. This discrepancy in findings might be due to differences in vitamin E status of subjects at baseline among the various studies. Subjects in the study by Lee and Wan⁴³ had significantly lower plasma vitamin E levels of 14.25 $\mu\text{mol/L}$ at baseline compared with vitamin E levels of 33.0 $\mu\text{mol/L}$ and 31.3 $\mu\text{mol/L}$ in the studies by De Waart et al.⁴⁶ and Pallast et al.,⁴² respectively.

Concern has been raised that consuming high amounts of vitamin E may negatively affect phagocytosis and bactericidal activity of polymorphonuclear cells or increase autoantibody formation. Administration of 1600 IU vitamin E for 7 days resulted in less effective killing of *Staphylococcus aureus* 502A.⁴⁹ The results of this study need to be interpreted with caution because there were only three subjects supplemented with vitamin E and no control group. In a recent study⁵⁰ on the effect of vitamin E supplementation on secondary bacterial infection following influenza infection, vitamin E supplementation did not have a significant effect on *S. aureus* infection alone. However, vitamin E supplementation prevented the priming effect of influenza infection on *S. aureus* infection. The cytotoxic ability of neutrophils against *Candida albicans* was not compromised after 4.5 months of supplementation with 60, 200, and 800 IU vitamin E in a double-blind, placebo-controlled study with 88 subjects.⁵¹ In addition, vitamin E supplementation did not increase the serum levels of two autoantibodies—anti-DNA and anti-thyroglobulin—in healthy elderly subjects.⁵¹ Richards et al.⁵² reported inhibition of oxidant generation by phagocytes after supplementation of cigarette smokers with 900 IU/d for 6 weeks. Oxidant generation was measured by the luminol-enhanced chemiluminescence response of phagocytes activated with PMA or *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine with cytochalasin B. Suppressed production of superoxide by polymorphonuclear cells was observed with both very low and high levels (by in vitro addition or intramuscular injection) of vitamin E.⁵³ Opsonized zymosan-stimulated superoxide generation in polymorphonuclear cells was detected by chemilu-

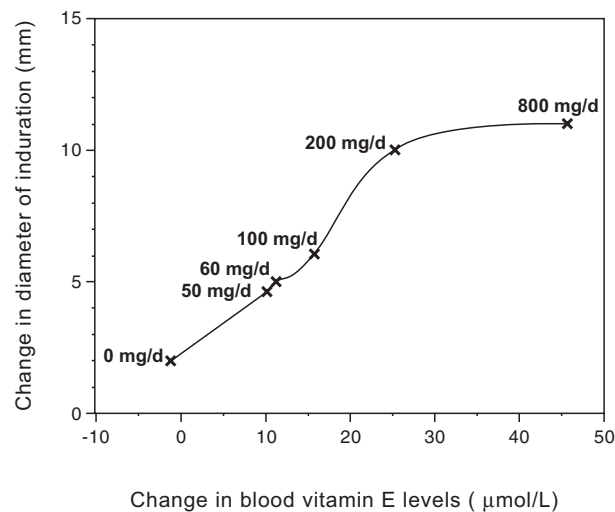


Figure 2. Relationship between changes in DTH and changes in blood vitamin E levels after different amounts of vitamin E supplementation. Data adapted from Meydani SN, Meydani M, Blumberg JB, et al. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *JAMA*. 1997;277:1380–1386 and Pallast EG, Schouten EG, deWaart FG, et al. Effect of 50- and 100-mg vitamin E supplements on cellular immune function in noninstitutionalized elderly persons. *Am J Clin Nutr*. 1999;69:1273–1281.

minescence using a *Cypridina* luciferin analog. However, supplementation of young adult subjects (24–31 years) with 600 mg/d for 3 months or premature infants with 40 mg/kg/d for 8 to 14 days did not affect chemiluminescence, indicating that oral administration of vitamin E does not impair polymorphonuclear cell function.

Is the Immunostimulatory Effect of Vitamin E Associated with Increased Resistance to Infectious Diseases?

The immunostimulatory effect of vitamin E has been shown to be associated with increased resistance against several pathogens in different species of animals.⁵⁴ A lower incidence of mortality from *E. coli* infection was observed in chicks supplemented with vitamin E at 300 mg/kg diet for 6 weeks.⁵⁵ Furthermore, a 37% reduction in incidence of clinical mastitis and a 44% reduction in the duration of clinical symptoms were observed in cows receiving 740 mg/d of vitamin E.⁵⁶ Vitamin E supplementation at 180 mg/kg diet for 4 weeks increased the survival of nonimmunized mice from 20% to 80% when they were challenged with 20 organisms of *Diplococcus pneumoniae* type I, and of immunized mice from 15% to 70% when they were challenged with 20,000 organisms. The increased protection against *D. pneumoniae* type I seemed to result principally from increased macrophage activity and antibody production.⁵⁷

Hayek et al.⁵⁸ showed that supplementation with vitamin E at 500 mg/kg diet for 6 weeks can lower the pulmonary viral titer in old mice infected with influenza virus. Old mice fed a diet high in vitamin E had a significantly lower lung viral titer than did those fed a diet containing an adequate level of vitamin E (30 mg/kg diet) on 2, 5, and 7 days after influenza virus infection (Figure 3). The mechanism for the antiviral effect of vitamin E was not fully described in this study; however, higher NK activity and preserved antioxidant nutrient status were found to contribute in part to the vitamin-E-induced reduction of viral titer. A subsequent study showed that IL-2 and IFN- γ production (Th1 response) by splenocytes increased significantly after influenza infection in vitamin-E-supplemented (500 mg/kg diet for 8 weeks) old mice, whereas old mice fed the control diet were unable to induce efficient Th1 response.⁵⁹ Old mice fed the vitamin E diet produced 100% more IFN- γ than did those fed the control diet. In addition, there was a significant inverse correlation between viral titer and IFN- γ production (Figure 4). Dysregulation of Th1 and Th2 functions are observed with aging; these changes in Th1/Th2 balance can contribute to the delayed clearance and recovery from influenza infection as Th1 clones are cytolytic in vitro and protective against lethal challenges in vivo, whereas Th2 clones are noncytolytic and not protective.⁶⁰ These studies indicate that the protective effect of vitamin E against

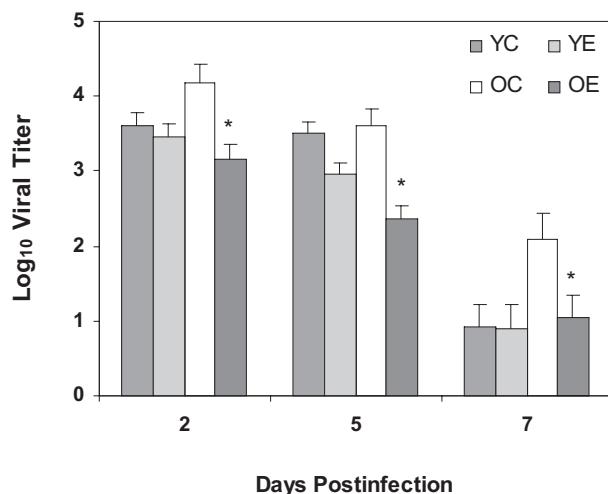


Figure 3. Pulmonary virus titers of young (4 months) and old (22 months) C57BL/6NIA mice fed 30 ppm (control) or 500 ppm (supplemented) vitamin E and given a total respiratory tract infection with H3N2 influenza virus. Mice were fed a diet for 6 weeks, infected intranasally, and killed 0, 2, 5, and 7 days after infection. Lungs were excised and homogenized, and virus titer was measured from the supernatants. Means \pm SE are shown. N = 4–8. Abbreviations: OC, old mice fed the control diet; OE, old mice fed the vitamin-E-supplemented diet; YC, young mice fed the control diet; YE, young mice fed the vitamin-E-supplemented diet. *Significantly lower virus titer compared with OC ($P < 0.05$). Adapted from Hayek MG, Taylor SF, Bender BS, et al. Vitamin E supplementation decreases lung virus titers in mice infected with influenza. *J Infect Dis.* 1997;176:273–276.

influenza infection is mediated through reducing the viral load partly by enhancing NK activity and partly by enhancing Th1 response. Vitamin E supplementation was also shown to prevent retrovirus-induced suppression of splenocyte proliferation and NK activity and to partially restore production of IL-2 and IFN- γ by splenocytes.⁶¹

The clinical significance of vitamin E supplementation in regard to protection against infectious diseases in humans has been investigated in several studies published recently (Table 7). Harman and Miller⁶² investigated the effect of vitamin E supplementation at 200 or 400 mg/d for 6 months on the incidence of infectious diseases in 103 patients in a chronic care facility. There was no effect of vitamin E on antibody titer to influenza vaccine or the incidence of pulmonary, urinary tract, and other infections. Because data on the subjects' health status, medication use, and other relevant variables were not reported, it is difficult to draw conclusions from this study. Furthermore, the number of subjects may not have been adequate to determine a significant difference.

In two separate articles published from the α -Tocopherol β -Carotene Cancer Prevention study,^{63,64} the long-term effects of vitamin E (50 mg/d) and β -carotene (20 mg/d) supplementation on the incidence of the common cold and hospital-treated pneumonia were evaluated. Vitamin E did not have an overall effect on the incidence of common cold episodes during a 4-year follow-up period in a cohort of 21,796 male smokers. Vitamin E did, however, show a beneficial effect in reducing the incidence of colds among older (>65 years) city dwellers who

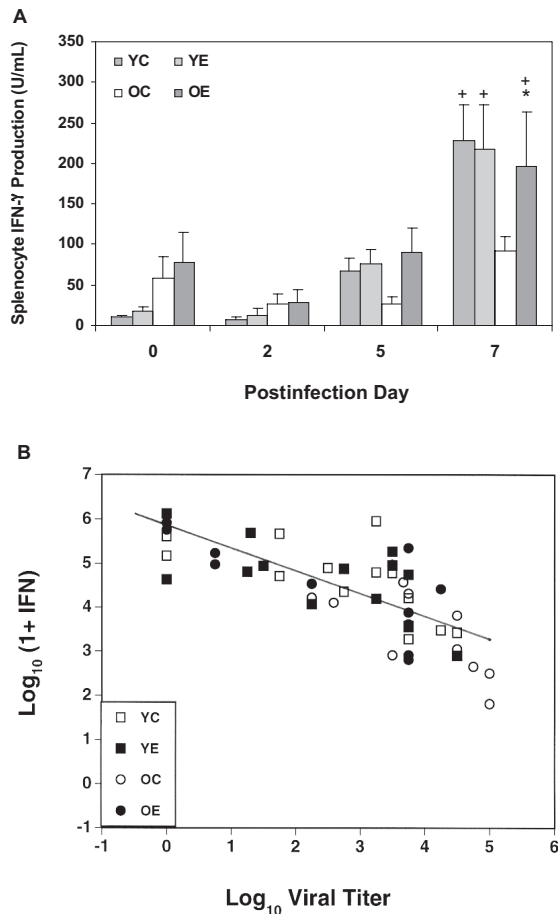


Figure 4. Interferon- γ (IFN- γ) production after influenza infection by splenocytes from young and old mice fed diets containing 30 ppm (control) or 500 ppm (supplemented) vitamin E for 8 weeks (A) and correlation between IFN- γ levels and pulmonary viral titers on days 5 and 7 (B). A: Splenocytes (5×10^6 cells/well) were stimulated with concanavalin A (ConA; 5 mg/L) for 24 hours. Values are means \pm SEM. $N = 4-9$. *Significantly different from mice of the same age fed the control diet by the Fisher least significant test at $P < 0.05$. Abbreviations: OC, old mice fed the control diet; OE, old mice fed the vitamin-E-supplemented diet; YC, young mice fed the control diet; YE, young mice fed the vitamin-E-supplemented diet. B: A significant inverse correlation was observed between IFN- γ levels and viral titers ($r = -0.721$; $P < 0.001$; $N = 49$) by Pearson correlation. Reproduced with permission from Han SN, Wu D, Ha WK, et al. Vitamin E supplementation increases T helper 1 cytokine production in old mice infected with influenza virus. *Immunology*. 2000;100:487-493.

smoked fewer than 15 cigarettes per day. The results from this study need to be interpreted with caution, as the dose of vitamin E used (50 mg/d) is not optimal for improving the immune response in the elderly. Furthermore, the data was based on self-reported incidence collected 3 times per year.⁶³ In another study,⁶⁴ vitamin E had no overall effect on the incidence of hospital-treated pneumonia during 6.1-year (median) follow-up in a cohort of 29,133 male smokers. Again, vitamin E did show a beneficial effect in a subpopulation of subjects, in this case, those who had initiated smoking at a later age (>21 years) with a relative risk of 0.65. These findings indicate that supplementation with 50 mg/d of vitamin E might

not be adequate to observe a beneficial effect in elderly smokers, as any beneficial effect of vitamin E was observed in subgroups who smoked less or had initiated smoking at a later age (fewer smoking years).

Graat et al.⁶⁵ reported the effect of vitamin E and multi-vitamin-mineral supplementation on the incidence and severity of acute respiratory tract infections in elderly, non-institutionalized individuals. A total of 652 individuals more than 60 years (mean age 73 years) were supplemented with placebo; 200 mg of vitamin E alone; multivitamin-mineral capsule containing retinol (600 μ g), β -carotene (1.2 mg), ascorbic acid (60 mg), vitamin E (10 mg), cholecalciferol (5 μ g), vitamin K (30 μ g), thiamin mononitrate (1.4 mg), riboflavin (1.6 mg), niacin (18 mg), pantothenic acid (6 mg), pyridoxine (2.0 mg), biotin (150 μ g), folic acid (200 μ g), cyanocobalamin (1 μ g), zinc (10 mg), selenium (25 μ g), iron (4.0 mg), magnesium (30 mg), copper (1.0 mg), iodine (100 μ g), calcium (74 mg), phosphorous (49 mg), manganese (1.0 mg), chromium (25 μ g), molybdenum (25 μ g), and silicon (2 μ g); or vitamin E in combination with the multivitamin-mineral supplement for 15 months. Mean incidence of infections per year was 1.53, 1.73, 1.48, and 1.63 for placebo, vitamin E alone, multivitamin-mineral, and vitamin E plus multivitamin-mineral groups, respectively. When effects of multivitamin-mineral or vitamin E supplements were analyzed according to the 2×2 factorial design, there was no significant effect of multivitamin-mineral supplementation on the incidence rate ratio (0.95) and severity of infections. There was no significant difference in incidence rate ratio between vitamin-E-supplemented groups (1.12) and no vitamin E groups (1.00). However, this study also has limitations because the incidence and severity of acute respiratory tract infections were self-reported (1024 reports by 443 subjects), and only a small portion of reported infections were confirmed by microbiologic tests. There was also a difference in risk factors pertinent to respiratory infections such as chronic obstructive pulmonary disease, asthma, allergy, and smoking status among placebo and intervention groups. Furthermore, no differentiation was made between upper and lower respiratory infections, which have different microbial causes (mainly viral for upper and bacterial for lower respiratory infections).

In a randomized, double-blind study, 617 people >65 years residing at 33 nursing homes in the Boston, Massachusetts area received either a placebo or 200 IU of vitamin E (*d*- α -tocopherol) daily for 1 year.⁶⁶ All participants received a capsule containing half the recommended daily allowance of essential vitamins and minerals. The results of this clinical trial showed that significantly fewer vitamin-E-supplemented subjects acquired ≥ 1 respiratory infections ($r^2 = 0.88$; $P = 0.04$) or upper respiratory infections ($r^2 = 0.81$; $P = 0.01$). However, supplementation with vitamin E had no significant effect on incidence or number of subjects acquiring lower respiratory infections. Further analysis on the foremost upper respiratory infection, the common cold, indicated that the vitamin E group had a lower incidence of common colds (0.66 vs. 0.83 per sub-

Table 7. Vitamin E Supplementation and Infectious Diseases in Humans

Subjects	Age (years)	Supplementation Dose	Duration	Results	Reference
Elderly nursing home residents (N = 617)	>65 (mean age 84–85)	200 IU (plus 0.5 dose of RDA of essential vitamins and minerals)	1 year	Lower incidence of common cold; no significant effect on lower respiratory infections	Meydani et al., 2004 ⁶⁶
Male smokers (N = 29,133)	50–69	50 mg/d vitamin E (2 × 2 factorial design: 20 mg/d β-carotene)	6.1 years	No overall effect on incidence of pneumonia; decreased risk of pneumonia in subjects who started smoking at a later age	Hemila et al., 2004 ⁶⁴
Noninstitutionalized elderly (N = 652)	>60 (mean age 73–74)	200 mg vitamin E (2 × 2 factorial design: multi-vitamin–mineral supplement)	15 months	No effect on respiratory tract infections	Graat et al., 2002 ⁶⁵
Male smokers (N = 21,796)	>50–69	50 mg/d vitamin E (2 × 2 factorial design: 20 mg/d β-carotene)	4 years	No overall effect on common cold incidence; lower incidence of colds in older subjects who smoked <15 cigarettes per day	Hemila et al., 2002 ⁶³
Adults in chronic care facility	24–104	200 or 400 mg/d	6 months	No effects on serum Ab titer to influenza vaccine; no effects on incidence of infectious diseases	Harman and Miller, 1986 ⁶²

Ab, antibody; RDA, recommended daily allowance.

ject-year, $r^2 = 0.80$; $P = 0.04$) and fewer subjects in the vitamin E group acquired ≥ 1 common colds (46% vs. 57%, $r^2 = 0.80$; $P = 0.02$). There was also a nonsignificant trend for shorter duration of the common cold in vitamin-E-supplemented subjects. In conclusion, the results of this clinical trial show that vitamin E supplementation significantly reduces the risk for acquiring respiratory infections in the elderly. In particular, vitamin E supplementation reduced the incidence rate of common colds and the number of subjects who acquire a cold among elderly nursing home residents. A nonsignificant reduction in the duration of colds was also observed. Because of the high rate and more severe morbidity associated with common colds in this age group, these findings have important implications for the well-being of the elderly and for the economic burden associated with their care.

Colds are common afflictions, accounting for 30% of absenteeism in the United States across all age groups.⁶⁷ Rhinoviruses and coronaviruses represent the majority of the documented causes of colds.⁶⁸ They exacerbate chronic obstructive pulmonary disease⁶⁹ and are known to be associated with lower respiratory infections in the el-

derly.^{68,70,71} For example, a prospective cohort study of community-based elderly found that rhinoviruses were associated with lower respiratory symptoms in nearly two-thirds of episodes: about one-fifth of patients were confined to bed, and 26% were unable to perform routine household activities.⁷¹ Constitutional and lower respiratory tract symptoms and signs have been reported to be more common in the elderly compared with younger adults infected with cold viruses.⁷⁰ Nursing home populations may also be at risk for epidemic outbreaks of rhinovirus infections.⁷² The common cold is generally less severe than influenza. However, its much higher incidence and its recognized morbidity in the elderly^{68,70-72} make it an important public health problem in this age group.⁷³ This is particularly relevant because at present no clinically useful vaccine or antiviral therapy is available to combat colds. The economic impact of non-influenza-related viral upper respiratory infections in general, and in the elderly in particular, has been overlooked. Because of their high attack rate, these diseases are responsible for an economic burden that approaches \$40 billion annually.⁷³ Thus, our finding

that E supplementation reduces the common cold by 22% has significant implications for the elderly in reducing the burden of diseases and associated health care costs. Currently, there are 34 million elderly in the United States. The observation that vitamin E reduced the risk for acquiring any respiratory infections by 20% will translate into approximately 7 million fewer elderly acquiring respiratory infections. Thus, the findings from this study could have significant impact for improving the health status of the elderly and need to be considered in relation to their vitamin E requirement.

Mechanisms for the Immunostimulatory Effect of Vitamin E

Several mechanisms are possible for the immunostimulatory effect of vitamin E: it can enhance the immune response by influencing membrane integrity, influencing signal transduction, reducing the production of suppressive factors such as PGE₂, or directly influencing T-cell functions.

Vitamin E is a potent peroxy radical scavenger that can prevent the propagation of free radical damage in biologic membranes. This antioxidant function of vitamin E can affect signal transduction pathways that are regulated by redox status.⁷⁴ Transcriptional factors such as nuclear factor κ B (NF- κ B) and activator protein-1 (AP-1) are important regulators of nuclear gene expression in immune cells and are sensitive to the antioxidant-oxidant balance. Reduction and oxidation can either up- or down-regulate DNA binding and transactivation activities in a transcriptional-activator-dependent and cell-type-dependent manner. In general, oxidants increase and reductants decrease NF- κ B activity, but AP-1 activity is dramatically increased by reductants.⁷⁵ NF- κ B is activated by various agents, including IL-1 and TNF- α , viruses, double-stranded RNA, endotoxins, phorbol esters, ultraviolet light, and hydrogen peroxide. Antioxidants may affect NF- κ B function through suppression of NF- κ B activation by various inducers.⁷⁶ The vitamin E derivatives α -tocopherol acetate and succinate inhibited NF- κ B activation induced by TNF- α in human Jurkat T cells.⁷⁷ In contrast, both oxidative and reducing signals can activate AP-1. Prooxidants (e.g., hydrogen peroxide, ultraviolet irradiation) can induce AP-1 activation. On the other hand, AP-1-dependent transactivation was strongly enhanced by thioredoxin, cellular protein oxidoreductase with antioxidant activity, and other structurally unrelated antioxidants such as pyrrolidine dithiocarbamate and butylated hydroxyanisole.⁷⁸ Vitamin E might also regulate cellular reaction through its nonantioxidant functions by inhibiting protein kinase C activity.⁷⁹

Many cytokines, including IL-1, IL-2, IL-6, and TNF- α , contain NF- κ B and AP-1 binding sites in the promoter and enhancer regions of the genes encoding them. AP-1, nuclear factor of activated T cells, octamer

proteins, and NF- κ B were shown to play integral roles in the regulation of the *IL-2* gene. The production of IL-2 by activated T cells is critical for T-cell proliferation and differentiation and the development of T-cell-dependent immune response.⁸⁰ Both animal^{30,32} and human⁴⁰ studies reported increased IL-2 production with vitamin E supplementation, but the effect of vitamin E on *IL-2* gene regulation was not tested.

PGE₂, the cyclooxygenase (COX) product of arachidonic acid metabolism, plays an important regulatory role in controlling immune function. PGE₂ has a direct inhibitory effect on the early stages of T-cell activation, resulting in decreased IL-2 production and decreased IL-2 receptor expression.⁸¹ In addition, PGE₂ can modulate Th1 and Th2 responses through its effect on IL-12, which plays a central role in increasing Th1 responses by promoting the differentiation of Th0 cells into a population of Th1 cells.⁸² In a co-culture study, the addition of PGE₂ at concentrations produced by macrophages from old mice decreased proliferation and IL-2 production by T cells from young mice; the addition of vitamin E decreased PGE₂ production and improved T-cell proliferation and IL-2 production.³³ Wu and collaborators⁸³ showed that in vivo vitamin E supplementation decreased PGE₂ production by LPS-stimulated macrophages from old mice (Figure 5). This effect of vitamin E was me-

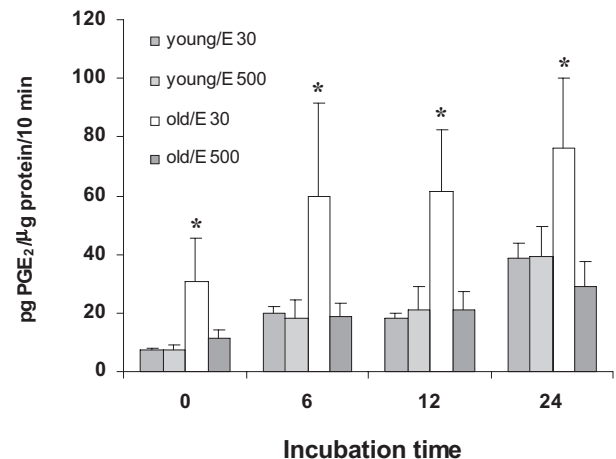


Figure 5. Cyclooxygenase (COX) activity in peritoneal macrophages isolated from young and old mice fed diets containing either 30 or 500 ppm vitamin E for 30 days. Peritoneal macrophages were isolated and cultured (5×10^5 cells/well) in the presence of lipopolysaccharide at 5 mg/L for 0, 6, 12, or 24 hours at 37°C. After removal of supernatants for assay of accumulated prostaglandin E₂ (PGE₂) production at each time point, cells were layered with 1 mL of endotoxin-free RPMI 1640 containing arachidonic acid at 30 μ mol/L. After 10 minutes of incubation at 37°C, aspirin (2.1 mmol/L) was added to stop reaction. Supernatants were then collected for PGE₂ analysis, cells were lysed with NaOH (1 mol/L), and total cell protein was measured with a protein assay kit. COX activity (means \pm SE for N = 10 in each age and diet group) is expressed as conversion of arachidonic acid to PGE₂ (pg/ μ g protein/10 min). *Significantly higher COX activity compared with young mice fed 30 or 500 ppm vitamin E containing diets and old mice fed 500 ppm vitamin E containing diet ($P < 0.05$). Adapted from Wu D, Mura C, Beharka AA, et al. Age-associated increase in PGE₂ synthesis and COX activity in murine macrophages is reversed by vitamin E. Am J Physiol. 1998;275:C661–C668.

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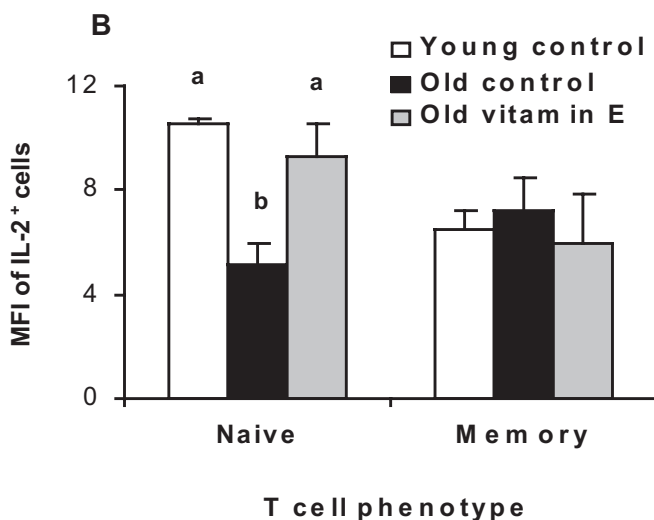
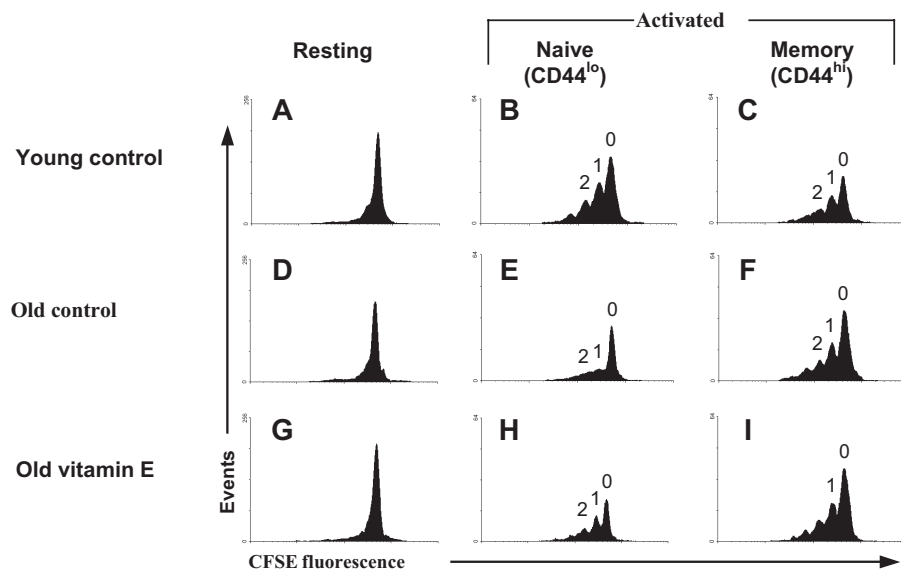


Figure 6. A. Effects of age and vitamin E on the progression of T cells through cell cycle division. Purified T cells were preincubated with 46 μ M vitamin E for 4 hours, labeled with carboxyfluorescein succinimidyl ester, and activated with immobilized anti-CD3 and soluble anti-CD28 mAbs for 48 hours. Cells were harvested, stained for CD44 expression, and analyzed on a flow cytometer. One representative histogram for each of young control (A, B, and C), old control (D, E, and F), and old preincubated with vitamin E (G, H, and I) are shown. Cell cycle division patterns are shown for unactivated T cells (A, D, and G), activated naive (CD44^{lo}) T cells (B, E, and H), and activated memory (CD44^{hi}) T cells (C, F, and I). Peaks representing cell division cycles 0, 1, and 2 are also indicated. B: Effects of age and vitamin E on intracellular IL-2 production by naive and memory T cell subsets. Purified T cells (N = 5) were preincubated with 46 μ M vitamin E for 4 hours and activated with immobilized anti-CD3 and soluble anti-CD28 mAbs for 48 hours. Cells were treated with monensin, an inhibitor of IL-2 secretion, for the last 10 hours 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 antigen, respectively. Cell fluorescence was measured on a flow cytometer. Bars represent the linearized mean fluorescence intensity of IL-2+ T cells. Bars with different letters within each phenotype are significantly different ($P < 0.05$) by an ANOVA followed by the Tukey HSD post-hoc procedure. Abbreviations: ANOVA, analysis of variance; HSD; highly significant difference; IL, interleukin; mAb, monoclonal antibody. Reproduced with permission from Adolfsson O, Huber BT, Meydani SN. 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. *J Immunol.* 2001;167:3809–3817.

diated mainly through inhibition of COX activity, the rate-limiting enzymes in PGE₂ production—a 60% decrease in Cox activity was observed with vitamin E supplementation. Vitamin E appears to affect COX activity post-translationally; there was no effect of vitamin E on protein or mRNA levels of COX. COX activity requires the presence of oxidant hydroperoxides for its activation.⁸⁴ It has been suggested that free radical nitric oxide is involved in the regulation of COX activity.⁸⁵ Nitric oxide can be further metabolized to peroxynitrite in the presence of superoxide, and peroxynitrite has been shown to increase the activity of COX without affecting its expression.⁸⁶ Vitamin E is an effective biologic antioxidant and a chain-breaking free radical scavenger and therefore may attenuate COX activity by scavenging the oxidant hydroperoxide necessary for COX activation. Dietary supplementation of vitamin E resulted in reduced production of nitric oxide in macrophages from old mice.⁸⁷ When a nitric oxide donor was added in the presence of superoxide to elevate peroxynitrite levels in the culture, vitamin-E-induced inhibition of COX activity in the macrophages from old mice was diminished. These results suggest that vitamin E reduces COX activity in old macrophages by decreasing nitric oxide production,

which results in lower production of peroxynitrite in macrophages from old mice.

Vitamin E was also shown to have a direct effect on T-cell functions independent of its effect on macrophage PGE₂ production.^{33,88} When purified T cells from old mice were incubated *in vitro* with vitamin E at 46 μM prior to activation, a significant improvement was observed in cell-dividing capability, total IL-2 production and the number of IL-2+ T cells, and the amount of IL-2 produced per naive T cell⁸⁸ (Figure 6). Preliminary results indicate that vitamin E may improve effective immune synapse formation in naive T cells from old mice.⁸⁹ In addition, it may also increase the expression of cell-cycle-related proteins.⁹⁰ Schematic mechanisms of vitamin E's influence on T-cell function are presented in Figure 7.

Conclusion

The recommended daily intake for vitamin E was recently increased from 10 mg/d⁹¹ to 15 mg/d of α-tocopherol for adults (ages >19 years).⁹² The current recommendation is based largely on induced vitamin E deficiency in humans and the correlation between hydrogen-peroxide-induced erythrocyte lysis and plasma α-tocopherol concentrations. The Food and Nutrition Board had acknowledged the growing body of evidence for beneficial effects of a high intake of vitamin E on some chronic diseases; however, they stated that clinical evidence is limited and not conclusive to warrant a recommendation for higher vitamin E intake.⁹²

Several studies in different species of animals have demonstrated an improvement in immune response when animals are supplemented with more than the recommended level of vitamin E. In addition, clinical trials in humans have shown significant improvement in the immune response of the elderly with vitamin E supplementation. The dose-response relationship shown in this age group suggests that 200 IU/d of α-tocopherol is the optimal level for improving the immune response in the elderly. This enhancement of immune response is associated with increased resistance to infectious diseases in animal models. Results from a recent clinical trial indicate that vitamin E supplementation reduces the risk of acquiring upper respiratory infections in elderly subjects. These findings should be taken into consideration when determining the vitamin E requirement of the elderly. Further studies are needed to determine if vitamin E supplementation is effective in improving the immune response and resistance to infectious diseases in young subjects as well.

Results from cellular and molecular mechanistic studies have shown that immunoregulatory effects of vitamin E are mediated indirectly by reducing the production of suppressive factors such as PGE₂ by macrophages and directly by increasing cell division capacity and IL-2 production by naive T cells.

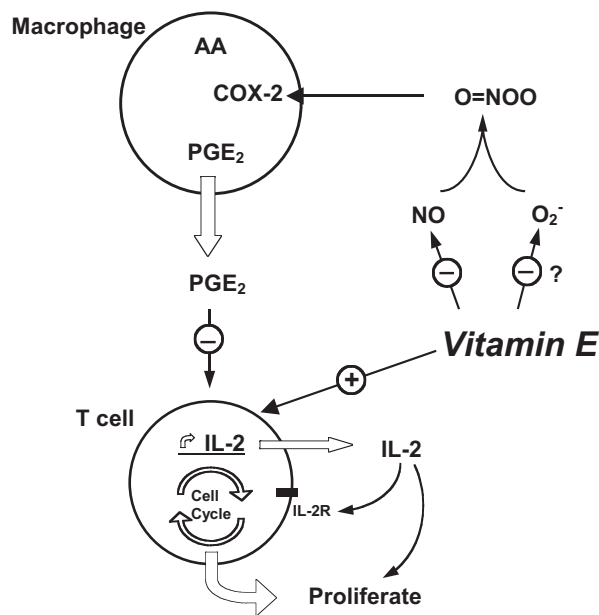


Figure 7. Mechanisms of vitamin E's influence on immune responses. Vitamin E reduces cyclooxygenase (COX) activity in old macrophages by decreasing nitric oxide production, which results in lower production of peroxynitrite in macrophages from old mice. Peroxynitrite has been shown to increase the activity of COX. Decreased COX activity by vitamin E results in decreased production of T-cell-suppressive prostaglandin E₂ (PGE₂) by macrophages, leading to enhancement of T-cell functions. Vitamin E can also directly (independent of its effect of PGE₂) enhance T-cell proliferation and IL-2 production by increasing cell-dividing capability, the number of IL-2+ T cells, and the amount of IL-2 produced per T cell. Vitamin E may also affect cell proliferation by affecting expression of cell-cycle-related molecules. AA, arachidonic acid; COX, cyclooxygenase; PGE₂, prostaglandin E₂; O=NOO, peroxynitrite; NO, nitric oxide; O₂⁻, superoxide; IL-2, interleukin-2; IL-2R, interleukin-2 receptor.

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