Nutrients and their role in host resistance to infection

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Abstract: Almost all nutrients in the diet play a crucial role in maintaining an "optimal" immune response, such that deficient and excessive intakes can have negative consequences on immune status and susceptibility to a variety of pathogens. Iron and vitamin A deficiencies and protein-energy malnutrition are highly prevalent worldwide and are important to the public health in terms of immunocompetence. There are also nutrients (i.e., glutamine, arginine, fatty acids, vitamin E) that provide additional benefits to immunocompromised persons or patients who suffer from various infections. The remarkable advances in immunology of recent decades have provided insights into the mechanisms responsible for the effects of various nutrients in the diet on specific functions in immune cells. In this review, we will present evidence and proposed mechanisms for the importance of a small group of nutrients that have been demonstrated to affect host resistance to infection will be presented. An inadequate status of some of these nutrients occurs in many populations in the world (i.e., vitamin A, iron, and zinc) where infectious disease is a major health concern. We will also review nutrients that may specifically modulate host defense to infectious pathogens (long-chain polyunsaturated n-3 fatty acids, vitamin E, vitamin C, selenium, and nucleotides). A detailed review of the effect of long-chain polyunsaturated n-3 fatty acids on host defense is provided as an example of how the disciplines of nutrition and immunology have been combined to identify key mechanisms and propose nutrient-directed management of immune-related syndromes. *J. Leukoc. Biol.* 71: 16–32; 2002.

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INTRODUCTION

"If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health."

—Hippocrates c. 460—377 B.C.

It has long been accepted that immunity (or susceptibility to disease) depends to some extent on nutrition. The interdependency between the disciplines of nutrition and immunology was formally recognized in the 1970s when immunological measures were introduced as a component of assessing nutritional status [1]. Our understanding of the effect of nutrients on immune function has been refined as the field of immunology has grown from a descriptive science to one in which diverse immune phenomena can be tied together coherently and explained in quite precise structural and biochemical terms.

Today protein energy malnutrition (PEM) is cited as the major cause of immunodeficiency worldwide [2]. This is not surprising because immune cells have a high requirement for energy and amino acids for cell division and protein synthesis. The influence of PEM on immune function has been reviewed extensively [3]. Our knowledge of the effects of nutrition on immune function now extends beyond clinical nutrient deficiency. A growing body of literature demonstrates the immune benefits of increasing the intake of specific nutrients. This article will review our current understanding of the role of several nutrients in maintaining host immune defense. An inadequate status of some of these nutrients occurs in many populations in the world (vitamin A, iron, and zinc) where infectious diseases are a major health concern. We will also review nutrients that may specifically modulate host defense to pathogens (long-chain polyunsaturated n-3 fatty acids, vitamin C, vitamin E, selenium, and nucleotides). We begin with a review of the effect of long-chain polyunsaturated n-3 fatty acids on host defenses to illustrate how the two disciplines of nutrition and immunology have been combined to identify key mechanisms and propose nutrient-directed management of immune-related syndromes. Because we are unable to review all nutrients that are needed to maintain immune function, the reader is directed to some excellent reviews presented in **Table 1** and to a recent book published on this topic [4].

LONG-CHAIN POLYUNSATURATED FATTY ACIDS (PUFA)

Adults in Western countries obtain 30–45% of their total caloric energy from dietary fat, a smaller proportion of which consists of long-chain PUFA. The essential fatty acids, linoleic (n-6) and α-linolenic (n-3), cannot be synthesized by mammalian cells and so must be obtained from the diet. Linoleic acid is found in most plant oils (e.g., corn, safflower, and sunflower), margarines, and animal fats, whereas α-linolenic acid is found

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TABLE 1. Nutrient/Food Compounds that Influence Immune Function

Nutrients[a]	Review or key references on immune effects
MACRONUTRIENTS	
Energy/Protein	[3]
Amino Acids	
Glutamine	[190]
Arginine	[190]
Fat	
Monounsaturated fats	[191]
α-linoleic acid (n-6 PUFA)	[10, 192]
γ linolenic acid (n-6 PUFA)	[193]
n-3 PUFA	[6, 7]
CLA	[194]
Fiber	[195]
VITAMINS	
Betacarotene	[196]
Folic Acid	[197]
Vitamin A	[51]
Vitamin B12	[198]
Vitamin B6	[199]
Vitamin C	[81]
Vitamin D	[200, 201]
Vitamin E	[95, 96]
MINERALS	
Copper	[202, 203]
Iron	[124–126, 130]
Selenium	
Zinc	[144–146]
Other Trace/micro nutrients	[196]
OTHER NUTRIENTS/FOOD COMPOUNDS[b]	
Alcohol	[204–206]
Nucleotides	[182, 188]

[a] All the nutrients in bold type are reviewed in this manuscript.
[b] Complete information on other nutrients can be found elsewhere [4].

in flaxseed, soybean, and canola oils. Long-chain n-3 PUFA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) can be synthesized from α-linolenic acid in humans but can be obtained preformed from marine fish oils. These lipids are important in brain development, cardiovascular disease, and cancer (see ref [5]), and there is now convincing evidence that dietary n-3 PUFA, particularly EPA and DHA, have a major impact on the function of many components of the immune system. Although various effects of dietary fat on immune functions have been reviewed previously (see refs [6–9]), in this review we will summarize the well-documented effects of the long-chained PUFA EPA and DHA on host resistance to infection and other diseases in which the immune system is important.

Clinical studies of the effects of feeding fish oil on immunological disorders

Fish oil administered in clinical trials and in animal models of rheumatoid arthritis, ulcerative colitis, psoriasis, and organ transplantation has resulted in measurable beneficial effects on the immune system (see refs [9, 10]). Similarly, feeding fish oil to animals reduces disease severity and prolongs survival in animal models of lupus (see ref [7]), but human trials have not been as encouraging, with studies showing positive [11, 12] or no effects [13]. These studies generally suggest that feeding fish oil (a source of EPA and DHA) suppresses inflammatory/autoimmune responses during conditions of partial immune system activation. Thus, one might conclude that these lipids would have a negative effect on the immune system of healthy humans or animals. Indeed, feeding high amounts of EPA/DHA has been shown to reduce survival or pathogen clearance in some animal models of infection; however, other studies demonstrate no effect or even protection against infection with EPA/DHA administration to infected animals (see ref [7]).

Feeding n-3 PUFA has been shown to decrease tumor growth, incidence, and/or metastasis in a large number of animal studies (reviewed in refs [5, 14]) and to prolong survival of cancer patients in a human clinical trial [15]. It is not clear whether these effects involve n-3 PUFA modulation of immune function. Although there are data to support an immune-independent mechanism [16], studies in our laboratory have shown that feeding n-3 PUFA to tumor-bearing animals enhances natural killer (NK) cell activity, CD8+ T-cell activation, and interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) cytokine production after mitogen stimulation [17]. We and others [17–19] have demonstrated that tumor burden itself can modulate/suppress the host's NK activity and the ability of T cells to respond to various stimuli, raising the possibility that the immune response to dietary n-3 fatty acids may differ in immune-suppressed hosts (i.e., tumor-bearing) compared with normal, healthy hosts.

Dietary n-3 fatty acids (EPA and/or DHA or fish oil) and the immune system

Feeding EPA and DHA has been shown to modulate specific functions of innate and acquired immunity. In general, feeding high levels (>10% of total fat) of n-3 PUFA (compared with diets high in n-6 PUFA) to healthy animals or human subjects results in suppression of the ability of lymphocytes to respond to mitogen stimulation, NK cell activity, and delayed-type hypersensitivity (DTH) reactions [8, 10]. Suppression of these functions has been demonstrated in studies supplementing as little as 180 mg EPA + DHA/day [20] or up to 6 g DHA/day [21] to the diet of humans or by feeding animals from 1% w/w purified EPA or DHA [22] up to 20% w/w fish oil (approximately 3.5% w/w EPA+DHA; ref [23]). Feeding long-chain n-3 PUFA was shown to significantly decrease the production of interleukin (IL)-1, IL-6, and TNF-α by peripheral blood mononuclear cells in humans and by peritoneal macrophages in animals after mitogen stimulation (see ref [9]). However, there is some discrepancy with respect to TNF-α production in animals, because many studies document increases in TNF-α production after stimulation when EPA/DHA were fed [9].

Conversely, a number of studies have shown that feeding more moderate amounts of n-3 PUFA (i.e., fed at <10% of fat to animals and <1 g EPA+DHA/day to humans) is not immunosuppressive [24, 25], and can even enhance immune functions such as lymphocyte proliferation/activation [26–28], NK cell activity [26, 29], macrophage activation [26], and IL-1, IL-2, and TNF-α production after mitogen stimulation [27, 28]. Recently, we have demonstrated that adding a small amount of DHA [and arachidonic acid (AA)] to infant formula alters the

maturation (expression of the CD45RO+ antigen on CD4+ cells) of T cells in preterm infants to be more similar to that of infants fed human milk [30]. These seemingly contradictory observations of the effect of n-3 fatty acids on immune function in healthy humans and animals may be a result of the overall content of polyunsaturated fat in the diet. We have found that n-3 PUFA reduce splenocyte proliferation in healthy rats when fed in a high polyunsaturated fat diet (unpublished results), but not when fed in a diet with a low polyunsaturated fat level (which is more representative of the diet consumed by most North Americans).

Mechanisms by which n-3 PUFA may modulate immune function

Several potential mechanisms have been proposed to explain the immunomodulatory effects of dietary n-3 PUFA, including effects on eicosanoid formation, signal transduction, gene expression, and lipid peroxidation (reviewed in refs [6, 31, 32]; **Table 2**).

i) Alterations in membrane lipid composition: Changing the fat composition of the diet changes membrane fatty acid composition of most cells of the body, including cells of the immune system [30, 33], and these changes can alter membrane-mediated functions such as eicosanoid production and signal transduction.

Modulation of eicosanoid synthesis

Dietary fat composition influences eicosanoid synthesis by affecting the supply of substrates (n-3 and n-6 fatty acids) for eicosanoid production. Increasing the n-3 content of the diet produces corresponding increases in the long-chain n-3 PUFA content of cell membranes at the expense of n-6 PUFA, particularly AA. n-3 Fatty acids compete with AA as substrates for cyclooxygenase (involved in eicosanoid production) and also directly suppress the activity of cyclooxygenase; thus, they inhibit AA metabolism to eicosanoids. Higher levels of n-3 PUFA in cell membranes reduce the production of proinflammatory eicosanoids (i.e., PGE_2, LTB_4, TXA_2) from n-6 PUFA and increase the production of eicosanoids from n-3 PUFA (PGE_3, LTB_5). It is important that eicosanoids formed from n-3 PUFA oppose or have weaker effects than eicosanoids formed from n-6 PUFA [34] (Table 2). The situation, however, is complicated because PGE_2 and LTB_4 have somewhat opposing effects; although both inhibit mitogen-stimulated lymphocyte proliferation, LTB_4 tends to enhance NK activity and T helper cell (Th)1-type cytokine production (IL-1, IL-2, IL-6, TNF-α, IFN-γ), whereas PGE_2 suppresses these functions. Thus, the outcome of reducing PGE_2 and LTB_4 by n-3 fatty acids is not clear and likely depends on the balance of the different mediators produced, the timing of their production, and the sensitivities of target cells to their actions. Because many studies indicate that dietary n-3 PUFA reduce the ability of lymphocytes to proliferate in vitro in response to mitogen stimulation [8], a function expected to increase with reductions in PGE_2 and LTB_4, other mechanisms besides eicosanoid formation probably are involved in n-3 PUFA immunomodulation.

Alteration of signal transduction

Evidence suggests that changes in membrane lipid composition can alter the binding of ligands, such as cytokines, to their receptors [35, 36]. Although support is limited, it is possible that an increase in n-3 PUFA in immune cell membranes may modify membrane properties and subsequently interfere with ligand-receptor interactions, leading to changes in receptor signal transduction. Another mechanism may involve the incorporation of n-3 PUFA into signaling molecules. Because all phospholipids and some of their second messengers, such as diacylglycerol (DAG) and ceramide, contain fatty acyl chains, it is possible that changing the fatty acid composition of these molecules may alter their function [32]. Indeed, it has been demonstrated that EPA and DHA are incorporated into signaling molecules such as DAG with the administration of n-3

TABLE 2. Mechanisms Responsible for the Role of Long Chain n-3 PUFA (EPA and DHA) on Immune Function

Function	Proposed Mechanisms	References
Lymphocyte proliferation/ activation	*Eicosanoid modulation*—EPA/DHA ↓ PGE_2 and ↑ T cell proliferation	[28]
	Lipid peroxidation—lymphocyte proliferation (otherwise suppressed by n-3 PUFA) is restored with adequate α-tocopherol +n-3 PUFA	[28, 207, 208]
	Altered signal transduction ↓ T cell function—fish oil may inhibit tyrosine kinase interaction with the membrane	[40]
	Altered signal transduction—EPA/DHA ↓ DAG and ceramide levels and ↓ IL-2 secretion and T cell proliferation	[209]
	Gene expression—EPA/DHA ↓ IL-2R mRNA levels	[210]
Lymphocyte maturation and differentiation	Mechanism(s) not established for observed effects	
Macrophage function	*Gene expression*—fish oil ↑ TNF-α mRNA transcription	[211]
	Gene expression—DHA ↓ iNOS mRNA transcription	[212]
	Altered signal transduction/gene expression—EPA ↓ MAP kinase and transcription factor (AP-1) activity, leading to lower TNF-α production	[45]
	Eicosanoid modulation—fish oil ↓ PGE_2 and ↑ TNF-α production	[213, 214]
NK cell function	*Eicosanoid modulation*—DHA ↓ LTB_4 which may have contributed to the ↓ NK activity	[21]
DTH reactions	Fish oil ↑ *lipid peroxidation* and ↓ DTH reactions	[25]
Neutrophil function (↓ chemotaxis)	*Altered signal transduction*—fish oil ↓ IP_3 second messenger levels by ↓ PLC function	[215]

PUFA in the diet [37, 38], and there is some evidence that n-3 PUFA-enriched DAG is less potent in activating protein kinase C (PKC) than n-6 PUFA-enriched DAG [39]. Finally, many signaling molecules, including some tyrosine kinases, are reversibly acylated during signaling, which targets them to the cell membrane where they interact with other signaling molecules. It has been suggested that changing the fatty acid content of the diet (or the culture media) may alter the acylation patterns of different signaling molecules [31, 40, 41], affecting their ability to interact with the membrane. Alternatively, it is possible that changes in membrane fatty acid composition induced by changes in diet could alter the physical nature of the membrane regions to which acylated signaling molecules bind [40]. Changes in early signal transduction events such as tyrosine kinase activation could be responsible for changes in other downstream signaling events that have been documented with n-3 PUFA administration (see Table 2).

ii) Alteration of gene expression: An increasing number of published studies indicate that n-3 fatty acids can modulate expression of various immune genes (see Table 2). Changes in gene expression induced by n-3 fatty acids may be the result of direct or indirect effects of fatty acids on the transcription factors that initiate gene expression. Recent work demonstrated that long-chain PUFA, including EPA, can bind to and activate the class of transcription factors known as the peroxisome proliferator-activated receptor (PPAR; refs [42, 43]), providing a mechanism by which n-3 PUFA could regulate directly gene expression. Some evidence indicates that PPARs are involved in immune cell function, because the activation of PPARγ with natural or synthetic PPAR agonists has been shown to inhibit macrophage activation and the production of TNF-α, IL-1, and IL-6 as well as activation of nitric oxide synthase (NOS) [31, 44]. It has also been shown that long-chain n-3 PUFA can modulate the activity of transcription factors such as nuclear factor κ-B (NF-κB) and activated protein-1 (AP-1) [45, 46]. In these studies, upstream signal transduction events were modified with n-3 PUFA treatment, which correlated with lowered transcription factor activity, suggesting that n-3 PUFA may affect early signal transduction events that lead to altered transcription factor activity (see Table 2).

iii) Lipid peroxidation: Long-chain PUFA are more sensitive to lipid peroxidation than are monounsaturated or saturated fatty acids. Thus n-3 PUFA incorporation into cell membranes may increase the host requirement for antioxidant nutrients [32]. Because lipid peroxides are toxic to cells, it is possible that the inhibitory effects of feeding large amounts of EPA/DHA on immune cell function could be a result of lipid peroxidation in the absence of adequate dietary antioxidants. There is some support for this mechanism (see Table 2), but other studies have found that supplying antioxidants does not reverse the immune effects of dietary n-3 PUFA [47, 48]. Further research is needed to establish a risk for peroxidation of immune cells when n-3 PUFA are fed.

Summary

It is well accepted that long-chain n-3 PUFA have immunomodulatory effects, but further work needs to be done to clarify their precise effects (e.g., immunosuppression vs. immunostimulation) in healthy subjects as well as in different disease conditions such as infection. Exciting work has been published recently identifying several mechanisms by which n-3 PUFA affect immune functions. Future studies pursuing the proposed mechanisms will likely provide further insight into the roles of n-3 PUFA in immunomodulation.

VITAMIN A

The importance of vitamin A in immune function and protection against infections is well-established [49, 50] and has been reviewed [51–53]. Vitamin A deficiency is a major public health problem in many developing countries; up to 10 million children show signs of deficiency and an estimated 100 million children experience subclinical depletion [54]. The different chemical forms of vitamin A (retinol, retinal, retinoic acid) all appear to be involved in its metabolic functions [50]. Vitamin A deficiency can affect host defenses directly through its essential functions in metabolism in the various immune cells [51] or indirectly through its role in epithelial cell differentiation and host barrier function [55].

Clinical and functional evidence for the essentiality of vitamin A to the immune system

The immune efficacy of supplementing vitamin A on infection rates has been examined in several randomized, double-blind, placebo-controlled trials of malnourished children in various regions of the developing world. Antibody-mediated immunity has been shown to be severely impaired in individuals with vitamin A deficiency [56]. Provinding vitamin A supplements has been found to improve the antibody titer response to measles vaccines [57], maintain gut integrity [58], lower the incidence of respiratory tract infections [59, 60], and reduce mortality associated with diarrhea and measles [54, 59–61] but not pneumonia [61]. There is clinical data suggesting that vitamin A deficiency in HIV-1-infected individuals contributes to mortality [62], disease progression [62], and maternal-infant disease transfer [63, 64]. This type of support has contributed to the World Health Organization's recommendation that vitamin A supplements be given to all individuals in developing countries who contract measles whether or not they have symptoms of vitamin A deficiency [54]. In animal studies, vitamin A deficiency inhibits mitogen-stimulated, T-cell proliferation [65–68], antigen-specific antibody production [66], and the ability to produce immunoglobulin (Ig)A [69] and IgG [70]. It also reduces the ability of CD4 cells to provide the B-cell stimulus for antigen (Ag)-specific IgG1 responses [70]; limits Th-2-type cytokine-gene expression [49]; decreases the ability of neutrophils to phagocytose infectious organisms (*Pseudomonas aeruginosa*) and generate active oxidant molecules [71]; and reduces the resistance to several infectious organisms [66, 68]. Most of these negative effects on host defense that have been associated with low vitamin A status appear to be reversible with restoration of vitamin A status [67, 68, 71, 72].

Proposed mechanisms for the effects of vitamin A on immune function

Indirect mechanisms

Vitamin A has been shown to control differentiation of epithelial cells by regulating the synthesis of keratin [50], and

deficiency results in altered epithelial structure (squamous metaplasia) and a reduced number of mucus-secreting cells [73] (**Table 3**). The rapidly dividing epithelia at mucosal surfaces (gut and lung) are especially susceptible to vitamin A deficiency, which results in a loss of gap junctions between epithelial cells [50], increasing the risk of bacterial translocation [58]. In addition, vitamin A deprivation has been shown to reduce the replication rate of basal and mucous cells and the proportions of preciliated and ciliated cells, which would further enhance the susceptibility to infection [55]. Because vitamin A is needed for glycoprotein synthesis [74], a deficiency of it would likely impair the synthesis of the many glycoproteins involved in the immune response (e.g., integrins, fibronectin, and globulins) [50].

Direct mechanisms

The role of vitamin A in lymphocyte proliferation likely occurs through activation of the retinoic acid receptor (RAR)-α, because provision of RA has been shown to increase mRNA levels of RAR-α in T lymphocytes [75, 76]. Substantial evidence supports a role for vitamin A in negatively regulating IFN-γ secretion, thus influencing the development of Th-2- versus Th-1-type responses (Table 3). Vitamin A deficiency in mice strongly favors the production of IFN-γ (a Th-1-type cytokine) [49, 77], but adding RA in vitro to T lymphocytes from vitamin A-deficient mice inhibits IFN-γ production [49, 77]. RA was shown to alter IFN-γ synthesis at the level of transcription [49], implicating direct effects of this vitamin on cytokine genes. The promotion of Th-1-type responses, via excessive IFN-γ production and limited Th-2 cell growth and differentiation, would contribute to the impaired humoral immune-response capacity observed in animals and humans deficient in vitamin A [70, 77].

Hypothesized immune effects of excessive vitamin A intake

Although the toxic effects of excess vitamin A have not been studied in humans, animal studies have shown excessive intakes to be associated with toxicity, including suppressed hematopoiesis [68], mitogen-induced T-cell proliferation [65, 76], antigen-specific antibody production [68], and an increased susceptibility to infectious organisms [66]. Although the mechanisms by which excessive vitamin A intake depresses immune responses are not known, some hypothesize that high circulation levels may down-regulate nuclear receptors for vitamin A (thus decreasing transcription and expression of several immune molecules such as cytokines), or that there is a direct toxic effect of the elevated retinyl esters in blood [67].

Summary

Vitamin A is essential for cells of the immune system. The considerable immune benefits (to cells of the innate and acquired immune system), which would contribute to reduce the risk of various pathogen-mediated diseases, warrants a recommendation to supplement individuals with minimal or poor vitamin A status. Today, however, there is not sufficient evidence to determine if there are immune benefits of providing additional vitamin A to those with sufficient status. Animal studies suggest that excessive intakes of vitamin A suppress various aspects of T- and B-cell function and may even increase the susceptibility to infectious diseases.

VITAMIN C

Ascorbic acid (vitamin C) is an essential component of every living cell. Vitamin C is highly concentrated in leukocytes and is used rapidly during infection (e.g., to prevent oxidative damage). Reduced concentrations of this vitamin in leukocytes is associated with reduced immune function [78]. In humans, the essentiality of vitamin C to the immune system is most clearly illustrated during the clinical deficiency disease, scurvy, where infections occur, and there is anergy (poor or immeasurable immune response) in almost every component of the immune system [73]. Indeed, a common method to assess vitamin C status is to measure the concentration of the vitamin in leukocytes [73].

Vitamin C intakes above the recommended daily intake and immune function

There has been a long-standing debate concerning the possible function of high doses of vitamin C in "boosting" immunity

TABLE 3. Mechanisms for the Essentiality of Vitamin A to the Immune System

Function	Proposed Mechanisms	References
I. Indirect		
Cell differentiation	Vitamin A controls differentiation of epithelial cells by regulating keratin synthesis	[50]
	Deficiency results in ↓ number of mucus-secreting cells and squamous metaplasia	[73, 216]
Glycoprotein expression	Vitamin A is a carrier of monosaccharides. Vitamin A (retinyl esters) become mannosylated and donate their attached mannose group to an appropriate glycoprotein acceptor during synthesis.	[74]
Gut structure	Deficiency ↓ gap junctions, leads to a loss of gut integrity	[217]
II. Direct		
Lymphocyte proliferation	Vitamin A enhances the level of mRNA for the retinoic acid receptor-α	[75, 76]
Th-1 vs Th-2 type responses	Vitamin A negatively regulates IFN-γ secretion by T cells *in vitro*	[77]
	Inhibition of IFN-γ production by retinoic acid occurs at the level of transcription	[49, 218]
	Vitamin A deficiency causes a shift from a Th2- to a Th1-dominated response, with high IFN-γ and low IL-4, IL-5 and IL-10 production	[49, 56]

(reviewed in ref [79]). A wealth of epidemiological studies suggest that higher intakes of vitamin C and other antioxidants are associated with a lower risk of chronic disease (such as cancer and cardiovascular disease, which involve the immune system to some extent), but there have been few intervention studies that measure specific components of the immune system. The results of animal studies and a few human studies have suggested that immune effects, such as antiviral resistance and anticarcinogenic effects, are increased with higher intakes of vitamin C [79]. However, most studies have complicated the interpretation of the results by administering a number of antioxidant nutrients in addition to vitamin C.

Because of the early literature review and sustained advocacy by Linus Pauling, subsequent studies on mega-dosing with vitamin C have not been shown unequivocally to prevent and/or treat colds and upper respiratory tract infections [80]. Some components of the study designs of several early trials demonstrating beneficial effects in this area have been challenged recently (reviewed in ref [81]). In a recent analysis of six large vitamin C supplementation (> or = 1 g/day) trials in Britain, four out of six studies (conducted on women) concluded that there was no evidence to support the claim that taking high doses of vitamin C decreases the incidence of colds [81]. However, four studies of vitamin C supplementation in male schoolchildren and students show a statistically significant reduction in the incidence of colds with vitamin C supplementation [81]. From this same analysis in yet another study in a group of males receiving vitamin C supplementation, the authors described a statistically significant reduction in recurrent common cold infections [81]. It has been suggested that physical stress and/or low nutritional intakes may have contributed to the positive effects of supplementation in some subjects [81], but the possibility of a beneficial effect of vitamin C on viral infections cannot be completely ruled out.

Despite the inconclusive results of the clinical trials, supplementation trials have demonstrated benefits of vitamin C supplementation on several immune functions (reviewed in ref [79]). For example, Delafuente et al. [82] demonstrated that providing 1 g of vitamin C (and 200 mg vitamin E) daily for 16 weeks resulted in a significant increase in the lymphoproliferative capacity (proliferative response to mitogens) and in the phagocytic functions of peripheral blood neutrophils (adherence to vascular endothelium, chemotaxis, and phagocytosis of latex beads and superoxide anion production) as well as a significant decrease of serum levels of lipid peroxides and cortisol, both in the healthy aged women and in the aged women with coronary heart disease.

Vitamin C and immune disorders

Vitamin C supplementation has also been shown to have some clinical usefulness in the treatment of several autoimmune diseases, allergy, asthma, phagocytic dysfunction disorders (Chediak-Higashi and granulomatous disease), and immunosuppressive disorders, including HIV (reviewed in ref [83]). After exposure to toxic environmental chemicals, a high oral dose of vitamin C restored the blastogenic responses of immune cells to T- and B-cell mitogens and enhanced NK activity tenfold in human subjects [79, 84]. Similarily, in animals, the toxic effects of cadmium on the immune system (phagocytic activity of polymorphonuclear leukocytes and monocytes and the percentage of active and total T lymphocytes in peripheral blood) were reduced by vitamin C supplementation [85].

Immune toxicity associated with high intakes of vitamin C?

Unlike many other dietary antioxidants, even when consumed at very high levels (5000 mg/day), vitamin C appears to be safe, and no negative or suppressive effects on immune cell function (e.g., NK cell activity, apoptosis, or cell cycle progression) or structure have been found [86, 87]. One might still remain cautious, however, because some in vitro experiments demonstrate that very high levels of vitamin C suppress T-cell proliferation (IL-2 production) and adhesion [88] and reduce the ability of neutrophils to phagocytose *Candida albicans* [88]. The effect of these in vitro changes on physiological function has not been established.

Proposed mechanisms

Many investigations have been undertaken to elucidate the mechanism by which vitamin C might enhance systemic immunity, particularly in defense of viral diseases (**Table 4**). The

TABLE 4. Proposed Mechanisms for Effects of Vitamin C on Immune Function

Function	Proposed Mechanisms	References
Antioxidant (prevents the level of ROI and their negative effect on various immune functions)	Prevents ROI-dependent activation of the transcription factor NFκB that regulates expression of proinflammatory cytokines such as IL-1 and TNF-α	[78]
	Reduces ROI mediated damage to DNA in lymphocytes	[90]
	Nonenzymatically reduces oxidized vitamin E, helps regenerate this important lipid soluble antioxidant (with established effects on immune function)	[91]
↓ T cell death (it is hypothesized that blocking apoptosis would boost immunity)	Ascorbic acid in culture decreases T cell death: Ag-induced T cell death, growth factor withdrawal-cell death, spontaneous and steroid-induced death	[79]
	Enhances effector T cell's ability to enter S phase	[79]
↑ NK activity (in suppressed individuals)	Upregulation of protein kinase C	[84]
Other immunostimulatory mechanisms proposed	↑ intracellular cyclic nucleotide levels	[92]
	Modulation of prostaglandin synthesis	[92]
	Antagonism of the immunosuppressive interaction between histamines and leukocytes	[92]

actions of vitamin C as a reducing agent and oxygen-radical quencher are well-established. Although frequently stated, exactly how this potent antioxidant enhances immune function is not well understood. The general belief is that reduction of free radicals will prevent DNA damage to immune cells, thereby maintaining their functional and structural integrity. Indeed, the immune system (which relies heavily on membrane receptors and signals) is particularly sensitive to oxidative stress [89]. Several cellular mechanisms have been identified with dietary supplementation or in vitro treatment of immune cells that might explain a role of vitamin C in antioxidant protection (Table 4). Ascorbic acid can reduce directly [90] or indirectly through the regeneration of vitamin E [91] damage to lymphocytes by reactive oxygen intermediates (ROI). It was suggested recently that ascorbate levels exert this effect by down-regulating ROI-dependent expression of proinflammatory IL genes via inhibition of transcription of NF-κB [78].

Vitamin C might "boost" T-cell capacity via several mechanisms. In vitro, three T-cell death pathways (growth factor withdrawal- , spontaneous- , and steroid-induced death) were inhibited when T cells were incubated with ascorbic acid [79]. Furthermore, this study demonstrated that activated and resting T cells were responsive to ascorbic acid because both populations were resistant to death stimuli when treated with ascorbic acid [79]. In addition, effector T cells were more likely to enter S-phase if treated with ascorbic acid [79]. Other potential immunostimulatory and anti-inflammatory mechanisms suggested for vitamin C are increasing intracellular nucleotide levels, modulation of proinflammatory cytokine synthesis, and decreasing the effect of histamines on leukocytes [92] (Table 4). Up-regulation of NK activity (important in destruction of virally infected cells) has been suggested to occur via a stimulatory effect of vitamin C on PKC activity.

Summary

Clearly the essentiality of vitamin C to cells of the immune system has been established. Although not all clinical data agree with an effect of vitamin C on viral infections, there is convincing evidence from feeding studies in humans and animals and experiments done on primary cultures that vitamin C has a positive effect on host defense. Unfortunately, we are far from being able to define the optimal levels of intake required to maintain an optimal immune response to prevent or treat viral or other infectious diseases.

VITAMIN E AND SELENIUM

Vitamin E (α-tocopherol) and the trace element selenium (Se) are discussed together as they function synergistically (by related but independent mechanisms) in tissues to reduce damage to lipid membranes by the formation of reactive oxygen species (ROS) during infections [93].

Influence of vitamin E on immune function

Several excellent reviews describe the effects of vitamin E on various immune parameters [94–96]. Vitamin E is a lipid soluble antioxidant, and deficiency results in increased free radical-induced membrane damage to red blood cells [73]. The effect of providing vitamin E to individuals at risk of deficiency (i.e., elderly) has been reviewed recently [97, 98]. Supplementation with vitamin E to those believed to have marginal status increased DTH skin test response, enhanced mitogen-induced lymphocyte proliferation and IL-2 production, and improved the antibody (Ab) responses to vaccines, while decreasing the synthesis of the immunosuppressive eicosanoid PGE_2 [99, 100]. Similarly, supplementing old rats with vitamin E alleviated the age-related impairment in some immune functions (e.g., lymphocyte proliferation, IL-2 production, and macrophage function) [101].

Providing vitamin E to healthy individuals was shown to increase the CD4/CD8 ratio, enhance T-cell proliferation, and lower measures of oxidative stress (urinary, plasma, and peripheral blood lymphocyte H_2O_2 production; ref [102]). However, supplementation of vitamin E to "healthy" individuals did not attenuate oxidative DNA damage in peripheral blood lymphocytes [103]. Animal studies support the immune benefits of supplemental vitamin E, which increased CD4+CD8− thymocytes and IL-2 production in rodents [104, 105], and improved responses to infection in swine [106].

The optimal intake of vitamin E required to provide immune benefits has not been established and likely depends on vitamin E status and the presence or absence of other conditions. Studies have demonstrated immune effects with 200–800 mg/day doses [94, 100]. Vitamin E is generally considered safe when supplemented in very high amounts [73]. However, providing 300 mg/day (considered megadose) of vitamin E for three weeks depressed the bactericidal activity and proliferation of peripheral leukocytes in humans [107] and reduced vaccine titers in animals (150 mg/kg) [108], indicating that there may be an upper limit above which immune impairment results.

Influence of Se on immune function

Se plays a role in balancing the redox state of the cell and removing reactive oxygen species, which likely contributes to its anti-inflammatory effects [109]. Se deficiency has been shown to decrease the production of free radicals and killing by neutrophils [110], IL-2R affinity and expression on T cells [111, 112], T-cell proliferation and differentiation [111, 113], and lymphocyte cytotoxicity [111, 114]. Se deficiency in vitro enhances neutrophil adherence to endothelial cells, an important preliminary event in inflammation [115]. These alterations in immune function likely contribute to the increased cancer susceptibility associated with Se deficiency and implicate Se deficiency in the pathogenesis and exacerbation of some chronic inflammatory and viral diseases [110].

Conversely, supplementation with Se increases lymphocyte proliferation, expression of the high-affinity IL-2R [116], cytolytic T lymphocyte (CTL) tumor destruction, and NK-cell function in humans [117] and increases lymphocyte proliferation, IL-2R expression, and macrophage and CTL tumor cytotoxicity in mice [111, 113, 118]. There is also substantial evidence for a benefit of providing Se during HIV-1 infection, where it has been demonstrated to reduce oxidative stress, modulate cytokine synthesis (increase IL-2; decrease TNF and IL-8), improve T-cell proliferation and differentiation, and reduce cy-

tokine-induced HIV-1 replication [119]. Recently, it was demonstrated that Se deficiency in the host enhances the mutation rate of coxsackievirus [120] and influenza A virus [121]. This suggests that the oxidative stress status of the host can alter the genome and pathogenicity of an infectious virus [121]. Although the amount of Se necessary for maximum immune benefit has not been established, it has been suggested that 200 mg/day may be sufficient [116]. However, excessive intakes of Se are toxic to many tissues and are associated with impaired cell-mediated and humoral immunity [110].

Proposed mechanisms for the effects of vitamin E and Se on immune function

Vitamin E is an oxidant scavenger that acts to protect cell membranes from damage by reactive oxygen species (**Table 5**). Immune cells are particularly susceptible to oxidative damage because of their highly unsaturated membranes [122] and their ability to produce large amounts of free radicals (i.e., during inflammation; ref [102]). The ability of vitamin E to scavenge lipid soluble-free radicals is dependent to some extent on the status of two other antioxidant compounds, vitamin C and glutathione, which are involved in reducing oxidized vitamin E back to a reusable (i.e., able to be oxidized) form [91]. Additionally, vitamin E may improve T-cell function by decreasing macrophage PGE_2 production by modulating the AA cascade initiated by lipoxygenase and/or cyclooxygenase [91, 94]. Furthermore, vitamin E influences lymphocyte maturation, possibly by stabilizing membranes and allowing enhanced binding of antigen-presenting cells (APC) to immature T cells via increased expression of intercellular adhesion molecule-1 (ICAM-1; Table 5) [105].

Se is essential for the function of several selenoproteins, because of the selenocysteine residues present at their active sites [110]. Glutathione peroxidase (GPX) is a selenoprotein that acts as an oxidant scavenger and protects against oxidative damage. Thioredoxin reductase is another selenoprotein that affects the redox regulation of a variety of key enzymes, transcription factors, and receptors, including ribonucleotide reductase, the glucocorticoid receptor, AP-1, and NF-κB [110]. In addition to reducing thioredoxin, this enzyme breaks down hydroperoxide and lipid peroxides in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) more efficiently than GPX [110], thus making it an effective protector against ROS.

In addition to its antioxidant role, Se may have additional immune properties involving membrane receptor expression. The stimulation of T-cell proliferation, CTL and macrophage cytotoxicity, and NK activity by Se may be a result of the ability of Se to enhance the expression of the α and/or β subunits of the IL-2R on these activated immune cells [111, 114, 123]. This results in a greater number of functional IL-2R/cell and in enhanced proliferation and clonal expansion of cytotoxic precursor cells [123]. Se deficiency causes in increased neutrophil adhesion and increased expression of E-selectin and ICAM-1 [115], suggesting that Se can down-regulate neutrophil activation.

Summary

Vitamin E and Se are essential to immune function and are supplemented routinely in the diets of domestic animals for their immune benefits [94]. These nutrients have long been known to have anticarcinogenic effects by means of their antioxidant properties. The potential of Se as a chemopreventive agent also involves its ability to enhance the clonal expansion of immunocompentent cells [NK cells, CTL, and lymphokine-activated killer (LAK)] [111]. Further research into the mechanisms of these nutrients would be beneficial, particularly in terms of how they influence expression of cell-surface molecules.

IRON (Fe)

Iron deficiency is estimated to affect 20–50% of the world's population, making it the most widespread nutritional defi-

TABLE 5. Proposed Mechanisms for the Effects of Vitamin E and Selenium on Immune Function

Function	Proposed Mechanisms	References
Vitamin E		
Antioxidant	Prevents lipid peroxidation and damage to cell membranes	[102, 107, 219]
Reduces immunosuppression	↓ PGE_2 formation by modulating the arachidonic acid cascade initiated by lipoxygenase and/or cyclooxygenase	[91, 94, 99]
Lymphocyte maturation	Stabilizes membranes, thus ↑ proportion of CD4+CD8− T cells through enhanced binding of antigen presenting cells to immature T cells via increased expression of ICAM-1	[105]
Selenium		
Antioxidant	Co-factor for glutathione peroxidase	[93]
	The selenoenzyme thioredoxin reductase can break down hydroperoxide and lipid peroxides in the presence of NADPH	[110]
Anti-viral	Deficiency results in enhanced mutation rate and pathogenesis of several viruses	[120, 121]
Anti-inflammatory	Deficiency is associated with ↑ neutrophil adherence (↑ expression of E-selectin and ICAM-1)	[115]
	Thioredoxin reductase is a selenoenzyme that acts alone or in conjunction with its substrate, thioredoxin, to affect the redox regulation of a variety of key enzymes, transcription factors and receptors, including the anti-inflammatory proteins AP-1 and NFκB	[110]
Lymphocyte and NK function	Se enhances the expression of the alpha and/or beta subunits of the IL-2R on activated lymphocytes and NK cells	[220]
Effector cells	Se ↑ IL-2R expression and thus ↑ ability of CTL & macrophages to destroy tumor cells, reverses ↓ proliferative potential of T cells	[111, 114, 221]

ciency in industrialized and developing countries. The physiological effects of iron deficiency are manifest in a large number of tissues including those of the immune system [124]. The interrelationship between iron deficiency, toxicity, and immunity is complex, and many excellent reviews have been published [124–127].

Iron and immune function

It is well-documented that iron regulates the function of T lymphocytes, and in most studies (in vivo and in vitro), a deficiency results in impaired cell-mediated immunity [124, 125, 127–129]. Iron deficiency may also delay the development of cell-mediated immunity [124]. Immune cells appear to differ from one another in their synthesis and use of iron-binding proteins and in the amount of iron they take up and store; this suggests that there would be differential effects of iron status on various immune functions [130]. Details on iron metabolism in immune cells have been reviewed elsewhere [128]. Numerous studies describe normal T-lymphocyte-proliferative responses to mitogens and limited effect on other immune functions in humans and animals with mild-to-moderate iron deficiency (reviewed in ref [124]). This suggests that the immune response may not be impaired by alterations in iron availability to the same extent as are other organs. Indeed, lymphocytes meet an increased iron requirement during proliferation or other conditions by increasing the synthesis and expression of surface transferrin receptors [131]. This, along with the clinical and experimental data, suggests that T cells may be able to sequester iron better than other cells when there is a limited iron supply, and only in a "deficient state" might the essentiality of iron to the immune system be evident. Humoral immunity may be less affected by iron deficiency than cellular immunity, because antibody production in response to immunization with most antigens is preserved in animals and humans with poor iron status (reviewed in refs [124, 125]).

Neutrophil function (decreased myeloperoxidase activity and bactericidal activity) and NK activity are impaired with iron deficiency [125]. Macrophage phagocytosis is generally unaffected by iron deficiency, but bactericidal activity of these cells is attenuated [132]. It has been proposed that the immunosurveillance role of macrophages may be mediated in part by modulation of iron status in cells [133]. Unlike other cells, macrophages acquire iron for metabolic use via phagocytosis of effete erythrocytes, which is subsequently released into the circulation, where it is bound to transferrin and available to other cells [124, 126]. When activated (i.e., during inflammation, possibly signaled by IL-1 and IFN-γ), macrophages increase their uptake of iron and bind it in the cells (via increased transferrin receptors and ferritin synthesis; refs [124, 126]). This sequestration of iron in macrophages has been proposed to be beneficial during the early, acute stages of infection with pathogens, because it would limit availability to microorganisms (particularly intracellular microorganisms); however, it would also limit availability to other immune cells, and this would impair host resistance [125, 126, 134].

Many of the immune abnormalities associated with iron deficiency appear to be reversible with iron repletion, but this has been difficult to demonstrate in clinical and observational studies [125]. Experimental and clinical data suggest that there is an increased risk of infection during iron deficiency, although a few studies indicate otherwise (reviewed in ref [125]). Interpretation of many of the human studies is confounded by the existence of multiple nutrient deficiencies and uncontrollable environmental factors associated with poverty [124]. Experimental studies in laboratory animals uniformly show reversible deleterious effects of iron deficiency on most measures of functional immunity (reviewed in ref [125]). Many of these effects appear to occur even in mild-to-marginal iron-deficient states in animal studies [125]. However, as discussed above, there is still little information available on whether mild or moderate iron deficiency influences immune effects in humans [125].

Iron supplementation and immune function

The major dilemma in alleviating iron deficiency revolves around the relationship between iron repletion/supplementation and increased morbidity from acute and chronic infections (reviewed in refs [124, 125, 135, 136]). Microbiology studies show a close relationship between the availability of iron and bacterial virulence (reviewed in ref [125]); one might conclude therefore that providing iron would benefit the infectious organisms. Indeed, it is well established that administration of parenteral iron has been shown in human and animal studies to be harmful when administered during infection [134]. This unresolved debate has been reviewed extensively [125], and it was concluded recently that there is little evidence that oral iron supplementation to deficient individuals inhibits immune function or increases the susceptibility to most infectious agents (with the possible exceptions of malaria-related disease, HIV, and tuberculosis) [125]. Animal studies of morbidity that have used a wide range of infectious organisms are even less consistent, and a recent study concluded that iron deficiency may be more likely to protect against intracellular (i.e., plasmodia, mycobacteria, and invasive salmonella) than extracellular pathogens [125]. This is likely a function of the ability of the organism to acquire iron from the host [125].

Mechanisms for the importance of iron to the immune system

The molecular and cellular mechanisms responsible for immune changes during iron deficiency are complex and remain unclear. This is because iron is important in several crucial, metabolic pathways in immune cells. Knowledge concerning the roles of iron and iron-binding proteins in lymphocyte physiology and pathology has developed rapidly over the last few years. The genes for the major iron-binding proteins have been cloned and sequenced and are now being studied regarding transcriptional and posttranscriptional regulatory mechanisms in T cells, B cells, macrophages, and NK cells [130]. Some of the known cellular and molecular changes associated with iron deficiency are summarized in **Table 6**.

Iron toxicity

Although iron is an essential nutrient, it can be potentially deleterious to cells [124]. Effects of iron overload (hemochromatosis) include decreased antibody-mediated and mitogen-stimulated phagocytosis by monocytes and macrophages, re-

TABLE 6. Mechanisms Responsible for the Effects of Iron Deficiency on Immune Function

Function	Fe-deficiency/sensitive alteration	References
Lymphocyte proliferation and cytokine production	↓ phosphatidylinositol 4,5 bis-phosphate (PIP_2) hydrolysis	[126, 127]
	↓ PKC activity, translocation and gene transcription	[126, 127]
	↓ ribonucleotide reductase activity, rate limiting step in DNA synthesis	[124, 126, 127]
	↓ electron transport and cellular respiration	[124]
	Altered progression through the cell cycle—cells arrested in the G_0-G_1 phase and reducing the proportion of the S and G_2-M phases	[127]
Lymphocyte maturation	Prevents thymocyte maturation into αβ-T-cells	[126]
Lymphocyte differentiation	↓ differentiation controlled by availability of Fe; iron is required for regulating gene transcription	[126]
Macrophage and neutrophil function	↓ transcription of NO synthase (iron dependent)	[124, 126]
	↓ myeloperoxidase activity (iron dependent)	[124]
	↓ NADPH oxidoreductase used to generate superoxide in neutrophils	[222]
	↑ NRAMP 1 which actively removes intracellular iron making it unavailable to microorganisms.	[125]
Others functions:		
Ability to acquire Fe	Some lymphocyte lines exhibit other specialized adaptations (use of transferrin-independent iron uptake or the up-regulation of transferrin) to increase iron uptake to support cellular function during limited iron supply	[126, 131]
Anticancer/antiviral effects through changes in iron availability	The production of NO by macrophages causes an efflux of non-heme iron from neoplastic and infected host cells and this cellular depletion of the metal can suppress DNA synthesis as well as the functioning of aerobic respiratory enzymes	[133]

duced neutrophil migration, alterations in T-lymphocyte subsets, modification of lymphocyte distribution in different compartments of the immune system, suppression of the complement system, and increased rate of infections [129, 137]. Convincing evidence shows that hydroxyl radicals, produced by the Fenton reaction or by the Fe-catalyzed Haber-Weiss reaction, are responsible for many of the damaging effects of iron [134]. Within minutes, however, the immune system, iron and its binding proteins have immunoregulatory properties, and shifting these immunoregulatory balances by providing too much iron may result in deleterious physiological effects [129]. In fact, the carcinogenic effects of excess iron have been attributed to the suppressive effect of excess iron on the host's immune system in addition to the formation of hydroxyl radicals and promotion of cancer cell multiplication [138].

Summary

Iron deficiency remains a public health nutrition problem affecting millions of children and women of child-bearing age. This nonexhaustive review supports the essential role of iron in immune function, the potential deleterious effects of supplementation during some infections, and the potential for iron toxicity.

ZINC (Zn)

Zinc is a dietary trace mineral that, in addition to its many essential functions in growth and development, is essential for the function of cells of the immune system [139]. Zn is required for the activity of more than 100 enzymes associated with carbohydrate and energy metabolism, protein degradation and synthesis, nucleic acid synthesis, heme biosynthesis. and CO_2 transport [139]. Zn deficiency impedes host-defense systems [140], leading to increased susceptibility to a variety of pathogens [141], and a deficiency of Zn is known to occur in many diseased states that involve the immune system [141]. These include alcoholism, renal disease, burns, and gastrointestinal tract disorders [142] as well as HIV and diarrhea [140]. The genetic Zn malabsorption syndrome, acrodermatitis enteropathica, is associated with frequent severe infections with fungi, viruses, and bacteria with concomitant thymic atrophy, anergy, reduced proliferative response, decreased T-helper cells, and deficient thymic-hormone activity [143]. The influence of Zn on immune function has been the subject of a number of excellent reviews [141, 142, 144–146].

Clinical and experimental evidence for the essentiality of zinc

A number of experimental trials have examined the ability of Zn to improve immune function during various diseases. For example, Zn supplementation resulted in a reduced duration and severity of cold symptoms [147]. However, other studies have shown that Zn compounds appear to have limited effectiveness for common cold treatment [148, 149], and a meta-analysis of eight published, randomized clinical trials showed that the evidence for effectiveness of Zn salts lozenges in reducing the duration of common colds is still lacking [150]. In patients with sickle-cell disease, Zn supplementation increased IL-2 production, decreased incidence of bacteriologically positive infections, decreased the number of hospitalizations, and decreased the number of vasoocclusive pain crises [151]. In young children, Zn supplementation reduced diarrhea duration [152–154], pneumonia [155], growth-stunting [154, 156, 157], acute lower respiratory infections and morbidity [157, 158], respiratory morbidity, incidence of dysentery, and altered intestinal permeability [112, 153]. Children receiving Zn supplementation had a significantly higher proportion of

CD4+CD3+ cells (CD3, CD4, and CD4/CD8 ratio) in peripheral blood and improved T-cell-mediated immunity (CMI) [159].

Animal studies have confirmed that Zn deficiency is associated with a significant reduction in T-helper cell function [160], impaired DTH responses [161], compromised B-cell development [145], low IgG production [162], decreased NK lytic activity [141, 146], and increased mortality to various infectious organisms [163]. Maternal Zn deprivation results in offspring with reduced thymus and spleen size, splenocyte numbers, mitogen responses [164], and antibody production [165]. However, the poor Ab-mediated response capacity and defective DTH could be restored by Zn supplementation [161, 165]. It is interesting that the effects of Zn deficiency may be immune cell-type specific, because one study suggests that myeloid cell numbers and function are not compromised by such a deficiency [166].

Proposed mechanisms for the immune essentiality of zinc

The proposed mechanisms by which Zn influences immune functions include generation of oxygen radicals, lymphocyte maturation, cytokine production, and the regulation of apoptosis and gene expression as described in **Table 7**.

During Zn deficiency, the presence of higher proportions of granulocytes (as much as 50%) and monocytes (almost twofold) [167] suggest that the myelopoietic environment of the marrow is more protected from, or even up-regulated during, Zn deficiency [140]. Although the numbers may not be compromised, the function of these cells may be. Zn-dependent enzymes or reactions are involved in the generation of oxygen radicals [168], and suboptimal levels of Zn have been demonstrated to lower the killing ability of internalized parasites by macrophages [169].

Furthermore, the capacity of macrophages to engulf and kill parasites can be restored after treatment with Zn [169]. Whether impaired killing ability of macrophages is a result of decreased production of H_2O_2 or of another Zn-related function or process remains to be established [168].

The decreased cell-mediated immune functions and the increased frequency of infection in Zn-deficient subjects [170] may be linked to the effects of Zn on cytokine production (decreased IL-2 production), a decrease in CD4+/CD8+ cell ratio, and a decrease in the production of antigen mature CD4+CD45R0+ cells, suggesting an effect on T-helper cell maturation (Table 7). Zn influences the activity of multiple enzymes at the basic level of replication and transcription [146]. For example, Zn is needed for the activity of thymidine kinase during the S-phase of cell growth [171] and for the activation of the Zn finger protein NF-κB that is involved in IL-2 and IL-2R expression [146]. In Zn-deficient cells, the activation, translocation, and binding of NF-κB to DNA are inhibited [146]. NK activity and cytotoxic T-cell precursors (CD8+CD73+) are decreased with Zn restriction [170, 172], which may be linked to decreased IL-2 production. Zn deficiency is also associated with an increase in plasma corticosterone, which can contribute to T-cell immunosuppression [160].

Some of the changes in T-cell maturation and function observed during Zn deficiency are likely related to decreases in

TABLE 7. Mechanisms Responsible for the Essentiality of Zn to Immune Function

Function	Proposed Mechanisms	References
Macrophage and neutrophil function: generation of oxygen radicals	Zn is required by Zn-dependent enzymes or reactions for production of oxygen radicals (e.g. peroxide superdismutase)	[168, 169]
Lymphocyte maturation	Important for formation of precursor T and B cells in the bone marrow	[140]
	Deficiency increases the CD4+CD45RA+ to CD4+CD45RO+ ratio, thus it appears that zinc is required for regeneration of new CD4+ memory T cells following antigen exposure	[170]
Thymulin production	Zn is required by this Zn-dependent thymus-specific hormone that binds to high-affinity receptors on T cells; thymulin induces several T cell markers, and promotes T cell function, including allogeneic cytotoxicity, suppressor function, and IL-2 production	[143]
Cytokine production	In humans, Zn deficiency caused an imbalance in cytokine production with ↓ IFN-γ, IL-2 & TNF-α (Th1-type), without changing the production of IL-4, IL-6 and IL-10 (Th2-type)	[170]
Apoptosis regulation	Low levels of Zn *in vitro* ↑ apoptosis in murine CD4+CD8+ thymocytes	[175]
	High levels of Zn *in vitro:*	
	↓ apoptosis by preventing activation of the endonuclease	[176]
	↓ apoptosis by inhibiting steroid binding to the glucocorticoid receptor, thus inhibiting binding of the receptor to specific glucocorticoid response elements in DNA that signal apoptosis	[223]
Gene regulation	Deficiency ↓ activation of NFκB, thus ↓ gene expression of IL2-IL2R	[146]
	Deficiency ↓ thymidine kinase activity delaying cell cycle and ↓ cell growth	[171]
	↓ activity of Zn-dependent metalloenzymes involved in cytokine production	[224]
Role in basic cellular functions	Many enzymes responsible for DNA replication, RNA transcription, cell division, and cell activation are Zn dependent	[139]
	Skin lesions, poor wound healing observed with deficiency	[142]
	Maintaining the barrier of the skin	[145]
	Zn itself has some antioxidant properties	[145]

Zn-dependent thymulin activity [143]. Zn deficiency in experimental animals results in atrophy of thymic and lymphoid tissue [143], with losses of precursor T and B cells in the bone marrow [140]. This is demonstrated by the dose-related decline in the number of pre-B cells (B220+) [173, 174], immature B cells (B220+IgM+IgD−) [173], and mature B cells (IgM+IgD+) [173].

In vitro, low concentrations of Zn have been shown to induce apoptosis in mouse CD4+CD8+ thymocytes, [175], whereas high zinc concentrations have been shown to block apoptosis [144]. In vitro, high concentrations of Zn blocked apoptosis by preventing activation of the endonuclease, which is involved in DNA fragmentation [176] and inhibited steroid binding (possibly by binding to the vicinal cysteines in the receptor-ligand-binding site) [144] to the glucocorticoid receptor during glucocorticoid-induced apoptotic death.

Summary

Although clinical Zn deficiency is more common in children and in elderly persons [140], it is estimated that a large proportion of the North American population has marginal intakes of Zn and may be at risk of deficiency. The evidence is quite convincing that ensuring an adequate intake of Zn is essential to optimal immune function and protection from infectious pathogens.

NUCLEOTIDES

Dietary nucleotides are obtained from nucleoprotein-rich foods, such as organ meats, fish, and poultry, and are especially high in human breast milk [177]. In general, de novo synthesis of nucleotides may be sufficient for normal growth and development in healthy persons, who typically consume <5% (1–2 g/day in adults) of their daily requirement for these compounds [178, 179]. Nucleotides may become conditionally essential during growth and immunological challenges when demand may exceed de novo synthetic capacity [180].

Animals fed nucleotide-free diets suffer impaired cellular and humoral immune function, including decreased NK cell and macrophage activity [181], lower DTH responses and cytokine production [182], decreased antibody production [178], and increased susceptibility to infections [182]. The addition of nucleotides to nucleotide-free diets has been shown to reverse or restore many of the changes observed with nucleotide deficiency, such as increasing Th1-type cytokines [183–185], increasing antibody production (IgG2a; refs [184, 186]), and increasing spleen cell proliferation [185]. In addition, human infants fed breast milk or formula supplemented with nucleotides had higher NK cell activity and IL-2 production compared with infants fed formula without nucleotides [181]. The beneficial effects of additional dietary nucleotides on immune function are supported by animal studies [187]. Clinical benefits (i.e., shorter hospital stays and reduced incidence of infection among critically ill patients) have also been demonstrated with the use of enteral formulas containing nucleotides [188]. Unfortunately, many studies examining nucleotide supplementation have fed mixtures that contain other "immunonutrients" (e.g., fish oil and amino acids), making it impossible to identify specific nucleotide effects on immune and clinical parameters.

The precise mechanism by which exogenous nucleotides modulate immune function is not known, but it is logical that they would contribute to the pool of nucleotides available to immune cells [181]. Nucleotides are building blocks for DNA and RNA synthesis and are involved in diverse cellular processes, serving as sources of chemical energy [e.g., 5′-triphosphate (ATP)] and intracellular signals (e.g., adenosine cyclic 3′,5′-adenosine monophosphate and cyclic 3′5′-guanosine monophosphate) [189]. Further research is needed to identify the specific functions and mechanisms and to define the importance of these nutrients, particularly in feeding situations such as enteral supplements and infant formula where the intake of nucleotides would be low.

CONCLUSIONS

Macronutrients and micronutrients in the diet are essential for maintaining the function of immune cells. Despite convincing evidence that nutrients can modulate many parameters of immune function, nutrient intake and status are rarely considered or even described in most immune function studies in humans or animals. One would predict that variations in nutrient intake contribute to the differences in immune responses between individuals and between studies. Also, much of our understanding of basic immunology has come from elaborate mechanistic studies performed in rodents who are fed diets where the level and source of a nutrient (e.g., fat and protein) are considerably different from that consumed by humans. Our knowledge of the effect of nutritional status on the functioning of the immune system has led to several practical applications. These include the use of immune tests as prognostic indexes for patients undergoing surgery and the use of immune parameters to assess nutritional status and to determine the efficacy and adequacy of nutritional therapy. As our understanding of the role of specific nutrients in host resistance to infectious diseases increases, it might be predicted that this will be used to formulate recommendations to achieve the immune response needed to prevent and treat specific infectious diseases in the population.

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