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Review

The Role of Nutrition in Enhancing Immunity in Aging

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ABSTRACT: Aging is associated with declined immune function, particularly T cell-mediated activity, which contributes to increased morbidity and mortality from infectious disease and cancer in the elderly. Studies have shown that nutritional intervention may be a promising approach to reversing impaired immune function and diminished resistance to infection with aging. However, controversy exists concerning every nutritional regimen tested to date. In this article, we will review the progress of research in this field with a focus on nutrition factor information that is relatively abundant in the literature. While vitamin E deficiency is rare, intake above recommended levels can enhance T cell function in aged animals and humans. This effect is believed to contribute toward increased resistance to influenza infection in animals and reduced incidence of upper respiratory infection in the elderly. Zinc deficiency, common in the elderly, is linked to impaired immune function and increased risk for acquiring infection, which can be rectified by zinc supplementation. However, higher than recommended upper limits of zinc may adversely affect immune function. Probiotics are increasingly being recognized as an effective, immune-modulating nutritional factor. However, to be effective, they require an adequate supplementation period; additionally, their effects are strain-specific and among certain strains, a synergistic effect is observed. Increased intake of fish or n-3 PUFA may be beneficial to inflammatory and autoimmune disorders as well as to several age-related diseases. Conversely, the immunosuppressive effect of fish oils on T cell-mediated function has raised concerns regarding their impact on resistance to infection. Caloric restriction (CR) is shown to delay immunosenescence in animals, but this effect needs to be verified in humans. Timing for CR initiation may be important to determine whether CR is effective or even beneficial at all. Recent studies have suggested that CR, which is effective at improving the immune response of unchallenged animals, might compromise the host's defense against pathogenic infection and result in higher morbidity and mortality. The studies published thus far describe a critical role for nutrition in maintaining the immune response of the aged, but they also indicate the need for a more in-depth, wholistic approach to determining the optimal nutritional strategies that would maintain a healthy immune system in the elderly and promote their resistance to infection and other immune-related diseases

Key words: Aging; Immunity; Infection; Nutrition

Aging is a complex process for living organisms. During the process of aging, the human body accumulates damage at the molecular, cellular, and organ levels, which results in diminished or dysregulated function and increased risk of disease and death. These age-related changes are well exemplified in the immune system. The immune system is a complex collection of different cells. Aging affects a majority of these cells to different degrees, often detrimentally; collectively, this is referred to as immunosenescence. While many aspects of immune function decline with aging, some become overactive, e.g., increased autoantibody production or upregulated inflammation state. Given that the main function of the immune system is to detect and guard the body against the invasion of foreign substances as well as the enemies within, e.g., neoplasia and autoimmunity, the consequences of immunosenescence are quite predictable. Indeed, it has been well-documented that the

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aged have increased susceptibility to and prolonged recovery from infectious disease, poor response to immunization, increased incidence of most types of cancers, and increased risk of developing certain autoimmune diseases.

Like the age-related changes that occur in other systems of the body, immunosenescence is a universal phenomenon, but there is considerable heterogeneity among individuals owing largely to the interaction of genetics, environment, life style, and nutrition. Nutrition as a modifiable factor in impacting immune function has been studied for a few decades, and this research has developed into a field called nutritional immunology. There is little argument that deficiency in both macronutrients and micronutrients can cause immune function impairment, which can be reversed by nutrient repletion. Nutritional deficiencies are prevalent among all age groups in less developed countries and are a main contributor to a high incidence of morbidity and mortality from infectious diseases. Even in developed countries, where general nutritional deficiencies are rare, specific nutrient deficiencies are observed among a significant portion of the elderly population. This is associated with a variety of factors seen more frequently in this population such as disability (physical, mental, dental), disease (digestive and metabolic disorders), disease and medicine-induced anorexia, poor food selection, and lower socio-economic status. Furthermore, the requirement for certain dietary components may need to be raised for the aged. For example, the elderly might need to consume more antioxidants to counteract increased oxidative stress and to compensate for reduced enzymatic antioxidant defense.

This review will examine the data representing the prominent nutritional approaches to delay/reverse immunosenescence and to improve the aged hosts' resistance to infection. Immunosenescence as discussed in this review is focused on defense-related immunity relevant to infection. However, this review will not cover age-associated low grade inflammation and increased risk for autoimmune disease, which also pertain to immunosenescence. The discussion of nutritional modulation of immunosenescence will be preceded by a brief review of age-related changes in the immune system. This will not be, by any means, a comprehensive review of the topic, given the fact that all the important aspects of immunosenescence are already covered in great detail by other authors in this special issue.

Alterations in immune system during aging

Most of the components in the immune system experience different degrees of change with advancing age. These age-associated alterations involve both arms of the immune system, i.e., innate and adaptive immunity. A negative impact of advancing age has been well documented on the adaptive immune system, but there appears to be no consensus on whether, how, and to what extent aging impacts the innate immune system. Within the adaptive immune system, both cell-mediated (T cells) and humoral (B cells) immune responses are affected by aging, but the most striking change is observed in T cells. A decline in T cell function is known to be the central defect in immunosenescence as evidenced by numerous animal and human studies [1-3]. Age-associated impairment in T cells involves both their early development in the thymus and their continued expansion, differentiation, and function in the peripheral lymphoid tissue. Chronic involution of the thymus is thought to be one of the major contributing factors to the loss of immune function with aging. Thymic involution leads to impaired T cell differentiation and maturation with aging [4] resulting in a significant decline in the output of new T cells [4, 5]. This drives a shift towards greater numbers of antigen (Ag)-experienced memory T cells and a smaller proportion of naïve T cells [6-8]. It has been argued that despite drastic thymic involution and low output, the number of peripheral T cells is well-maintained with aging except for a moderate reduction in the population of naïve T cells. Recent research suggests that to maintain this relatively stabilized T cell population, naïve T cells in the aged must live longer, and as a result, they may accumulate defects that make their function less optimal [9, 10], and it also causes a reduced T cell receptor (TCR) diversity [11-13].

Age-related defects in T cell function have been repeatedly demonstrated, either determined *in vitro* as proliferative responses to stimulation with mitogens, Ag, and anti-CD3 antibody (Ab), or determined *in vivo* as T cell proliferation responding to immunization in adoptive transfer animal models, delayed-type hypersensitivity (DTH) response, Ab production to T cell-dependent Ag, and increased incidence of infectious disease. Aging is associated with declined interleukin (IL)-2 production and expression of its receptor, which often result in diminished proliferation of T cells [6, 14-16]. The defect in proliferation and IL-2 production appears more specific to naïve rather than to memory T cells [14]. Since IL-2 is essential to drive T cell expansion, which is required for mounting an effective immune response [17], the age-related defect in IL-2 production is probably a major contributing factor responsible for declined T cell function in aged mice [16, 18, 19]. T cell activation signaling through TCR is needed for IL-2 production and subsequent T cell expansion. Several age-related defects have been identified in the early

signaling events of T cell activation including tyrosine and serine/threonine phosphorylation [20-22], calcium mobilization [21, 22], MAPK activity [23, 24], and activation of nuclear transcription factors NFAT [25], AP-1 [26, 27], and NF-κB [28, 29]. Ag engagement with T cells through formation of an immune synapse between Ag-presenting cells (APC) and T cells triggers redistribution of several signaling molecules to the proximity of TCR. An impaired immune synapse formation and reduced distribution of key signaling molecules including Zap70, LAT, Vav, Lck, and PLCγ have been reported in T cells from old compared to young mice [30-32]; this defect in immune synapse formation is more pronounced in naïve than in memory T cells [31]. Another important feature of T cell senescence is the loss of co-stimulation molecule CD28 expression. CD28 provides the major co-stimulatory signal that complements T cell receptors and is critical for T cell activation, proliferation, and survival. Thus, the loss of CD28 expression on T cells with aging is expected to significantly contribute to impaired T cell function. Indeed, CD28- T cells manifest defects in T helper (Th) cell function such as assisting B cell proliferation and immunoglobulin (Ig) production and Ag-specific cytotoxic CD8 T cell function. On the other hand, CD28- T cells are shown to promote the survival of autoreactive T cells, suppress APC function of dendritic cells (DC), and produce large amounts of interferon (IFN)-γ, which together seem to play a proinflammatory role (reviewed in [33, 34]).

In addition to intrinsic changes in T cells with aging, other non-T cell factors, particularly suppressive factors produced by macrophages (MΦ), have been shown to contribute indirectly to the decline of T cell function with aging. Among them, prostaglandin (PG)E_2 has been consistently shown to directly inhibit T cell proliferation. MΦ and spleen cells from old mice [35-37] and peripheral blood mononuclear cells (PBMC) from elderly human subjects [38] produce significantly more PGE_2 than their young counterparts. Increased PGE_2 production observed in MΦ from old compared to young mice is mainly caused by increased cyclooxygenage (COX)-2 mRNA and protein expression and thus, increased COX-2 activity when stimulated with lipopolysaccharide (LPS) [39, 40]. It has been suggested that ceramide mediates the age-associated increase in COX-2 expression and consequently, PGE_2 production [39]. In a subsequent study, Wu et al. have shown that ceramide-induced up-regulation of COX-2 in old MΦ was a result of its induction of NF-κB activation, a key transcription factor in COX-2 regulation [41].

B cell-mediated humoral immune response is believed to be compromised during aging. Age-related impairment in T cell helper function contributes to the compromised humoral immune response in the aged; however, studies have clearly shown that intrinsic defects occur in B cells during aging. Despite substantially decreased pro-B cells and pre-B cells exported from the bone marrow of aged mice [42, 43], the numbers of peripheral B cells remain relatively constant in a manner somewhat similar to what happens in T cell homeostasis during aging. This suggests increased longevity of peripheral B cells [44]. Although blood Ig concentrations generally remain unchanged or even increased, specific Ab response decreases with aging [45]. The quality of humoral immune response is less optimal in the aged as characterized by lower levels of effective and specific Ab, lower Ab affinity, higher levels of non-specific Ab, and decreased IgG isotype class switching in response to vaccination [46-48]. Furthermore, while specific Ab response declines with age, there is an increase in autoantibody levels [49, 50], which may explain the increased risk of developing autoimmune diseases in the elderly. No clear explanation exists presently for increased autoantibody levels with aging. One interesting theory proposes that increased autoantibody production with aging might be a consequence of the autoreactive recall response of memory B cells [51]. According to this theory, existing autoreactive memory B cells, which have been maintained in a tolerant state, become re-activated at a later age due to triggering events. These events include the change in B cell repertoires, reduced self-tolerance with a detection bias, loss of tissue integrity yielding neo-self antigens, and re-exposure to infectious agents that mimic the molecular composition of self tissues.

The innate immune system is the first line of defense for the host. It is composed of a variety of cell types and functional molecules produced by these cells. The impact of aging on the innate immune system is complex and has been discussed in several recent reviews [52-54]. While some innate immune responses are diminished with aging, others remain unchanged or even elevated. Unfortunately, there have been many discrepancies between studies on several aspects of innate immunity. As an example, the term “dysregulation” is often used to describe an altered innate immune response rather than “increase” or “decrease” given that some innate immune responses go down while others go up with aging. For instance, inflammation is a protective response to injury signals so that the body can eliminate the injury, whether it is an invading microorganism or dead tissue. However, excessive, long-lasting inflammation can be harmful. Although consensus has not yet been reached, some studies suggest that aging is associated with a chronically upregulated inflammation state, a

phenomenon often called "inflammaging." This theory is mainly supported by studies showing higher peripheral levels of inflammatory cytokines and acute-phase reaction proteins from the liver such as C-reactive protein in old compared to young subjects [1, 55-59]. It has been estimated that the aged have a 2-4-fold increase of these inflammatory mediators in their serum, which predicts mortality independent of pre-existing morbidity [3]. This inflammation state has been implicated in the pathogenesis of several common and disabling diseases, most of which have a clear connection to advancing age including cardiovascular disease, type 2 diabetes, Alzheimer's disease, Parkinson's disease, osteoporosis, and rheumatoid arthritis.

Natural killer (NK) cells, a key component of innate immunity, are involved in the recognition and lysis of tumors and virally-infected cells. NK cell numbers have been shown to remain unchanged or to increase with aging. However, evidence from animal and human studies indicates that NK cell cytotoxicity on a per cell basis and the cells' ability to produce cytokines and chemokines are impaired with aging (reviewed in [53, 54, 60, 61]). Furthermore, aging can also affect NK cell activity indirectly via diminished production of NK cell-promoting Th1 cytokines (IL-2, IFN-α, and IFN-γ). Therefore, decreased NK activity with aging may contribute to a higher incidence of viral infection and cancer in the elderly.

Neutrophils are another primary innate immune defenses against pathogens, particularly bacteria, yeasts, and fungi. They function through phagocytosis and microbicidal mechanisms, which involve the generation of reactive oxygen and nitrogen species, proteolytic enzymes and anti-microbial peptides as well as the recruitment of other immune cells. While the total number of neutrophils remains constant, some studies have demonstrated that aging is associated with declined neutrophil activity including chemotaxis, phagocytosis, oxidative burst, and intracellular killing as summarized in several reviews [53, 54, 60].

DC, the major APC in the innate system, is present in peripheral tissue including epithelial tissues. They capture and process Ag, and they mature while migrating toward the lymphoid organs where they present Ag to T cells, initiating a specific immune response. By doing this, DC function as a bridge linking the innate to the adaptive immune response. Limited information in the literature indicates a lack of consensus regarding the changes in DC function with aging (reviewed in [53, 54, 60]).

Nutritional intervention to delay/reverse immunosenescence

Apart from genetics, environmental factors have a significant impact on the composition and function of the immune system. Nutrition is a well-recognized, modifiable factor in this regard. Nutritional interventions are an effective, logistically feasible, and cost-effective approach to tackle immunosenescence and its associated diseases. Compelling evidence suggests that a suboptimal status of essential nutrients contributes to the immunological defects observed with aging. Furthermore, evidence indicates that increased intake of some nutrients above the recommended levels is needed to maintain proper function of the immune system and to reduce the incidence of infection in the elderly. Also, a variety of non-nutrient, dietary components have been shown to affect immune cell function, though with varying qualitative and quantitative efficacy. In this section, we will discuss published studies on nutritional interventions involving macronutrients (dietary lipids such as n-3 polyunsaturated fatty acids, PUFA), micronutrients (vitamin E, zinc), functional foods (probiotics), and total calorie control (caloric restriction, CR). Some studies, such as those on vitamin E and zinc, provide information regarding nutrients' effectiveness in the elderly, with or without comparison to their younger counterparts, whereas other studies, such as those on n-3 PUFA and CR, do not present much information focusing specifically on aged animals or elderly humans. Our discussion of each topic has been distributed according to the abundance and relevance of the information pertaining to the theme of this review: nutritional modulation of immunosenescence. While we have mainly focused on the studies that address the factor of age, we have also included some of the studies that do not since the immunologic changes reported in those studies have significant relevance to immunosenescence and age-related diseases and thus, deserve special attention for their potential applications in the elderly.

1. Vitamin E

1) Vitamin E and immune function

Vitamin E, a very effective chain-breaking, lipid-soluble antioxidant present in the membrane of all cells, is particularly enriched in the membrane of immune cells, which protects them from oxidative damage related to high metabolic activity, as well as high PUFA content in these cells [5, 62]. Vitamin E is considered one of the most effective nutrients enhancing immune function. A number of animal and human studies have indicated that vitamin E deficiency impairs both humoral and cell-mediated immune functions [63, 64]. Conversely, supplementation with vitamin E above recommended

levels, especially in the aged, has been shown to enhance immune response, which is possibly associated with increased resistance against several pathogens [37, 38, 65, 66]. Vitamin E is a generic term for all tocopherols and tocotrienols that exhibit the biological activity of α-tocopherol. There are eight naturally occurring forms of vitamin E: α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols. Chemically synthesized α-tocopherol contains eight stereoisomers and is designated all-rac-α-tocopherol (historically and incorrectly called dl-α-tocopherol), while the naturally-occurring stereoisomer of α-tocopherol is RRR-α-tocopherol (also called d-α-tocopherol). α- and γ-Tocopherols are the main forms of vitamin E in the common Western diet. α-Tocopherol is the most bioavailable and its plasma concentration is about 10-fold higher than γ-tocopherol, while other forms of vitamin E are very low or undetectable. α-Tocopherols, both synthetic and natural forms, are used in a majority of published studies (Table 1), and no consistent evidence suggests a difference in the immunologic effect between these two forms of α-tocopherol.

In an animal study testing vitamin E's effect on immune response, Meydani et al. [37] fed young and old mice diets containing either 30 ppm (control) or 500 ppm (supplementation) vitamin E for 6 wk and found that vitamin E supplementation significantly enhanced DTH response, lymphocyte proliferation to concanavalin A (Con A), and IL-2 production in old mice. This effect of vitamin E was associated with a decrease in PGE_2 production. These findings were later confirmed in rats by Sakai and Moriguchi [67] who reported that vitamin E supplementation (585 mg/kg diet) for 12 mo significantly improved T cell-mediated function (proliferation and IL-2 production) compared with rats fed a control diet containing 50 mg vitamin E/kg.

This immuno-enhancing effect of vitamin E in aged animals is applicable to elderly humans as evidenced by several double blind, placebo controlled clinical trials. Meydani et al. [38] showed that healthy elderly (≥60 y) who received vitamin E supplementation (800 mg/d) for 1 mo displayed a significant improvement in DTH response, *ex vivo* T cell proliferation, IL-2 production, and a significant decrease in plasma lipid peroxide concentration and PGE_2 production compared to values before supplementation. These parameters remained unaltered in elderly subjects receiving a placebo. In a subsequent study by the same group [66], the authors tested whether lower doses of vitamin E could also be effective. They gave free-living elderly (≥65 y) 60, 200, or 800 mg/d of vitamin E, or a placebo for 4.5 mo. All three vitamin E groups showed a significant increase in DTH response after supplementation compared to their baseline levels. The subjects receiving 200 mg/d of vitamin E showed a significantly greater increase in median percent change of DTH compared to those receiving the placebo as well as a significant increase in Ab titers to hepatitis B and tetanus vaccines (T cell-dependent Ag) compared to their baseline. In a similar study, Pallast et al. [68] found that healthy elderly subjects (65-80 y) administered 50 or 100 mg/d of vitamin E for 6 mo had a significant increase in DTH (induration diameter and number of positive responses) compared with their own baseline values. Notably, a change in the number of positive DTH responses tended to be greater in the 100 mg/d group than in the placebo group ($p=0.06$). A significantly greater improvement in the cumulative DTH score and the number of positive DTH responses was observed in a subgroup of subjects who received 100 mg vitamin E and had a low baseline DTH reactivity. To this end, a more recent study [69] further supported this finding by showing that elderly subjects receiving 200 mg/d vitamin E for 3 mo had higher levels of lymphocyte proliferative response to phytohemagglutinin (PHA), Con A-stimulated IL-2 production, NK activity, and neutrophil chemotaxis and phagocytosis but lower levels of neutrophil adherence and superoxide anion production. Although this study was not placebo-controlled, this drawback is reduced by the fact that when the subjects were tested again 6 mo after ending supplementation, the majority of improvements were reduced to the baseline levels. Based on combined results from several studies, Meydani et al. proposed that changes in plasma vitamin E levels up to 25 μmol/L are linearly associated with a change in DTH and that further increase in plasma vitamin E levels does not seem to be associated with an additional improvement in DTH [70]. It is estimated that a 25 μmol/L increase in plasma vitamin E can be achieved by consuming 200 mg/d of vitamin E [70]. Thus, it appears that 200 mg/d is an optimal dose for improving T cell-mediated function in the elderly.

Results from animal studies have helped determine the mechanism by which vitamin E has an immune-enhancing effect in the aged. In general, 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 PGE_2 by MΦ as summarized in previous reviews [70, 71]. The direct effect of vitamin E on T cell response was examined in T cells purified from the spleens of young and old mice using *in vitro* and *in vivo* supplementation methods. The results indicate that vitamin E can reverse the age-associated reduction in activation-induced T cell division and IL-2 production in naïve but not memory T cells [14]. How vitamin E

Table 1. Effect of vitamin E on immune function

Subjects	N	Intervention	Findings	Reference
Animals				
Male C57BL/6J mice, 24 mo	10 (C) 10 (E)	C: 30 mg/kg diet E: 500 mg/kg diet as dl-α-tocopheryl acetate for 6 wk	-Increase DTH response -Increase lymphocyte proliferation in response to Con A, and LPS -Decrease PGE_2 production -Increase Con A-stimulated IL-2 activity	Meydani et al. 1986 [37]
Male Fisher rats, 12 wk	5 (C) 5 (E)	C: 50 mg/kg diet E: 585 mg/kg diet as dl-α-tocopheryl nicotinate for 12 mo	-Increase lymphocyte proliferation in response to Con A, and PHA -Increase Con A-stimulated IL-2 activity measured by proliferation of IL-2 dependent CTLL-2 cells	Sakai and Moriguchi, 1997 [67]
Male C57BL/6 mice, 4 mo and 24 mo	Young 4 (C), 4 (E) Old 4 (C), 4 (E)	C: 30 mg/kg diet E: 500 mg/kg diet as dl-α-tocopherol acetate for 4 wk	-Increase IL-2 gene expression in young and old T cells -Decrease IL-4 gene expression in old T cells -Increase cell cycle-related gene Ccnb2, Cdc2, and Cdc6 in old T cells	Han et al. 2006 [249]
Male C57BL/6 mice, 22-26 mo	5 (C) 5 (E)	C: 30 mg/kg diet E: 500 mg/kg diets as dl-α-tocopheryl acetate for 8 wk	-Increase redistribution of LAT and Vav and a trend towards a increase in Zap70 (p=0.095) into the immune synapse	Marko et al. 2007 [31]
Male C57BL/6 mice, 23 mo	11-13 (C) 11-13 (TE)	C: 107 mg/kg diet as dl-α-tocopherol TE: 107 mg/kg diets as dl-α-tocopherol + 393 mg/kg as tocotrienols for 6 wk	-Increase lymphocyte proliferation in response to Con A, PHA, anti-CD3, or anti-CD3 and anti-CD28 (anti-CD3/CD28) -Increase LPS-induced IL-1β in splenocytes and LPS-induced IL-6 in MΦ -No effect on IL-2, IFN-γ, IL-4, IL-10 in splenocytes in response to either Con A or anti-CD3/CD28	Ren et al. 2010 [250]
Humans				
Healthy elderly, ≥60 y	14 (P) 18 (E)	P: soybean oil provided as capsule E: 800 mg/d as dl-α-tocopherol acetate in soybean oil provided as capsule for 30 d	Compared to basal value (before supplementation), -Increase Con A-stimulated lymphocyte proliferation and IL-2 production -Increase DTH response -Decrease PGE_2 and plasma lipid-peroxide concentration	Meydani et al. 1990 [38]
Free living healthy elderly, ≥65 y	19 (P) 20/20/19 (E)	P: capsule containing soybean oil E: 60, 200, or 800 mg/d as dl-α-tocopherol in soybean oil provided as capsule for 235 d	Compared to basal value (before supplementation), -Increase DTH with 60, 200, 800 mg/d, Increase Ab titer to hepatitis B with 200, 800 mg/d, Increase Ab titer to tetanus toxoid with 200 mg/d -No effect on serum Ig level (IgA, IgM, IgG), lymphocyte subsets (T cells, B cells, CD4+, CD8+), Ab production to diphtheria vaccine, ability of neutropils to kill C. albicans -No additional effect on IgG Ab against pneumococcal vaccine	Meydani et al. 1997 [66]
Free living elderly, 65-80 y	50 (P) 54/53 (E)	P: soybean oil provided as capsule E: 50 or 100 mg/d as dl-α-tocopheryl acetate in soybean oil provided as capsule for 6 mo	-Increase DTH response in all groups including two vitamin E supplementation group compared to their own baseline -A trend towards an increased DTH in 100 mg vitamin E/d group compared to placebo (p=0.06) -A trend towards a greater increase in cumulative DTH score (p=0.07) and the number of positive DTH response (p=0.08) in 100 mg vitamin E/d group with a low baseline DTH reaction -Increase IL-2, IL-4 and decrease IFN-γ in response	Pallast et al. 1999 [68]

			to PHA compared to their own basal values -A trend towards an increase in IFN-γ in 50 mg/d groups (p=0.07) compared to those in placebo	
Healthy adults, 25-35 y	26 (E)	400 mg/d as dl-α-tocopherol provided as capsule for 28 d	-Decrease PMA-stimulated hydrogen peroxide production -Increase PHA or LPS-stimulated lymphocyte proliferation -Increase % T cells and CD4+ T cells, but no change in % CD8+, NK cells, CD25+ cells -Decrease urinary 8-hydroxy-2'deoxyguanosine and malondialdehyde	Lee and Wan, 2000 [251]
Healthy elderly, mean age 70.4 y	33 (E)	200 mg/d as dl-α-tocopherol for 3 mo	-Increase PHA-stimulated lymphocyte proliferation, Con A-stimulated IL-2 production, NK cell activity -Increase neutrophil chemotaxis and phagocytosis	De la Fuente et al. 2008 [69]
Nursing home residents, >65 y	63 (P) 47 (E)	P: multivitamin and mineral (50% of RDA) provided as capsule E: 200 IU/d as dl-α-tocopherol + multivitamin and mineral (50% of RDA) provided as capsule for 1 y	-A significant interaction between vitamin E treatment and baseline cytokine production for follow-up production of IFN-γ (both Con A and PHA elicitation), TNF-α, IL-1β and IL-6 (LPS elicitation); a significant decrease cytokine production in subjects with low basal levels of cytokine production while a significant increase cytokine production in subjects with high basal levels of cytokine production -Subjects with the A/A and A/G genotype at TNF-α-308G>A who were treated with vitamin E had lower TNF-α production than those with the A allele treated with placebo -TNF-α-308G>A had a significant effect on TNF-α production at baseline in whole blood elicited with LPS	Belisle et al. 2008 [82] Belisle et al. 2009 [83]
Healthy adults, 20-50 y	17 (P) 15 (E) 16 (E+TE)	P: placebo tablet E: 200 mg/d as dl-α-tocopherol or 200 mg/d (70% tocotrienol +30% tocopherol) for 56 d	-Plasma vitamin E concentration, 8 μg/ml at basal and 18 μg/ml after vitamin E supplementation -No change in lymphocyte phenotype (% CD4+, CD8+, B, NK cells) -No effect on IL-4, IL-10, and IFN-γ production in response to Con A	Radhakrishnan et al. 2009 [252]
Healthy women, 18-25 y	108 into (P) or (TE)	P: soy oil provided as capsule TE: 400 mg/d as tocotrienol + 183.2 mg/d as dl-α-tocopherol for 56d	-After tetanus toxoid (TT) vaccination, increase plasma anti-TT IgG in both placebo and TE, but more significant increase in TE group -Increase Con A-stimulated IFN-γ production in both placebo and TE group, but more significant increase in TE group -Increase TT-stimulated IFN-γ production in TE group -Increase Con A-induced IL-4 in both groups and no difference between P and TE -Increase TT-stimulated IL-4 in TE -Decrease LPS-stimulated IL-6 in TE	Mahalingam et al. 2011 [253]

(C) control, (P) placebo, (E) vitamin E supplementation, (TE) tocotrienol supplementation

improves cell division and IL-2 production remains to be elucidated. The available information suggests that vitamin E may improve the early events in T cell activation including formation of effective immune synapses known to be impaired in aged animals and humans. For example, Marko et al. [31] showed that both *in vivo* and *in vitro* vitamin E supplementations improved effective immune synapse formation and restored defective redistribution of signaling molecules Zap70, LAT, Vav, and PLCγ in the immune synapse formed between APC and naïve CD4+ T cells from old mice. This effect of vitamin E was found in old naïve

CD4+ T cells and not in old memory or young CD4+ T cells of either subset [31]. The phosphorylation of LAT is required for recruitment of adaptor and effector proteins including Grb2, Gads, SLP76, Vav1, PLCγ1, and phosphoinositide 3-kinase [72, 73]. In line with reduced LAT distribution in immune synapse in old mice [31], LAT phosphorylation was reduced in CD4+ T cells, and this was reversed by vitamin E supplementation [74].

In addition to its direct effect on T cells, vitamin E can enhance T cell function by reducing production of PGE_2, a well known potent T cell suppressor. Studies have shown that MΦ and spleen cells from old mice and PBMC from elderly human subjects synthesize significantly more PGE_2 than their young counterparts [35-38], which contributes to the age-associated decline in T cell function [75, 76]. Wu et al. [77] reported that dietary supplementation with 500 ppm of vitamin E for 30 d reduced LPS-stimulated PGE_2 production by MΦ from old mice to a level comparable to that which is seen in young MΦ. They further showed that vitamin E exerted its effect through inhibiting COX activity but without altering COX-1 or COX-2 expression at either protein or mRNA level, which was later confirmed by other investigators [78, 79]. While it is still not well understood how vitamin E inhibits COX activity, it has been suggested that it may occur through reduction of peroxynitrite production [80]. Peroxynitrite is a molecule that has been shown to upregulate COX-2 activity without changing its expression [81].

Although vitamin E generally improves cell-mediated immune function in the elderly, it has been well recognized that response to vitamin E supplementation varies among individuals depending on several factors, such as baseline levels of immune response and genetic background. For example, Belisle et al. examined the interaction between the response to vitamin E treatment (200 IU/d for 1 y) in cytokine production (IFN-γ, TNF-α, IL-1β, and IL-6) and the baseline levels of these cytokines in an elderly population. They concluded that the effect of vitamin E supplementation on cytokine production depended on pre-supplementation cytokine levels [82]. This group [83] also proposed that single nucleotide polymorphisms may influence how an individual responds to vitamin E treatment in terms of cytokine production. They found that only the participants with the A/A and A/G genotypes at TNF-α-308G > A who were treated with vitamin E had lower TNF-α production than those with the A allele treated with placebo. Since the A allele at TNF-α-308G>A has been shown to be associated with higher TNF-α levels [84, 85], these results suggest that the anti-inflammatory effect of vitamin E may be specific to those genetically predisposed to higher inflammation. Table 1 provides a summary for the effects of vitamin E supplementation on immune responses demonstrated in animal and human studies.

2) Vitamin E and infection

The elderly have not only an increased incidence of infection but also prolonged infection recovery times. These complications result in high mortality rates among the elderly [86-88], and one of the underlying causes might be their compromised immune response [89, 90]. Thus, it is anticipated that supplementing the elderly with vitamin E would be a useful strategy to enhance a human host's resistance to infection by improving immune function. Indeed, several animal and human studies have reported a protective effect of vitamin E against infection (Table 2). Several of these studies have determined the effect of vitamin E on influenza infection in mice because host resistance to influenza infection has been used as a relevant outcome to test the changes in host immunity after nutrient intervention, especially those nutrients that affect T cell functions. As such, Hayek et al. [91] reported that old mice had higher viral titers after infection with influenza A/Port Chalmers/1/73 (H3N2) compared to young mice. Vitamin E supplementation (500 mg/kg diet) reduced viral titers in mice of both age groups but more significantly in old mice. Additionally, the authors noticed that the age-related decline in NK cell activity was restored by vitamin E, suggesting a possible mechanism for the protective effect of vitamin E in influenza infection. In a similar study, Han et al. [92] examined the involvement of cytokines in the effect of vitamin E on mouse influenza infection. They confirmed the protective effect of vitamin E in reducing viral titers in old mice and further showed that old mice, compared to young mice, produced less IL-2 and more PGE_2 before infection and had an impaired IFN-γ response to infection. All of these age-related changes were prevented by vitamin E supplementation. The authors also indicated that IL-4 production was not affected by age, infection, or vitamin E whereas the change in IFN-γ production significantly correlated with the decrease in viral titer. These results suggest that enhancement of the Th1 response is one of the mechanisms through which vitamin E provides protection against influenza infection in old mice.

Few investigators have directly examined the effect of vitamin E on resistance to infection in humans. While a majority of human studies have investigated the effect of nutritional intervention (including vitamin E) on immune response as primary outcome measures, these results are often used as surrogate markers for clinical endpoints. A retrospective study [93] in healthy persons

Table 2. Vitamin E in prevention and treatment of infections

Subjects	N	Intervention	Disease	Findings	Reference
Animals					
Male C57BL/6 mice, 22 mo	13 (C) 18 (E)	C: 30 mg/kg diet E: 500 mg/kg diet as dl-α-tocopherol acetate for 6 wk	Influenza Infection	-Decrease lung virus titers on day 2, 5, 7 post-infection -Tend to increase/restore NK activity -No effect on pulmonary cytotoxic T lymphocyte activity	Hayek et al. 1997 [91]
Male C57BL mice, 22 mo	6-9 (C) 6-9 (E)	C: 30 mg/kg diet E: 500 mg/kg diet as dl-α-tocopherol acetate for 8 wk	Influenza infection	-Decrease lung virus titer on day 5 and 7 post-infection -Increase IL-2 and IFN-γ productions -Inhibit transient increase of TNF-α on day 2 post-infection -No effect on IL-4	Han et al. 2000 [92]
Male C57BL/6 mice, 22-24 mo	NA	C: 30 mg/kg diet E: 500 mg/kg diets as dl-α-tocopherol acetate for 4 wk	Influenza/ S. aureus infection	-No effect on primary pulmonary Staphylococcus aureus (S. aureus) infection -Protect against secondary S. aureus infection after influenza infection	Gay et al. 2004 [254]
Humans					
Noninstitutionalized individuals, ≥ 60 y	153 (P) 164 (E) 163 (M) 172 (E+M)	E: 200 mg/d as dl-α-tocopherol M: vitamin (RDA) +mineral (25-50% RDA) E+M: 200 mg/d as dl-α-tocopherol + vitamin (RDA) +mineral (25-50% RDA) for median of 441 d	Respiratory Infection (RI)	-No effect on incidence and severity of acute RI in E group -Increase the number of symptoms, the duration of illness, % participants with fever, and restriction of activity in (E and E+M) compared to (P and M)	Graat et al. 2002 [98]
Male smokers, 50-69 y	21796 into (P), (E), (B), (E+B)	E: 50 mg/d as dl-α-tocopherol B: 20 mg/d as β-carotene E+B: 50 mg/d as dl-α-tocopherol + 20 mg/d as β-carotene during 4 y follow-up	Common colds	-Lower incidence of common cold in (E and E+B) compared to (P and B) who live in a city, smoke <15 cig/d, and aged ≥65 y	Hemila et al. 2002 [255]
Male smokers, 50-69 y	7287 (P) 7286 (E) 7282 (B) 7278 (E+B)	E: 50 mg/d as dl-α-tocopherol B: 20 mg/d as β-carotene E+B: 50 mg/d as dl-α-tocopherol + 20 mg/d as β-carotene for median of 6.1 y	Pneumonia	-Lower incidence of pneumonia in (E and E+B) compared to (P and B) who initiated smoking >21 y	Hemila et al. 2004 [96]
Nursing home residents, >65 y	220 (P) 231 (E)	P: multivitamin and mineral (one half of RDA) provided as capsule E: 200 mg/d as dl-α-tocopherol + multivitamin and mineral (one half of	RI	-No effect on incidence and number of days with infection for all, upper, or lower RI -Fewer participants ≥ 1 RI or upper RI -Lower incidence of common colds and fewer subjects ≥ 1 colds	Meydani et al. 2004 [94]

		RDA) provided as capsule for 1 y			
Male smokers, 50-69 y	14573 into (P), (E)	E: 50 mg/d as dl-α-tocopherol B: 20 mg/d as β-carotene E+B: 50 mg/d as dl-α-tocopherol + 20 mg/d as β-carotene during 4 y follow-up	Common colds	Due to interaction between vitamin E and β-carotene supplementation, mentioned in [256], they only analyzed E group compared to P group -Reduce incidence of common cold in subjects who live in cities, smoke <15 cig/d, and aged ≥69 y -Increase incidence of common cold in subjects who live away from cities, smoke ≥15 cig/d	Hemila et al. 2006 [97]
Male smokers, 50-69 y	7287 (P) 7286 (E) 7282 (B) 7278 (E+B)	E: 50 mg/d as dl-α-tocopherol B: 20 mg/d as β-carotene E+B: 50 mg/d as dl-α-tocopherol + 20 mg/d as β-carotene for median of 6.1 y	Tuberculosis (TB)	-Increase TB risk with dietary vitamin C ≥90 mg/d in (E and E+B) compared to (P and B) who smoke heavily (≥ 20 cig/d)	Hemila and Kaprio, 2008 [257]
Male smokers who initiated smoking ≤20 y, 50-69 y	10784 into (P), (B) 10873 into (E), (E+B)	E: 50 mg/d as dl-α-tocopherol B: 20 mg/d as β-carotene E+B: 50 mg/d as dl-α-tocopherol + 20 mg/d as β-carotene for median of 6.1 y	Pneumonia	-No difference in risk of pneumonia in subjects with BW from 70-89 kg between (E and E+B) and (P and B) -Increase pneumonia risk in subjects with BW <60 kg, or >100 kg in (E and E+B) compared to (B and B) whose dietary vitamin C ≥ median (75.3 mg/d)	Hemila and Kaprio, 2008 [258]
Nursing home residents, >65 y	246 (P) 254 (E)	P: multivitamin and mineral (one half of RDA) provided as capsule E: 200 mg/d as dl-α-tocopherol + multivitamin and mineral (one half of RDA) provided as capsule for 1 y	RI	Three way interactions between vitamin E supplementation, sex, and IL-10-819G>A -Fewer total RI and lower RI among women with G/G genotype who received vitamin E -Fewer total RI and lower RI among women with A/A who received placebo -Women with A/A genotype, more total RI and lower RI in vitamin E group than placebo	Belisle et al. 2010 [259]
Male smokers, 50-69 y	29133 into (P), (E), (B), (E+B)	E: 50 mg/d as dl-α-tocopherol B: 20 mg/d as β-carotene E+B: 50 mg/d as dl-α-tocopherol + 20 mg/d as β-carotene for median of 6 y	Pneumonia	-Decrease pneumonia risk in subjects who had the least exposure to smoking (smoking initiation ≥21 y, 5-19 cig/d) and exercised during leisure time in (E and E+B) compared to (P and B) -Increase pneumonia risk in subjects who had the highest exposure to smoking (smoking initiation ≤20 y, ≥20 cig/d) and did not exercise in (E and E+B) compared to (P and B)	Hemila and Kaprio, 2011 [95]

(C) control, (P) placebo, (E) vitamin E supplementation, (M) multivitamin-mineral, (E+M) vitamin E plus multivitamin-mineral, (B) β-carotene, (E+B) vitamin E plus β-carotene, NA: non applicable.

(≥60 y) showed that plasma vitamin E levels were negatively related to the number of past infections in these subjects despite an absence of such a correlation between the vitamin status and the measurements of immune function indices (T cell subsets, PHA-induced lymphocyte proliferation, and DTH). In a study by Meydani et al. [66], vitamin E supplementation was shown to improve immune response (DTH and response to vaccines) in healthy elderly. In addition, the authors noticed a non-significant ($p<0.09$), 30% lower incidence of self-reported infections among the groups supplemented with vitamin E (60, 200, or 800 mg/d for 235 d) compared with the placebo group. Since infection was not the primary outcome, the study did not have enough power to detect significant differences in the incidence of infections. The research team later addressed this question in a larger, double-blind, placebo-controlled trial that determined the effect of one-year's supplementation with 200 mg/d of vitamin E (optimal dose based on their previous study) on objectively recorded respiratory infections (RI) in 617 elderly nursing home residents (>65 y) [94]. Results showed significantly fewer participants acquiring one or more RI or upper RI in vitamin E-supplemented *vs.* the placebo-treated subjects and a lower incidence of common colds in the vitamin E group. These studies suggest that the immunostimulatory effect of vitamin E is associated with improved resistance to RI in the aged.

In contrast to this study, previous studies on vitamin E and infection in the elderly have demonstrated mixed results, largely owing to the various confounding factors present, particularly the difference in the health conditions of participants and the intervention protocols. For example, the results from the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) study showed a positive, no effect, or even a negative effect of vitamin E on pneumonia and the common cold depending on the age, smoking history, residence, and exercise, among other factors, of the subjects [95-97]. It is worth mentioning that the ATBC study used a small dose (50 mg/d) of vitamin E in combination with 20 mg/d of β-carotene, which makes it difficult to compare this study's results with those reported by Meydani et al. Likewise, a double-blind trial in the Dutch elderly cohort living in the community reported no effect of 200 mg/d of vitamin E on the incidence of all RI and even reported a worsening in the severity of infections [98]. Notably, however, there are several differences in the study design and data analysis between this study, conducted with free living participants, and the one conducted in managed nursing homes by Meydani et al, which might have contributed to different outcomes of the two studies. Taken together, the data obtained thus far suggest that the immune-enhancing effect of vitamin E in the elderly might be associated with increased resistance to RI in nursing home residents. However, further studies are needed to confirm these results in elderly living independently.

2. Zinc

1) Zinc and immune function

Zinc is a trace element essential for the growth and development of all organisms, and its impact on immune system has been a topic of intensive study [99-101]. The importance of zinc to immune function has been well-documented in studies on zinc-deficient humans and experimentally-induced animal models. Zinc deficiency affects multiple immune cell types involved in both innate and adaptive immunity. For instance, zinc deficiency is known to cause thymus involution and reduction in Th1 cells leading to an imbalance of Th1/Th2. Further, this deficiency causes a reduction in an array of immune functions such as lymphocyte proliferation, IL-2 production, DTH response, Ab response, NK cell activity, MΦ phagocytic activity, and certain functions of neutrophils (reviewed in [61, 101-105]). One of the best demonstrated examples is acrodermatitis enterophathica, a rare, inheritable metabolic disorder in which zinc deficiency is caused by zinc-specific malabsorption. Patients of this disease have thymic atrophy and lymphopenia as well as intercurrent infection due to impaired cell-mediated immune function, which can be reversed by zinc supplementation [102, 106]. These zinc-related impairments in immune function are similar to those observed in the elderly. Not surprisingly, like the elderly, zinc deficient subjects have greater susceptibility to a variety of pathogens (reviewed in [107]).

In contrast to the relatively clear association of zinc deficiency with impaired immune function, the beneficial effects of zinc supplementation on immune response has not been demonstrated in a consistent manner, particularly in human studies. In animal models, zinc supplementation can reverse thymic involution as demonstrated by an increase in thymulin activity, thymus weight, absolute number of T cells in thymocytes, and thymic output in middle-aged (12 mo) [108] or aged mice (22 mo) [109, 110]. In addition to its effect on the thymus, zinc supplementation can increase PHA- or Con A-stimulated lymphocyte proliferation and NK cell activity in old mice [109].

Several studies have investigated the efficacy of zinc supplementation on cell-mediated immunity and humoral response in the elderly human population. In some early studies, zinc supplementation was shown to improve

DTH response [111-114]. In one of those studies [112], institutionalized healthy elderly (>70 y, n=30) receiving zinc supplements (440 mg of zinc sulfate/d) or control for 1 mo also had improved IgG Ab response to tetanus vaccine, along with an increased proportion of circulating T cells. More recently, Prasad et al. showed that the elderly who consumed 45 mg of zinc/d as gluconate for 6 mo had increased mRNA expression of IL-2 and IL-2 receptor (IL-2R)α mRNA in PBMC [115]. When interpreting the results from early studies, however, caution is needed since these studies were usually done on small numbers of subjects without a placebo control and thus, it is possible that the improved DTH response over time may be due to repeated DTH administration. The studies reported by Bogden et al. may exemplify this point [116, 117]. They gave healthy elderly (60-89 y) a placebo, 15 mg, or 100 mg of zinc/d in combination with multivitamin/mineral supplements for 12 mo, and tests were conducted every 3 mo. At 3 mo, they found increased serum zinc levels in only the 100 mg of zinc/d group, but observed no significant change in immune function including DTH and *in vitro* PBMC proliferation [117]. When this study ended at 12 mo, they found that zinc supplementation at 100 mg/d increased plasma zinc levels and *in vitro* PBMC proliferation, but the increased NK cell activity found at 3 mo was no longer present [116]. DTH response was progressively improved over time in all groups, but this increase was significantly greater in the placebo group than in zinc-supplemented groups [116]. The authors suggested that this might have been due to the multivitamins and minerals consumed by the placebo group.

Results on the effect of zinc supplementation on lymphocyte population are varied. For example, Fortes et al. [118] reported that institutionalized healthy elderly (≥65 y) showed increased numbers of activated (HLA-DR+) CD4+ and cytotoxic T lymphocytes (CTL) after 3 mo of zinc supplementation (25 mg/d as zinc sulfate), while Kanmann et al. [119] showed a reduction in activated (CD25+) CD4+ and no difference in Th2/Th1 (CCR4+/CCR5+) in the free-living elderly (65-82 y) who had been supplemented with zinc (10 mg/d as zinc aspartate for 7 wk).

Serum concentrations of active thymulin decline with aging as evidenced in both mice and humans [120, 121]. Thymulin, a zinc-containing thymic hormone, needs zinc to exert its biological activity [122]. Similar to the results obtained from animal studies [108, 109], the elderly who received zinc supplementation showed increased concentration of active thymulin [114, 123, 124]. *In vitro* administration of thymulin has been shown to improve the reduced NK cell activity of spleen cells from old mice [125]. In line with this, an observational study on healthy subjects (>90 y) found a strong association between serum zinc levels and the proportion of NK cells [126]; also, zinc supplementation increased NK cell cytotoxicity in healthy elderly (12 mg/d for 1 mo) [124] and in zinc-deficient elderly (10 mg/d for 7 wk) [127]. However, it is not certain that this effect of zinc is sustainable. Bogden et al. [117] reported that zinc supplementation at 100 mg of zinc/d enhanced NK activity transiently after 3 mo but this effect was no longer present at later time points (6 mo and 12 mo). 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. As an example, a recent study showed that elderly carrying the GG genotype in the IL-6-174G/C locus displayed some differences in the relationship between zinc status (low plasma zinc, erythrocyte zinc, and nitric oxide-induced release of zinc in PBMC) and immune function (impaired NK cell cytotoxicity) when compared to those carrying GC and CC genotypes [127]. In a subgroup analysis based on plasma zinc levels, elderly with GC/CC alleles with plasma zinc >10.5 μmol/L had higher NK cell activity compared to those with GG allele, while subjects with plasma zinc ≤ 10.5 μmol/L showed the lowest NK cell cytotoxicity regardless of IL-6 polymorphism. Subjects with low zinc levels (≤10.5 μmol/L) benefited from zinc supplementation (10 mg of zinc/d for 7 wk) as demonstrated by improved plasma zinc levels and NK cell cytotoxicity. Among zinc-deficient elderly (60-83 y, serum zinc ≤11 μmol/L), those subjects receiving 10 mg of zinc/d for 7 wk and carrying MT1a+647GG and IL-6-174GC/CC alleles appeared to benefit more from zinc intervention than those bearing other genotypes as indicated by the largest elevation in plasma zinc and NK activity as well as reduction in plasma IL-6 and monocyte chemotactic protein-1 (MCP-1) levels in this group [128].

Zinc supplementation has also been tested for its potential enhancement of low vaccination efficacy observed in the elderly; the results thus far are inconclusive. In an observational study [129] determining the effect of host immune responses and plasma micronutrient levels on vaccination efficacy of trivalent influenza vaccine, the authors found that the elderly subjects (mean 81 y, n=61) had a lower immune response, a lack of post-vaccination increase in immune response, and lower Ab titers compared to young subjects (mean 27 y, n=27) even though plasma zinc levels were similar in young and old subjects before and after vaccination. In a controlled clinical trial [130], the elderly (64-100 y, n=160) supplemented with 400 mg of

zinc sulfate/d for 60 d starting 15 d before influenza vaccination did not show any difference in Ab titers compared to those receiving the placebo (n=190), and no change was found in lymphocyte phenotype (CD3+, CD4+, and CD8+) as a result of either vaccination or supplementation. Zinc's lack of a positive effect cannot be clearly interpreted, but it might be related to the high dose used (zinc sulfate 400 mg/d is equal to element zinc 90 mg/d) compared to lower doses used in other studies. Of note, a previous study showed that oral zinc administration of 300 mg/d for 6 wk caused an inhibition in PHA-induced lymphocyte proliferation as well as chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes (PMN) [131]. Results from some *in vitro* zinc supplementation studies support this speculation. For example, zinc supplementation at physiological concentration (15 μmol/L) boosts IFN-α production from leukocytes of elderly subjects [132], but zinc at higher concentrations (≥0.1 mmol/L) directly inhibits IL-1-dependent T cell proliferation through IL-1 receptor-associated protein kinase [133] and IL-2 production, expression of IL-2 and IL-2Rα mRNA, and NF-κB activation [134, 135]. The main findings from animal and human studies for zinc's immunomodulating effect are summarized in Table 3. Taken together, the efficacy of zinc supplementation in enhancing vaccination response in the elderly needs further investigation. In future studies, researchers should use a multi-dose design given the dose-dependent, biphasic effect of zinc on immune responses.

2) Zinc and host resistance against infection

Several human studies have investigated zinc's effect on infection (Table 4), and some of them indicate that zinc supplementation may provide protection against infection. Guatemalan children (6-9 mo) receiving 10 mg of zinc/d as sulfate for a mean of 7 mo had a reduced prevalence of diarrhea by 22% although no effect was observed in the incidence of RI [136]. In sickle-cell disease (SCD) patients, whose zinc status is commonly deficient, zinc supplementation (75 mg of zinc/d as acetate) for 3 mo reduced the total number of infections and upper RI, and it lowered the incidence of the common cold compared to those in the placebo group [137]. These SCD patients had a lower production of IFN-γ and a trend toward lower IL-2, but they had a higher production of TNF-α and IL-1β, compared to healthy subjects, representing a profile similar to that found in the elderly. Of note, zinc supplementation not only increased IL-2 and IFN-γ but also reduced TNF-α and IL-1β compared to the placebo group. These results might be relevant to zinc supplementation in the elderly.

Several studies have investigated the effect of zinc supplementation when it is started during the course of natural infection in the elderly population. In a controlled clinical trial (n=81) [138], institutionalized elderly (>65 y) had a significant decrease in the mean number of RI during a 2-y supplementation period with 20 mg of zinc and 100 μg of selenium. In a larger (n=725), controlled intervention trial [139], low-dose zinc and selenium supplementation (20 mg of zinc/d and 100 μg of selenium/d) for 2 y significantly increased humoral response (Ab titer and seroprotection) in institutionalized elderly (>65 y) after vaccination and tended to (p=0.06) reduce the incidence of RI, but these doses had no effect on DTH response during the 2-y period. Although these studies suggest a protective effect of zinc against RI, selenium's contribution must be considered. In a controlled trial with elderly persons (55-87 y), of whom 35% were zinc-deficient [140], Prasad et al. found that zinc supplementation (45 mg of zinc/d) for 1 y significantly increased plasma zinc levels over time in the zinc group (n=24) but not in the placebo group (n=25), and the zinc group tended to have fewer common colds (p=0.067) as well as fewer infections and fevers during the study. Recently, an observational study by Meydani et al. [141] showed that 29% of nursing home residents (>65 y) had low serum zinc levels (<70 μg/dL) despite supplementation that included 7 mg of zinc/d for over one year. Subjects with normal serum zinc concentrations of >70 μg/dL had lower pneumonia incidence, reduced total antibiotic use (by almost 50%), and shorter duration of pneumonia and antibiotic use (by 3.9 and 2.6 d, respectively) compared to those with plasma zinc concentrations of <70 μg/dL. The authors suggested that a double-blind, placebo controlled study is needed to determine the efficacy of zinc supplementation on elderly immune response. Observations from a large controlled trial in children further supports this finding by showing that zinc supplementation (70 mg, weekly) in children (<2 y, n=706) reduced the incidence of pneumonia compared to the placebo group (n=768) [142].

In summary, zinc deficiency induces immunological changes similar to those observed in the elderly. A significant percentage of the elderly have low serum zinc levels due to inadequate intake, impaired metabolism, infection, inflammation, etc. Evidence from the literature indicates that the elderly, particularly those with low serum zinc levels, might benefit from optimizing their serum zinc status through adequate intake, but further studies to determine optimal zinc intake in the elderly are needed. These studies should consider variations in individual genetic background in order to maintain optimal immune response and to document the efficacy

Table 3. Effect of zinc on immune function

Subjects	N	Intervention	Findings	Reference
Animals				
Male C57BL/6 mice, 12 mo	12 (C) 12 (Z)	117 mg/kg of zinc for 3 mo or 6 mo	-Increase serum zinc, thymulin activity, thymus weight, viable thymocytes, absolute number of T cells (DN, DP, SP) in thymocyte without altering frequency	Dardenne et al. 1993 [108]
Male Balb/c mice, 22 mo	10 (C) 10 (Z)	18 μg zinc/ml in the drinking water as sulphate for 1-3 mo	-Increase plasma zinc, thymulin activity, thymus weight, number of thymocytes, absolute number of T cells (DN, DP, SP) and % of CD4+CD8- in thymocyte -Increase absolute number of spleen T cells, PHA- or Con A-induced lymphocyte proliferation -Increase NK cell activity and IFN-γ-induced NK cell activity	Mocchegiani et al. 1995 [109]
Male C57BL/6 mice, 22 mo	8 (C) 8 (Z)	300 mg/kg of zinc as zinc carbonate for 25 d	-Increase plasma zinc level, thymic output -Decrease immature DN thymocytes, negative stem cell factor (SCF)	Wong et al. 2009 [110]
Humans				
Institutionalized healthy, >70 y	15 (C) 15 (Z)	440 mg zinc sulfate for 1 mo	-Increased number of circulating T cells, DTH, IgG Ab response to tetanus vaccine compared to control -No effect on number of total circulating leukocytes or lymphocytes and lymphocyte proliferation stimulated with PHA, Con A, or PWM.	Duchateau et al. 1981 [112]
Anergic to DTH, 64-76 y	5 (Z)	55 mg zinc/d as zinc sulfate for 1 mo	-Improvement in DTH response (assessed before and after supplementation)	Wagner et al. 1983 [113]
Free living healthy, 60-89 y	36 (P) 36 (Z, 15) 31 (Z, 100)	15 mg zinc/d or 100 mg zinc/d as zinc acetate plus vitamin and mineral vs vitamin and mineral (placebo) for 3 mo	-Increase in the zinc concentration in plasma at 100 mg zinc supplementation, but not in 15 mg while no change was observed in zinc concentration in other cells -No effect on DTH and lymphocyte proliferation at 3 mo	Bogden et al. 1988 [117]

Zinc deficient, 65-78 y	8 (Z)	60 mg zinc/d as zinc acetate for 4.5 mo	-Increase in the zinc concentration in plasma as well as lymphocytes and neutrophils -Improvement in DTH response	Cossack 1989 [111]
Free living healthy, 60-89 y	24 (P) 20 (Z, 15) 19 (Z, 100)	15 mg zinc/d or 100 mg zinc/d as zinc acetate plus vitamin and mineral vs vitamin and mineral (placebo) for 1 y	-Improvement in DTH response over time in all groups, but DTH response was greater in control group than either zinc groups -Transient enhancement in NK cell activity at 3 mo, but not at 6 mo and 1 y supplementation	Bogden et al. 1990 [116]
Institutionalized, 73-106 y	44 (P)/(Z) crossover	20 mg zinc/d for 8 wk and placebo for 8 wk	-Increase in active thymulin concentration	Boukaiba et al. 1993 [123]
Zinc deficient, 50-80 y	13 (Z)	30 mg zinc/d as zinc sulfate for 6 mo	-Increase in the zinc concentration in plasma as well as lymphocytes and granulocyte -Increase in serum thumulin activity, IL-1 production -Improvement in DTH response	Prasad et al. 1993 [114]
Institutionalized, 64-100 y	190 (C) 160 (Z)	400 mg zinc sulfate/d for 60 d	-No effect on influenza vaccine -No phenotypic change in lymphocyte population (CD3+, CD4+, and CD8+)	Provinciali et al. 1998 [130]
Institutionalized healthy, ≥ 65 y	30 (P) 31 (A) 28 (Z) 29 (Z+A)	A: 800 μg retinol palmitate/d Z: 25 mg zinc/d as zinc sulfate Z+A: 25 mg zinc/d as zinc sulfate and 800 μg retinol palmitate/d for 3 mo	-Tend to increase lymphocyte proliferation in response to Con A (p=0.052) and PHA (p=0.091) -No phenotypic change in lymphocyte population (CD3+, CD4+, CD8+, and NK cells) *In comparison between (Z and Z+A) and (P+A)* -Increase number of CD4+DR+ T cells (p=0.016) and cytotoxic T lymphocytes (p=0.005)	Fortes et al. 1998 [118]
Healthy, 65-85 y	12 (P) 15 (Z)	12 mg zinc/d for 1 mo	-Increase NK cell cytotoxicity and thymulin activity	Mocchegiani et al, 2003 [124]
Free-living, 65-82 y	19 (Z)	10 mg zinc/d as zinc aspartate for 7 wk	-Increase serum zinc level -Reduce activated T helper cells (CD4+/CD25+), but no effect on Th2/Th1 (CCR4+/CCD5+)	Kahmann et al. 2006 [119]
Institutionalized	12 (P) 12 (Z)	45 mg zinc/d as zinc gluconate for 6 mo	- Increase IL-2 and IL-2Rα mRNA in response to PHA	Prasad et al. 2006 [115]
Free living healthy, 55-70 y	31 (P) 28/34 (Z)	15 mg zinc/d or 30 mg zinc/d as zinc gluconate for 6 mo	-No effect on NK cells or CRP levels -Transiently decrease B cells with 30 mg zinc (3 mo) and	Hodkinson et al. 2007 [260]

			increase the ratio of CD4 to CD8 with 15 mg zinc (6 mo)	
Zinc deficient, 60-84 y	110 (Z)	10 mg zinc/d as zinc aspartate for 7 wk	-Increase plasma zinc level, NK activity, IL-6 production	Mocchegiani et al. 2008 [127]
Zinc deficient, 60-84 y	39 (Z)	10 mg zinc/d as zinc aspartate for 7 wk	-Increase plasma zinc level, NK activity, IL-6 production, and reduce MCP-1 production	Mariani et al. 2008 [128]

(C) control without supplementation, (P) placebo, (Z) zinc supplementation, (Z+S) zinc+selenium supplementation, (A) vitamin A supplementation, (Z+A) zinc+vitamin A supplementation, CRP: C-reactive protein, DN: double negative (CD4-CD8-), DP: double positive (CD4+CD8+), SP: single positive (CD4+CD8-, CD4-CD8+), PWM: pokeweed mitogen

of zinc supplementation in reducing infection, particularly pneumonia in the elderly.

3. Probiotics

1) Probiotics and immune function

Probiotics are defined as live microorganisms that reach the intestinal tract in sufficient numbers and exert health benefits on the host [143]. Probiotic microorganisms are typically members of the genera *Lactobacillus* (*L.*), *Bifidobacterium* (*B.*), and *Streptococcus* (*S.*). Aging is associated with a reduction in 1) beneficial microbes in the colon including bifidobacteria, countered by a rise in proteolytic bacteria, 2) intestinal Ag-specific secretory IgA response, which is important in mucosal-associated immunity and helps protect the host by binding a variety of Ag from bacteria, viruses, and fungi [144-146], and 3) gut-associated lymphoreticular tissues as manifested by a size reduction in Peyer's patches (PP), a decline in the number of lymphocytes in PP (especially, naïve CD4+ T cells and follicular DC) and mesenteric lymph nodes, a decreased ability of lamina propria T cells to proliferate and produce IL-2 [147], and an impaired T cell response such as Ag-specific Th function and CTL activity in PP (reviewed in [148]).

Probiotics are believed to modulate immune function in the gastrointestinal (GI) tract through interaction with intestinal epithelial cells [149, 150], M-cells in the PP [151, 152], and DC [153, 154]; thus, probiotics may affect other mucosal surfaces, including the upper respiratory tract, via mucosal immune systems. An increasing body of information now suggests that probiotics can also positively impact the systemic immune system [155-157]. Since mucosal and systemic immune functions are known to decline with aging, it is therefore expected that the aged would most likely benefit from consumption of probiotics. Indeed, studies have shown that splenocytes from old mice have a lower capacity to produce IFN-α and IFN-γ in response to mitogens, compared to splenocytes in young mice; this was reversed after administration of viable *L. bulgaricus* and *S.thermophilus* [$8x10^8$ colony forming units (CFUs)/d] for 7 d [152]. Likewise, administration of *B. bifidum* ($5x10^8$ CFUs/d) for 8 wk significantly increased Con A-induced IL-2 and IFN-γ production in splenocytes from old mice. Additionally, elevated serum levels of TNF-α and IL-6 in old mice were reduced by *B. bifidum* treatment, suggesting that it might be beneficial in reducing age-associated inflammation [158].

In addition to intrinsic age-related decline in immune response, malnutrition in the elderly can intensify compromised immune function, infection susceptibility, and ultimately, infection-related mortality [159, 160]. In aged mice with experimentally-induced protein-energy malnutrition (PEM) [161], it was evident that some immune responses were decreased compared to aged mice fed a complete diet. These compromised immune responses included diphtheria toxin (DT)-specific IgG in serum, lymphocytes' proliferative response to Con A, and percentage of CD8+ cells; importantly, all of these parameters were improved after consumption of *L. johnsonii* La1 for 14 d. To a lesser extent, probiotics supplementation also increased DT-specific IgA and total IgA in fecal extract, and they decreased TNF-α production in LPS-induced splenocytes in old mice fed a complete diet [161]. While it is unclear how La 1 improved the impaired immune responses under PEM condition, it is noteworthy that La 1 consumption prevented the decrease in serum albumin levels and body weight in PEM mice, which the authors speculated was due to improved nutrient absorption by La 1 [161]. Thus improved nutritional status appeared to contribute to preserving positive immune responses seen in La 1 fed aged mice. Furthermore, studies have also suggested that probiotics may modulate innate immunity and DC APC function. Two *B.* strains isolated from healthy centenarians enhanced NK cell activity and phagocytic activity of MΦ in young mice [162]. Two other strains (*L. fermentum* strain PL9005 and *L. plantarum* strain PL9011) of probiotics were also shown to enhance the phagocytic capacity of peritoneal leukocytes [163].

Table 4. Zinc in prevention and treatment of infections

Subjects	N	Intervention		Findings	Reference
Human					
Young Guatemalan children, 6-9 mo	44 (P) 45 (Z)	10 mg zinc/d as zinc sulfate for 7 mo (on average)	Diarrhea, RI	-22% reduction in prevalence of diarrhea -no effect on incidence of RI	Ruel et al. 1997 [136]
Institutionalized elderly, >65 y	(P) (Z+S)	20 mg zinc/d as sulfide plus 100 μg selenium/d as sulfide for 2 y	Infection	-Decrease the mean number of RI	Girodon et al. 1997 [138]
Institutionalized elderly, >65 y	182 (P) 182 (Z+S)	20 mg zinc/d as sulfide plus 100 μg selenium/d as sulfide for 2 y	Infection	-Increase humoral response -A tendency for higher number of subjects without RI (p=0.06) -No effect on DTH response	Girodon et al. 1999 [139]
Severe pneumonia patients, 2-23 mo	149 (P) 150 (Z)	20 mg zinc sulfate/d during hospitalization	Pneumonia	-Increase duration of pneumonia in the hot season	Bose et al. 2006 [261]
Institutionalized elderly, 55-87 y	25 (P) 24 (Z) 6 (P) 6 (Z)	45 mg zinc/d as zinc gluconate for 1 y 45 mg zinc/d as zinc gluconate for 6 mo	Infection	-Less infection and fever, a tendency for fewer common cold and upper RI -Reduce *ex vivo* TNF-α production in response to LPS -Increase IL-2 mRNA in a separate 6 mo-zinc supplementation study	Prasad et al. 2007 [140]
Institutionalized elderly, >65 y	420 (Z)	7 mg zinc/d as zinc sulfate with vitamin and mineral	Infection	-Lower pneumonia incidence, reduced total antibiotic use, and shorter duration of pneumonia and antibiotic use in subjects with adequate blood zinc (>70 mg/dL) post-intervention	Meydani et al. 2007 [141]
Sickle-cell disease patients	18 (P) 18 (Z)	75 mg zinc/d as zinc acetate for 3 mo	Infection	-Lower incidence of total number of infection, upper RI, a trend in common cold (p=0.074) -Increase IL-2 and IFN-γ and decrease TNF-α and IL-1β ex vivo production -Increase IL-2 and IL-2Rα mRNA and decrease TNF-α and IL-1β mRNA levels	Bao et al. 2008 [137]
Severe pneumonia patients, 2-23 mo	146 (P) 149 (Z)	20 mg zinc sulfate/d during hospitalization	Pneumonia	-A longer hospital stay and a slower recovery in suspected bacterial pneumonia (CRP>40 mg/dL) -No difference in suspected non-bacterial pneumonia	Coles et al. 2007 [262]
Pulmonary tuberculosis (PTB) patients, >15 y	116 (P) 117 (Z)	90 mg zinc/wk as sulfate for 9 wk	PTB	-No effect on sputum conversion rate, resolution of radiographic abnormalities, cough, and fever	Lawson et al. 2010 [263]
Malnourished PTB patients, 15-55 y	86 (P) 76 (Z)	15 mg zinc/d as sulfate for 6 mo	PTB	-No effect on plasma zinc and sputum conversion rate	Pakasi et al 2010 [264]

(P) placebo, (Z) zinc supplementation, (Z+S) zinc plus selenium supplementation, PTB: pulmonary tuberculosis

Tsai et al. [164] showed that supplementing mice with *L. paracasei* NTU 101 (10^8 CFUs/d) for 6 or 9 wk (but not 3 wk) upregulated expression of DC maturation markers (MHC-II^{hi}, CD80+, and CD86+) and NK group-2D (NKG2D) and promoted lymphocyte proliferation in response to *L. paracasei* Ag. These results suggest that probiotics may enhance specific immunity by promoting APC function. Indeed, Vidal et al. [157] showed that following a vaccination protocol with keyhole limpet hemocyanin (KLH), old mice supplemented with *L. paracasei* NCC2461 ($1x10^9$ CFUs/d) for 44 d had an improved KLH-specific CD4+ T cell response including anti-KLH IgG2a production and DTH response, compared to the control aged mice. Since the proportions of lymphocytes including CD4+, CD44^{high} (memory T cells), CD44^{low} (naïve T cells) and NK cells remained unaltered [157], it is likely that probiotics administration improved the functionality of CD4+ T cells in response to Ag. Given the observed impairment in generating Ag-specific CD4+ T cell immunity in old mice [165, 166], probiotics could potentially be used as a practical strategy to boost protective immunity in the elderly.

Similar to animal study findings, certain strains of probiotics have been shown to influence innate immunity such as phagocytosis and cytotoxicity in humans. Administration of *B. lactis* ($3x10^{11}$ CFUs/d) to healthy elderly for 6 wk resulted in a significant increase in PMN cell phagocytic and bactericidal activity to *Staphylococcus aureus* challenge [167]. Dietary supplementation for 3 wk with *L. rhamnosus* HN001 ($5x10^{10}$ CFUs/d) or *B. lactis* HN019 ($5x10^9$ CFUs/d) in the elderly (60-84 y) increased both the peripheral blood proportion of NK cells and their tumoricidal activity. Of note, subjects over 70 y showed more pronounced improvement in tumoricidal activity [168]. In a subsequent study by the same group [169], healthy elderly were supplemented with two different doses of HN019 (low dose $5x10^9$ CFUs/d or typical dose $5x10^{10}$ CFUs/d) for 3 wk, and both doses were similarly effective in increasing phagocytic activity of PBMC and PMN cells as well as NK cell activity. The researchers also noticed a greater improvement in those subjects who had a lower pretreatment immune response. Furthermore, probiotics were shown to upregulate expression of the receptors in neutrophils important for their phagocytic activity including CR1, CR3, FcγRIII, and FcαR in healthy individuals [170, 171]. It appears that probiotics have a general immuno-enhancing effect irrespective of age: study participants who were selected from a broad range of ages (41-81 y [172]; 44-80 y [173]; 51-58 y [174]) all benefited from treatment with a variety of strains of probiotics (*L. rhamnosus*, $5x10^{10}$ CFUs/d [173]; *L. casei* DN114001 [174]; *L. lactis*, $3.4x10^{10}$ CFUs/d [170]; *L. GG*, $2.6x10^8$ CFUs/d [171]).

A striking feature of immunosenescence is inefficient response to vaccines, which reduces a vaccine's protective effect against infections such as flu in the elderly. Based on a review of 48 studies [175], vaccine response in the elderly was about one-fourth to one half of the Ab response in young adults. Therefore, increasing vaccine efficacy through probiotics is one strategy that may enhance immunity to infectious diseases in the elderly. It has been reported that healthy elderly (>70 y) residing in nursing homes displayed improved humoral response (Ab titer against influenza vaccine and seroconversion) after 13 wk of supplementation with the daily consumption of a product containing *L. casei* DN114001 ($2x10^{10}$ CFUs/d) and *S. thermophilus* and *L. bulgaricus* ($2x10^{10}$ CFUs/d). However, a shorter time period of 7 wk of supplementation failed to induce this protective effect [176]. Similarly, 7 d supplementation with *L. GG* or *L. lactis* did not affect humoral response induced by *Salmonella typhi* oral vaccine in healthy adults [170]. Several studies have indicated that probiotics may induce production of proinflammatory cytokines, which support an adequate immune response against infection. On the other hand, anti-inflammatory cytokines are also induced, ameliorating excessive inflammatory reaction (reviewed in [145, 150, 177]). However, the immunomodulatory effects of probiotics on cytokine production appear to be strain-dependent. For example, probiotics taken orally induced IFN-α (*B. lactis* HN019, [167]), reduced TNF-α (*L. rhamnosus GG*, [178]) and IL-2 (*B. animalis ssp. Lactis Bb12*, [178]), and had no effect on IFN-γ, IL-1β, and IL-2 (*L. casei*, [179]). Immunomodulating effects of probiotics reported in the literature are summarized in Table 5. In short, studies thus far indicate that probiotics have the potential to modulate different aspects of immune function in the elderly. Whether this property of probiotics ultimately conveys a clinically-relevant benefit in terms of reducing infection in the elderly is summarized in Table 6 and discussed below.

2) Probiotics and resistance against infection

Several studies have demonstrated that probiotics administration could protect young mice from infection including *Salmonella typhimurium* [180, 181] and *Listeria monocytogenes* [182]. Results from these studies indicated that dietary probiotics reduced pathogen translocation to spleen and liver while increasing phagocytic activity, lymphocyte proliferation, and mucosal Ab response [180-182]. Data obtained from young mice raise the possibility that probiotics may exert

Table 5. Effect of probiotics on immune function

Subjects	N	Intervention	Findings	Reference
Animals				
Female Balb/c mice, 7 wk and 19 mo	8/10 (C) 8/10 (PB)	Live L. bulgaricus +S.thermophilus ($4x10^8$ CFUs, each) for 7 d	-Increase IFN-α production by LPS-induced splenocytes and IFN-γ production by Con A-stimulated splenocytes	Muscettola et al. 1994 [152]
Balb/c mice, 6 wk	5 (C) 5x6 (PB)	Live W. kimchii, L. fermentum, L. plantarum (10^7 CFUs/d, each or 10^9 CFUs/d, each) for 3 wk	-Enhance phagocytic capacity of peritoneal leukocytes against FITC-labeled E. coli -Heat-killed PB did not increase the phagocytic activity	Lee and Lee, 2005 [163]
Male C57BL/6 mice, 20 mo	Complete 6 (C) 6 (PB) Low protein 8 (C) 8 (PB)	L. johnsonii La1 (≥$1x10^9$ CFUs/d) for 14 d	*In complete diet* -Increase DT specific IgA and total IgA in fecal extract -Decrease TNF-α production by LPS-stimulated splenocytes -No effect on serum DT-specific IgA and IgG, splenocytes proliferation, proportion of CD4+ and CD8+ *In low protein diet* -Increase DT specific IgA and total IgA in fecal extract -Increase serum DT-specific IgG, total IgA, -Increase % CD8+, Con A-induced lymphocyte proliferation	Kaburagi et al. 2007 [161]
Balb/c mice, 6-8 wk	6 (C) 6x6 (PB)	L. paracasei subsp. paracasei NTU 101 (10^8 CFUs/d) for 3, 6, 9 wk	-Upregulate DC maturation markers (MHC-II, CD80, CD86) -Increase serum IgG -Increase NKG2D involved in NK cell cytotoxicity and lymphocyte proliferation	Tsai et al. 2008 [164]
Male C57BL/6 mice, 21 mo	10 (C) 15 (PB) 15 (S)	PB: L. paracasei NCC2461 ($1x10^9$ CFUs/d) S: L. paracasei NCC2461 ($1x10^9$ CFUs/d) + fructo-oligosaccharide+inulin, given in the drinking water for 44 d	Immunized on day 15 and challenged on day 22 with keyhole limpet hemocyanin (KLH) -Increase Th1 cell-dependent immune responses such as anti-KLH IgG2a and DTH response and a trend towards increased anti-KLH IgG1 -No effect on splenocyte proliferation, T cell subsets, systemic total IgG, mucosal total IgA, Con A-stimulated cytokine production (IL-2, IFN-γ, IL-4) -No additional effect of fructo-oligosaccharide and inulin	Vidal et al. 2008 [157]
Balb/c mice, 6-9 wk	10 (C) 6x10 (PB)	Live B. adolescentis BBMN23, B. longum BBMN68 ($2x10^7$, $2x10^9$ or $2x10^{11}$ CFUs /kg BW) for 4 wk	Two Bifidobacterium strains isolated from healthy centenarians in China -Increase NK cell activity in BBMN23 starting $2x10^9$ CFUs/kg BW and in BBMN68 starting at $2x10^7$ CFUs/kg and lymphocyte proliferation in all BBMN23 and BBMN68 groups -Increase serum lysozyme and phagocytic activity of MΦ in all PB groups	Yang et al. 2009 [162]
Female Kunming mice, 2 mo and 20 mo	Young 20 (C) Old 20 (C) 20 (PB)	B. bifidum ($5x10^8$ CFUs/d) for 8 wk	-Increase thymus weight relative to the final BW -Decrease serum TNF-α and IL-6 -Increase IL-2 and IFN-α production in Con A-stimulated splenocytes	Fu et al. 2010 [158]
Female C57BL/6 mice, 4-6 wk	12 (C) 2x12 (PB)	Heat killed L. bulgaricus OLL1181 (10^9 CFUs/d) or L. caseri MEP222701 (10^9 CFUs/d) for 8-11 d starting 7 d prior to colitis induction	-L. bulgaricus OLL1181 increases percentage of survival from dextran sodium sulfate-induced colitis and COX-2 mRNA expression in the colon -L. bulgaricus OLL1181 decreases inflammation score, TNF-α and myeloperoxidase mRNA expression in the colon	Takamura et al. 2011 [265]

Humans				
Healthy male, 40-65 y	10 (P) 10 (PB)	P: unfermented milk PB: L. casei (>10^{11} CFUs/d) fermented milk for 4 wk	-No change in lymphocyte population, humoral parameters, NK activity, cytokine production (IFN-γ, IL-1β, IL-2), phagocytic capacity, oxidative burst and DTH response	Spanhaak et al. 1998 [179]
Healthy adults, 22-50 y	9 into (P), (PB)	P: milk PB: L. GG ($2.6x10^8$ CFUs/d) for 1 wk	-Increase expression of phagocytosis receptors (CRI, CRI3, FcγRIII, and FcαR) on neutrophil	Pelto et al. 1998 [171]
Healthy adults, 20-50 y	9 (P) 10/10 (PB)	L. GG ($4x10^{10}$ CFUs/d) or L. lactis ($3.4x10^{10}$ CFUs/d) for 7 d	All subjects received attenuated Salmonella typhi oral vaccine on day 1, 3, and 5 -No effect on humoral response (IgA, IgG and IgM) -Increase CR3 receptor expression on neutrophils in L. lactis group	Fang et al. 2000 [170]
Healthy, 41-81 y	27/23 (PB)	B. lactis HN019 ($5x10^{10}$ CFUs/d) in low fat milk/or in lactose-hydrolysed low fat milk for 3 wk	-Increase PMN cell phagocytic activity and NK cell tumor killing activity -Greater effect of B. lactis in lactose-hydrolysed low fat milk on phagocytic activity and NK cell cytotoxicity	Chiang et al. 2000 [172]
Healthy elderly, >60 y	12 (P) 13 (PB)	Live B. lactis HN019 ($3x10^{11}$ CFUs/d) for 6 wk	-Increase IFN-α production by Con A-stimulated PBMC -Enhance phagocytic activity of PMN cells	Arunachalam et al. 2000 [167]
Healthy, 44-80 y	25/27 (PB)	L. rhamnosus ($5x10^{10}$ CFUs/d) in low fat milk /or in lactose-hydrolysed low fat milk for 3 wk	-Increase PMN cell phagocytic activity and NK cell tumor killing activity -Greater effect of L. rhamnosus in lactose-hydrolysed low fat milk on NK cell activity	Sheih et al. 2001 [173]
Healthy elderly, 60-84 y	27 (PB)	L. rhamnosus HN001 ($5x10^{10}$ CFUs/d) or B. lactis HN019 ($5x10^9$ CFUs/d) for 3 wk	-Increase NK cell in PBMC -Increase tumoricidal activity of PBMC -More pronounced improvement in tumoricidal activity of PBMC isolated from subjects >70 y	Gill et al. 2001 [168]
Healthy elderly, 64-84 y	29 (PB)	B. lactis HN019 (low dose, $5x10^9$ CFUs/d or typical dose, $5x10^{10}$ CFUs/d) for 3 wk	-Increase percentage of CD3+, CD4+, CD25+, NK cells, but no change in CD8+, B cells, MHC-II bearing APC -Increase phagocytic activity of PBMC and PMN cells -Greater improvement in phagocytic activity in subjects supplemented with typical dose and poor immune response before intervention	Gill et al. 2001 [169]
Healthy adults, 51-58 y	22 (P) 23 (PB)	L. casei DN114001 for 8 wk	-Increase oxidative burst capacity of monocytes and NK cell tumoricidal activity	Parra et al. 2004 [174]
Healthy adults, 18-62 y	20 (P) 7x9 (PB)	B. lactis Bi-07, B. lactis Bl-04, L. acidophilus La-14, L. acidophilus NCFM®, L. plantarum Lp-115, L. salivarius Ls-33, L. paracasei Lpc-37; Each group received one of 7 different strains (10^{10} CFU/d) for 3 wk	-No difference in specific IgA against oral cholera vaccine in saliva samples among groups -Increase serum IgG in B. lactis Bl-04 group and L. acidophilus La-14 group	Paineau et al. 2008 [266]
Healthy adults, 23-58 y	16 (P) 13/16/17 (PB)	L. rhamnosus GG ($1.6x10^{10}$ CFUs/d), B. animalis ssp. Lactis Bb12 ($3.5x10^{10}$ CFUs/d), P. freudenreichii ssp. Shermanii JS ($3.3x10^{10}$ CFUs/d) for 3 wk	-No effect on lymphocytes population, serum cytokine, serum Ig and secretory IgA in saliva -Decrease *ex vivo* TNF-α production in response to Streptococcus in L. GG group and *ex vivo* IL-2 production in response to Influenza in Bb12 group	Kekkonen et al. 2008 [178]
Nursing home elderly, mean age 84.3±0.98 y	86 (C) 67 (P) 56 (PB)	C: commercial fermented oat drink containing B. animalis ssp lactis Bb-12 (10^9 CFUs/d)	-Modulate fecal B. species -Increase TGF-β1 in all three groups -Negative correlations between levels of B. species	Ouwehand et al. 2008 [267]

		P: oat-based drink PB: oat-based drink with B longum 2C and 46 (10^9 CFUs/d, each) for 6 mo	and cytokines (TNF-α and IL-10)	
Elderly, regularly using non-steroidal anti-inflammatory drugs, >65 y	23 (P) 24 (PB)	L. acidophilus (≥10^{10} CFUs/d) + lactitol for 2 wk	-Transient increase in B. levels during intervention -Increase feacal IgA, but no difference between P and PB -Increase faecal PGE_2 -No effect on faecal TNF-α	Ouwehand et al. 2009 [268]
Healthy nursing home elderly, >70 y	44 (P) 42 (PB) 113 (P) 109 (PB)	P: non-fermented control dairy product for 7 wk or 13 wk PB: L. casei DN-114 001 (≥2x10^{10} CFUs/d) + S. thermophilus and L. bulgaricus (≥2x10^{10} CFUs/d) as fermented dairy drink (Actimel®) for 7 wk or 13 wk	-Tend to improve humoral response after 7 wk supplementation, but not significant -Improve humoral response (Ab titer against influenza vaccine and seroconversion) after 13 wk supplementation	Boge et al. 2009 [176]
Healthy elderly, 61-94 y	13 SNH (PB) 23 SPH (PB)	PB: L. johnsonii La1 (10^9 CFUs/d) in yoghurts for 4 wk	-Reduce endotoxin concentration in plasma of SNH (a trend in SPH) -Reduce soluble CD14 in SPH -Decrease basal phagocytic activity of monocytes and neutrophil in both SNH and SPH -Upon LPS stiumulation, monocytes from SPH produced less IL-1β and IL-10 than those prior to supplementation, but PB supplementation increased the capacity of monocytes similar to SNH and the capacity of neutrophils to release of reactive oxygen species in SPH	Schiffrin et al. 2009 [269]

(C) control, (P) placebo, (PB) probiotic supplementation, (S) symbiotic supplementation, SNH: subjects with negative hydrogen breath test, SPH: subjects with positive hydrogen breath test

a protective effect in the elderly population since they are more vulnerable to infection. In a study by Bunout et al. [183], free-living elderly (≥70 y) were provided with nutritional supplementation including *L. paracasei* (NCC 2461) and prebiotic (fructo-oligosaccharides) for 1 y and vaccinated against influenza and pneumococcus during the intervention period. The elderly receiving the nutritional supplementation (n=28) reported less infection, particularly RI, than those without supplementation (n=28). However, problems with the study design make it impossible to attribute the effect specifically to probiotics. First, the study was not blinded; further, the formulation contained several other active components such as protein, vitamin E, vitamin B12, folic acid, and a prebiotic (cited above) in addition to *L. paracasei*. Another study [184] reported that hospitalized enterally-fed elderly receiving *L. johnsonii* La1 and *S. thermophilus* for 12 wk had a significantly shorter duration of infection compared to the control group. Although these data suggest that probiotics may be able to modulate the risk of infection in the elderly, enterally-fed patients who are bed-ridden may not represent the general elderly population. In a larger controlled intervention [185], healthy, independently living elderly (≥70 y) who were supplemented with a fermented dairy drink (Actimel®) containing *L. casei* DN114001 as well as *S. themophilus* and *L. bulgaricus* for 3 mo (including the winter season) reported a shorter cumulative duration (7 *vs* 8 d in control group) and an average duration per episode (6.5 *vs* 8 d in control group) for all common infectious diseases (CID) compared to the placebo group, which consumed a non-fermented and acidified dairy drink. On average, probiotic supplementation reduced the duration of CID by 1-1.5 d. Reductions in both the number of episodes and cumulative duration were also significant for upper RI, accounting for 30.3% CID, and rhinopharyngitis, accounting for 50.3% CID. In contrast, probiotic consumption did not alter CID severity, fever, pathogen, medication, and immune parameters tested (oxidative burst activity in monocytes, cytotoxic activity and number of NK cells in blood, levels of serum cytokine including IL-1, IL-6, IFN-α, β, and γ, IL-12, IL-10, TNF-α, and IL-8). Similarly, Makino et al [186] reported that

the elderly who were supplemented with yoghurt (fermented with *L. bulgaricus* and *S. thermophilus*) for 8 wk (Mar to May) or 12 wk (Nov to Feb) had a significant reduction in common cold or influenza virus infection compared to the placebo group consuming milk. Depending on the intervention period, NK cell cytotoxicity remained either unchanged (8 wk) or increased (12 wk), but both 8 wk and 12 wk interventions significantly improved NK cell cytotoxicity in the subjects with low basal NK cell activity. Again, as with immune response, the probiotics' protective effect against infection is not limited to the elderly population. Healthy subjects (18-67 y) who received *L. gasseri, B. longum, B. bifidum* in addition to vitamins and minerals for 3 mo or 5.5 mo showed a 21.5% reduction in the mean duration of common cold episodes (1.9 d) along with increased CD8+ T cells, compared to the placebo group, which took only vitamins and minerals [187, 188]. Intake of *L. plantarum* HEAL9 and *L. paracasei* for 12 wk was shown to decrease incidence of acquiring one or more common colds in subjects aged 18-65 y [189].

In summary, probiotics are an active food component, and interest in determining their impact on the immune response of the elderly has grown in recent years. The research to date suggests a potential for using probiotics to improve age-related defects in the immune system and to reduce the high incidence and severity of infectious diseases in the elderly. However, studies in this field have presented many inconsistent and discrepant results. The possible reasons include: unequal baseline conditions between treatment and control groups, short intervention periods, underpowered designs, and lack of precise documentation of infections. In addition, it is important to note that there are many different probiotics, and their effects may be strain specific. Furthermore, interaction among probiotics occurs so that it is possible to obtain various synergistic effects by combining different strains.

4. Fish oil and n-3 PUFA

1) N-3 PUFA and immune function

Dietary lipids are not only fundamental energy-providing nutrients, but also greatly impact specific cell functions. There are different classifications and categorizations for dietary fatty acids (FA) including: essential (further divided into n-3 and n-6 families), saturated, monounsaturated, and PUFA. Mammals depend on food to obtain essential FA from plants or animals, and each source has a unique FA composition. Most plants and land animals provide us with n-6 PUFA, some plants are sources of shorter chain n-3 PUFA, and marine animals are virtually the sole source of long chain n-3 PUFA. Marine animal-derived n-3 PUFA (mainly eicosapentaenoic acid or EPA, and docosahexaenoic acid or DHA) have the most significant effect on immune cell functions compared to other FA. In fact, n-6 FA are often used as the control fat in studies determining the effect of n-3 PUFA. Consumption of long chain n-3 PUFA or fish oil has been shown to have beneficial effects on several prevalent, age-related diseases such as cardiovascular disease, degenerative neurological diseases, inflammatory and autoimmune diseases, and age-related macular degeneration. A common mechanism of the beneficial effect of n-3 PUFA on these diseases is their anti-inflammatory property. Although still controversial, n-3 PUFA are largely known to suppress both innate (mainly inflammation) and adaptive (mainly T cell-mediated) immune responses, and thus may impair the host's defense against infectious and neoplastic diseases. Given that aged individuals already have impaired immune responses, it is important to carefully assess the advantages and drawbacks of n-3 PUFA supplementation in the elderly population. To date, however, only a very limited number of studies have compared the effect of dietary supplementation with fish oil on immune responses of young and aged animals or humans. We are therefore not limiting our literature review to only those studies that address the age issue but rather, we will also discuss the effect of n-3 PUFA on immune functions that are known to change with age. This evaluation has potential clinical relevance for the elderly since they are the population that is particularly encouraged to, and in fact do take more n-3 PUFA to mitigate age-related diseases.

There are a number of recent excellent reviews on the effect of n-3 PUFA on inflammatory and immune responses [190-196]. In general, n-3 PUFA are anti-inflammatory, having been shown repeatedly to inhibit production of inflammatory mediators including eicosanoids (PGE_2, 4-series leukotrienes), proinflammatory cytokines (IL-1β, TNF-α, IL-6), chemokines (IL-8, MCP-1), adhesion molecules (ICAM-1, VCAM-1, selectins), platelet activating factor, and reactive oxygen and nitrogen species. In addition, more recent work indicates that n-3 PUFA have a pro-resolution effect by serving as precursors for the production of pre-resolving mediators resolvins, protectins, and marensins [197, 198]. These effects of n-3 PUFA apparently contribute to their efficacy in alleviating inflammatory and autoimmune diseases, some of which have an increased incidence with aging.

It is widely agreed that the most dramatic, consistently observed change in the immune system during aging is defective T cell response. A majority of

Table 6. Probiotics in prevention and treatment of infections

Subjects	N	Intervention	Infection	Findings	Reference
Mice					
Male Balb/c mice, 6-8 wk	21/35 (C) 21/36 (PB)	C: skim milk for 2 wk starting 1 wk before S. typhimurium infection PB: B. lactis HN019 (10^9 CFUs/d) in skim milk for 2 wk starting 1 wk before S. typhimurium infection	Salmonella typhimurium infection	-10 fold increase in survival rate -Reduce pathogen translocation to spleen and liver and increase phagocytic activity of blood and peritoneal cells, lymphocyte proliferation in spleen and mesenteric lymph node, and peyer's patch (negative correlation between bacterial translocation and immune function) -Increase mucosal Ab response against S. typhimurium	Shu et al. 2000 [181]
Male Balb/c mice, 6-8 wk	35/21 (C) 36/21 (PB)	C: skim milk for 2 wk starting 1 wk before S. typhiumrium infection PB: L. rhamnosus HN001(10^9 CFUs/d) in skim milk for 2 wk starting 1 wk before S. typhimurium infection	Salmonella typhimurium infection	-Maintain high general health score -Increase survival rate -Reduce pathogen translocation to spleen and liver (control>concurrent>pre-supplementation) and increase phagocytic activity of blood and peritoneal cells -Increase intestinal and serum Ab titers against S. typhimurium	Gill et al. 2001 [180]
Male Wistar rats, 6 wk	4 (C) 3x4 (PB)	Live Lactobacillus strains Utr-1 (10^9 CFUs/d), Utr-2 (10^9 CFUs/d), Utr-3 (10^9 CFUs/d) for 3 d or 8 wk starting 7 d after infection	Listeria monocytogenes infection	-Decrease DTH response in 3-d supplementation with Utr-3 -Reduce pathogen translocation to spleen and liver in re-infected rats after 8 wk supplementation of Utr-2 or Utr-3 showing improved acquired cellular resistance towards Listeria re-infection	De Waard et al. 2002 [182]
Human					
Free-living elderly	180 (P) 180 (PB)	Milk fermented with yoghurt cultures and L. casei DN-114001 for 3 wk	GI infection RI	-No effect on the incidence of infections -Decrease duration of all pathologies and maximal temperature	Turchet et al. 2003 [270]
Free living elderly, ≥70 y	28 (C) 28 (S)	L. paracasei NCC2461 (10^9 CFUs/d) + fructo-oligosaccharides + vitamin+ protein for 1 y	Infection	- Change in NK cell activity (value at 4 mo-baseline), significantly higher in supplemented group compared to control group. -At 2 mo after vaccination against influenza and pneumococcus, NKT cells and PHA-induced IL-2 production decreased in controls and did not change in supplemented group -Increase Flu antigen-stimulated IFN-γ production in both groups, but no difference in humoral response 2 mo after vaccination -During the 1 y follow-up, less infection (especially, RIs) in S supplemented group, no effect on DTH	Bunout et al. 2004 [183]
Day-workers and three shift workers, mean age 44 y	87 (P) 94 (PB)	L. reuteri (10^8 CFUs/d) for 80 d	Short term illness due to RI and GI infection	-Reduce number of subjects reporting sick-leave and frequency of sick-days -More remarkable decrease in proportion of subjects reporting sick among three shift workers	Tubelius et al. 2005 [271]

Healthy adults, 18-67 y	232 (P) 230 (PB)	P: tablet PB: tablet containing L. gasseri ($4x10^8$ CFU/d) + B. longum ($5x10^7$ CFUs/d) + B. bifidum ($5x10^7$ CFUs/d) plus vitamin and mineral for 3 mo or 5.5 mo	Common colds	-13.6% reduction in incidence of common colds -Reduce headache (30%), conjunctivitis (49%), and days with fever (54%) -Tend to decrease duration of common colds (p=0.19), influenza symptom (p=0.09), and total symptom score (p=0.12) -Increase leukocytes, lymphocytes, T cells, CD4+, CD8+, and monocytes (analyzed after 14 d supplementation) and tend to increase NK cells (p=0.18) -No change in T cells activation and phagocytic activity	Winkler et al. 2005 [272]
Healthy adults, 18-67 y	229 (P) 225 (PB)	P: tablet containing vitamin and mineral PB: tablet containing L. gasseri ($4x10^7$ CFU/d) + B. longum ($5x10^6$ CFUs/d) + B. bifidum ($5x10^6$ CFUs/d) plus vitamin and mineral for 3 mo or 5.5 mo	Common colds	-21.5% reduction in mean duration of common cold episodes (1.9 d) -Reduce bronchial symptoms and days with fever and tend to decrease nasal symptoms (inclusive sneezing) (p=0.053), pharyngeal symptoms (p=0.051), and total symptom score (p=0.056) -Increase CD8+ T cells and tend to increase CD4+ T cells (p=0.081), but not other markers including NK cells, T cell activation, and phagocytic activity	De Vrese et al. 2005 [187] De Vrese et al. 2006 [188]
Elderly patients with total enteral nutrition, >70 y	12 (C) 12 (PB)	L. johnsonii La1 (10^9 CFUs/d)+ S. thermophilus (10^8 CFUs/d) in fermented milk for 12 wk	Infection	-Shorter duration of infection during 12 wk intervention -Decrease in TNF-α and increase phagocytic activity whose basal level lower than 90% [6 (C), 6 (PB)] after 4 wk supplementation -No effect on lymphocytes population	Fukushima et al. 2007 [184]
Healthy adults	105/75 (P) 114/78 (S)	S#1: 3 strain of L. plantarum (10^{10} CFUs/d) + L. rhamnosus (10^{10} CFUs/d) + B. lactis (10^{10} CFUs/d) + fructo-oligosaccharide for 3 mo S#2: 5 strain of L. plantarum ($5x10^9$ CFUs/d) + L. rhamnosus ($5x10^9$ CFUs/d) +B. lactis ($5x10^9$ CFUs/d) +fructo-oligosaccharide for 3 mo	RI	*S#1* -Decrease number of episodes in total acute RI and influenza-like illness -Reduce severity of total acute RI, upper RI and influenza-like illness -Reduce duration of total acute RI (-0.84 d) and upper RI (-1.94 d) *S#2* -Decrease number of episodes and severity in total acute RI, cold, and influenza-like illness -Reduce duration of total acute RI, cold, and cough -More significant decrease in the number of episodes, severity, and duration even with half dose of 5 probiotic strains compared to 3 strains	Presgliasco et al. 2008 [273]
Patients with chronic inflammatory rhinosinusitis, 15-70 y	35 (P) 37 (PB)	L. rhamnosus R0011 (10^9 CFUs/d) for 4 wk, 8 wk	Chronic inflammatory rhinosinusitis	-As adjunctive, improve scores from the 20-item sinu-nasal outcome test (SNOT-20) after 4 wk, but not 8 wk compared to baseline -No difference between P and PB	Mukerji et al. 2009 [274]
Hospitalized children, >12 mo	366 (P) 376 (PB)	L. rhamnosus GG (10^9 CFUs/d) during hospitalization	Nosocomial RIs and GI infection	-Reduce number of patients with GI infections (RR=0.45, 95% CI 0.25-0.7) and RI (R=0.38, 95% CI 0.18-0.85) -Reduce vomiting episodes, diarrheal episodes, episodes of GI infection that lasted >2 d and episodes of GI that lasted >3 d	Hojsak et al. 2010 [275]

Healthy free living elderly, ≥70 y	535 (P) 537 (PB)	P: non-fermented, acidified, sweetened, flavoured dairy drink PB: L. casei DN-114 001 ($\geq 2x10^{10}$ CFUs/d) + S. thermophilus and L. bulgaricus ($\geq 2x10^{10}$ CFUs/d) as fermented dairy drink (Actimel®) for 3 mo	Common infectious disease (CID)	-Transient increase in L. casei during intervention -Reduce cumulative and episode duration in all CID, all upper RI, and rhinopharyngitis -No effect on CID severity and other immune parameters (oxidative burst activity in monocytes, NK cell number and cytotoxicity, serum cytokine levels)	Guillem ard et al. 2010 [275]
Healthy adults, 18-65 y	137 (C) 135 (PB)	L. plantarum HEAL9 + L. paracasei 8700:2 (10^9 CFUs/d) for 12 wk	Common cold	-Decrease incidence of acquiring one or more common cold episode (67% for control vs 55% for probiotics) -Reduce paryngeal symptom -Reduce B lymphocytes	Berggre n et al. 2010 [189]

(C) control, (P) placebo, (PB) probiotic supplementation, (S) symbiotic supplementation

work on the immunomodulating effect of n-3 PUFA has focused on T cell-mediated immunity, but the research on this topic has not reached a clear consensus. In general, the results from animal studies are relatively less discrepant, which is mainly due to the ability to control genetic background, habitual diet, and feeding regimen. Further, high doses of n-3 PUFA can be used in animal models. Although some studies have reported no significant effect [199, 200] or even an enhancing effect [201-203] of n-3 PUFA supplementation on immune response, more studies have shown a suppression in cell-mediated immune function resulting from n-3 PUFA supplementation as summarized in the reviews mentioned above. Consumption of fish oil or n-3 PUFA has been shown to inhibit *ex vivo* T cell mitogen- or TCR activation-induced lymphocyte and CD4+ T cell proliferation, IL-2 production, and IL-2R expression, or specific Ag-driven CD4+ T cell expansion both *ex vivo* and *in vivo* [204, 205], as well as the *in vivo* immune response indicated by DTH skin test [206]. Mechanistic studies have revealed the cellular and molecular basis for n-3 PUFA-induced effects. For example, n-3 PUFA can inhibit several important events in the TCR activation cascade including phosphorylation of signaling molecules and their translocation in the immunologic synapse [194, 195, 207]. This proposed association between n-3 PUFA and T cell function is further reinforced by the recent work using *fat-1* mice, a transgenic mouse model that can endogenously synthesize n-3 PUFA [208, 209].

Few studies have particularly examined how n-3 PUFA supplementation affects immune response in aged persons with respect to their young counterparts. Meydani et al. [210] reported that n-3 PUFA supplementation (1.68 g EPA and 0.72 g DHA/d) for 3 mo significantly inhibited T cell mitogen-induced PBMC proliferation and IL-2 production in older (51-68 y), but not young (23-33 y), female subjects. They also observed a decreased production of inflammatory cytokines (L-1β, IL-6, TNF-α) in both age groups, but the effect was larger in older than in young subjects. In another study, Bechoua et al. [211] showed that elderly subjects (70-83 y) consuming habitual amounts of n-3 PUFA (30 mg EPA and 150 mg DHA/d) for 6 wk had decreased lymphocyte proliferation in response to Con A, PHA, and OKT3. In a later study, Rees et al. [212] compared the change in innate immune function in young and older men after they consumed 1.35, 2.7, or 4.05 g EPA/d for 12 wk. They found that EPA treatment dose-dependently decreased neutrophil respiratory burst in only the older men. These results suggest that elderly individuals are more sensitive to the immunologic effects of n-3 PUFA, which can be an advantage (in the case of inflammatory and autoimmune disorders) or a disadvantage (in the case of combating pathogens and cancers). Future studies should be designed to address the clinical relevance of the differential effect of n-3 PUFA in different age groups.

2) N-3 PUFA and infection

Since n-3 PUFA can attenuate inflammatory and T cell-mediated immune responses, which are key components in the body's defense against microbial infection, it is important to know if increased intake of n-3 PUFA can actually compromise the host's defense against infection. Because data from human studies are very limited, preliminary knowledge is largely from animal studies utilizing a variety of infection models or from very limited epidemiological studies. Of relevance to this issue, a prospective study of 83,165 participants (27-44 y) in the Nurses' Health Study II showed that women in the highest quintile of EPA and DHA intake had a 24% greater risk of community-acquired pneumonia than did those in the lowest quintile [213]. However, the Health Professionals Follow-Up Study cohort of 38,378 men (44-79 y) found no association between EPA and DHA intakes and pneumonia risk [214]. It is not clear if and how much the difference in age and gender is attributable to the discrepancy between the two studies. Regarding animal studies, although information is relatively more abundant, the picture is not much more explicit. Outcomes are roughly even between reported adverse effects, no effects, or even beneficial effects of n-3 PUFA intake on the host's resistance to infections. As summarized by Anderson and Fritsche [215], many animal studies have shown both improved and impaired host's resistance to a number of pathogens following increased consumption of n-3 PUFA. Importantly, studies have shown impaired host's resistance to several infectious pathogens that are prevalent in infections in the elderly. For example, fish oil fed mice infected with influenza had a higher lung viral load, more weight loss, lower cytotoxicity of lung virus-specific T cells, and reduced production of lung IFN-γ, serum IgG, and lung IgA-specific Ab [216, 217]. These results were confirmed by a more recent study showing that feeding fish oil to mice resulted in higher mortality, higher lung viral load, and longer recovery period after influenza infection, and all these changes were at least in part associated with fewer CD8 T cells and inflammatory cytokine expression in lungs [218]. n-3 PUFA is also related to lower host resistance to infection from *mycobacterium tuberculosis* (TB). As another example, n-3 PUFA fed to guinea pigs [219, 220], mice [221], and transgenic *fat-1* mice, which can endogenously synthesize n-3 PUFA [222], have reduced survival and impaired bacterial clearance after TB infection. Impaired resistance to TB infection is associated with reduced oxidative burst, less robust inflammatory response manifested by lower production of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, and diminished Ag-specific T cell function in response to TB infection. Likewise, n-3 PUFA supplementation was also shown to impair infection resistance in mice infected with several other pathogens such as *Listeria monocytogenes* [223] and *Salmonella typhimurium* [224]. In both cases, the animals fed fish oil had much lower survival rates and delayed bacterial clearance from spleens. Conversely, some studies have reported a beneficial effect of n-3 PUFA against infection by increasing host survival after infection. In those cases, it is possible that the overwhelming inflammation induced by pathogens or pathogen-released endotoxin is the major cause of fatality; therefore, n-3 PUFA could be protective given their ability to inhibit production of PGE_2 and pro-inflammatory cytokines [196].

Given all the potential beneficial and adverse effects of n-3 PUFA reported in the literature, the data suggest that n-3 PUFA do not improve immunosenescence in defense of the host against pathogens. Although this might discourage the elderly from taking advantage of the beneficial effects of n-3 PUFA, the advantages of taking n-3 PUFA may outweigh the potential adverse effects under certain circumstances. For example, increasing n-3 PUFA intake might be beneficial to treat inflammatory and autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis or diseases in which inflammation is an underlying factor of pathogenesis such as cardiovascular disease, type 2 diabetes, and Alzheimer's disease. In addition, dose and adequate provision of other nutrients such as vitamin E can influence the nature and magnitude of the effect of n-3 PUFA on immune response.

5. Caloric restriction (CR)

1) CR and immune function

In the 1930's, McCay et al. [225] presented the first evidence suggesting that long term CR increases life span. Since then, compelling, supporting evidence has accumulated to show that reduced caloric intake without malnutrition not only extends average and maximum life spans in a wide array of species (from yeast to rodents, and possibly non-human primates, NHP) [226-228], but also preserves a variety of body functions at a youthful age and delays the onset or decreases the severity of several age-associated diseases. Importantly, we have learned that CR can intervene in the body's age-related changes that come with immunosenescence. The majority of studies related to CR and immune response have been conducted in rodents, particularly mice. These investigations show that CR can reverse most of the manifestations of immunosenescence including defects in hematopoietic stem cells [229], decreased naïve T cell and increased memory T cell population [230], reduced T cell proliferative response to mitogens or Ag,

decreased IL-2 production, NK activity, and CTL generation [230-234], age-related increase in serum levels of TNF-α and IL-6 [235], and in autoimmune diseases [236, 237]. More recently, the impact of CR has been studied in NHP. These studies take advantage of their higher degree of similarity to humans and serve as an alternative to long term CR tests on humans. For the most part, CR studies in NHP have generated similar results to those seen in the rodent studies [238-241] regarding effect on immune response and several other age-related disorders [226]. Since conducting long term CR to determine its effect on human longevity is not feasible, many surrogate outcome measures on a variety of body functions have been used to assess CR's health benefit. Recently, Ahmed et al. [242] reported the first human study determining the effect of CR on immune response. In this study, a small number of overweight men and women (20-40 y) were subjected to 10% or 30% CR for 6 months. The authors found that CR at both levels enhanced T cell-mediated immune response, which was measured *in vivo* by DTH and *ex vivo* by T cell proliferation. Also, 30% CR reduced LPS-stimulated *ex vivo* production of T cell-suppressive eicosanoid PGE_2. While these results still need to be confirmed in larger human trials, current observations strongly suggest that CR's beneficial effects on immune function in experimental animal models may also apply to humans.

Although animal studies and limited human studies indicate a positive effect of CR on immunosenescence, many questions remain. Apart from needing more human studies, the issues of when to initiate CR and how long to maintain it still must be addressed. It is generally agreed that CR should not be recommended for individuals in growing and developmental stages, but there is no agreement on when to initiate CR during adult years. To this end, a recent study by Messaoudi et al. [239] has provided interesting, valuable information. In their study, the authors started CR at early, adult, and late life stages in primates and found that CR induced beneficial effects in maintaining youthful T cell function in the adult-onset animals, but this benefit was lost in both early- and late-onset animals. These findings caution us about the importance of timing since there might be window of time when initiating CR might be ineffective or even harmful. These results need to be verified and, if confirmed, we will need to learn if and to what extent this phenomenon is applicable to humans.

2) CR and infection

Resistance to infection is one of the most important outcomes for testing the efficacy of an intervention on a host's immune function. The question that logically follows is: are all the reported beneficial effects of CR on selected parameters of immune function sufficient to afford a host, in particular an aged one, better protection from pathogenic infection? It is well known that the elderly have a particularly higher rate of morbidity and mortality from influenza infection; therefore, influenza infection and vaccination are commonly used outcomes to determine the efficacy of immune enhancing interventions. An early study by Effros et al. [243] showed that aged CR mice had an improved immune response to influenza infection with enhancement in Ag presentation, T cell proliferation, and Ab production compared to the control mice. Since no information on the clinical signs of the disease or viral titer was provided, it is not clear if CR-induced enhancements were associated with better resistance to influenza infection. However, a recent study by Gardner et al. [244] reported that after influenza infection, aged CR mice had lower NK activity, decreased survival, and delayed viral clearance (higher viral titers) compared to *ad-libitum* fed mice. The same CR effect was also reported by this group in a later study of young adult mice [245] suggesting that this effect of CR is independent of age. Further, this group showed that short-term re-feeding of the young adult mice, after they had been subjected to CR regimen, restored their body weight and improved their survival and NK cell function [246]. There are only two other reported studies that have examined how CR impacts the host's response to infection by intact pathogen. One study showed that adult CR mice had a higher mortality from polymicrobial sepsis induced by cecal ligation and puncture [247]. The CR mice had impaired MΦ function, indicated by lower production of inflammatory cytokines IL-6 and IL-12, lower expression of toll like receptor (TLR)2 and TLR4 mRNA, reduced phagocytic capacity, and diminished class II (I-A(b)) expression compared to the control mice [247]. In the other study [248], the author initially hypothesized that CR mice would be less susceptible to the intestinal parasite *heligmosomoides bakeri* (a rodent model for human hookworm infection) through enhancing both innate and adaptive immune responses. However, contrary to the predictions, CR mice not only had more worms, but the worms from the CR mice produced more eggs compared to the control mice. This occurred even though CR caused no change in the number of eosinophils and even increased serum IgG1 levels. These studies indicate that further investigation is needed to more precisely delineate the overall impact of CR on infection. In the meantime, caution should be taken before recommending CR to the frail elderly, and those who are at high risk of infection.

Concluding remarks

Since human lifespan and the proportion of elderly are increasing worldwide, immunosenescence has become an increasingly prominent topic given its relationship to increased incidence of infectious disease and cancer. The intrinsic defects that develop in the immune system during aging are further intensified by the absolute or relative deficiency in the elderly of several nutrients with known immune-enhancing properties. Therefore, nutritional intervention has been recognized as a practical, cost-effective approach to attenuating age-associated decline in immune function, vaccination efficiency, and resistance to infectious and neoplastic diseases. Although research in this field has continued to make significant progress, the impact of virtually all the nutritional agents studied on both immune response and related clinical endpoints remains controversial. It is well-acknowledged that being able to favorably modulate immune cell functions would not necessarily translate into a corresponding change in clinical outcome. Further, for those nutrients or food components with a relatively strong clinical efficacy, reproducibility continues to be a significant issue. The literature review summarized above indicates that substantial variation exists among nutritional interventions in the nature and relative potency of their immunomodulating activity. For nutrients like vitamin E, increased intake at 5-10 folds above recommended levels may be needed for optimal effect, whereas for others such as zinc, increased intake above recommended levels might not be beneficial and even might cause harm due to the narrow range of safe doses. For probiotics, strain, dose, and combined use of different strains are all important in determining their immunomodulatory effect. While some nutrients clearly benefit one aspect of immune function, they may adversely affect another. As an example, increased intake of n-3 PUFA is recommended to prevent and ameliorate inflammatory and autoimmune diseases as well as several age-related diseases in which inflammation plays a role; however, the reported immunosuppressing effect on T cells and increased risk of certain infections cautions against a general "one-fits-all" recommendation. Similarly, CR, an intervention shown to have broad health benefits including immunoenhancement, still needs further study in humans since it may be ineffective or even harmful under certain circumstances. Taken altogether, strong evidence generally supports the notion that proper nutrition is critical for optimal immune response and host defense against infection. Future studies are needed to determine the effectiveness and optimal conditions for various nutritional intervention regimens to improve the function of the aged immune system. In designing these studies, the following factors need to be considered: 1) optimal dose, 2) characteristics of targeted populations including their nutritional status, health condition, and genetic background, and 3) selection of clinically relevant, sensitive, reproducible, and feasible endpoints or surrogate readouts for reliable assessments of intervention efficacy. It is hoped that future studies will further enrich our knowledge to help us develop more effective and directed nutritional strategies to undertake the challenges of immunosenescence.

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Abbreviations

Ab: antibody; Ag: antigen; APC: antigen-presenting cells; Con A: concanavalin A; CR: caloric restriction; CTL: cytotoxic T lymphocytes; DHA: docosahexaenoic acid; DTH: delayed-type hypersensitivity; EPA: eicosapentaenoic acid; Ig: immunoglobulin; IL: interleukin; IL-2R: IL-2 receptor; LPS: lipopolysaccharide; MΦ: macrophages; PBMC: peripheral blood mononuclear cells; PHA: phytohemagglutinin; PMN: polymorphonuclear leukocytes; PUFA: polyunsaturated fatty acids; RI: respiratory infections; TB: mycobacterium tuberculosis; TCR: T cell receptor; Th: T helper; TLR: toll like receptor; TNF: tumor necrosis factor

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