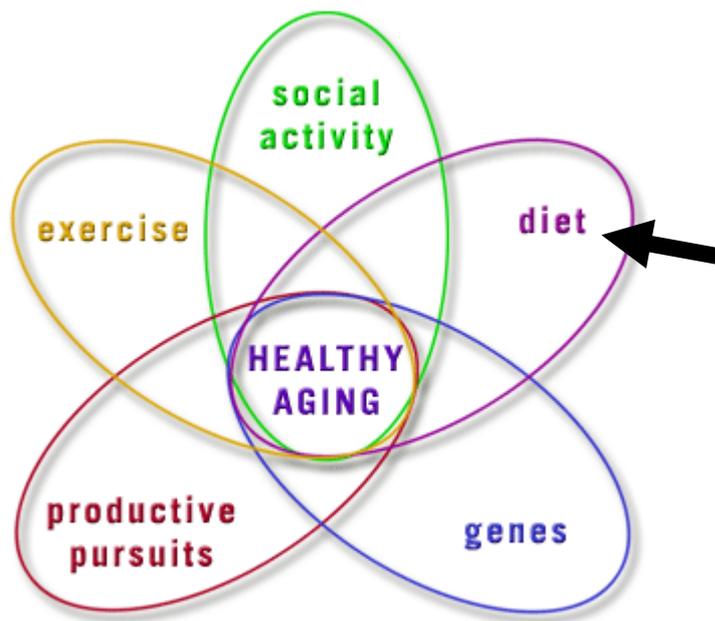


The effects of supplementation of vitamin E on immune senescence



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Abstract

Immune senescence is the deterioration of both the adaptive and the innate immune system with increasing age. This leads to a higher incidence of infections and auto-immune diseases and lower efficiency of vaccination in aged individuals. There is no consensus yet whether nutrition or nutritional supplements can positively affect immune senescence. The effects of supplementation of vitamin E on immune senescence are assessed in this article. Vitamin E has been reported to influence immune responses in the elderly. In addition, promising results have shown that vitamin E might stimulate the immune response to infections and autoimmune diseases and might enhance the efficacy of vaccinations. Mechanistically, vitamin E might stimulate aged T cells directly by various mechanisms or indirectly by inhibiting prostaglandin (PG)E₂ production by macrophages, which might lead to improved immune responses to infections and vaccinations. In addition, vitamin E, as a lipid-soluble antioxidant, might delay the onset of or improve the development of auto-immune diseases by preventing damage from reactive oxygen species (ROS). More research is needed to investigate the effects of vitamin E supplementation to healthy elderly. Future studies should focus on elucidating the mechanisms of vitamin E and identifying responsive subgroups, optimal vitamin E dose and potential adverse effects of long-term supplementation.

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1. Introduction

Aging is a universal process, which affects all living organisms. Many physiological functions deteriorate during aging, including the host defense function of the immune system. Immune senescence is the decline of the immune system with increasing age. Both the innate and the adapted immunity are affected by immune senescence. Until now, most research has focused on the loss of functionality of T cells. However, B cells, natural killer (NK) cells, dendritic cells and monocytes/macrophages also decline in function in the elderly. Immune senescence is associated with a higher risk of infections, cancer and autoimmunity and with less efficiency of vaccinations. In addition, elderly people have increased serum levels of pro-inflammatory cytokines. This subclinical pro-inflammatory state has been called 'inflamm-aging' (Franceschi *et al.*, 2000).

It is not exactly clear which factors are causing the dysfunction of the immune system. Individuals do not age at the same pace, indicating that inter-individual differences affect the process of aging. These differences might be genetic, environmental or a combination of both. Nutrition is an important part of the environment and has an influence on the immune system (Pae *et al.*, 2012). Differences in nutrition might therefore account for differences in aging and immune senescence. However, there is no consensus yet whether and by which mechanisms nutrition or nutritional supplements can positively affect immune senescence.

In this article, the effects of supplementation of vitamin E on immune senescence will be assessed. Vitamin E is an effective chain-breaking lipid-soluble antioxidant in the membrane of cells. The membranes of immune cells are particularly rich in vitamin E, since they experience much oxidative stress due to their high metabolic activity and contain a high amount of poly unsaturated fatty acids (PUFAs) (Pae *et al.*, 2012).

Vitamin E is the collective term for all the tocopherols and tocotrienols that possess the biological activity of α -tocopherol. Eight naturally occurring forms of vitamin E exist, namely α -, β -, γ -, δ -tocopherol and α -, β -, γ -, δ -tocotrienol (see figure 1). The naturally occurring stereoisomer of α -tocopherol is RRR- α -tocopherol, while chemically synthesized α -tocopherol contains eight stereoisomers and is called all-rac- α -tocopherol (formerly dl- α -tocopherol) (Meydani *et al.*, 2005). One International Unit (IU) of vitamin E is defined as 1 mg of the synthetic form for practical purposes (Serafini, 2000).

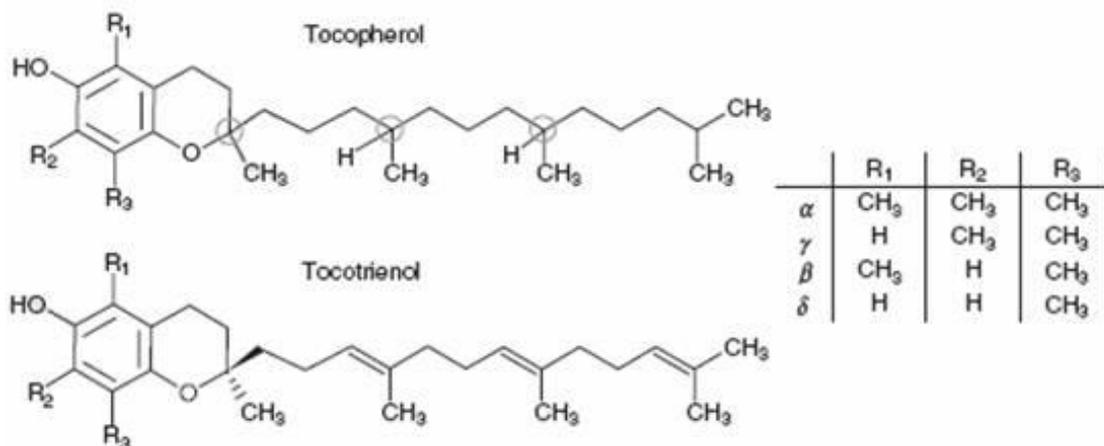


Figure 1. Chemical structure of naturally occurring forms of vitamin E, α -, β -, γ -, δ -tocopherol and α -, β -, γ -, δ -tocotrienol. The circles mark the three chiral centers in tocopherols (Manolescu *et al.*, 2008).

Several studies showed that vitamin E deficiency impairs the immune response (Beharka *et al.*, 1997). Supplementation of vitamin E has been reported to have beneficial effects on immune function (as reviewed by (Pae *et al.*, 2012)) and to influence diseases with a high prevalence in older people. It has been reported that administration of vitamin E decreases the risk of infections in aged rats (Gay *et al.*, 2004, Han *et al.*, 2000, Hayek *et al.*, 1997b) and in the elderly (Meydani *et al.*, 2004), but some studies found no or even a negative effect of vitamin E on the incidence and severity of infections (Graat *et al.*, 2002, Hemila *et al.*, 2002). Furthermore, evidence suggests that vitamin E can increase the response to vaccinations in healthy adults (Mahalingam *et al.*, 2011) and in aged individuals ((Meydani *et al.*, 1997)), even though mixed results have been found in animals (Panousis *et al.*, 2001, Petersson *et al.*, 2010). Studies also suggest an effect of vitamin E on the severity of auto-immune diseases in animal models (De Bandt *et al.*, 2002, Hsieh & Lin, 2005a, Hsieh & Lin, 2005b, Tidow-Kebritchi & Mobarhan, 2001) and in humans (Tidow-Kebritchi & Mobarhan, 2001).

The evidence for a role of vitamin E in decreasing the risk of infections and autoimmunity and increasing the efficacy of vaccinations will be discussed in this article. Finally, some possible mechanisms by which vitamin E could influence immune senescence will be elaborated. Vitamin E has been reported to stimulate T cells directly (Molano & Meydani, 2012) and indirectly (Meydani *et al.*, 2005). Some possible mechanisms behind this vitamin E-mediated stimulation of the response to infections and vaccinations will be explained. Mechanisms by which vitamin E might impact autoimmune diseases will be assessed as well.

2. Aging of the immune system

2.1. Aging of the adaptive immune system

Both the innate and the adaptive system are affected by immune senescence. The normal functions of different immune cells and age-related changes are depicted in figure 2. The changes are indicated by arrows, which are explained in the text below. Regarding the adaptive immune system, much evidence indicates that T cells decline in function with increasing age. A remarkable event during immune senescence is the involution of the thymus, the organ where T cells mature. Thymocyte progenitor cells enter the thymus and go through the double-negative stage and double-positive stage until they become single-positive CD4 or CD8 naïve T cells and migrate to peripheral lymphoid tissues (Rymkiewicz *et al.*, 2012). During aging, the functional thymus cells are replaced by fat cells and the total organ size decreases. This process starts early in life and is almost completed at age 40 - 50 (Weiskopf *et al.*, 2009). Therefore the amount of new CD4+ T cells and CD8+ T cells decreases with age.

Several functional defects have been found in naïve T cells from old people. They have a restricted T cell receptor (TCR) repertory, shorter telomeres, produce less interleukin (IL)- 2 and are less able to expand and differentiate into effector cells (Weiskopf *et al.*, 2009). The decreased IL-2 production might be caused by defects in TCR signaling. IL-2 is required for T cell expansion, which is necessary for an effective immune response. It has been suggested that naïve T cells decline in function, because they must live longer to maintain the total number of peripheral T cells, which allows mutations to accumulate (Pae *et al.*, 2012).

In the elderly the balance shifts from mainly naïve to mainly memory and effector cells. The accumulation of memory CD8+ cells correlates with the impaired antibody response after vaccination of old people (Weiskopf *et al.*, 2009). Old effector cells undergo phenotypic changes and for instance lose CD28, the most important co-stimulatory molecule that complements the TCR (Weng *et al.*,

2009). CD28 is important for the activation, proliferation and survival of T cells and the loss of CD28 contributes to impaired T cell function. CD28⁻ T helper cells are less able to assist antigen-specific cytotoxic CD8 T cell function (Pae *et al.*, 2012). The loss of CD28 also coincides with lower expression of CD40L in T helper cells. CD4⁺ CD28⁻ cells are therefore less able to induce B cell proliferation and antibody production (Weiskopf *et al.*, 2009). This contributes to the impaired antibody response to infections and vaccinations in elderly people. Besides, CD28⁻ T cells promote the survival of autoreactive T cells, suppress antigen-presenting by dendritic cells and produce interferon (IFN)- γ , which might contribute to the pro-inflammatory state observed in old people (Pae *et al.*, 2012).

The balance between different subsets of CD4⁺ cells also changes with increasing age, particularly the T helper 1 (Th1) – T helper 2 (Th2) balance. Th1 cells stimulate the defense against intracellular pathogens and produce IFN- γ and IL-2. Th2 cells are involved in the defense against extracellular pathogens as well as allergies and asthma and they produce IL-4 and IL-10 (Zhu & Paul, 2008). Lymphocytes of the elderly produce less IFN- γ and more IL-4 and IL-10 than lymphocytes of young subjects (Rink *et al.*, 1998). This indicates that the Th1- Th2 balance shifts to the Th2 side.

Thymic involution can also decrease the output of regulatory T cells (Tregs). Suppression by Tregs has been found to decrease after the age of 50, which might play a role in increased inflammation and autoimmunity with advanced age (Tsaknaridis *et al.*, 2003).

The humoral immune response declines with increasing age too. On one hand, this is caused by defective interactions between B cells and other immune cells. B cell activation requires assistance from other immune cells. As previously mentioned, aged T helper cells express less CD40L and are less able to assist B cell proliferation and antibody production. Furthermore, follicular dendritic cells stimulate B cells from aged individuals 70% less efficiently than B cells from young adults (Weiskopf *et al.*, 2009).

On the other hand, functional defects in B lymphocytes of aged individuals have been observed. The output of pre- and pro- B cells from the bone marrow of senior individuals is decreased, comparable to the reduced output of naïve T cells from the thymus (Pae *et al.*, 2012). As in T cells, the total number of B cells in the periphery does not decline, but the portion of naïve B cells, characterized by the absence of CD27, is lowered in the elderly (Weiskopf *et al.*, 2009). Aged B cells express less CD27 and CD40, important co-stimulatory molecules. In aged individuals, memory B cells with a lower susceptibility to apoptosis accumulate and clones of certain B cell specificities expand. This might lead to a reduced B cell repertoire, which possibly explains the impaired response to vaccination in elderly individuals (Weiskopf *et al.*, 2009). Serum antibody levels of aged persons remain stable, but the quality of the immune response is impaired by lower levels of specific and effective antibodies, higher levels of non-specific antibodies, lower affinity and less isotype class switching to IgG in response to vaccination (Pae *et al.*, 2012). Besides, levels of autoreactive antibodies are increased during aging, which might explain the higher risk of autoimmune diseases in aged individuals (Lindstrom & Robinson, 2010).

2.2. Aging of the innate immune system

There is also evidence that the aged innate immune system declines in function. The innate immune system provides the first line of defense against pathogens and alarms the adaptive immune system. It consists of several cell types and soluble mediators such as cytokines. In elderly individuals, a decreased function of epithelial barriers of several organs has been observed, which enables pathogens to invade mucosal tissues (Weiskopf *et al.*, 2009). Some studies suggest that aging is associated with a chronically upregulated pro-inflammatory state called 'inflamm-aging' (Franceschi

et al., 2000). Particularly, aged individuals have higher serum levels of acute-phase proteins and pro-inflammatory cytokines such as IL-6, IL-1 β and Tumor Necrosis Factor (TNF)- α than young subjects (Pae *et al.*, 2012).

Neutrophils have a short lifespan and help in the defense against bacterial and fungal infections and during acute inflammation. The number of neutrophils remains the same during aging but neutrophils from aged individuals are reported to be less capable of phagocytosis of opsonized bacteria such as *Staphylococcus aureus* (Wenisch *et al.*, 2000). This might explain the higher susceptibility for *S. aureus* in the older population. Besides, neutrophils from the aged express less of the Fc γ receptor CD16 and the superoxide production mediated by the Fc receptor is significantly reduced (Weiskopf *et al.*, 2009). There is also evidence for reduced chemotaxis and intracellular killing (Pae *et al.*, 2012).

Natural Killer (NK) cells play a role in the recognition and lysis of virus-infected and tumor cells. The number of NK cells remains stable or even increases during aging. However, it has been reported that the lytic activity and production of chemokines and cytokines per cell is decreased. Decreased NK activity in elder individuals might lead to a higher incidence of cancer and viral infections (Pae *et al.*, 2012).

Macrophages are 'pathogen sensors', and help initiating inflammatory responses, eliminating pathogens and repairing damaged tissue (Weiskopf *et al.*, 2009). They also regulate the adaptive response and present peptides to T cells. Aging macrophages seem to express less MHC II molecules, which might inhibit CD4 $^{+}$ responses. Macrophages from senior individuals also produce less superoxide anions and chemokines and have an impaired phagocytic function (Weiskopf *et al.*, 2009). Besides, macrophages from aged subjects produce more prostaglandin (PG)E $_2$, which inhibits T cells (Pae *et al.*, 2012).

Dendritic cells (DC) are the link between the innate and the adaptive immune system. DC capture and process antigens and migrate to the lymphoid organs during their maturation. There they present antigens to T cells. It is still largely unclear how aging affects DC, but studies suggest that aging might impair micropinocytosis (a form of endocytosis), phagocytosis and migration to lymphoid organs (Weiskopf *et al.*, 2009).

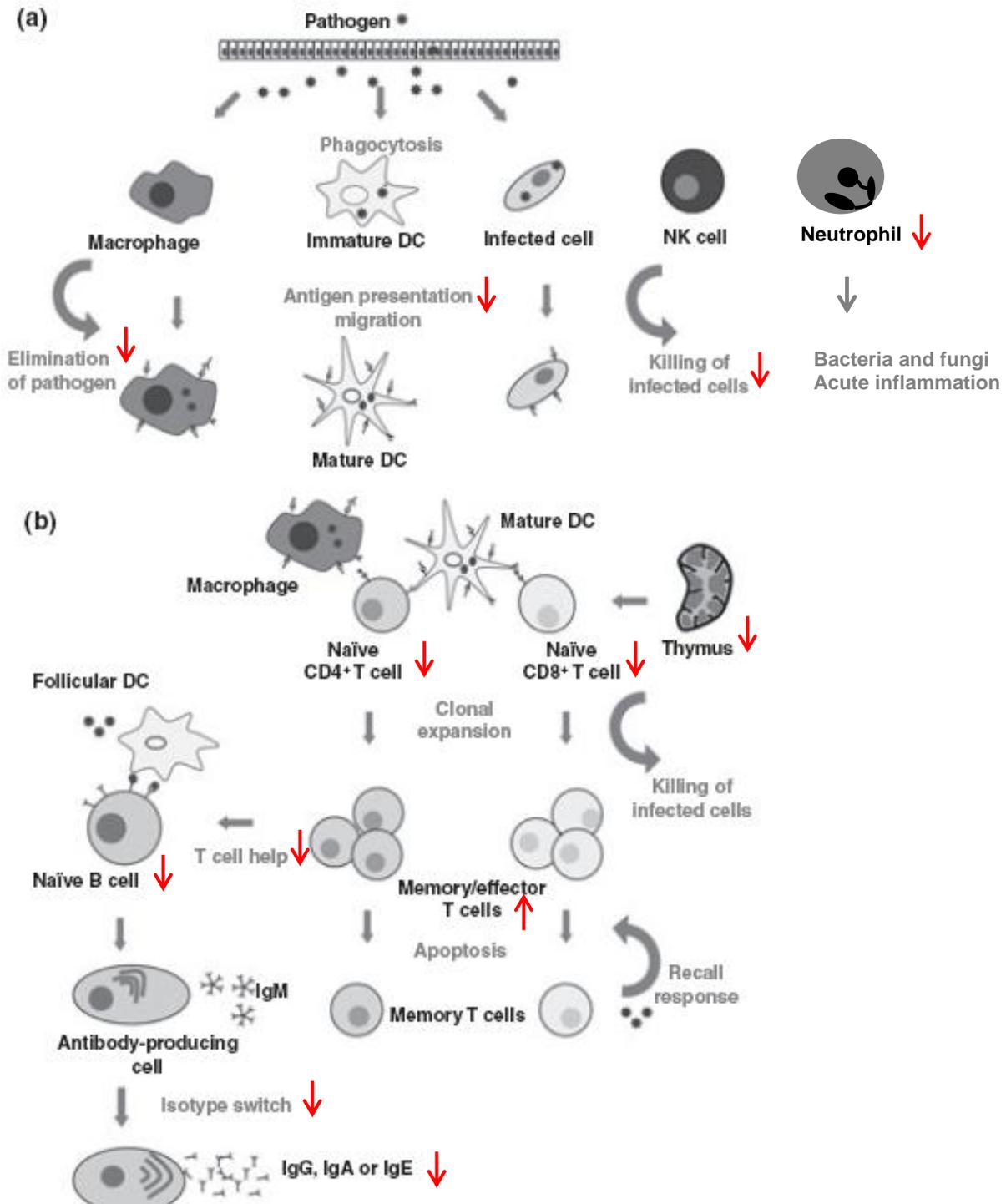


Figure 2. The innate (a) and adaptive (b) immune response and age-related changes. (a) Innate immune responses are induced at the site of infection by invading pathogens. Antigen presenting cells, such as macrophages and dendritic cells, take up the pathogen and present it in lymph nodes. Natural killer cells recognize and eliminate infected cells. Neutrophils are involved in the defense against bacterial and fungal infections and in acute inflammatory responses. (b) Antigen-presenting cells induce activation and clonal expansion of naïve CD4+ and CD8+ T cells, which differentiate into memory T cells and effector T cells. Antigen and CD4+ T cells induce the activation and differentiation of naïve B cells, which differentiate into memory B cells and antibody-secreting cells. Memory T and B cells in the blood and lymph nodes provide long-term immunity. The red arrows indicate the age-related changes in number or function of immune cells, which are described in the text. Adapted from Weiskopf et al. (Weiskopf *et al.*, 2009).

3. Higher incidence of diseases among the elderly

It has been reported several times that aged subjects have a higher incidence and mortality of infections. For instance in 2004, 4357 people died from 'other intestinal infections' in the US. The majority, i.e. 3937 were aged 65 years and older. 418 out of 657 persons who died from tuberculosis were aged 65 years and older; 944 out of 1100 deaths from influenza were over 65 years old; and 35816 out of 58564 deaths from pneumonia were aged 65 years and older (Minino *et al.*, 2007).

There is also much evidence that elderly individuals exhibit lower responses to vaccination than young people. Reber and colleagues for example evaluated studies about the antibody response of elderly individuals to influenza vaccination. They quoted several studies that showed that influenza vaccine effectiveness ranged from 47% to as high as 86% in young people, but in older individuals influenza vaccines were estimated to be 17% to 53% effective. They concluded that the vaccination response of aged subjects is decreased compared to young people (Reber *et al.*, 2012).

Aged individuals have a higher risk of developing autoimmune diseases. The tendency for autoimmune reactivity usually increases with age and age is a risk factor for several autoimmune diseases in which the adaptive immunity plays an important role (Goronzy & Weyand, 2012). Rheumatoid arthritis for instance occurs mostly in older adults in both genders (Doran *et al.*, 2002).

4. Influence of vitamin E on diseases

4.1. Vitamin E and infections

Several animal studies have been conducted to investigate the effect of vitamin E on infections in aged individuals. Hayek *et al.* reported that aged mice (22 months) fed 30 parts per million (ppm) vitamin E for 6 weeks had significantly higher lung influenza titers than young mice (4 months) fed 30 ppm vitamin E, indicating a decrease in anti-viral immunity (Hayek *et al.*, 1997b). Administration of 500 ppm vitamin E in aged mice significantly reduced lung influenza titers compared to aged mice fed 30 ppm vitamin E. Young mice fed 500 ppm vitamin E showed only a reduction in influenza titers on day 5, but on that day the decrease in aged mice was higher than in young mice. NK cell activity significantly decreased in old mice fed 30 ppm vitamin E and administration of 500 ppm vitamin E to aged mice tended to enhance NK cell activity to the level of young mice fed 30 ppm vitamin E. Serum anti influenza hemagglutinin titers were significantly lower in aged mice compared to young mice. Supplementation of vitamin E tended to increase antibody titers, but not significantly. Hayek and colleagues suggested that vitamin E might restore NK cell activity, improve the antioxidant status of aged mice or modulate cytokines involved in the pathogenesis of influenza virus (Hayek *et al.*, 1997b).

Han and colleagues investigated whether supplementation of vitamin E influenced these cytokines (Han *et al.*, 2000). Just like Hayek *et al.*, they reported higher pulmonary influenza titers in aged mice (24 months) compared to young mice (6 months). Again, supplementation of 500 ppm vitamin E for 8 weeks reduced the age effect (see figure 3). IL-2 and IFN- γ production was significantly lower in aged mice than in young mice, but supplementation of 500 ppm vitamin E increased IL-2 and IFN- γ production. Macrophages stimulated with lipopolysaccharide from old mice on the control diet produced more prostaglandin (PG)E₂ than macrophages from old vitamin E supplemented mice and both young groups. Young respectively old mice fed 500 ppm vitamin E had significantly lower IL-1 β and TNF- α production respectively lower TNF- α production than their age-matched controls. According to the authors, this indicated that vitamin E possibly mediates the

decrease in influenza viral titer through enhancement of Th1 cytokines, potentially by decreasing the PGE₂ production (Han *et al.*, 2000).

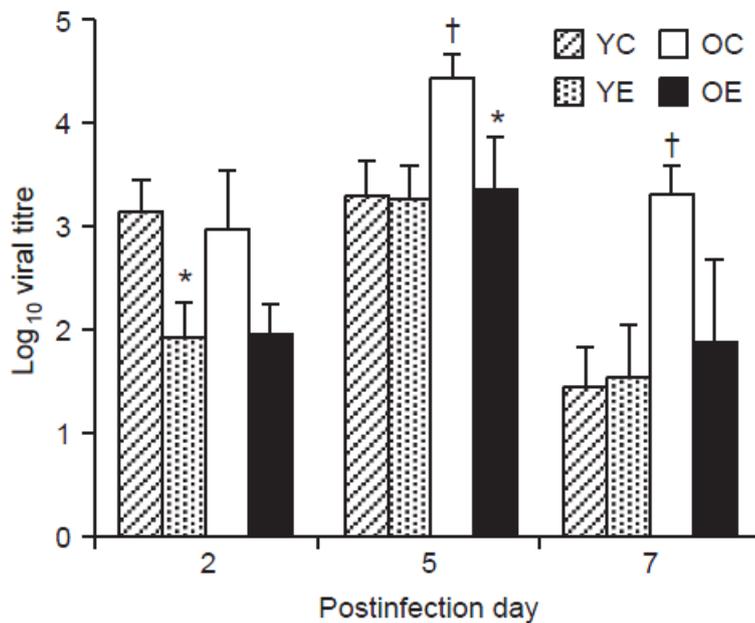


Figure 3. Pulmonary viral titres after infection with influenza virus in young (6 months) and old (24 months) mice fed diets containing 30 ppm (control) or 500 ppm (supplemented) of dl- α -tocopheryl acetate (vitamin E) for 8 weeks. Values are expressed as mean + SEM, n=6±9. † Significantly higher than young mice fed the control diet, at P<0.05. *Significantly different from mice of the same age fed the control diet, at P<0.05. YC, young mice on control diet; YE, young mice on vitamin E-supplemented diet; OC, old mice on control diet; OE, old mice on vitamin E supplemented diet (Han *et al.*, 2000).

These studies indicated that vitamin E reduces influenza infections in aged mice. Gay *et al.* investigated whether supplementation of vitamin E influenced secondary bacterial infection after influenza infection in young and aged mice fed 30 or 500 ppm vitamin E (Gay *et al.*, 2004). Mice fed control diet and primed with influenza had significantly increased *Staphylococcus aureus* lung levels. Age did not significantly influence *S. aureus* infection alone or secondary infection with *S. aureus* after influenza infection. Supplementation of vitamin E did not affect *S. aureus* infection alone, but inhibited the priming effect of influenza on *S. aureus*. However, Gay and colleagues did not investigate the potential mechanism by which vitamin E could exert this effect (Gay *et al.*, 2004).

Unfortunately, these promising results have not always been reproduced in humans. Supplementation of vitamin E (0,200 or 400 mg/day α -tocopherol acetate) in a small trial (N=103) did not influence the incidence of infections in the lungs, urinary tract or other infections in institutionalized patients aged 24-104 years (Harman, D. & White Miller, R., 1986). In contrast, a retrospective study among self-sufficient healthy individuals aged 60 and over (N=209) showed that subjects with high vitamin E plasma concentrations reported less infections in the past three years than those with medium or low levels (Chavance *et al.*, 1989).

More recently, Graat *et al.* studied the effect of maximum 15 months of daily multivitamin (25-50% of recommended daily allowances) and vitamin E supplementation (200 IU/day dl- α -tocopherol acetate) on the incidence and severity of acute respiratory tract infections in Dutch non-institutionalized individuals aged 60 years and older (N=652) (Graat *et al.*, 2002). Remarkably, they found a negative effect of vitamin E. Incidence did not differ significantly between vitamin E and non-vitamin E, but severity was worse in the vitamin E group: they experienced longer duration of illness

duration, more symptoms, and a higher frequency of fever and restriction of activity. The adverse effects of vitamin E could be explained by the low frequency (1.3 %) of patients who had suboptimal concentrations of vitamin E at baseline. The authors suggested that high levels of vitamin E might have adverse effects in the elderly by acting as pro-oxidants (Graat *et al.*, 2002).

Meydani and colleagues assessed the effect of 1 year of daily multivitamin (50% of recommended dietary allowances) and vitamin E supplementation (200 IU/ day α -tocopherol) on the incidence and duration of respiratory tract infections in American institutionalized individuals aged 65 and older (N=617)(Meydani *et al.*, 2004). Vitamin E had no effect on the incidence or duration of all respiratory tract infections. In contrast, less persons in the vitamin E group had one or more respiratory tract infections or upper respiratory tract infections. A post hoc subgroup analysis showed that vitamin E group participants who completed the study had significantly less common colds and a 20% lower risk of having a cold compared to the placebo group (Meydani *et al.*, 2004).

Hemila *et al.* used data from the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study to assess the influence of 5-8 years (median 6.1 years) of supplementation of vitamin E (50 mg/day) and beta-carotene (20 mg/day) on the incidence of common cold infections in Finnish male smokers, aged 50-69 years at the start of the trial (N=29133) (Hemila *et al.*, 2002). Long-term supplementation of vitamin E had no overall effect on the incidence of common colds as assessed by self reports. However, vitamin E supplementation slightly reduced the risk of colds among subjects aged 65 years or older. Smoking and residential area influenced the effect of vitamin E among these older subjects: the incidence of common cold was reduced only among participants living in cities and smoking 5-14 cigarettes a day (Hemila *et al.*, 2002). The same authors later analyzed whether age, residential neighborhood and smoking modified the effect of vitamin E on the incidence of the common cold (Hemila *et al.*, 2006). They found a reduced incidence of the common cold in subjects who live in cities, smoke less than 15 cigarettes a day and are aged 69 years or older. Vitamin E however increased the incidence of the common cold in subject who live away from cities and smoke more than 15 cigarettes a day (Hemila *et al.*, 2006).

Using the same cohort, Hemila *et al.* also analyzed whether vitamin E or beta-carotene influenced the incidence of hospital-treated pneumonia in older male smokers. Supplementation of vitamin E had no overall effect, but decreased the risk of pneumonia among subjects who started smoking at the age of 21 years or older (Hemila *et al.*, 2004). When Hemila *et al.* analyzed the effect of body weight on the influence of vitamin E on the incidence of pneumonia in participants who started smoking at age <21 years, they found that vitamin E supplementation had no effect in participants with a body weight of 70- 89 kg. However, vitamin E increased the risk of pneumonia in participants who weighed less than 60 kg or who weighed more than 100 kg with high dietary vitamin C intake (>90 mg/day) (Hemila & Kaprio, 2008a). Hemila and colleagues also examined the effect of age of smoking initiation, level of smoking at baseline of trial and exercise during leisure time on the influence of vitamin E on the incidence of pneumonia. Vitamin E decreased the risk of pneumonia by 69% among participants who smoked the least and exercised during leisure time. In the group that smoked the most and did not exercise, vitamin E increased the pneumonia risk by 79% (Hemila & Kaprio, 2011).

Furthermore, the same authors looked at the incidence of hospital-treated tuberculosis in the same cohort. Supplementation with vitamin E had no overall effect. Vitamin E increased the risk of tuberculosis in the first year after the start of supplementation in participants with high dietary vitamin C intake levels (>90 mg/day) and smoking 20 or more cigarettes a day at baseline (see figure 4). This increased incidence was not caused by new infections in the participant but by reactivation of

old infections. Most of the participants were born in the 1920s and 1930s and in the 1930s about 80% of the Finnish population was infected by *Mycobacterium tuberculosis* (Hemila & Kaprio, 2008b).

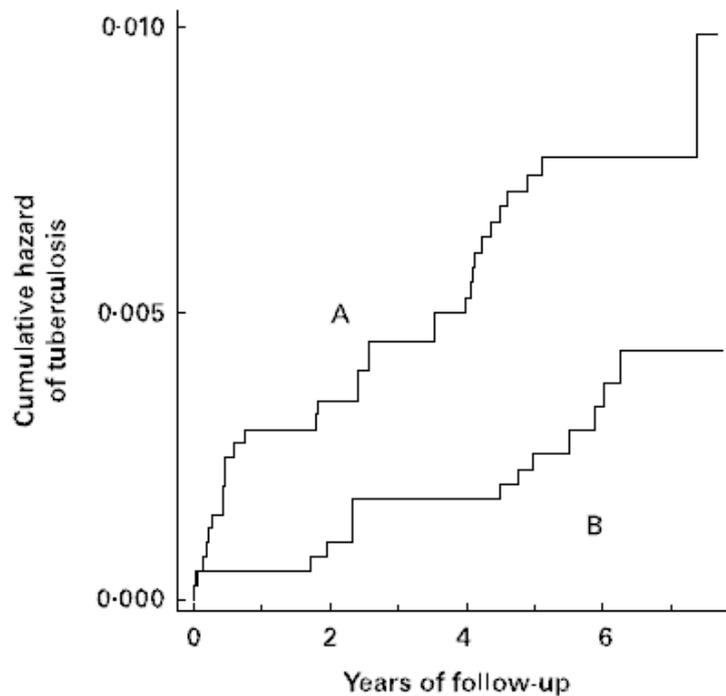


Figure 4. Vitamin E supplementation and tuberculosis risk in Alpha-Tocopherol Beta-Carotene Cancer Prevention Study participants smoking twenty or more cigarettes a day with dietary vitamin C intake higher than 90 mg/day (n 8172). Nelson–Aalen cumulative hazard functions for vitamin E (A) and no-vitamin E (B) groups are shown. Each step represents one case of tuberculosis (Hemila & Kaprio, 2008b).

It is difficult to compare these studies with each other. Graat et al. studied free-living, probably less frail elderly individuals, while Meydani and colleagues investigated institutionalized aged subjects. Institutionalized elderly have a higher risk of infections, since they share sources of air, food, water, and medical care, which facilitates both the introduction and subsequent transmission of certain infectious agents among vulnerable residents (Strausbaugh *et al.*, 2003). The subjects in the studies of Hemila et al. were all male. In addition, the participants all smoked, and smoking might influence the duration of symptoms of the common cold (Huttunen *et al.*, 2011). These results are therefore hard to compare with the results from studies among non-smoking elderly individuals of both sexes. Besides, it is difficult to generalize the outcome of these clinical trials for the whole elderly population.

There were differences in the study design as well, such as data analysis and diagnostic methods. Graat et al. used self reports of the elderly, while infectious disease specialists documented the clinical outcomes in the study of Meydani et al. and Hemila et al. (except in the study of respiratory infections by the latter). However, these self reports were tested by home visits, telephone calls from nurses, and microbiological and serological testing in subsets of patients (Graat *et al.*, 2002). Besides, all groups were assessed by self reports, indicating that the differences between them are still valid. Graat et al. also did not differentiate between types of infection or between respiratory tract infections and allergies, and might have overlooked any effect of vitamin E on upper respiratory tract infections (Meydani *et al.*, 2004). Furthermore, Hemila et al. did not measure α -tocopherol levels at baseline or other time-points, unlike Graat et al. and Meydani et al. It

is therefore not clear how many participants had subclinical concentration of vitamin E at baseline and whether the supplementation of 50 mg/day, which is lower than in the study of Graat et al. and Meydani et al., significantly influenced the α -tocopherol levels. However, calculated dietary intake of vitamin E did not modify the effect of vitamin E supplementation on the incidence of pneumonia (Hemila *et al.*, 2004).

Even though several studies have reported a positive influence of vitamin E on the immune response, also in aged individuals (as recently reviewed by Pae et al.) (Pae *et al.*, 2012), these changes might not always translate into hard end-points such as infections, as the conflicting results of Graat et al. and Meydani et al. indicate. The findings of Hemila et al. all suggest that the effect of supplementation of vitamin E may be harmful to some subgroups and helpful to others, depending on individual characteristics. Belisle and colleagues, who analyzed the same data as Meydani et al., suggested that this individual variation in the response to vitamin E might depend on sex and *IL-10* genotypes (Belisle *et al.*, 2010). All in all, promising results of supplementation of vitamin E on infections in healthy elderly have been reported. However, since the effect of vitamin E on infections is not clear for the entire aged population, future research should focus on identifying the subgroups vitamin E might affect beneficially.

4.2. Vitamin E and vaccinations

The effect of vitamin E on vaccination efficacy has been tested in animal studies with ambiguous results. Some studies reported positive effects of vitamin E on antibody titers after vaccination in healthy animals (Barber *et al.*, 1977, Muir *et al.*, 2002, Sahoo & Mukherjee, 2002, Zhao *et al.*, 2011) or only in immunocompromised animals (Sahoo & Mukherjee, 2002), while others reported positive effects of vitamin E in healthy animals in combination with selenium (Droke & Loerch, 1989, Kandil & Abou-Zeina, 2005, Panousis *et al.*, 2001) or arginine (Ruiz-Feria & Abdokalykova, 2009). In contrast, some studies did not find an effect of vitamin E on antibody titers in healthy animals (Daniels *et al.*, 2000, Hatfield *et al.*, 2002, Petersson *et al.*, 2010) and aged animals (Petersson *et al.*, 2010). In these studies, several different animal species, ages of animals, vaccines and vitamin E doses were used.

A limited numbers of studies have assessed the effect of supplementation of vitamin E on vaccination efficacy in humans. Mahalingam and colleagues investigated the effect of 56 days supplementation of vitamin E (400 mg/day tocotrienol-rich fraction) on the antibody titers against tetanus toxoid (TT) vaccine in healthy Asian women aged 18-25 years (N=108). The participants were given three standard meals a day to minimize the effect of diet on overall vitamin E absorption. Supplementation of vitamin E increased the anti- TT IgG production (see figure 5). The vitamin E supplemented group also showed increased production of IFN- γ and IL-4 and decreased production of IL-6 compared to the placebo group (Mahalingam *et al.*, 2011).

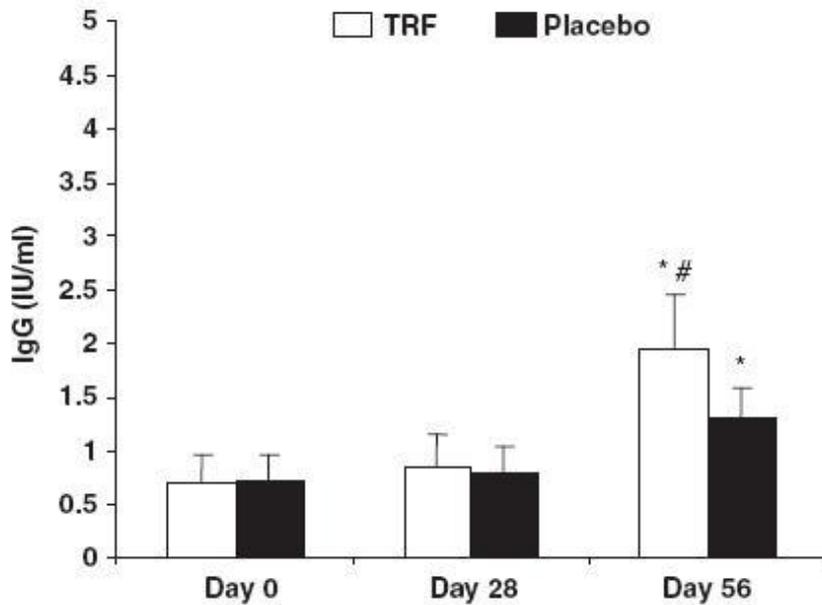


Figure 5. Anti-tetanus toxoid (TT) IgG concentrations in the plasma of 400 mg/day TRF- or placebo-supplemented healthy female volunteers aged 18-25 years. There was significant difference in the production of IgG between day 0 and day 56 * ($P < 0.001$). There was also a Day \times Group interaction effect $\#$ ($P < 0.001$), tested by the Greenhouse-Geisser method (Mahalingam *et al.*, 2011).

In the elderly, very few studies have investigated the direct effect of vitamin E on the response to vaccination. Gardner *et al.* assessed the association between the immune response to influenza vaccine and plasma concentration of α -tocopherol of healthy elderly (Gardner *et al.*, 2000). They evaluated the immune responses in American healthy seniors from continuing care retirement communities (N=61, mean age: 81 years) and young adults (N=27, mean age: 27 years). Post-vaccination titers of elderly were lower than those of young people, even though elderly had higher plasma α -tocopherol levels. Plasma levels of α -tocopherol and other micronutrients were also comparable for elderly with or without intact antibody responses after vaccination. The authors suggested that differences in plasma levels of α -tocopherol and other micronutrients are not necessary to observe decreased immune responses to vaccination of healthy elderly compared to young people and are not associated with differences in antibody responses among healthy elderly (Gardner *et al.*, 2000).

Fülöp *et al.* investigated the association between immune response to influenza vaccination and nutritional status of institutionalized elderly subjects (N=23) (Fulop *et al.*, 1999). Eleven elderly subjects were responsive and 12 were not. Several nutritional parameters, including vitamin E levels, as well as the dehydroepiandrosterone (DHEA) level and the cellular immune response were significantly lower in the nonresponsive group compared to the responsive group. According to these authors, they might be associated with responsiveness to influenza vaccine of institutionalized elderly subjects (Fulop *et al.*, 1999).

Some studies assessed the effect of micronutrient supplementation on the immune response to vaccines. These studies found limited (Wouters-Wesseling *et al.*, 2002) or no influence (Bunout *et al.*, 2004, Girodon *et al.*, 1999) of supplements on immune response to vaccination against influenza and pneumococcus. Even though all supplements contained a small amount of vitamin E, the composition of the used nutritional supplements differed and they also contained many other ingredients. This hinders comparisons between the studies. In addition, the effects found in these

studies cannot easily be ascribed to vitamin E.

Meydani et al. conducted the only randomized controlled trial in healthy elderly subjects with vitamin E that also looked at response to vaccination. Free-living healthy elderly aged 65 or older (N=88) consumed vitamin E (60, 200 or 800mg/day) for 235 days. Meydani and colleagues observed several immunological changes after supplementation with vitamin E. Subjects consuming 200 mg/day of vitamin E had a higher increase in antibody titer to hepatitis B compared to placebo, 60 mg/day and 800 mg/day. The 200 mg/day group had a significant increase in antibody titer to tetanus vaccine as well. Subjects with the highest serum α -tocopherol concentration (>48.4 micromol/L [2.08 mg/dL]) after supplementation had higher antibody response to hepatitis B. Vitamin E supplementation had no effect on antibody titer to diphtheria and pneumococcus and did not affect immunoglobulin levels or levels of T and B cells.

Overall, animal studies of the effect of vitamin E on vaccinations have found ambiguous results. Very few studies have been conducted in elderly individuals and among them was just one randomized controlled trial, which did find a positive effect of vitamin E. Despite promising results, more randomized controlled trials with healthy seniors are needed to investigate whether the results of the small study of Meydani et al. can be confirmed. Importantly, it has been suggested that antibody titers correlate poorly with protection in the elderly and that T cell responses seem to correlate better with immune protection in older adults (Reber *et al.*, 2012). Future studies should also take T cell responses into consideration.

4.3. Vitamin E and autoimmune diseases

Autoimmune diseases also affect the elderly more often than young persons. Several animal studies have reported a link between vitamin E and autoimmune diseases. Pesillo and colleagues found lower serum vitamin E concentrations and higher lipid peroxidation in dogs with immune-mediated hemolytic anemia compared to healthy control dogs, indicating increased oxidative stress (Pesillo *et al.*, 2004). Some animal studies have reported an effect of vitamin E on the onset of autoimmune diseases. Vitamin E did not reduce incidence of Type 1 diabetes mellitus in non-obese diabetic mouse at the age of 30 weeks, but it did significantly delay the onset of the disease (Beales *et al.*, 1994). In addition, four months of 500 IU/ kg diet vitamin E enhanced the effects of a fish oil- enriched diet in autoimmune- prone MRL/lpr mice (a model for rheumatoid arthritis) and might delay the onset of autoimmunity (Venkatraman & Chu, 1999).

Furthermore, Weimann et al. found that 0.4 mg/day vitamin E had a beneficial effect on the development of systemic lupus erythematosus (SLE)- like autoimmune disease in MRL/lpr mice (Weimann & Hermann, 1999). Supplementation of 550 mg/kg diet vitamin E in NZB/W F1 mice (a model for lupus) fed oxidized oil diet diminished autoantibody production and inflammation and thus delayed the development of autoimmune disease and prolonged the lifespan of NZB/W mice. These data indicated that vitamin E might not only act as an antioxidant, but also as an immunomodulator in autoimmune-prone mice under oxidative stress (Hsieh & Lin, 2005a). However, the same authors also reported that low doses of vitamin E had positive effects on the survival of MRL/lpr mice, whereas a high dose of 500 mg/kg diet vitamin E had negative effects on survival (Hsieh & Lin, 2005b). They explained this by the opposite effect of high and low doses of vitamin E on the Th1/Th2 cytokines balance. According to them, extra high vitamin E intake should be avoided unless under extra oxidative stress (Hsieh & Lin, 2011).

Evidence for a link between vitamin E and autoimmune diseases has been reported in several patient studies of autoimmune diseases as well. Patients with autoimmune hepatitis have lower

serum vitamin E levels, elevated lipid peroxidation and a decreased antioxidant capacity compared to controls (Pemberton *et al.*, 2004, Saron *et al.*, 2009). However, a recent review found no convincing evidence to support or refute vitamin E supplementation to treat (among others) autoimmune liver diseases (Bjelakovic *et al.*, 2011).

Furthermore, one year of supplementation of vitamin E in combination with vitamin C in children suffering from Type 1 Diabetes mellitus improved diabetes control and reduced markers of oxidative stress in comparison with non-supplemented diabetic children (Varvarovska *et al.*, 2004). In addition, 200- 1200 IU/ day vitamin E was reported to improve dermatological symptoms in scleroderma patients, even though it did not improve non-dermatological aspects of the disease (Gaby, 2006). Moreover, Maeshima *et al.* reported that supplementation of 150-300 mg/day vitamin E and prednisolone in SLE patients reduced autoantibody production compared to SLE patients who only took prednisolone, via an antioxidant- independent pathway (Maeshima *et al.*, 2007). It has also been reported that supplementation of high doses of vitamin E reduced pain in rheumatoid arthritis patients (Tidow-Kebritchi & Mobarhan, 2001).

These findings suggest that vitamin E plays a role in autoimmune diseases, probably by mediating the redox balance. Some studies suggest that vitamin E might delay the onset or improve the development of certain autoimmune diseases, but evidence supporting this is limited. Whether vitamin E decreases the risk or severity of autoimmune diseases in the elderly is however not entirely clear yet. The previously mentioned studies did not especially investigate effects of vitamin E on immune responses in the elderly. Randomized controlled clinical trials that assess the effect of vitamin E on the incidence and mortality of autoimmune diseases should be executed.

5. Possible mechanisms of vitamin E

5.1. Direct effects on T cells

Supplementation of vitamin E to aged individuals to influence infections, vaccinations and autoimmune diseases seems promising. Vitamin E has been reported to support the aged immune system (as reviewed by (Pae *et al.*, 2012)). There are several mechanisms by which vitamin E could influence the immune system and in this case infections and vaccinations. Most of them focus on the impact of vitamin E on aged T cells, since they produce less IL-2 and are therefore less able to elicit an effective immune response. Some possible mechanisms by which vitamin E can influence T cells, both directly and indirectly, will be described (see figure 6). Furthermore, a possible mechanism of the impact of vitamin E on the inappropriately strong immune reaction against self pathogens in autoimmune diseases will be assessed.

Vitamin E has been reported to directly influence several functional defects in aged T cells. Supplementation of vitamin E positively influenced critical signaling/adaptor proteins of the TCR and enhanced immune synapse formation (see figure 6), which is important for T cell activation and proliferation (Marko *et al.*, 2007).

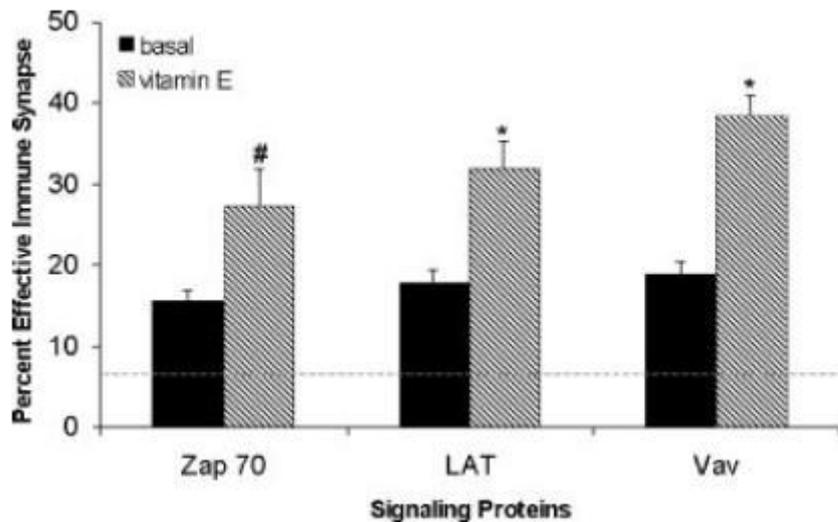


Figure 6. In vivo vitamin E supplementation of old mice increases redistribution of signaling molecules (Zap 70, LAT and Vav) to the immune synapse. Old mice were fed diets containing 30 ppm or 500 ppm vitamin E (dl- α -tocopheryl acetate) for 8 wk. Bars show mean \pm SEM of percentage of CD4 T cells forming immune synapse, which were positive for the indicated signaling molecule. * $p < 0.05$; # $p < 0.1$ by Kruskal- Wallis one-way ANOVA. The broken line represents the fraction (average 8%) of conjugates that scored false positives using rabbit IgG in place of the primary Ab (Marko *et al.*, 2007).

Vitamin E also restored IL-2 production, the expression of several cell cycle control proteins and proliferation of CD4⁺ cells (Molano & Meydani, 2012). The mechanisms by which vitamin E does this are not fully understood. Reactive oxygen species (ROS) are known to be involved in the aging of T cells. Vitamin E is a lipophilic antioxidant capable of preventing the propagation of polyunsaturated fatty acid peroxidation and might therefore neutralize ROS- associated damage of membrane lipids or associated adapter proteins/kinases (Molano & Meydani, 2012). Another possibility is that vitamin E modulates certain properties of lipid rafts and indirectly influences the enzymes involved in signal transduction or phosphatases which downmodulate these enzymes (Molano & Meydani, 2012). Furthermore, vitamin E might also bind to a possible 'vitamin E receptor', which transduces its activities. Some tocopherols resemble agonists of the peroxisome proliferator-activated receptor (PPAR)- γ . Low and high dose of vitamin E have been reported to oppositely influence the PPAR- γ receptor; low concentration increases IL-2 gene expression, while high dose has the opposite effect, associated with the upregulated PPAR- γ pathway (Hsieh *et al.*, 2006). In addition, a microarray analysis suggested that vitamin E had a significant effect on the expression of various genes involved in the cell cycle control in T cells from aged mice (Han *et al.*, 2006). All in all, it is possible that some effects of vitamin E could involve direct or indirect interactions with intracellular receptors (Molano & Meydani, 2012).

5.2. Prostaglandin production by macrophages

Aged macrophages have been found to produce more prostaglandin (PG) E_2 . PGE $_2$ is necessary for T cell function, but it inhibits T cell proliferation at higher concentrations. PGE $_2$ inhibits Th1 cytokine IL-2 and IFN- γ production as well as IL-2 receptor expression. Depending on the conditions of stimulation, PGE $_2$ increases or has no effect on the production of Th2 cytokines IL-4, IL-5, and IL-10. Hence, PGE $_2$ promotes a shift in T-cell cytokine production from Th1 type to Th2 type (Meydani *et al.*, 2005). Vitamin E inhibited the increased PGE $_2$ production of aged macrophages and improved T cell proliferation and IL-2 production in vitro and in vivo (Meydani *et al.*, 2005). The mechanism is not yet

fully understood, but evidence suggests the following (see figure 7):

The fatty acid arachidonic acid (AA) is converted into PGE₂ in several steps by the enzyme cyclo-oxygenase (COX). Isoform COX-1 is constitutively expressed and helps to maintain physiological functions, while the inducible isoform COX-2 is involved in inflammatory responses and several disorders (Meydani *et al.*, 2005). Old macrophages showed higher COX-2 activity (Hayek *et al.*, 1997a). This was due to a higher rate of transcription of COX-2 mRNA mediated by ceramide, a sphingolipid second messenger involved in the regulation of cell differentiation, proliferation, and apoptosis through multiple signaling pathways (Claycombe *et al.*, 2002). Ceramide levels are increased during aging. Ceramide stimulated PGE₂ production and COX-2 expression by inducing NF-κB, a transcription factor of COX-2 whose activity is also increased in aged macrophages compared to young (Wu & Meydani, 2008).

Vitamin E has been shown to decrease PGE₂ production by inhibiting COX-2 activity via a post-translational mechanism (Wu *et al.*, 1998). Free-radical nitric oxide (NO) is involved in the regulation of COX activity. NO can be further metabolized to peroxynitrite (ONOO) in the presence of superoxide, and ONOO has been shown to increase the activity of COX without affecting its expression (Landino *et al.*, 1996). Aged macrophages produced more NO, but vitamin E reduced the age-associated increase in NO production in vitro and in vivo (Beharka *et al.*, 2002). These results suggest that vitamin E reduces COX activity in old macrophages by decreasing NO production, which leads to lower production of ONOO in macrophages from old mice (Wu & Meydani, 2008). In this way, vitamin E increases T cell function by inhibiting PGE₂ production.

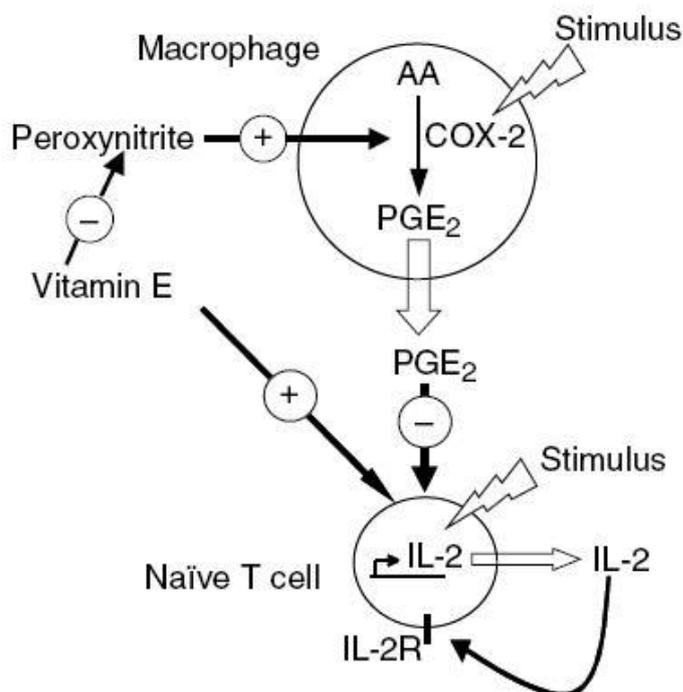


Figure 7. Direct and indirect effect of vitamin E on T cells. Vitamin E might stimulate T cells by reducing PGE₂ production of macrophages, or stimulate T cells directly as described in the text above (Meydani *et al.*, 2005).

5.3. Antioxidant in autoimmune diseases

Free radicals have been associated with the pathogenesis of various disorders, for instance autoimmune diseases. Oxidative stress can damage cells and tissues, by causing membrane damage,

fragmentation or random cross-linking of molecules like DNA, enzymes and structural proteins and can even lead to cell death induced by DNA fragmentation and lipid peroxidation. The consequences of oxidative stress construct the molecular basis of several disorders, such as autoimmune diseases (Ratnam *et al.*, 2006). Vitamin E is a lipid-soluble antioxidant and might prevent membrane damage by ROS. A possible mechanism is described by Margutti *et al.* They identified antibodies to the C-terminus of Ral binding protein 1 (RLIP76), a protein that catalyzes the ATP-dependent transport of glutathione (GSH), in the serum of a significant percentage of patients with various diseases characterized by immune-mediated endothelial dysfunction, including SLE. These autoantibodies induced oxidative stress-mediated endothelial cell apoptosis, but vitamin E counteracted endothelial cell demise *in vitro* (Margutti *et al.*, 2008). In this way, vitamin E may prevent ROS-mediated inflammation and tissue destruction.

6. Conclusion

The immune system deteriorates with increasing age, leading to higher incidence of infections and auto-immune diseases and lower efficiency of vaccinations. Both the innate and the adaptive immune system are affected. Supplementation of vitamin E is an example of a nutritional intervention that might benefit the health of the elderly. Earlier, it has been reported that vitamin E improves immune function in aged animals and humans. In this review, the effects of vitamin E on infections, vaccinations and autoimmune diseases in the elderly have been assessed. All in all, the results seem promising, but more research is necessary.

Although vitamin E has been reported to influence immune responses, it is less simple to translate these results into hard end-points. Vitamin E had a positive effect on infections in both animal models and in randomized controlled trials with humans. However, negative effects have also been found. Several factors that modify the effect of vitamin E on infections have been reported: age, residence, smoking (age at initiation as well as the amount of cigarettes smoked a day), body weight, exercise, vitamin C intake, sex and *IL-10* genotypes. This clearly indicates that vitamin E possibly influences infections in the elderly, although subgroups of elderly exist that might benefit from or might be harmed by supplementation of vitamin E.

Regarding vaccinations, supplementation of vitamin E had ambiguous results in animal studies using different animals, vaccines and doses of vitamin E. In humans, a positive effect was found in a randomized controlled trial among young healthy women. Furthermore, conflicting results have been reported about associations between vaccination efficiency and serum levels of vitamin E. In the elderly, micronutrient supplementation studies found no or a limited effect on vaccination response. Only one small randomized controlled trial in aged individuals has been conducted, which showed positive results of especially 200 mg/day of vitamin E on the vaccination response of elderly. Potentially, supplementation of vitamin E might improve vaccination efficiency. Larger randomized controlled trials in senior individuals are needed to confirm these first promising results.

Animal studies showed positive effects of vitamin E on the onset and development of several autoimmune diseases. In humans, associations have been found between lower vitamin E plasma levels and autoimmune diseases, and vitamin E supplementation improved several aspects of different autoimmune diseases. However, it has not yet been investigated whether vitamin E supplementation lowers the risk of developing autoimmune diseases or just modulates the symptoms of autoimmune diseases, i.e. decreases oxidative stress. The pathogenesis of autoimmune diseases is often very complex and it is unknown whether vitamin E can affect this. Therefore, randomized controlled trials should be executed in healthy elderly which look at autoimmune

diseases as end-points.

Mechanistically, vitamin E might stimulate aged T cells directly by various mechanisms or indirectly by inhibiting $PG(E)_2$ production by macrophages, which might lead to improved immune responses to infections and vaccinations. In addition, vitamin E, as a lipid-soluble antioxidant, might delay the onset of or improve the development of auto-immune diseases by preventing damage from ROS.

All in all, vitamin E potentially improves responses of the immune system to infections, vaccinations and autoimmune diseases. It is important to further elucidate the mechanisms behind these effects of vitamin E on immune senescence. Research is also needed to investigate which subgroups benefit from vitamin E supplementation, since many factors seem to modify the effect of vitamin E. In addition, future research should further focus on finding the right dose of vitamin E. Many studies used a dose of 200 mg/day, but a meta-analysis suggested that doses higher than 150 mg/day might increase all-cause mortality (Miller *et al.*, 2005). Graat *et al.* hypothesized that too high doses of vitamin E might act as pro-oxidants. Besides, conflicting effects of high and low doses of vitamin E on survival of rats have been found. Vitamin E deficiency also impairs immune function. It is therefore important to maintain the balance of vitamin E. Thus, future research should focus on finding the optimal dose for vitamin E supplementation of healthy elderly. Finally, trials in elderly should be executed that look at long-term effects of vitamin E supplementation. It is important to follow elderly for several years and critically review whether vitamin E supplementation is also beneficial on the long term. In conclusion, healthy elderly potentially benefit from vitamin E supplementation, but more research is needed.

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