Nutritional modulation of age-related changes in the immune system and risk of infection

Munkyong Pae¹, Dayong Wu²

¹Department of Food and Nutrition, Chungbuk National University, Chungdae-ro 1, Seowon-gu, Cheongju 28644, Republic of Korea

²Nutritional Immunology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA

* Corresponding authors:
Munkyong Pae and Dayong Wu

Dr. Munkyong Pae
Department of Food and Nutrition, Chungbuk National University
Chungdae-ro 1, Seowon-gu, Cheongju 28644, Republic of Korea
Phone: +82-43-261-2745
Fax: +82-43-267-2742
Email: mpae@chungbuk.ac.kr

Dr. Dayong Wu
Nutritional Immunology Laboratory
Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University
711 Washington Street
Boston, MA 02111, USA
Phone: 617-556-3368
Fax: 617-556-3224
Email: dayong.wu@tufts.edu

Abbreviations

25-(OH)D, 25-hydroxyvitamin D;
1,25-(OH)₂D, 1,25-dihydroxyvitamin D;
APC, antigen presenting cells;
Con A, concanavalin A;
COX-2, cyclooxygenase-2;
DC, dendritic cells;
DTH, delayed type hypersensitivity;
HAI, haemagglutinin inhibition;
IFN, interferon;
Ig, immunoglobulin;
IL, interleukin;
LPS, lipopolysaccharide;
MΦ, macrophages;
mDC, myeloid dendritic cells;
NK cells, natural killer cells;
PAMPs, pathogen-associated molecular patterns;
PBMC, peripheral blood mononuclear cells;
pDC, plasmacytoid dendritic cells;
PGE₂, prostaglandin E₂;
PHA, phytohemagglutinin;
PMN, polymorphonuclear cells;
RDA, recommended daily allowance;
SNP, single nucleotide polymorphism;
TLR, toll-like receptor;
VDR, vitamin D receptor

Abstract

The immune system undergoes some adverse alterations during aging, many of which have been implicated in the increased morbidity and mortality associated with infection in the elderly. In addition to intrinsic changes to the immune system with aging, the elderly are more likely to have poor nutritional status, which further impacts the already impaired immune function. While the elderly often have low zinc serum levels, several manifestations commonly observed during zinc deficiency are similar to the changes in immune function with aging. In the case of vitamin E, although its deficiency is rare, the intake above recommended levels is shown to enhance immune functions in the elderly and to reduce the risk of acquiring upper respiratory infections in nursing home residents. Vitamin D is a critical vitamin in bone metabolism, and its deficiency is far more common, which has been linked to increased risk of infection as demonstrated in a number of observational studies including those in the elderly. In this review, we focus on zinc, vitamin E, and vitamin D, the three nutrients which are relatively well documented for their roles in impacting immune function and infection in the elderly, to discuss the findings in this context reported in both the observational studies and interventional clinical trials. A perspective will be provided based on the analysis of information under review.

Keywords

Aging; Immune function; Infection; Nutrition

1. Introduction

It is well documented that aging is associated with adverse alterations in the immune system, a phenomenon often termed as “immunosenescence.” Although both arms of the immune system, innate and adaptive, are affected by aging, this impact is not equal; instead, certain components of the immune system are more dramatically affected than others with advancing age.

Consequently, the elderly with their impaired immune response are more susceptible to infection and have a substantially high morbidity and mortality from infection. Further, it has been repeatedly evidenced that a normal nutritional status is essential for optimal immune function. However, the prevalence of malnutrition is generally higher among the elderly, especially in the community-dwelling and nursing home residents [1]. It is believed that malnutrition or inadequate intake of certain nutrients found in the elderly constitutes another adverse factor further contributing to the dysregulation of immune function developed with aging. Based on the reported animal and human studies, zinc, vitamin E, and vitamin D stand out as the nutritional components known to be substantially important in maintaining and enhancing immune function in the elderly population and thus, they are the primary topics in this review.

There are recommended daily allowances (RDA) for intake of major nutrients. The RDA for zinc for adults (19 y and older) is currently set at 11 mg/d for men and 8 mg/d for women whereas those recommendations are slightly lower in the United Kingdom and the European Community [2]. In one study, 20% of free living elderly and 85% of institutionalized elderly were reported to consume less than 10 mg of zinc per day [3]. In the third National Health and Nutrition Examination Survey (NHANES), data on the intake of several nutrients including zinc indicated that 35-45% of participants aged ≥ 60 years had inadequate dietary intake, below 9.4 mg/day for men and 6.8 mg/day for women [4]. However, other studies have reported a low

prevalence of zinc deficiency in healthy, free-living older Europeans, e.g., below 11% based on the intake [5-6], and less than 6% by zinc status [5]. The RDA for vitamin E in individuals 19 y and older is 15 mg/day of α -tocopherol. It is documented that certain populations such as the elderly are at greater risk of inadequate dietary intake of vitamin E [7-8]. At present, no special RDA exists for the elderly for zinc and vitamin E. In contrast, the RDA for vitamin D is set at 600 IU (15 μ g) for those aged 1 to 70 y and 800 IU (20 μ g) for those > 70 y. According to several studies, 40 to 100% of U.S. and European elderly men and women still living in the community are deficient in vitamin D [9-10]. There is little doubt that restoring physiological concentrations from deficient status will improve immune function, however, it is a great challenge to identify the exact needs and to create effective supplementation regimen meet the needs in the individuals with greatly varied genetic, nutritional, and health conditions.

To help the readers better understand the discussion as to how these nutrients influence immune function in the elderly, we first provide a brief introduction on the age-associated dysregulation of immune function, i.e., immunosenescence, before moving on to the discussion of studies reporting on the roles of zinc, vitamin E, and vitamin D in modulating immune response and resistance to infectious diseases in the elderly population.

This review will examine the data representing the prominent nutritional approaches to delay and/or reverse immunosenescence and to improve host's resistance to infection in the aged. It is not the authors' intention to cover age-associated low grade inflammation and increased risk of autoimmune diseases that are also pertinent to immunosenescence, but rather, this review focuses on the immunity against infection in the aged. That said, other food nutrients and components which are known to impact immune functions, such as selenium, vitamin A, vitamin C, ω -3 polyunsaturated fatty acids, and a variety of phytochemicals, but have not been

specifically evaluated in the context of age-associated difference, are beyond the scope of this review.

2. Immunosenescence: age-associated dysregulation of immune function

2.1. Innate immune system

Upon recognizing molecular patterns of pathogens, the innate immune system initiates an immediate immune response, mainly through neutrophil granulocytes, monocytes/macrophages, and natural killer cells. These cells directly attack pathogens and also secrete cytokines and chemokines that stimulate the cellular reaction of innate immunity resulting in a process called inflammation.

2.1.1. Neutrophils

After an invasion of pathogens, neutrophils, the most abundant polymorphonuclear leukocytes (also referred to as granulocytes), migrate into the infected sites and provide the first line of host defense by phagocytosis and oxidative burst. While the numbers of circulating neutrophils at baseline and during acute inflammation are largely maintained, these cells' chemotaxis (directional movement in response to a gradient of a stimulus) and antimicrobial function are known to be impaired in the older individuals (reviewed in [11-12]).

2.1.2. Monocytes/Macrophages

Monocytes represent another important cell type in innate immunity. They migrate into the infected tissue shortly after neutrophils where they differentiate into macrophages (MΦ). They mediate host defense not only by engulfing the pathogens and generating reactive oxygen species

and cytokines, but also by serving as antigen presenting cells (APC). While the number of monocytes is mostly unchanged in older adults [13-14], increased prostaglandin E₂ (PGE₂) production and cyclooxygenase-2 (COX-2) expression with aging have been reported in mouse and rat MΦ [15]. Similarly, peripheral blood mononuclear cells (PBMC) from elderly human subjects synthesize significantly more PGE₂ than their young counterparts [16]. A series of studies with murine MΦ revealed that a T cell suppressive factor PGE₂ was upregulated with aging, mainly owing to NF-κB-mediated COX-2 activation [15, 17-18], indicating that increased PGE₂ in MΦ can contribute to T-cell defects with aging. However, the research on effect of aging on phagocytosis has produced mixed results. Some reported preserved phagocytosis of bacterial targets by aged mouse MΦ, whereas the others showed reduced phagocytosis of apoptotic cells by mouse MΦ, and an age-associated impairment in phagocytosis in human monocytes (reviewed in [12]).

2.1.3. Natural killer cells

Natural killer (NK) cells are lymphocytes that are essential for maintaining the innate immune response and host defense against viral infections and tumors. They are characterized by the expression of CD56 and/or CD16 on the surface. While the number or percentage of NK cells was preserved or even increased with age, decreased function of NK cells, as manifested by decreased cytotoxicity, has been found in both aged mice and older adults (reviewed in [19-20]). In particular, decreased NK cell activity is associated with increased incidence of and mortality from infection in elderly individuals [21-22] whereas a preserved NK function is associated with lower incidence of respiratory tract infection and better response to influenza vaccination in the vaccinated elderly people [23]. These results suggest that developing strategies to prevent, delay,

or reverse NK cell immunosenescence may be a potential approach for countering high risk of infection in the elderly.

2.1.4. Dendritic cells

Dendritic cells (DC) are considered professional APC because of their ability to capture, process, and present antigen to T cells, thus serving to bridge the innate to the adaptive immune system. DC can be divided into two subsets: myeloid (mDC) and plasmacytoid (pDC) dendritic cells. mDC (also known as conventional DC) facilitate adaptive T cell responses, in part through Toll-like receptor (TLR)-induced interleukin (IL)-12 production, whereas pDC are the main producers of type I interferon (IFN) in response to viral infection (reviewed in [20]). No consensus has been reached regarding how aging impacts the percentages and numbers of mDC or pDC, but it appears that cytokine production upon activation is reduced in mDC or pDC, while basal cytokine production is increased with aging (reviewed in [12, 24]).

2.2. Adaptive immune system

The adaptive immunity, also known as the acquired immunity, is carried out by T-lymphocytes (T cells) and B-lymphocytes (B cells). These cells recognize and distinguish different antigens and are responsible for specificity and memory. B cells are so called because they are bone marrow-derived lymphocytes. T cells also originate from the bone marrow, but they migrate to the thymus (T for thymus) where they become mature cells. B cells produce antibodies, which are proteins called immunoglobulins (Ig). The antibodies circulate in the blood stream and permeate other body fluids, where they bind to the foreign antigens for neutralization (humoral

immune response). T cells can either help other cells to eliminate microbes (CD4+ T cells) or kill the infected cells (CD8+ T cells) to fulfill the cell-mediated immune responses.

2.2.1. T cells

Although dysregulation of the innate immune system components has been described, the most significant changes occur in T cells. A consistently observed change during aging in the T cell profile is decreased naïve cells and increased memory cells. Since the size of the naïve T cell pool is governed by output from the thymus and not by replication, thymic involution with aging decreases the output of naïve T cells, contributing to a shift towards a greater numbers of antigen-experienced memory T cells and a smaller proportion of naïve T cells [25]. In addition, T cells from aged animals and elderly individuals have decreased proliferation and production of IL-2 in response to mitogens [26-28]. This decline in T cell function is in part attributed to alterations in the signaling pathways including a defect in forming the effective immune synapse [29-30]. These age-associated changes in T cells are more specific to naïve T cells [27, 30].

Notably, aged CD4+ T cells contribute significantly to the impaired humoral response by promoting low-affinity antibody production [31] as well as producing less IL-2 and expressing less CD40L, critical for T-B cell interaction [32-33]. CD8+ T cell responses specific to influenza virus are diminished with aging in both mice [34] and humans [35]. It is obvious that intrinsic defects occur within T cells, but studies with MΦ from old mice suggest that extrinsic factors such as PGE₂ also contribute to the decline of T cell function with aging.

2.2.2. B cells

B cells play an essential role in the humoral immune response by producing antibodies. Although the numbers of peripheral B cells remain relatively constant during aging [36], B cells from elderly individuals exhibit a limited repertoire [37], generate antibodies of lower affinity due to a shift in isotypes from IgG to IgM [38], and are less efficiently responsive to stimulation than those from young subjects [39]. In addition, age-associated defects in T cells significantly influence the decline of B cell specific functions and reduce intact antibody response [40-41].

3. Evaluation of the immune responses in the elderly

3.1. Source of immune cells

In the studies reviewed here, one of the frequently used measurements is the phenotypic changes in circulating immune cells determined by the use of flow cytometry. When peripheral whole blood is drawn from human subjects, it is often isolated to obtain peripheral blood mononuclear cells (PBMC), which consist of lymphocytes (T cells, B cells, NK cells, DC) and monocytes, but without polymorphonuclear cells (PMN) including neutrophil granulocytes.

3.2. Phenotype and *in vitro* response

Immune cell populations in whole blood or PBMC are commonly defined based on the expression of their respective, specific surface markers, which can be detected by fluorescence-labeled antibodies that bind to them. As stated above, decrease of naïve cells and increase of cells with memory phenotype are one of the most characteristic age-associated alterations found in T cells. Along with phenotypic changes, *in vitro* responsiveness (i.e., cell proliferation and cytokine production) to antigen or mitogen stimulation by way of receptor activation can be measured in whole blood or PBMC cultures. Anti-CD3/anti-CD28 antibodies and T cell

mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) are commonly used for T cells, pokeweed mitogen for both T and B cells, and lipopolysaccharide (LPS) for B cells. Additionally, pharmacologic agents such as ionomycin and phorbol myristate acetate are used to activate immune cells through triggering intracellular signaling while bypassing surface receptors.

Regarding innate immunity, NK cell tumoricidal activity is typically assessed in PBMC by target lysis against NK cell-specific tumor cells. Concomitantly, phagocytosis is measured in whole blood, PBMC, PMN cultures by uptake of microbes (bacteria, yeasts) or inert particles.

3.3. DTH response

In order to detect immunity *in vivo*, one of the commonly used immune measures is the delayed type hypersensitivity (DTH) skin test. DTH test involves subcutaneous injection of a small amount of antigens such as *Mycobacterium tuberculosis*, tetanus, and *Candida* species followed by measurements of induration at 48 – 72 h post-injection. Considering the central role of CD4⁺ T cells in DTH response and age-associated defects in T-cells, it is expected that aging would decrease DTH response. Indeed, the elderly individuals exhibit impaired DTH response (the number of positive responses and/or diameter of induration) [42-43], which are correlated with their greater morbidity and mortality [44-46].

3.4. Response to vaccination

Another indicator of *in vivo* immunity is antibody production after vaccination. It is well documented that antibody response declines with aging. This decline is observed in response to hepatitis B and influenza vaccines [47-48], tetanus toxoid vaccine (T-cell dependent) [49], and

pneumococcal vaccine (T-cell independent) [50]. In a meta-analysis of 31 vaccine antibody response studies, antibody response in the elderly is considerably lower than in younger adults (adjusted odds ratio: 0.24 - 0.59 in terms of three influenza antigens used in the vaccines) [51]. The hemagglutinin inhibition (HAI) antibody titer is widely used in vaccination studies, and the host responsiveness is determined by 1) seroconversion – the percentage of subjects with a 4-fold increase in antibody titers, 2) seroprotection – the percentage of subjects with HAI antibody titers $\geq 1:40$ post-vaccination, and 3) the post-vaccination geometric mean titer (post-GMT).

3.5. Infections

Infections, particularly respiratory infections, are common in the elderly, resulting in decreased daily activity, prolonged recovery times, increased health care service utilization, and more frequent complications including death [52-54]. For these reasons, risk of infection is often measured as a main outcome of clinical studies in elderly populations. However, some studies have employed the method of self-reporting, which is susceptible to reporting biases. Thus, diagnosis by physician in addition to serological and molecular diagnosis is recommended to minimize false results.

4. Nutritional intervention for improving immune function and resistance to infections in the elderly

4.1. Zinc

Zinc is a trace element essential for membrane integrity, DNA synthesis, and cell proliferation; thus, it is a particularly important nutrient for cell functions in the immune system [55-56]. Since there is no specialized zinc storage system in the body, zinc deficiency can rapidly impair zinc

supply to immune cells resulting in compromised immune function [57]. It has been shown that zinc deficiency can profoundly alter immuno-homeostasis which involves both innate and adaptive immunity causing impaired phagocytosis and intracellular killing activity of phagocytes, decreased NK cell activity, thymus involution and decreased thymic output, and decreased lymphocyte proliferation, IL-2 production, DTH response, and antibody response to vaccines [58-61], all manifestations similar to those seen in the aged immune system. In agreement with these findings, decreased zinc intake and low zinc status have been reported in the elderly [3, 62-64], and low zinc status in the elderly has been shown to contribute to age-associated dysregulation of the immune response [65-66]. While this similarity in altered immune function seen in zinc deficiency and aging should not lead to assumption that immunosenescence represents causal relationship between the two and simply providing zinc would rescue aged immune system, it is a reasonable to expect that appropriately formulated zinc supplementation may serve as an effective strategy to minimize the adverse impact of aging on immune system and ultimately, prevent infectious diseases in the elderly.

4.1.1. Immune function

In one of the early studies, institutionalized healthy elderly (>70 y) who received 1 mo of daily supplementation with 440mg zinc sulfate (corresponding to 100 mg of elemental zinc) had a significant increase in the proportion of circulating T cells, DTH response, and anti-tetanus toxin IgG titers, all of which depend on T cell response [67]. Similarly, 60 mg zinc supplementation (as acetate) for 4.5 mo significantly increased plasma zinc concentrations and improved DTH response in zinc deficient elderly (n=8) who demonstrated an anergic response to skin antigen tests [68]. In contrast, Bogden et al. did not detect any difference in DTH response

nor lymphocyte proliferation between placebo and zinc-supplemented group (15mg or 100mg) [69]. However, repeated DTH administration (every 3 mo) increased DTH response over time even in the placebo group, which may be due to an immune memory carried over from the first DTH administration at baseline leading to a larger than normal response; in addition, the co-administration of multivitamins/minerals (5-250% of RDA) including 30 mg of vitamin E to all the study participants may have masked zinc effect. It is also noteworthy that the subjects' mean values of plasma zinc levels were normal (85µg/dL) prior to supplementation [69].

Several studies that have determined effect of zinc on immune cell phenotype have proved to be inconclusive. For example, free-living elderly receiving zinc at 10mg/d for 7 wk showed a reduction in activated (CD25⁺) CD4⁺ T cells [70], whereas institutionalized healthy elderly who consumed 25 mg/d zinc for 3 mo increased the number of activated (HLA-DR⁺) CD4⁺ T cells and cytotoxic T cells [71]. In another study, the number of circulating T cells were increased in a group of zinc deficient nursing home elderly (serum zinc <70 µg/dL) that had been supplemented with 30 mg zinc for 3 mo, which were accompanied by increased lymphocyte proliferation with T cell stimulants (PHA, anti-CD3/anti-CD28 antibodies) [72]. Since participants with serum zinc ≤60 µg/dL failed to attain adequate concentrations of serum zinc (≥70 µg/dL) despite of supplementation [72], those with low zinc status may need a larger dose or longer duration of supplementation to achieve higher zinc levels adequate to reverse T-cell immunosenescence.

With increasing age, the response to vaccination decreases, including the case of T-cell dependent tetanus and influenza vaccination, the relevant markers of *in vivo* immunity that are shown to have close clinical relevance to infection in the elderly. When antibody response to vaccination was investigated, one study found a greater IgG antibody response to tetanus

vaccination administered at the end of 1-mo of supplementation with 100 mg zinc [67], but the other reported no difference on antibody titer to influenza strains [73]. However, in the latter the supplementation with 90 mg zinc started 15 d prior to the influenza vaccination and thus it is possible that short duration of supplementation prior to vaccination may not be sufficient to restore cellular immunity in the host. To support this assumption, zinc supplementation at 20 mg/d for 15 – 17 mo prior to influenza immunization has been shown to result in a better response in elderly individuals [74]. However, given the fact that zinc was given together with selenium, caution should be exercised in interpreting these results.

4.1.2. Infections

In the research of determining zinc's impact on infection in the elderly population, early evidence arose from a 2-y randomized controlled trial in which 20 mg zinc and 100 µg selenium led to a significant reduction in infectious events in institutionalized elderly among whom 61% were zinc deficient (<70 µg/dL) [75]. A subsequent study also reported that the number of subjects with respiratory infections was 64 in the zinc plus selenium group versus 82 in the placebo group, a nearly significant decrease by 22% ($p=0.056$) [74]. Apparently, this protection could be achieved by zinc alone since daily supplementation with 45 mg zinc for 1 y showed fewer infections and fevers with a trend toward a lower incidence of the common cold ($p=0.067$) [76]. In line with these findings, a post-hoc analysis showed that 29% of nursing home elderly had low serum zinc concentration (<70 µg/dL) despite of daily supplementation of 7 mg zinc together with other micronutrients for 1 y and these subjects had a significantly higher incidence and longer duration of pneumonia, and more antibiotic use compared to those with serum zinc ≥ 70 µg/dL [77].

Based on these results, in the majority of studies zinc deficiency was defined as a serum or plasma concentration below 70 µg/dL (10.7 µM). Although there is a clear trend towards suboptimal zinc intake and lower blood zinc concentrations with advancing age [78], other factors may also influence suboptimal zinc levels in the elderly, which are not necessarily limited to the reduced intake, as will be discussed later in this review.

4.1.3. Mechanisms

Given the fact that zinc can impact expression of hundreds of gene in immune cells [79-81], and functions of different types of cells in immune system, the mechanisms for zinc's immunomodulating effect are believed to be multifaceted and complicated. The most recognized factor in understanding the mode of zinc's action is its role as a signaling ion. Free intracellular zinc can reversibly bind to regulatory sites in signaling proteins to affect immune cell signal transduction (reviewed in [82-83]). Upon stimulation, intracellular zinc increases within minutes [84], and this response is known to control the regulation of T cell receptor signaling and thus, T cell activation [85]. Therefore, it is conceivable that zinc deficiency would reduce availability of intracellular zinc ion causing impaired intracellular signaling events and immune cell activation. There are several age-associated defects in T cell signaling pathways, including tyrosine and serine/threonine phosphorylation [86-88], MAPK activity [88-89], and activation of nuclear transcription factor NFAT [90], AP-1 [91-92], and NF-κB [93-94]. Additionally, the impaired immune synapse formation and reduced distribution of the key signaling molecules for T cell activation such as Zap70, LAT, Vav, Lck, and PLCγ were observed in T cells from aged mice compared with those of young mice [30, 95-96]. Among these factors, zinc has been shown to facilitate recruitment of Lck to T cell receptor signaling complex and its activation by bridging

protein interface sites and Lck homodimerization [82, 97-98], to promote PKC's binding to the plasma membrane as well as the kinase activity [83, 99-100]. However, zinc is also shown to suppress certain T cell activity. For example, T cell receptor/CD28-mediated calcium signaling activates phosphatase calcineurin which in turn promotes IL-2 transcription through NFAT activation, and zinc has been shown to inhibit calcineurin activation to block NFAT-induced IL-2 mRNA and protein expression [101-103]. Together, these studies suggest an important role of zinc in maintaining immune cell homeostasis rather than a universal immuno-enhancer.

Since the most significant age-associated changes occur in T cells, one way in which zinc could affect T cell-mediated immunity in the aged is to serve as a cofactor of thymulin, a hormone secreted by thymic epithelial cells and critical for differentiation and function of T cells [82]. Thymulin exists in two forms, a zinc-bound active form and a zinc-free, inactive form. Zinc-deficient mice had lower levels of active thymulin in circulation [104], and *in vitro* and *in vivo* zinc supplementation enhanced thymulin activity [105]. Consistent result was found in zinc-deficient adults [106]. Of note, serum concentrations of active thymulin decline with aging [107-109], which can be improved by zinc supplementation [62, 109].

While zinc deficiency is more prevalent in the elderly based on plasma levels, the actual zinc status may be even more so given that low intracellular zinc ion availability may occur in those with blood zinc in the normal range. The intracellular concentration of free zinc is regulated by two main classes of proteins, zinc transporters and metallothioneins. Zinc transporters include two families, Zip and ZnT where Zip transporters increase cytosolic zinc bioavailability by increasing zinc influx while ZnT transporters function by decreasing cytosolic zinc [110]. For example, zinc influx is mediated by zinc transporter Zip6, and Zip6 gene silencing is found to inhibit T cell activation [85]. Recent study showed that compared to young,

aged mice had lower intracellular zinc levels in immune cells, which was associated with less Zip6 mRNA levels upon activation [111]. Lower intracellular zinc levels may occur during aging due to increased expression of metallothioneins, a major storage protein for zinc, resulting in increased sequestration of zinc [112]. Therefore, age-associated dysregulation in zinc transporter and metallothioneins may in part attribute to reduced intracellular zinc levels, leading to immune dysfunction.

Genetics may also influence an individual's response to zinc supplementation, and this may possibly be one of the contributing factors to the discrepancies reported in the literature. Mocchegiani et al proposed that single nucleotide polymorphism (SNP) at IL-6 gene is associated with zinc bioavailability and immune function [113]. Elderly subjects carrying GG genotypes (C^- carriers) in IL-6-174G/C locus had higher metallothioneins, low zinc status (plasma zinc and intracellular zinc ion availability) and impaired innate immune response (NK cell cytotoxicity) compared to those carrying GC and CC genotypes (C^+ carriers). It is likely that old C^- carriers benefit from zinc supplementation to a larger extent than old C^+ carriers. Indeed, a short term (7 wk) zinc supplementation (10 mg/d) in old C^- carriers restored NK cell cytotoxicity to values present in old C^+ carriers and considerably improved zinc status coupled with diminished metallothioneins [113]. In another study, the authors found that there was a significant interaction of -174 IL-6 and +647 MT1A polymorphisms, and IL-6 $C^+/MT1a C^-$ carriers could benefit from zinc supplementation more than other types [114]. The examples of other relevant genes affecting zinc bioavailability via their polymorphisms include the report that heat shock protein-70 +1267 AA genotype was an independent factor associated with Zn plasma concentrations [115]. Thus, genetic background is a factor to be considered for achieving the best outcome of zinc supplementation.

4.2. Vitamin E

As a lipid-soluble antioxidant, vitamin E plays a central role in protecting the integrity of cell membranes from oxidative damage. Vitamin E is a generic term for all tocopherols and tocotrienols, each having 4 homologs designated as α , β , γ , and δ -. Among them, α -tocopherol is the major form of vitamin E found in diet and it has the highest bioavailability and tissue concentrations. For this reason, α -tocopherol is predominantly used in the study on vitamin E, almost exclusively in the interventional trials. However, studies have suggested that tocotrienols may be superior regarding antioxidant activity [116-117] and possess distinct biological properties not shared by tocopherols [118], which are the subject of continuing investigations, but will be discussed in this review.

Since vitamin E is particularly enriched in the membrane of immune cells, it is not surprising that vitamin E deficiency impairs both humoral and cell-mediated immune functions [119-120]. Conversely, vitamin E supplementation has a beneficial effect on the immune system, particularly in the aged individuals who have compromised immune function. It has been shown that vitamin E supplementation increased lymphocyte proliferation [121-122], IL-2 production [121-123], and DTH response in old mice [121]. In addition, old mice had an impaired response to infection such as declined NK cell activity, IFN- γ production, and neutrophil recruitment [124], as well as the associated higher viral titers [125-126], which are all restored by vitamin E supplementation.

4.2.1. Immune function

Given the observed efficacy of vitamin E in restoring cell-mediated immunity as well as improving innate immunity in the aged animals, several double blind, placebo controlled clinical trials have been conducted to confirm these effects in the elderly. In one study, short-term (1 mo) supplementation of vitamin E with 800 mg/d significantly improved DTH response, T cell proliferation, IL-2 production while decreasing plasma lipid peroxide and PGE₂ production in healthy individuals (≥ 60 y) [16]. A subsequent study with lower doses for a longer period (4.5 mo) showed that supplementing vitamin E at 200 mg/d compared to 0, 60 or 800 mg/d was the most effective in increasing DTH response and antibody titers to hepatitis B and tetanus vaccine in free living, healthy elderly (≥ 65 y), all of which indicate an improvement in T cell-mediated immunity [127]. Notably, there is a linear association between a change in DTH response and changes in plasma vitamin E levels up to 25 μ M prior to leveling off [128]. It is suggested that a 25 μ mol/L increase in plasma vitamin E can be achievable by consuming 200 mg/d of vitamin E [128]. Thus, supplementing healthy elderly subjects with 50 and 100 mg of vitamin E may not be sufficient to achieve the optimal vitamin E levels since subjects show a mean increase of 10–15.8 μ M after supplementation, and there is only a trend towards an increased DTH with 100 mg in the vitamin E group [129]. Additionally, their baseline vitamin E status was higher than the studies that reported positive results [16, 127], suggesting that those who have low plasma vitamin E levels at baseline may benefit more after receiving vitamin E supplementation. Moreover, elderly subjects consuming 200 mg/d of vitamin E for 3 mo showed significant improvement in T cell mitogens-induced lymphocyte proliferation and IL-2 production, NK cell activity, and neutrophil function (chemotaxis and phagocytosis), compared to the respective baseline values [130]. From all these findings, it appears that vitamin E intake above recommended levels has a beneficial effect on the immune system; however, this is not

supported by all studies, and some investigators have called caution against consuming large doses of vitamin E.

A meta-analysis on 19 trials showed that vitamin E supplementation at doses above 400 IU/d was associated with a small increase in all-cause mortality [131]; for high-dosage vitamin E trials (≥ 400 IU/d, corresponding to approximately 260 mg of natural α -tocopherol), the all-cause mortality risk difference was 39 per 10000 persons ($p=0.035$). However, high-dosage trials were often small and enrolled more participants with chronic diseases. Similarly, a long-term (3.5 y) supplementation of vitamin E at 300 mg/d increased the risk of developing heart failure in post-infarction patients [132]. Although it is still in debate regarding the cutoff dose of vitamin E for preventing potential adverse effects, the suggested optimal dose at 200 mg/d based on the beneficial effect on immune function in the elderly seems to be reasonable and beyond concern nevertheless, as this dose has a distance far from any of those higher doses suspected to cause adverse effects.

4.2.2. Infections

Since available evidence suggests beneficial effects of supplemental vitamin E on immune function in the aged, its clinical relevance has been tested in elderly individuals. Early evidence showed that free-living, healthy elderly who received vitamin E supplementation (60, 200, or 800mg/d) for 235 d had 30% lower incidence of self-reported infections in comparison to placebo ($p=0.10$), but the study was too small and not designed to measure change in numbers of infections [127]. In a subsequent larger, double-blind, placebo-controlled trial, long term supplementation of vitamin E (200 IU/d for 1 y) in nursing home residents (>65 y) reduced the number of participants who acquired 1 or more upper respiratory tract infections by 12% (risk

ratio, 0.88; 95% CI, 0.75–0.99) and the incidence of common colds by 20% (rate ratio, 0.80; 95% CI, 0.64–0.98). In the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) study, supplementation with vitamin E at lower dose (50 mg/d) in combination with 20mg of β -carotene showed positive, neutral, or negative effects on pneumonia and the common cold in participants, depending on smoking history, residence, exercise, and other factors [133-135]. In a cohort of Dutch well-nourished noninstitutionalized elderly individuals, neither daily vitamin E at 200 mg nor multivitamin-mineral supplementation showed a favorable effect on incidence and severity of acute respiratory tract infections [136]. Instead, the adverse effects of vitamin E on illness severity were observed.

The discrepancies among these studies may be in part attributed to the differences in the study design such as supplemental dose, whether given singly or in combination with other nutrients, and selection of subjects (age, sex, nutritional & physical status, and genetic variations). For example, in the ATBC study, a small dose of vitamin E (50 mg/d) in combination with β -carotene (20 mg/d) was used in middle-aged and older male smokers (50 – 69 y) [133-135]; in the Dutch study, participants were well-nourished noninstitutionalized elderly individuals (≥ 60 y) [136], which is in contrast to the study conducted in institutionalized nursing home elderly residents (65–103 y) [137]. Accumulating evidence suggests that genetic differences may play a critical role for the success of vitamin E intervention, which will be discussed next in the mechanism section.

4.2.3. Mechanisms

The working mechanisms underlying the immuno-modulating effect of vitamin E have been studied mainly with experiments in cell cultures and animal models. Overall, vitamin E can

enhance T cell-mediated function by directly influencing membrane integrity and signal transduction in T cells or indirectly, by reducing production of suppressive factors such as PGE₂ by MΦ (reviewed in [128, 138]). Pre-incubation with vitamin E at 46 μmol/L (average plasma levels of human taking 200 IU/d) in purified T cells from the spleens of young and old mice was shown to increase both cell-dividing and IL-2 producing capacity of naïve T cells from old mice [27]. Vitamin E was further shown to enhance the formation of effective immune synapses between APC and naïve CD4⁺ T cells as well as redistribution of signaling molecules (Zap70, LAT, Vav, and PLCγ) in the immune synapse [30]. It seems that age-associated decline in effective immune synapse formation, especially of naïve CD4⁺ T cells that represent a T cell subset exhibiting the greatest age-related defects, can be reversed by vitamin E supplementation, leading to enhanced cell division and IL-2 production. In addition to its direct effect on T cells, vitamin E is able to reverse the age-associated increase in PGE₂ synthesis by MΦ, a potent T cell-suppressive, pro-inflammatory lipid mediator [121]. While the working mechanism for this effect of vitamin E is only partly understood, available evidence indicates that vitamin E-induced change in PGE₂ production is due to its effect on COX activity without altering COX-1 and COX-2 expression [139], probably by inhibiting peroxynitrite production [140].

Results from murine infection model have expanded our understanding of how aging affects immunosenescence with clinical implications and how vitamin E functions during the process. Upon infection with influenza A/Port Chalmers/1/73 (H3N2), old mice exhibited higher lung viral titers [125-126] and impaired IL-2 and IFN-γ response [126], all of which were improved by vitamin E supplementation (500 mg/kg diet) for 8 wk. It appears that the protective effect of vitamin E against influenza virus may be associated with enhancement in Th1 cytokine production. More recently, Bou Ghanem et al. demonstrated that old mice were more susceptible

to *Streptococcus pneumonia* than young mice, along with higher pulmonary bacterial burden, lethal septicemia, and lung inflammation (neutrophil infiltration), which were reduced by supplemental vitamin E (500 mg/kg diet) for 4 wk [124]. These findings suggest that vitamin E can enhance resistance of aged mice to bacterial pneumonia by modulating the innate immune response.

However, the results from clinical studies of vitamin E intervention thus far have been inconsistent or even contradictory. This may be in part owing to the varied conditions in vitamin E administration regimen and subject characteristics. On the subject side, both baseline vitamin E status, and individual's specific genetic background should be considered. It has been reported that polymorphisms of genes including apolipoprotein E, SR-BI scavenger receptor, CD36 scavenger receptor, α -tocopherol transfer protein, and lipoprotein lipase, may influence the bioavailability and cellular activity of vitamin E (see the review [141]). Thus, it is plausible that the polymorphisms of the genes related to vitamin E bioactivity may affect the effects of vitamin E supplementation; however few studies have investigated vitamin E-gene interactions in immune function, especially in the elderly. In the context of polymorphisms, Belisle et al. reported that elderly subjects with the A/A and A/G genotypes at TNF- α -308G>A treated with vitamin E had lower TNF- α production than those with the A allele treated with placebo [142]. Since the A allele at TNF- α -308G>A is associated with higher TNF- α levels [143], these results suggest that the anti-inflammatory effect of vitamin E may be specific to those genetically predisposed to higher inflammation. As such, the interaction of vitamin E with genes related to its bioactivity and immune response should be further explored in elderly population, which may help to discern the contradictions and provide a better understanding of vitamin E's efficacy in restoring age-associated immune response.

4.3. Vitamin D

Like vitamin E, Vitamin D is a lipid-soluble vitamin; however, unlike vitamin E, vitamin D is primarily produced in the skin during sun exposure rather than absorbed from the diet.

Synthesized or ingested vitamin D is transported to the liver where it is hydroxylated to 25-hydroxyvitamin D [25-(OH)D], the major circulating form of vitamin D, and further metabolized mainly in the kidney to the active form, 1,25-dihydroxyvitamin D [1,25-(OH)₂D] by 1 α -hydroxylase (CYP27B1). 1,25-(OH)₂D circulates to various target tissues to exert its endocrine actions that are mediated by the vitamin D receptor (VDR). A long-recognized role of 1,25-(OH)₂D involves calcium homeostasis and bone health. However, extraskelatal functions of vitamin D has been revealed along with the discovery of the VDR in tissue and cells that are not involved in maintaining mineral and bone homeostasis. One of the most prominent effects is found on immune cells. As the majority of immune cells possess VDR and some of them express 1 α -hydroxylase, localized activation of vitamin D can act on immune cells with VDR in an autocrine or paracrine manner. Thus, higher circulating 25-(OH)D levels are likely required for optimal intracrine actions of 1,25-(OH)₂D, whereas insufficient vitamin D levels may be linked to dysregulated immune function and possibly infectious diseases. Although no consensus has been reached, 25-(OH)D levels between 20 and 30 ng/mL (corresponding to 50 and 75 nM) tend to be considered as vitamin D insufficiency, but not necessarily vitamin D deficiency, which is defined as 25-(OH)D level below 20 ng/mL (50 nM). Vitamin D insufficiency is common in community-dwelling elderly and more so among institutionalized elderly [10].

4.3.1. Immune function

Numerous *in vitro* studies have demonstrated significant importance of vitamin D as an important modulator in both innate and adaptive immunity. For example, 1,25-(OH)₂D stimulates differentiation of precursor monocytes to mature phagocytic MΦ [144-145]. In addition, human MΦ are able to synthesize 1,25(OH)₂D when stimulated with IFN-γ [146]. Furthermore, both VDR and CYP27B1 mRNA were induced when monocytes were stimulated with LPS or *M. tuberculosis*-derived lipopeptide [147]. In particular, 1,25-(OH)₂D supplementation can enhance the production of the antimicrobial peptides cathelicidin by MΦ [147] and β- defensin by endothelial cells [148]. T cells can also upregulate VDR upon antigenic activation [149]. However, 1,25-(OH)₂D inhibits T cell proliferation [150], in particular T helper 1 (Th1) cells, which are a subset of CD4⁺ effector T cells capable of producing IL-2 and IFN-γ and activating MΦ [151]. Therefore, vitamin D may help to limit the potential tissue damage associated with Th1 cellular immune response. It appears that other CD4⁺ effector cells can also be modulated by Vitamin D. Studies reported that 1,25-(OH)₂D can suppress pro-inflammatory Th17 cells [152] while increasing regulatory T cells (Treg) [153], which may be a pivotal mechanism for the reported potential of vitamin D in mitigating autoimmune disorders.

The immunosuppressive mechanism of vitamin D on T cells may be in part explained by its influence on DC; 1,25-(OH)₂D was shown to inhibit the maturation of monocyte-derived DC, thereby suppressing their capacity to present antigen to T cells [153]. Based on these cellular studies, it appears that vitamin D can stimulate innate immune responses, which can help eliminate invading bacteria and viruses whereas the regulatory effect of vitamin D on T cells can be useful under a number of conditions such as T cell-mediated autoimmune inflammatory diseases. Since older people are at greater risk for poor vitamin D status, mainly due to restricted

sunlight exposure, reduced ability of the skin to produce vitamin D, and reduced vitamin D intake [154-156], it is plausible that low vitamin D status in elderly individuals may also contribute to their compromised immune response and their increased prevalence of infection, which can be alleviated by adequate intake of vitamin D.

Epidemiologic studies have shown an association between low 25-(OH)D levels and chronic diseases including infections [9, 157], but few have evaluated immune function in elderly subjects. One study in 34 healthy females over 60 y reported that those with high 25-(OH)D levels (>75 nM) had lower percentages of naïve CD8⁺ T cells and higher percentage of effector CD8⁺ T cells, suggesting that increased vitamin D may accelerate CD8⁺ T cell senescence; however, 25-(OH)D status influenced neither T cell proliferative response nor the serum levels of inflammatory cytokine IFN- γ , IL-1, IL-6, and IL-17, as well as the Th2 cytokine IL-4 [158]. Another study showed no correlation between 25-(OH)D and humoral immune response against seasonal influenza, vaccine, or changes in subpopulations of immune cells, and cytokine/chemokine response in adults ≥ 50 y [159]. However, since the latter study included middle-aged subjects, it may not be an ideal evidence for supporting or disputing the association between serum vitamin D status and immune response in the elderly. Currently there is essentially a lack of cohort studies or interventional trials with detailed data of vitamin D intake/status and immune profiling to help with establishing a link between vitamin D and immune function in the elderly, or even in general population. Thus we still don't know whether and to what extent vitamin D supplementation, beyond the context of correcting deficiency, would help in improving the aged immune system, nor do we know whether vitamin D's effect on immune function would vary depending on individual's age. More controlled studies are needed to fill the gap in this regard.

4.3.2. Infections

In contrast to vitamin E, more vitamin D studies have been conducted to determine its effect on infection than on immune function. Many observational studies with participants of various ages predominantly reported an association between low 25-(OH)D levels and increased risk of infection (reviewed in [160]). Insufficient vitamin D status has been linked to more days of absence from duty due to respiratory infection in young men [161], increased risk of acute respiratory infection [162-163] and upper respiratory infection in adults [164] as well as higher mortality rate in patients with community-acquired pneumonia [165]. When restricted to elderly subjects, one study reported an inverse association between serum 25-(OH)D and respiratory disease in participants (≥ 65 y, $n=2070$) [166] and the other study showed a lower serum level of 1,25-(OH)₂D correlated with a decreased DTH response and increased susceptibility to respiratory infection in institutionalized elderly subjects (mean 80.9y, $n=32$) [167].

Given the suggested benefit of vitamin D intake in reducing respiratory infection based on the epidemiologic studies, the preventive effect of vitamin D supplementation has been tested. In a meta-analysis including 11 placebo-controlled studies in 5660 patients, vitamin D supplementation was shown to have an overall protective effect against respiratory tract infection (OR, 0.64; 95% CI, 0.49 to 0.84) [168]. However, results from randomized controlled trials in the elderly are very limited and less inconclusive. Vitamin D supplementation was shown to reduce the number of subjects with cold and influenza symptoms in postmenopausal African American women aged 50 to 75y [169]. While it appears undisputable that compared to younger adults, the elderly has higher incidence, severity, and mortality of infection, in particular respiratory tract infection, the research has not provided adequate data to clarify whether vitamin

D supplementation is particularly more effective in preventing/improving respiratory infection in the elderly. Some study outcomes have suggested that this effect of vitamin D may be age-related. For example, in vitamin D intervention study, 620 Australian residents (60-84 y) received monthly doses of a placebo (n = 205), or 30,000 (n = 210) or 60,000 (n = 205) IU oral cholecalciferol for ≤ 12 mo. In the post hoc analysis of the data from this study, the authors found an insignificant reduction in vitamin D group vs. placebo group in antibiotic use; however, after stratified by age, this effect of vitamin D was significant in subjects aged ≥ 70 y but not in those aged < 70 y (RR, 1.07; 95% CI, 0.58 to 1.97) (P-interaction = 0.1) [170]. In an one-year clinical trial, elderly patients (≥ 65 y, mean age 84 y) with recent hip fracture were randomized to receive 800 IU or 2000 IU vitamin D, the authors found that the higher dose of vitamin D decreased the risk of hospital readmission due to infection (1% in higher dose group vs 11% in lower dose group) [171]. It is also reported that long term of vitamin D supplementation ($1\alpha(\text{OH})\text{D}_3$, 1 $\mu\text{g}/\text{d}$ for 20 y) reduced *H. pylori* infection in healthy female nursing home residents (aged 70-99 y) [172].

The reported efficacy of vitamin D intervention varies widely and this may be related to initial vitamin D status as well as the achievement of adequacy post-intervention. A systemic review showed that protective effect of vitamin D in protecting against acute airway infections had high heterogeneity; however, the significant efficacy of vitamin D was observed in the three trials with initial 25-(OH)D levels < 50 nmol/L, or the subgroup with < 40 nmol/L in another trial [173]. In one study in which 25% of participants are vitamin D deficient and over 70% had insufficient vitamin D status, vitamin D supplementation at 800-2000 IU for 3 y still left 40% of participants in vitamin D insufficient [169]. Thus, those with low vitamin D status may need larger doses to attain desired serum 25-(OH)D concentrations. A dose algorithm study suggests

that 3800 IU are needed for those with 25-(OH) $\geq$ 55 nM, while 5000 IU for those less than 55 nM to achieve sufficient levels of vitamin D [174]. These results indicate that serum vitamin D status at baseline should be an important factor to consider for identifying the vulnerable population and for determining the appropriate doses needed to induce sustained elevation of serum 25-(OH)D levels. Monitoring the vitamin D status at follow-up will help validate the efficacy of vitamin D supplementation.

4.3.3. Mechanisms

As discussed above, vitamin D has extensive influences on multiple aspects of immune system, enabled by the expression of the VDR and the enzyme 1 α -hydroxylase present in most immune cells [146-147, 175-176]. Vitamin D has been shown to enhance several innate immune functions necessary for fighting against microbial infections. Local synthesis of 1,25(OH)₂D, the functional form of vitamin D, plays a critical role in respiratory infection. Both airway epithelial cells and lung M Φ express VDR and contain 1 α -hydroxylase. Upon infection, pathogen-associated molecular patterns (PAMPs) on pathogens trigger pathogen recognition receptors called toll-like receptors (TLR) in host [177]. TLR when triggered result in induction of the gene for 1 α -hydroxylase (CYP27B1), which in turn induce local production of 1,25-(OH)₂D. Notably, 1,25-(OH)₂D is a direct inducer of antimicrobial peptide gene expression [147-148]. For example, TLR activation in human M Φ has been shown to up-regulate expression of VDR and vitamin D-1-hydroxylase genes leading to increased 1,25(OH)₂D production and its binding to VDR, which promotes synthesis of the antimicrobial peptide cathelicidin and killing of intracellular *M. tuberculosis* [147]. Cathelicidin has direct antiviral activity against several major respiratory viruses including influenza [178], respiratory syncytial virus [179], and rhinovirus [180]. Studies

also suggest that vitamin D-induced production of reactive oxygen species and activation of antibacterial autophagy may represent additional factors in the innate immunity against pathogens [181-182]. Furthermore, it can be suggested that vitamin D's beneficial effect in infection may be related to its anti-inflammatory property based on the reasoning that follows. Excessive and dysregulated inflammatory response is known to worsen the disease symptoms and mitigate pathogen clearance in infections; vitamin D has been shown to inhibit production of pro-inflammatory cytokines (IL-6, IL-8, IL-12, IFN- γ , TNF- α) in the innate immune response, and in the adaptive immune response, it inhibits T-cell activation and modulates CD4⁺ T-cell differentiation by favoring polarization toward Th2 and Treg phenotypes while suppressing Th1 and Th17 differentiation [152, 183-186]. Another possible mechanism proposed involves direct inhibition of pathogen replication by vitamin D in the host, but there is no solid evidence supporting this speculation; in fact, an in vitro study showed no direct effect of vitamin D on rhinovirus replication in epithelial cells [187].

Since vitamin D regulates immune function through the VDR, genetic variants in VDR could affect the function of VDR, which would modulate the biological effects of vitamin D. Indeed, VDR genetic variants have been implicated in several infectious diseases, including tuberculosis [188], rhinovirus lower respiratory tract infections [189], and pneumonia [190]. Additionally, several single nucleotide polymorphisms (SNPs) in vitamin D pathways have been found to associate with serum 25-(OH)D concentration [191-192]. In older persons, one study reported a significant association between three SNPs in the PPARG gene and serum 25-(OH)D levels in a 159 healthy subjects (50-74y) [159], whereas the other study found that none of the 15 SNPs in 6 genes (DBP, DHCR7, CYP2R1, CYP27B1, CYP27A1, VDR) were independently associated with serum 25-(OH)D in 222 subjects (48-94y) [193]. More studies are needed to

address how epigenetic regulation of VDR and VDR-related genes affect plasma vitamin D levels, and an individual's response to vitamin D supplementation, which can be linked to the host's susceptibility to infection.

The current recommendations for vitamin D intake are primarily based on bone health and mortality, but not for infection prophylaxis. More specifically designed, controlled clinical trials are called for to definitely characterize the effects of vitamin D supplementation on immune function and risk of infection in the elderly with different basal serum levels of 25-(OH)D and to determine optimal doses for achieving sustained adequacy in vitamin D status. Before such information is available, however, given the high prevalence of vitamin D deficiency/insufficiency and well documented correlation between vitamin D deficiency and infection incidence, vitamin D intake should be raised to 1000 IU/d for majority of people in order to achieve circulating levels > 50 nmol/L as suggested [173, 194]. Regardless of the baseline levels, the dose 1000 IU/d should be considered safe given the large room from the current tolerable upper intake level (4000 IU/d).

7. Concluding remarks and perspectives

Aging-associated impairment in the immune system is well documented and is a main factor contributing to the increased morbidity and mortality associated with infection in the elderly. Optimal immune function depends on a normal, well-balanced nutritional status; however, prevalence of malnutrition is generally higher among the elderly, which further deteriorates the aged immune system. Thus, nutritional intervention may have a promising potential in mitigating the aging's negative impact on immune function, thus improving resistance to infection in the

elderly population. In this context, zinc, vitamin E and vitamin D have emerged as prominent candidates.

There is substantial similarity between immunosenescence and changes of the immune function associated with zinc deficiency. Supplementation with zinc could increase DTH response, thymulin levels, vaccination efficacy, T cell numbers, and thus may help prevent infections in the elderly. Although vitamin E deficiency is rare, vitamin E supplementation beyond recommended levels can enhance immune function and reduce the risk of upper respiratory infections, particularly the common cold in the elderly. However, the results of some studies do not support these beneficial effects of zinc or vitamin E. While the discrepancy remains to be resolved, investigators have identified the factors that may be attributable and thus should be controlled in the future studies.

Among such factors are the differences in the studied populations and their health and nutrition status, in the supplementation regimen including supplement form, dosage, and duration, and in the methods used to measure outcome variables. There is a linear association between DTH response and net increase in plasma vitamin E levels up to a span by 25 $\mu\text{mol/L}$. In addition, elderly with higher plasma vitamin E levels had a better response to the hepatitis B vaccine than those with lower levels. Similarly, subjects with normal final serum zinc concentration had a lower incidence of pneumonia as well as shorter duration of pneumonia and antibiotic use relative to those with low zinc levels. Thus, zinc supplementation could improve immune function in those with zinc deficiency, but it may be less effective in healthy, zinc-replete elderly.

Similarly, while it is quite convincing that vitamin D supplementation is effective in reducing the risk of colds or influenza in the elderly subjects who had low 25-(OH)D levels,

there is no consistent evidence to support that the same is true in those with adequate baseline levels. It remains to define how vitamin D supplementation impacts immune function and risk of infection in the elderly with different basal serum levels of 25-(OH)D.

Taken together, the interaction of aging, immunity, infection and nutrition is a dynamic area of research. Based on what we have known, nutrition is a modifiable factor that may allow us to mitigate age-associated defects in the immune system leading to improved defense against infection. Although epidemiologic studies have suggested an association of certain nutrients and dietary components with risk of mortality and morbidity related to infections, the results of interventional studies have been inconsistent, and very limited success have achieved in this regard. This indicates that effective nutrients and dietary components may work together generating additive and synergistic effects, which cannot be achieved by taking single nutritional supplements. Therefore, it is recommended that intake of nutrients should focus first on consumption of an adequate and balanced daily diet. However, it is worth emphasizing that higher amounts of certain nutrients above the currently recommended intake levels may be needed for elderly persons, especially those who are frail, ill, on medication, or in a lower socioeconomic status.

The information from the previous studies and the new methods/techniques increasingly available can be utilized for implementing and developing the future studies in this area. Note that the discrepancy in the previous study findings is not only related to the use of different methods and protocols in measuring the study outcome variables, but more importantly, it may also reflect wide variation in subjects' characteristics which affect their response to intervention. For the nutrients reviewed here as well as many others, the nature of their impact on health (beneficial, neutral, or harmful) and efficacy can vary depending on whether and to what extent

they are really needed. Therefore, the future studies should use multiple doses, specifically targeted populations or large size general population with adequate power allowing for analysis of stratified sub-populations. In addition, studies need to consider the subjects' genetic and epigenetic background that potentially affects their response to a given nutrient. The “omics” information, such as genomic and epigenetic regulations (alleles, single nucleotide polymorphism, methylation), post-transcriptional regulation (microRNA), and proteomics screening for isomeric forms of protein and post-translational modification, can be used to identify the intrinsic inter-personal differences and design personalized interventions to achieve optimal results. Combined with the conventional assays, these new approaches will provide a more comprehensive and insightful understanding of whether and how nutritional intervention impacts aged immune system as well as the potential response to pathogen challenges. Finally, the current dietary guidelines for the nutrients reviewed here and also some others are not based on immune health and have not considered that additional intake, at least for some of them, may be required to help the elderly individuals to mitigate the adverse changes in immune system during the aging process. More research in this area is necessary so that specific recommendations could be made for this vulnerable population.

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Figure legend

Figure 1. Age-related changes in immune system (immunosenescence) and effects of zinc, vitamin E, and vitamin D. ?, not conclusive; N.D., not determined yet

Table 1. Effect of zinc on immune function and infection

Author, Year [Ref.]	Subjects (age range)	No. of participants	Daily dose (form of zinc)	Duration of intervention	Control for comparison	Immune-related results
Duchateau et al 1981 [67]	Institutionalized healthy elderly (>70y)	30	100 mg (sulfate)	1mo	No supplement	↑ % T cells, DTH and antibody response to tetanus ⇔ Leukocytes and lymphocytes numbers ⇔ Lymphocyte proliferation
Bogden et al 1988 [195]	Free living healthy elderly (60-89y)	103	15, 100 mg (acetate)	3mo	Placebo	⇔ DTH ⇔ Lymphocyte proliferation
Cossack 1989 [68]	Zinc deficient elderly (65-78y)	8	60 mg (acetate)	4.5mo	Pre-intervention	↑ DTH ⇔ Lymphocyte numbers
Bogden et al 1990 [69]	Free living healthy elderly (60-89y)	63	15, 100 mg (acetate)	1y	Placebo	↑ DTH ↑ Lymphocyte proliferation (PHA) with 15 mg and placebo ↑ NK cell activity with 100mg at 3 mo
Boukaïba et al 1993 [196]	Institutionalized elderly (73-106y)	44	20 mg (gluconate)	8wk	Placebo	↑ Thymulin activity
Prasad et al 1993 [62]	Zinc deficient elderly (50-80y)	13	30 mg (acetate)	6mo	Pre-intervention	↑ DTH ↑ Thymulin activity ↑ IL-1 production (PHA)
Kajanachumpol et al 1995 [197]	Diabetic patients (60-80y)	25	50 mg (sulfate)	12wk	Placebo	↑ % CD4 in lymphocytes ⇔ % CD8, CD4/CD8
Girodon et al 1997 [75]	Nursing home residents (≥ 65y)	81	20mg (sulfate)	2 y w selenium	Placebo	↓ Infectious events (12/20 vs placebo 35/20)
Fortes et al 1998 [71]	Retirement home residents (≥ 65y)	118	25 mg (sulfate)	3mo w/wo vitamin A	Placebo w/wo vitamin A	↑ Lymphocyte proliferation (ConA, PHA) ↑ CD4+, CD8+ T cells
Provinciali et al 1998 [73]	Institutionalized elderly (64-100y)	350	90mg (sulfate)	60d	No treatment	⇔ Antibody titer to influenza vaccine ⇔ Number of leukocytes, neutrophils and lymphocytes

						⇔ Lymphocyte phenotype (% CD3+, CD4+, CD8+)
Girodon et al 1999 [74]	Nursing home residents (≥ 65y)	364	20mg (sulfate)	2 y w selenium	Placebo	⇔ Antibody titer to influenza vaccine ⇔ DTH ↓ Number of subjects with respiratory infections (64/182 vs placebo 82/182) (p=0.056) ⇔ Number of subjects with urogenital infection (32/182 vs placebo 36/182)
Mocchegiani et al 1999 [198]	Elderly with chronic obstructive bronchitis (63-75y)	29	12mg	1mo	Placebo	↑ CD4+ T cell numbers ↓ Infections relapses (2 relapses/15 patients vs 6 relapses/14 patients)
Mocchegiani et al 2003 [108]	Healthy elderly (63-75y)	27	12mg	1mo	Placebo	↑ NK cell activity
Kahmann et al 2006 [70]	Healthy elderly (65-82 y)	19	10mg (aspartate)	7wk	Pre-intervention	⇔ CCR4/CCR5 ↓ CD25+/CD4+
Hodkinson et al 2007 [199]	Healthy individuals (55-70y)	93	15, 30mg (gluconate)	6mo	Placebo	↓ B cell counts with 30 mg at 3 mo ↑ CD4+/CD8+ with 15mg at 6mo
Meydani et al 2007 [77]	Nursing home elderly (≥ 65y)	420	7mg (sulfate)	1y w vitamin E	End-of-study serum zinc < 70 µg/dL	↓ Incidence and duration of pneumonia, number of new antibiotic prescription as well as days of antibiotic use for pneumonia among subjects with end-of-study serum zinc ≥ 70 µg/dL
Prasad et al 2007 [76]	Healthy elderly (55-87y)	49	45mg (gluconate)	12mo	Placebo	↓ Incidence of infections ↓ Incidence of common cold (p=0.067) and fever ↓ TNF-α production (LPS) ↑ IL-2 mRNA among zinc-deficient elderly subjects at 6 mo
Barnett et al 2016 [72]	Nursing home elderly (≥ 65y)	25	30mg (gluconate)	3mo	Placebo	↑ Lymphocyte proliferation (PHA, anti-CD3/CD28) ↑ T cell numbers

Table 2. Effect of vitamin E on immune function and infection

Author, Year [Ref.]	Subjects (age range)	No. of participants	Daily dose*	Duration of intervention	Control for comparison	Immune-related results
Meydani et al 1990 [16]	Healthy elderly (≥ 60 y)	32	800 mg	30d	Placebo	$\uparrow$ Lymphocyte proliferation (Con A) $\uparrow$ IL-2 production $\uparrow$ DTH $\downarrow$ PGE ₂ production $\Leftrightarrow$ Lymphocyte proliferation (PHA) $\Leftrightarrow$ IL-1 production (LPS), serum IgG, IgM, IgA
Meydani et al 1997 [127]	Free-living healthy elderly (≥ 65 y)	78	60, 200, 800 mg	235d	Placebo	$\uparrow$ DTH and antibody titer to hepatitis B and tetanus with 200 and 800 mg $\Leftrightarrow$ % T cell, CD4, CD8, B cells $\Leftrightarrow$ IgA, IgM, IgG $\Leftrightarrow$ Antibody titer to diphtheria
Pallast et al 1999 [129]	Free living elderly (65-80y)	156	50, 100 mg	6mo	Placebo	$\Leftrightarrow$ DTH, IL-2 production
Graat et al 2002 [136]	Noninstitutionalized elderly (≥ 60 y)	317	200 mg	15mo	Placebo	$\Leftrightarrow$ Incidence and severity of acute respiratory tract infections
Hemila et al 2004 [134]	Male smokers (50-69y)	7469	50 mg (<i>dl</i> - α -tocopherol)	median 6.1y w/wo β -carotene	Placebo w/wo β -carotene	$\uparrow$ Incidence of pneumonia among subjects who had initiated smoking at a later age (>21 y)
Meydani et al 2004 [137]	Nursing home residents (≥ 65 y)	451	200 IU (<i>dl</i> - α -tocopherol)	1y	Placebo	$\Leftrightarrow$ Incidence and number of days with infection for all, upper, or lower respiratory infection $\downarrow$ Number of subjects with all or upper respiratory infection $\downarrow$ Incidence of common cold $\Leftrightarrow$ Antibiotic use
Hemila et al 2006 [135]	Male smokers (50-69y)	14573	50 mg (<i>dl</i> - α -tocopherol)	mean 4.7y w/wo β -carotene	Placebo w/wo β -carotene	$\downarrow$ Risk of common cold among the subjects smoking 5-14 cigarettes per day at baseline and living in cities $\uparrow$ Risk of common cold among the subjects smoking more and living away from cities
De la Fuente	Free-living	33	200 mg	3mo	Pre-	$\uparrow$ Lymphocyte proliferation (PHA)

el al 2008 [130]	healthy elderly (70.4±5.1y)		(<i>dl</i> - α -tocopherol)		intervention	↑ IL-2 production ↑ NK activity ↑ Chemotaxis and phagocytosis
Belisle et al 2009 [142]	Nursing home residents (≥ 65y)	110	182 mg (<i>dl</i> - α -tocopherol)	1y	Placebo	↓ TNF- α production with A/A and A/G genotypes at TNF α -308G>A
Hemila et al 2011 [133]	Male smokers (50-69y)	29133	50 mg (<i>dl</i> - α -tocopherol)	median 6.1y w/wo β -carotene	Placebo w/wo β - carotene	↓ Incidence of pneumonia among subjects who had the least exposure to smoking and exercised during leisure time

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

Table 3. Effect of vitamin D on immune function and infection

Author, Year [Ref.]	Subjects (age range)	No. of participants	Daily dose*	Duration of intervention	Control for comparison	Immune-related results
Kawaura et al 2006 [172]	Healthy nursing home resident, female (70-99y)	34	40 IU (1 α -OH cholecalciferol)	20y	No supplement	↓ Rates of <i>H. pylori</i> infection by serology
Aloia and Li-Ng 2007 [169]	Postmenopausal African American women (50-75y)	208	800 IU (2y) +2000 IU (1y)	36mo w calcium	Placebo w calcium	↓ Number of subjects with cold and influenza symptoms (8/104 vs 26/104)
Avenell et al 2007 [200]	Elderly ($\geq 70y$)	3444	800 IU	24-62mo w calcium	Placebo w calcium	↔ Infection (300/1740 vs 321/1704) ↔ Antibiotic use (11/1737 vs 128/1703)
Bischoff-Ferrari et al 2010 [171]	Elderly with recent hip fracture ($\geq 65y$)	162	2000 IU	3mo w calcium	800 IU w calcium	↓ Number of subjects with hospital readmission due to infections (1% vs 11%)
Tran et al 2014[170]	Elderly (60-84y)	620	30,000 or 60,000 IU	12mo	Placebo	↓ Antibiotic use in those $\geq 70y$ with 60,000 IU (RR=0.53)
Asamura et al 2010 [167]	Nursing home residents (mean 80.9y)	32	-	-	1,25-(OH) ₂ D low group	↓ DTH ↓ Respiratory infection (22% vs 64%) ↔ Pneumonia (6% vs 21%)
Hirani 2013 [166]	Non-institutionalized elderly ($\geq 65y$)	2070	-	-	25-(OH)D, 49-63.9 nmol/L	↓ Self-reported respiratory infection (OR=0.61)
Hwang et al 2013 [158]	Healthy female ($>60y$)	34	-	-	25-(OH)D < 75 nmol/L	↓ Naïve(CD28+CD95-) CD8+ T cells ↔ Lymphocyte proliferation (PHA) ↔ Serum IL-1, IL-4, IL-6, IL-17, IFN- γ
De Vita et al 2014 [201]	Elderly ($>60y$)	867	-	-	25-(OH)D < 31.4 nmol/L	↓ IL-6, hsCRP, spg130, TNF- α
Sadarangani et	Healthy	159	-	-	25-(OH)D	↔ Antibody response to influenza vaccine

al 2016 [159]	subjects (50-74y)				levels	⇔ Influenza virus-specific IgG secreting B cells ⇔ Immune cell phenotype (DC, CD4+, CD8+ T cells)
Li-Ng et al 2009 [202]	Adults (18-80y)	148	2000 IU	3mo	Placebo	⇔ Incidence of upper respiratory infection symptoms ⇔ Duration and severity of upper respiratory symptoms
Laaksi et al 2010 [203]	Young men undergoing military training (18-28y)	164	400 IU	6mo	Placebo	↑ No days absent from duty ⇔ Days of absence from duty due to acute respiratory infection ⇔ Self-reported symptoms
Urashima et al 2010 [204]	School-children (6-15y)	334	1200 IU	120d	Placebo	↓ Incidence of influenza A ⇔ Incidence of influenza B ⇔ Other influenza-like illness
Yusopov et al 2010 [205]	Adults (18-80y)	148	2000 IU	3mo	Placebo	⇔ Serum levels of GM-CSF, IFN- γ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, and TNF- α
Jorde et al 2012 [206]	Adults (32-84y)	569	1111-6800 IU	6mo	Placebo	⇔ Risk of influenza-like illness
Murdoch et al 2012	Healthy adults (mean 47y)	322	Bolus dose of 100,000-200,000 IU/mo	18mo	Placebo	⇔ Number of upper respiratory infections ⇔ Duration and severity of upper respiratory infections
Urashima et al 2014 [207]	High-school students (15-18y)	148	2000 IU	2mo	Placebo	⇔ Incidence of influenza A ⇔ Incidence of influenza B
Martineau et al 2015 [208]	Sheltered accommodation residents and carers (67.5±12.8y)	240	Residents: 10 μ g daily + 2.4mg once every 2mo Carers: 3mg once every 2mo	1y	Residents: 10 μ g daily + placebo once every 2mo Carers: placebo once every 2mo	↑ Risk of median time to first upper respiratory infection ↑ Duration of upper respiratory infection symptoms (median 7 vs 5d) ⇔ Risk and duration of lower respiratory infection

*Supplemented with cholecalciferol unless indicated.

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pathogen

Innate Immunity

Neutrophils

NK cells

Macrophages

Dendritic cells

Aging

Nutrients

Chemotaxis	↓	
Bacterial killing	↓	↑ 1,25-(OH) ₂ D
Cytotoxicity	↓	↑ Zn, VitE
PGE ₂	↑	↓ VitE
COX-2	↑	↓ VitE
Phagocytosis	?	
1α-hydroxylase	N.D.	↓ 1,25-(OH) ₂ D
Cathelicidin	N.D.	↑ 1,25-(OH) ₂ D
Maturation	↓	↑ 1,25-(OH) ₂ D

Apolipoprotein E
SR-B1 scavenger receptor
CD36 scavenger receptor
← VitE

VitD
Liver
↓
Kidney
↓
1,25-(OH)₂D

Adaptive Immunity

T cells

CD4
T cells

B cells

CD8
T cells

Naïve T cells	↓	↑ VitE
Proliferation	↓	↑ Zn, VitE
IL-2	↓	↑ Zn, VitE
IFN _γ	↓	↑ VitE
Lck recruitment	↓	↑ Zn, VitE
Memory T cells	↑	
Treg cells	?	↑ 1,25-(OH) ₂ D
Th17 cells	?	↓ 1,25-(OH) ₂ D
Response to influenza virus	↓	↑ Zn, VitE
Low affinity Ab	↑	↓ Zn

Metallo-thionein ↑

Aging