Contribution of Selected Vitamins and Trace Elements to Immune Function

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Abstract
Adequate intakes of vitamins and trace elements are required for the immune system to function efficiently. Micronutrient deficiency suppresses immune functions by affecting the innate T-cell-mediated immune response and adaptive antibody response, and leads to dysregulation of the balanced host response. This increases the susceptibility to infections, with increased morbidity and mortality. In turn, infections aggravate micronutrient deficiencies by reducing nutrient intake, increasing losses, and interfering with utilization by altering metabolic pathways. Insufficient intake of micronutrients occurs in people with eating disorders, in smokers (both active and passive), in individuals with chronic alcohol abuse, in patients with certain diseases, during pregnancy and lactation, and in the elderly. With aging a variety of changes are observed in the immune system, which translate into less effective innate and adaptive immune responses and increased susceptibility to infections. Antioxidant vitamins and trace elements (vitamins C, E, selenium, copper, and zinc) counteract potential damage caused by reactive oxygen species to cellular tissues and modulate immune cell function through regulation of redox-sensitive transcription factors and affect production of cytokines and prostaglandins. Adequate intake of vitamins B₆, folate, B₁₂, C, E, and of selenium, zinc, copper, and iron supports a Th1 cytokine-mediated immune response with sufficient pro-
duction of proinflammatory cytokines, which maintains an effective immune response and avoids a shift to an anti-inflammatory Th2 cell-mediated immune response and an increased risk of extracellular infections. Supplementation with these micronutrients reverses the Th2 cell-mediated immune response to a proinflammatory Th1 cytokine-regulated response with enhanced innate immunity. Vitamins A and D play important roles in both cell-mediated and humoral antibody response and support a Th2-mediated anti-inflammatory cytokine profile. Vitamin A deficiency impairs both innate immunity (mucosal epithelial regeneration) and adaptive immune response to infection resulting in an impaired ability to counteract extracellular pathogens. Vitamin D deficiency is correlated with a higher susceptibility to infections due to impaired localized innate immunity and defects in antigen-specific cellular immune response. Overall, inadequate intake and status of these vitamins and minerals may lead to suppressed immunity, which predisposes to infections and aggravates malnutrition.

Introduction
The profound interactions among nutrition, infection, and health were recognized many decades ago [1]. Nutrients have been demonstrated in both animal and human studies to be required at appropriate amounts for an efficient and adequate immune response. Undernutrition and nutrient deficiency affect the innate, adaptive and cellular immune responses and suppress immune functions, leading to deregulation of normally coordinated...
host response to infection, and thereby enhancing the virulence of pathogens [2–4]. In turn, infections aggravate micronutrient deficiencies by reducing nutrient intake, increasing losses, and interfering with utilization by altering metabolic pathways [5]. These interactions are of particular significance in populations with insufficient intake of multiple micronutrients, which is occurring more frequently than single deficiency due to poor nutrition, especially in developing countries. However, vitamin/mineral undernutrition is also observed in industrialized countries, especially among people with restricted dietary intake due to disease or dieting [6], smokers (both active and passive) [7], individuals exposed to environmental stress [8], individuals with chronic alcohol abuse [9], patients with colds and infectious diseases [10], teenagers, pregnant and lactating women [11], and among the elderly [12–14]. Furthermore, a variety of changes are observed in the immune system with increasing age, which translate to less effective innate and adaptive immune responses, increased reactivity to self-antigens in vivo, and an increased susceptibility to infections (immune senescence). The mechanisms underlying age-related changes in immune function are not fully understood, but there is growing support that nutritional factors including vitamins and minerals play a role in age-related declines in immune response [4, 15–17].

In the past decade substantial research has focused on the role of nutrition and especially on the contribution of vitamins and minerals to an optimum functioning of the immune system. This overview compiles the current knowledge on the effects of selected vitamins (vitamins A, B6, B12, C, D, E, and folate) and minerals (selenium, zinc, copper, and iron) on immune function.

**Overview on the Immune System**

Excellent reviews on the immune system are available [18–20]. The immune system fights bacteria, viruses, fungi, protozoans, and reacts against cancer cells and foreign substances or matter such as organ transplants. The immune response to invaders can be divided into two interactive main systems (fig. 1) [21]: innate or non-specific immunity and adaptive or specific/acquired immunity.
Adaptive immunity takes over if the innate immunity cannot clear the infection in a short time. Moreover, the second time an intruder tries to invade the body, B and T memory cells help the immune system to activate much faster. Innate immunity consists of epithelial barriers, a cellular component (macrophages, polymorphonuclear leukocytes, natural killer (NK) cells, and dendritic cells (DCs)), and a non-cellular component with recognition molecules (C-reactive protein, serum amyloid protein, mannose-binding protein, complement). Engagement of the latter helps to differentiate invading pathogens from host cells and triggers phagocytosis and the elimination of the invader.

Activated phagocytic cells increase production of inflammatory mediators such as proinflammatory cytokines (interleukin (IL)-1, 6, and 12) and tumor necrosis factor-α (TNF-α), prostaglandins, and leukotrienes, and secrete oxygen and nitrogen radicals (reactive oxygen species, ROS). Cytokines are a group of proteinaceous signaling compounds that like hormones are used extensively for inter-cell communication and differentiation. They are critical for the regulation of immune and inflammatory responses.

The generation of ROS is part of the physiological function of cells involved in host defense, especially during chemotactic locomotion, phagocytosis and microbicidal activity. A decrease in antioxidant status or an enhanced cellular oxidative stress results in escalating inflammation. The immune system is particularly vulnerable to oxidative stress, since immune cells rely on cell–cell communication via membrane receptors. Interference with the signaling system is deleterious and results in an impaired immune response. NK cells can lyse tumor and virus-infected cells without prior antigenic stimulation. They are activated in response to interferons or macrophage-derived cytokines. They serve to contain viral infections while the adaptive immune response is generating antigen-specific cytotoxic T cells that can clear the infection. DCs constantly sample the surroundings for pathogens such as viruses and bacteria. These cells are activated by endogenous danger signals, such as the release of interferon-γ (IFN-γ) from virally infected cells, and finally act as antigen-presenting cells as stimulators of naïve T cells to initiate immune responses for which immunological memory had not been established. DCs endocytose extracellular antigens complexed with major histocompatibility complex (MHC) class II.

Specific immunity is divided into cell-mediated and antibody-mediated immunity. The majority of the cells of specific immunity are T lymphocytes (T cells originating from bone marrow and maturing in the thymus) and B lymphocytes (B cells originating and maturing in the bone marrow). These cells carry receptors on their surfaces that distinguish self from non-self. Each T and B cell recognizes antigens specific to particular infectious agents. Following maturation these cells enter the pool of naive cells in the peripheral lymph nodes where they will respond to foreign pathogens.

There are three kinds of T cells. Cytotoxic T cells directly kill invaders. Helper T cells aid B and other T cells to do their jobs. Suppressor T cells suppress the activities of B and other T cells so they do not overreact. Cytotoxic T lymphocytes express a surface protein marker, CD8+, and kill cells infected with viruses and tumor cells. T-helper (Th) cells are recognized by the glycoprotein surface marker, CD4+. CD4+ cells function as Th cells type 1 (Th1) by producing immunomodulatory cytokines that promote T-cell growth and stimulate CD8+ T-cell division and cytotoxicity (IL-2, IL-15), and activate microbioidal action of macrophages (IFN-γ, TNF-α), or as Th2 cells producing cytokines that could deactivate macrophages (IL-10, transforming growth factor-β) and promote humoral immune responses by stimulating B-cell growth and antibody production (IL-4, IL-1). With regard to the overall immune response the balance between Th1- and Th2-type activities is considered to be of utmost importance and guarantees an adequate and efficient immune response [22]. Following clonal expansion and elimination of the pathogen, many of the cells die through programmed cell death (apoptosis), but some remain as memory cells, providing a rapid response in case of a re-infection with the same pathogen. T cells are only able to recognize antigens displayed on cell surfaces. Upon recognition of a foreign substance or pathogen, the antigen is complexed with MHC class I (endogenous pathogens) or MHC class II (exogenous antigens). Antigen-presenting cells are DCs, B cells, and macrophages. Antigens complexed with MHC class I molecules activate CD8+ cytotoxic T cells, and antigens complexed with MHC class II molecules activate CD4+ T cells.

Humoral immunity refers to the branch of immunity that is mediated by secreted antibodies produced in the B cells. Humoral immunity is particularly effective against extracellular pathogens. Secreted antibodies bind to antigens on the surfaces of invading pathogens presenting them to macrophages for destruction.

A very recent review identified current immune function assays commonly used as markers in human intervention studies and evaluated their biological significance with regard to clinically relevant endpoints, sensi-
tivity, and practical feasibility [23]. Vaccine-specific serum antibody production, delayed-type hypersensitivity (DTH) response, vaccine-specific or total secretory IgA in saliva or other relevant fluids and the response to attenuated pathogens were classified as highly suitable in vivo markers. Ex vivo markers, such as NK-cell cytotoxicity, oxidative burst of phagocytes, lymphocyte proliferation and cytokine pattern generated by activated immune cells, are considered of medium suitability based largely upon the lack of a sufficient association between a change in immune marker and a change in susceptibility to infection. These authors therefore suggest the use of combinations of immune markers such as T-cell proliferation, production of Th1, Th2 and regulatory-type cytokines (e.g. IFN-γ, IL-2, IL-4, IL-5, IL-10, and transforming growth factor-α) in addition to clinical outcome in future clinical human nutrition intervention studies.

The objective of this overview is to demonstrate whether an inadequate or deficient micronutrient status influences the balance between the Th1 and Th2 response pattern and thus impairs the body’s overall ability to combat infections.

Role in Immune Function: Water-Soluble Vitamins

Vitamin B<sub>6</sub>

Vitamin B<sub>6</sub> is essential in nucleic acid and protein biosynthesis by providing one-carbon units used in the production of purines and deoxythymidylate affecting DNA and mRNA synthesis. Hence, an effect of vitamin B<sub>6</sub> on immune function is logical, since antibodies and cytokines build up from amino acids and require vitamin B<sub>6</sub> as a coenzyme in their metabolism. Human studies demonstrated that vitamin B<sub>6</sub> deficiency impairs lymphocyte maturation and growth, and antibody production and T-cell activity. Lymphocyte mitogenic response was impaired by dietary vitamin B<sub>6</sub> depletion in elderly subjects and restored by administration of vitamin B<sub>6</sub>. The effects of deficiency were seen in a decreased antibody response of DTH, IL-1-β, IL-2, IL-2 receptor, NK-cell activity, and in lymphocyte proliferation [24–26].

Marginal vitamin B<sub>6</sub> deficiency induced by 11 weeks of intake of 30 and 50% of the recommended dietary allowance altered the percentage of Th cells (IL-2-producing cells) and slightly decreased the serum immunoglobulin (Ig) D concentration. The observed effects were suggested to be caused by a reduction of one-carbon units and a reduced capability to synthesize nucleic acids and proteins, and a lower activity of the pyridoxal 5'-phosphate-dependent serine hydroxymethyl transferase [27]. Marginal vitamin B<sub>6</sub> deficiency in the elderly was found to be associated with decreased numbers and function of circulating T lymphocytes which could be corrected by short-term (6 weeks) supplementation with 50 mg vitamin B<sub>6</sub>/day [28]. Decreased IL-2 production, T-lymphocyte numbers, and T-lymphocyte proliferation were observed in subjects undergoing vitamin B<sub>6</sub> depletion, indicating that vitamin B<sub>6</sub> deficiency suppressed a Th1 and promote a Th2 cytokine-mediated activity, whereas repletion reversed it [29].

The effect of increased intakes of vitamin B<sub>6</sub> on lymphocyte proliferation and IL-2 concentration was studied in young women who consumed a constant diet containing 1 mg vitamin B<sub>6</sub>/day for 7 days, followed by three 14-day periods with intakes of 1.5, 2.1, and 2.7 mg vitamin B<sub>6</sub>/day. Lymphocyte proliferation in response to phytohemagglutinin (PHA) increased significantly by 35% with intakes of 2.1 mg/day, compared to intakes of 1.5 mg/day. With higher intakes there was no additional increase. Five of seven participants had increased IL-2, but this increase was not significant. Lymphocyte proliferation was correlated with vitamin B<sub>6</sub> intake (p < 0.05; r = 0.757). The authors concluded that there is a vitamin B<sub>6</sub> intake or a range of intakes that may provide an optimal immune response in humans, as indicated by lymphocyte proliferation, and may require intakes higher than the current recommended dietary allowance [30].

Folate

Folate plays a crucial role in nucleic acid and protein synthesis by supplying in concert with vitamins B<sub>6</sub> and B<sub>12</sub> one-carbon units, and therefore a deficiency or lack of folate significantly alters the immune response. Folate deficiency modulates immune competence and resistance to infections and affects cell-mediated immunity by reducing the proportion of circulating T lymphocytes and their proliferation in response to mitogen activation. This effect in turn decreases resistance to infections [31]. Further, folate deficiency induced in PHA-activated human T lymphocytes cell cycle arrest in the S-phase, induced apoptosis, and increased the level of uracil in DNA. Folate deficiency also increased the ratio of CD4+ to CD8+ T cells due to a marked reduction in CD8+ cell proliferation. All these effects were reversible in vitro by either folate addition or nucleotide repletion, and suggest that folate status may affect the immune system by inhibiting the capacity of CD8+ T-lymphocyte cells to proliferate in response to mitogen activation. If these effects could also be shown in vivo, it might explain the observa-
Vitamins and Trace Elements and Immune Response

Since it is not possible to study the action of vitamin B12 in artificially deficient humans, some studies used patients with vitamin B12-deficient disorders, and evaluated the alterations in immunological indicators following administration of vitamin B12 (methylcobalamin). In 11 patients (aged 36–83 years) with pernicious anemia or post-gastrectomy megaloblastic anemia (vitamin B12 serum concentrations <85 pg/ml), a significant decrease (p < 0.01) was found in the number of lymphocytes and CD8+ cells, and in the proportion of CD4+ cells. In addition, findings showed an abnormally high CD4+/CD8+ ratio, and suppressed NK cell activity. Intramuscular injection of methylcobalamin (500 μg/day; every other day for 2 weeks) significantly decreased the CD4+/CD8+ ratio in the patients to the level in controls. The augmentation of CD8+ cells by methylcobalamin treatment was observed even in the control subjects, which supports a potential anti-tumor effect of vitamin B12, and may partly explain the high risk of gastric cancer in pernicious anemia. The decreased level of NK cell activity was restored but did not reach control levels, but restoration to control levels could be achieved after 1 to 2 years of follow-up with methylcobalamin injections of 1,000 μg every 3 months. The results indicate that vitamin B12 may act as an immunomodulator of cellular immunity, especially in relation to CD8+ and NK cells [37].

In a trial with 60 healthy subjects (aged >70 years) who, in addition to the regular diet, received over 4 months a special nutritional formula providing, among other nutrients, 400 μg folic acid, 120 IU vitamin E, and 3.8 μg vitamin B12, NK cell cytotoxicity increased in supplemented subjects and decreased in nonsupplemented participants, and supplemented subjects reported fewer infections (p = 0.02), suggesting that this nutritional supplement increased innate immunity and provided protection against infections in elderly people [35].

**Vitamin B12**

Vitamin B12 is involved in carbon-1 metabolism and there are interactions with folate metabolism. In a vitamin B12-deficient state the irreversible reaction that forms 5-methyltetrahydrofolate results in an inactive form of folate if it is not de-methylated by methionine synthase. The ‘trapping’ of 5-methyltetrahydrofolate may result in a secondary folate deficiency with impairments in thymidine and purine synthesis and subsequently in DNA and RNA synthesis, leading to alterations in immunoglobulin secretion [36].

**ROS**

ROS, generated by activated immune cells during the process of phagocytosis, can be scavenged by non-enzymatic antioxidants, such as ascorbic acid or glutathione,
or by enzyme action (superoxide dismutase (SOD) or glutathione reductase). Whereas ROS play essential roles in intracellular killing of bacteria and other invading organisms, the immune system and other biomolecules (lipids, proteins) may be vulnerable to oxidative attack. If ROS are produced in high concentrations, this can cause oxidative stress and lead to impaired immune response, loss of cell membrane integrity, altered membrane fluidity, and alterations in cell–cell communication. These alterations could contribute to degenerative disorders such as cancer and cardiovascular disease [2, 40, 41]. The immune-enhancing role of vitamin C has recently been reviewed [42].

Vitamin C is highly concentrated in leukocytes and is used rapidly during infection, e.g. to ameliorate oxidative damage. Vitamin C was found in vivo to be a stimulant of leukocyte functions, especially of neutrophil and monocyte movement, and supplementation of healthy adults (1–3 g/day) and children (20 mg/kg/day) enhanced neutrophil chemotaxis [43]. It has been shown to stimulate the immune system by enhancing T-lymphocyte proliferation in response to infection increasing cytokine production and synthesis of immunoglobulins [44]. Vitamin C dose-dependently inhibited the lipopolysaccharide-stimulated number of monocytes producing the proinflammatory cytokines IL-6 and TNF-α and the number of lymphocytes producing IL-2 in vitro. These data confirm that vitamin C may play a significant role in the regulation of the inflammatory response. Mechanisms considered for this immunomodulating effect of vitamin C may be the protection from dysregulation of the immune-inflammatory response by inhibition of the initial expression of proinflammatory cytokines via the oxidant-sensitive transcription factor, nuclear factor κB (NF-κB), or the modulation of the immune system by inhibiting T-cell apoptosis-signaling pathways [45].

Administration of vitamin C resulted in improvement in several components of human immune response such as anti-microbicidal and NK cell activities, lymphocyte proliferation, chemotaxis, and DTH response [46–48]. The effect of dietary vitamin C at different intakes (5–250 mg/day) on immune function was studied in young, healthy non-smokers, who consumed a vitamin C-deficient diet. Plasma and leukocyte vitamin C concentrations were decreased with the deficient diet by about 50%, and the DTH response to several antigens was decreased, whereas lymphocyte proliferation was not affected. With higher doses (60 and 250 mg/day) the DTH response was normalized [47]. Vitamin C supplementation (500 mg/day for 1 month) resulted in a significant increase in the proliferative response of T lymphocytes to PHA and concanavalin A in older people (>70 years) known to have reduced vitamin C plasma and leukocyte concentrations, even if free living [49].

In a cross-sectional study of 3,258 men aged 60–69 years with no history of cardiovascular disease or diabetes, a significant inverse association of dietary and plasma vitamin C and fruit and vegetable intakes with biomarkers of inflammation (C-reactive protein; tissue plasminogen activator) was reported. The authors concluded that vitamin C has anti-inflammatory effects and is associated with an attenuation of endothelial dysfunction [50]. On the other hand, a review of available prospective studies with vitamin C (250–500 mg/day) indicated no anti-inflammatory response and it was concluded that vitamin C is not anti-inflammatory [51].

Limited evidence suggests that ascorbic acid has antiviral activity in humans [52]. Topical application in patients with herpes simplex virus infections decreased the duration of the lesions and viral shedding [53]. Based on its immune-stimulating properties (stimulant of leukocyte function; enhancement of chemotaxis) [43], vitamin C was postulated to be effective in ameliorating symptoms of upper respiratory tract infections, especially the common cold. Further, plasma and leukocyte vitamin C concentrations fall rapidly with the onset of the infection and return to normal with the amelioration of the symptoms suggesting dosage with vitamin C could be beneficial for the recovery process [54]. A review of the large numbers of studies on a potential effect of vitamin C on the common cold and respiratory infections concluded that administration of >1 g/day had no consistent effect on the incidence of common colds, but supported a moderate benefit on duration and severity of symptoms which may also be of economic advantage [55].

Ultra-marathon runners supplemented with 600 mg/day of vitamin C showed a significantly decreased incidence of post-race upper respiratory infections and were better able to cope with the oxidative stress from strenuous exercise [56]. A cocktail of antioxidants (including 120 mg vitamin C, selenium, and α-tocopherol succinate) helped to preserve the antioxidant system (alleviation of muscle damage) during an overloaded training-induced stress in subjects with initially low antioxidant intake [57]. A more recent single-blind placebo-controlled study (n = 7 in each group) in healthy subjects showed that supplementation with antioxidant vitamins (500 mg/day ascorbic acid and 400 IU/day RRR-α-tocopherol) attenuated the release of the proinflammatory IL-6 into plasma from contracting skeletal muscle during exercise [58]. On
the other hand, short-term supplementation with vitamin C alone had either a negligible or only a minor effect on exercise-induced IL-6 response [59–61].

A summary on the role of the water-soluble vitamins on the immune system is given in Table 1.

### Table 1. Role of water-soluble vitamins on the immune system and effects of deficiency and supplementation

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Role in the immune system</th>
<th>Effects of deficiency and supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Interferes with immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B&lt;sub&gt;12&lt;/sub&gt; and folate</td>
<td>Deficiency in humans is accompanied by a suppression of a Th1 response and promotion of a Th2 response (decreased lymphocyte growth and proliferation, decreased NK activity, decrease in antibody response (DTH), and decrease in proinflammatory cytokines IL-1β, IL-2, IL-2 receptor) Supplementation (repletion) reverses the immune response (Th1 response); required intakes to obtain optimal lymphocyte proliferation may be higher than the current RDA High intravenous doses of pyridoxal phosphate may be beneficial in the treatment of patients with autoimmunity and HIV</td>
</tr>
<tr>
<td>Folate</td>
<td>Interferes with immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B&lt;sub&gt;12&lt;/sub&gt; and vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Deficiency causes an impaired immune response and resistance to infections (reduction in circulating lymphocytes, decreased proliferation; increased CD4+/CD8+ ratio, decreased DTH, and NK cell activity) (impaired Th1 response) The reduction in CD8+ cell proliferation may be related to the finding of an increased carcinogenesis due to reduced cytotoxic activity Supplementation of elderly individuals improves overall immune function by altering the age-associated decrease in NK cell activity (impaired killing of virus-infected cells and tumor cells) supporting a Th1 response providing protection against infections Very large intakes of folic acid were shown in one study to possibly impair NK cytotoxicity</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Interferes with immune function through its involvement in nucleic acid and protein biosynthesis in concert with vitamin B&lt;sub&gt;6&lt;/sub&gt; and folate</td>
<td>Human studies in vitamin B&lt;sub&gt;12&lt;/sub&gt;-deficient patients showed an abnormally high CD4+/CD8+ ratio, and suppressed NK cell activity, which could be restored by injection of methyl vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Effective antioxidant contributing to the maintenance of the redox integrity of cells and protection against ROS generated during respiratory burst and inflammatory response Regenerates other antioxidants (e.g. vitamin E) Stimulates leukocyte functions (neutrophil, monocytes movement) Role in antimicrobial and NK cell activities, lymphocyte proliferation, chemotaxis and DTH response</td>
<td>Impaired leukocyte functions, decreased overall NK cell activity and lymphocyte proliferation Rapid decline in plasma and leukocytes during stress and infection Low vitamin C concentrations in elderly predictive of all-cause and cardiovascular disease mortality Supplementation improves antimicrobial and NK cell activity, chemotaxis, lymphocyte proliferation, and DTH response (Th1 response)</td>
</tr>
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</table>

DTH = Delayed-type hypersensitivity; RDA = recommended dietary allowance; ROS = reactive oxygen species.

### Role in Immune Function: Fat-Soluble Vitamins

**Vitamin A**

Vitamin A, acting via all-trans retinoic acid, 9-cis retinoic acid, or other metabolites and nuclear retinoic acid receptors, plays an important role in the regulation of immune function, both in innate immunity and cell-mediated immunity and in humoral antibody response [62, 63]. In vitamin A deficiency the integrity of mucosal epithelium was found to be altered, mainly due to the loss of mucous-producing goblet cells. As a consequence, an increased susceptibility to various pathogens in the eye and in the respiratory and gastrointestinal tracts was observed. Vitamin A-deficient children were shown to have an increased risk of developing respiratory disease [64] and an increased severity of diarrheal disease [65]. A meta-analysis of large clinical trials showed that vitamin A supplementation reduced the severity of diarrheal disease, but had little impact on pneumonia [66, 67]. Where-as in a recent study, vitamin A supplementation, together with zinc, was shown to increase the risk of diarrheal dis-
ease and respiratory tract infections in young children aged 6–15 months [68], earlier trials of supplementation with vitamin A and zinc showed a reduction in both diarrheal and respiratory infections [69, 70]. On the other hand, vitamin A supplementation has been shown to be beneficial in a number of inflammatory conditions. It is believed that supplementation could improve the inflammatory state induced by vitamin A deficiency [71]. Further, vitamin A deficiency has been shown to impair innate immunity by hindering normal regeneration of mucosal epithelial barriers during infection and diminishing resistance to infection by pathogens. Vitamin A supplementation improved the regeneration process of the mucosal barrier in children recovering from diarrhea as compared to children receiving placebo [72].

Vitamin A deficiency has been associated with a diminished phagocytic and oxidative burst activity of macrophages activated during inflammation [73], and a reduced number and activity of NK cells [74]. The increased production of IL-12 (promoting T-cell growth) and proinflammatory TNF-α (activating the microbicidal action of macrophages) in a vitamin A-deficient state may promote an excessive inflammatory response, but supplementation with vitamin A was shown to reverse these effects [75].

Lymphocyte proliferation is caused by activation of retinoic acid receptors and therefore vitamin A is playing an essential role in the development and differentiation of Th1 and Th2 lymphocyte subsets [76]. Vitamin A maintains the normal antibody-mediated Th2 response by suppressing the IL-12, TNF-α, and IFN-γ production of Th1 lymphocytes. As a consequence, in vitamin A deficiency there is an impaired ability to defend against extracellular pathogens [77]. Antibody-mediated immunity was shown to be strongly impaired in vitamin A deficiency, which is consistent with a dominant Th1 cytokine response and the reduction in Th2 anti-inflammatory cytokines (IL-4, IL-10, IL-13), which stimulate the B-cell production of IgG1, IgE, and IgA [29]. Oral vitamin A supplementation increased DTH in infants which may reflect vitamin A-related upregulation of lymphocyte function [78]. In humans, vitamin A supplementation has been shown to improve the antibody titer response to measles vaccine, to increase the serum antibody response to tetanus toxin and diphtheria vaccine [79, 80]. The benefits of vitamin A supplementation in reducing the morbidity and mortality from acute measles in infants and children, diarrheal diseases in preschool children in developing countries, acute respiratory infections, malaria, tuberculosis, and infections in pregnant and lactating women have recently been reviewed [81].

**Vitamin D**

Besides its effect in calcium and bone metabolism, vitamin D and especially its biologically active metabolite 1,25-dihydroxycholecalciferol (1,25(OH)2D3) have been shown to act as powerful immunoregulators [82–84]. The discovery of significant quantities of vitamin D receptors (VDRs) in monocytes, macrophages, and thymus tissue suggests a specific role of vitamin D and its metabolites in the immune system. The highest concentration of VDRs is found in immature immune cells of the thymus and in mature CD8+ T lymphocytes, whether or not they were activated, whereas CD4+ T lymphocytes and macrophages were shown to contain relatively small amounts of VDR, and B cells do not have appreciable quantities of VDR [85]. Macrophages activated by IFN-γ can synthesize 1,25(OH)2D3, provided these cells have enough substrate in the form of 25(OH)2D3. 1,25(OH)2D3 stimulates the maturation of DCs [86]. Activated and VDR expressing T lymphocytes are considered direct targets of 1,25(OH)2D3 and may respond through altered gene expression and function, such as increased apoptosis or decreased IL-2 and chemokine synthesis. 1,25(OH)2D3 decreased the proliferation of purified Th cells, impaired the production of IFN-γ, TNF-α, and IL-2, whereby CD4+ T cells were the preferred target of 1,25(OH)2D3-mediated inhibition during in vitro activation. However, when stimulated with cutaneous antigens in the presence of 1,25(OH)2D3, naïve CD4+ T cells adopted the Th2 cell response with increased IL-4, IL-5 and IL-10 production [83, 87]. Activated B lymphocytes can inactivate 1,25(OH)2D3 through enzymatic hydroxylation to 25(OH)2D3. There is little information available regarding an interaction of NK cells and 1,25(OH)2D3 [83].

The maturation of DCs, a specialized subtype of antigen-presenting cells derived from monocytes, has recently been shown to be inhibited in vitro by treatment with 1,25(OH)2D3 through a VDR-dependent mechanism. In these cells secretion of the immunostimulatory IL-12 was found to be downregulated, secretion of the immunosuppressive IL-10 was increased, and antigen-presenting capacity was inhibited in the presence of 1,25(OH)2D3. As a consequence, the capacity of DCs to induce T-cell activation, proliferation, and cytokine production was substantially impaired. It is believed that, through this indirect action, 1,25(OH)2D3 is modulating the function of CD4+ T cells to a Th2 response [88, 89]. This is supported by observations that vitamin D deficiency was found to be correlated with a higher incidence...
of infections due to an impaired localized innate immune response [90]. This view is supported by studies in vitamin D-deficient or vitamin D-undenourished human subjects [82].

Further, there is evidence from human epidemiological studies that vitamin D status influenced the occurrence of Th1-mediated autoimmunity diseases which is in accordance with 1,25(OH)_2D_3 to inhibit maturation of DCs and downregulate production of the immunostimulatory IL-12, and the observed increase in immunosuppressive IL-10 [91, 92]. Human epidemiological studies indicated supplementation with 1,25(OH)_2D_3 as an independent protective factor influencing the occurrence of Th1-mediated autoimmunity [93, 94].

Vitamin D supplementation has been suggested as a nutritional intervention strategy to prevent progression of congestive heart failure. Whereas in earlier studies with 10 μg vitamin D/day included in a preparation of other micronutrients, either no [95] or only a modest effect was seen, with supplementation of 25 μg vitamin D/day [96] an effect on inflammatory cytokines was observed. A very recent double-blind, randomized, placebo-controlled trial with 50 μg vitamin D/day (2,000 IU/day over 9 months) produced substantial effects on inflammatory cytokine concentrations in men with congestive heart failure (mean age 55 years). Serum concentrations of the proinflammatory cytokine TNF-α decreased with vitamin D treatment, and concentrations of the anti-inflammatory cytokine IL-10 were shown to be increased substantially by 43% compared to placebo. These changes suggest that vitamin D has protective effects on the heart itself and on the atherosclerosis that may precipitate congestive heart failure and that higher doses of vitamin D seem to have enhanced effects on regulatory substances of the immune system [97]. This finding is in line with an earlier study linking vitamin D deficiency with an increase in heart disease [98].

**Vitamin E**

Free radicals and lipid peroxidation are immunosuppressive, and due to its strong lipid-soluble antioxidant activity vitamin E was shown to optimize and enhance the immune response. Supplementation with vitamin E increased lymphocyte proliferation in response to mitogens, increased production of IL-2, enhanced NK cell cytotoxic activity, and increased phagocytic activity by alveolar macrophages, and caused an increased resistance against infectious agents indicating that higher vitamin E intake is promoting a Th1 cytokine-mediated response and suppressing a Th2 response [99].

Immune function in humans declines with age (immuno-senescence). Alterations include impaired T-cell-dependent functions such as T-cell proliferation to mitogens, antibody response after primary immunization with T-cell-dependent antigens, impaired DTH and IL-2 production, whereas IL-4 and IL-6 are elevated. These findings could be interpreted that, as a consequence of the aging process, a shift from a proinflammatory Th1 to a more anti-inflammatory Th2 cytokine response occurs [15, 16, 100]. Since dysregulation of the responses with age is associated with a higher morbidity and mortality from infections and neoplastic diseases, vitamin E has been investigated in human studies with regard to its potential to improve the overall immune response, especially in the elderly [101–110]. Further support for a more specific role of vitamin E was provided by the finding that vitamin E supplementation increased IL-2 production of T cells and enhanced a Th1 response, and increased gene expression of the IL-2 and IL-1 receptor antagonist, shown to be involved in the upregulation of a Th1 response, and decreased the expression of IL-4, a stimulator of Th2 response. Other studies indicate that vitamin E causes a shift toward greater proportions of antigen-experienced memory T cells with fewer naïve T cells [111].

Recent reviews comprehensively covered the role of vitamin E and immunity in humans, especially in the elderly [99]. In a study of 88 healthy elderly (>60 years old) either placebo or 60, 200 or 800 mg/day of vitamin E for 235 days were administered by a double-blind, randomized design. All 3 dosages of vitamin E significantly enhanced DTH skin response. The median percent change in the subjects with 200 mg/day was 65% and significantly higher than that in the placebo group (17%). A significant increase in antibody response to hepatitis B was observed in the subjects with 200 and 800 mg/day, but not in the 60 mg/day group, suggesting that supplementation with at least 200 mg/day may represent the optimum intake of vitamin E in the elderly [101]. In another study it was reported that vitamin E supplementation (400 IU/day) was sufficient to improve DTH of skin tests, mitogen-stimulated lymphocyte proliferation, and IL-2 activity in healthy elderly [102]. These findings of an optimum effect of about 200 mg/day are consistent with results from a study in The Netherlands investigating supplementation with either 50 or 100 mg/day over 6 months in 161 healthy elderly men and women where the results indicated possible beneficial effects of 100 mg supplementation, especially on DTH reactivity (p = 0.06) [103]. In a randomized, double-blind, and placebo-controlled trial
on the effect of vitamin E supplementation (200 IU/day over 1 year) on respiratory tract infections in 617 elderly nursing home residents aged >65 years, there was no significant reduction in the incidence or duration of respiratory tract infections, but participants receiving vitamin E experienced less multiple infections (R = 0.88; p = 0.048), especially upper respiratory infections (R = 0.84; p = 0.05) and common colds (R = 0.80; p = 0.04) [104]. Contrary to other investigations, a randomized double-blind placebo-controlled trial in 652 Dutch men and women (aged >60, mean 73 years) given a placebo, a multivitamin/mineral supplement, 200 mg vitamin E, or both supplements, over a mean duration of 441 days, showed infection severity (duration of infection, presence of fever) to be enhanced in the supplemented group (p = 0.03–0.09), but an evaluation of immune parameters was not performed, and no difference in incidence was seen [105]. This study, however, was criticized as data collection was based on self-reports by the elderly and only 10% of all episodes were checked by laboratory tests and, if tested, only 58% of the reported infections could be confirmed [112].

The immune response of aged women (72 ± 6 years old) suffering from major depression disorders or having coronary heart disease and receiving a combination of antioxidants with 1 g vitamin C and 200 mg vitamin E daily over 16 weeks was measured. A significant increase in the lymphoproliferative capacity and in the phagocytic functions of polymorphonuclear neutrophils as well as a significant decrease in serum concentrations of lipid peroxides were reported [106]. A study in 3 different age groups of Korean women (young women 33 ± 5 years; middle-aged women 47 ± 5 years; elderly women 68 ± 5 years) receiving daily supplementation of 400 IU vitamin E for 6 weeks showed lowered peroxidation of plasma lipids and a significantly increased radical scavenging activity of red blood cells, but a modulatory effect of vitamin E supplementation on humoral immunity could not be shown [107]. Also others could not demonstrate a modulation of the humoral immune response by vitamin E [108].

In elderly nursing home residents in Japan the immune response to influenza vaccine was shown to be significantly and positively associated with the nutritional status of vitamin E [109]. In healthy Chinese adults (<35 years) a significant increase in the proliferative response of T lymphocytes to PHA or lipopolysaccharide, an improvement in the CD4+/CD8+ ratio, and a significant decrease in parameters indicative of oxidative stress (plasma malondialdehyde; urinary DNA adduct 8-hydroxy-2’-deoxyguanosine) were seen after supplementation with 233 mg/day vitamin E for 28 days [110].

On the other hand, several studies in healthy subjects have shown that oral supplementation with antioxidants may attenuate the exercise-induced increase in IL-6 in plasma [61, 113]. A recent study showed that supplementation with vitamins C (500 mg/day) and E (400 IU/day) attenuated the systemic IL-6 response to exercise by about 50% compared to the control when measured in arterial blood during peak appearance. This effect was primarily due to inhibition of the IL-6 protein release from the contracting muscle. The authors suggest that the use of high intakes of antioxidants might be less desirable, since they may attenuate the normal physiological response to concentric exercise [58].

In the rare case of vitamin E deficiency in humans, a patient showed impaired T-cell functions that improved after repletion by intramuscular injection of vitamin E [114]. Patients suffering from tropical sprue over 8–10 years showed a decreased immune response (DTH) besides polyneuropathy. Injection with vitamin E improved these conditions as well [115].

With regard to the mechanism of the immunostimulatory effect of vitamin E, in addition to its role as a protective antioxidant, vitamin E is associated indirectly by reducing the production of the T-cell-suppressive factors such as PGE 2 by macrophages [99]. PGE 2 synthesis, which increases with aging, has been consistently shown at higher concentrations to inhibit Th1 cytokines IL-2 and IFN-γ production and the expression of IL-2 receptor, but upregulate or have no effect on the production of Th2 cytokines IL-4, IL-5, and IL-10. The indirect effect of vitamin E was shown to be due to a reduction in cyclooxygenase activity in macrophages by an impairment of the age-associated increase in the free radical nitric oxide (NO), known to be involved in the regulation of cyclooxygenase, and as a consequence, lowered production of PGE 2 [99].

A summary on the role of the fat-soluble vitamins on the immune system is given in table 2.

**Role in Immune Function: Trace Elements**

**Selenium**

Selenium is essential for an optimum immune response and influences the innate and acquired immune systems. It plays a key role in redox regulation and antioxidant function, and contributes to membrane integrity and protection against DNA damage. The antioxidant effect is mediated through glutathione peroxidases (GSPX) that remove an excess of potentially damaging lipid hy-
Vitamin D Potent immune system modulator when metabolized to 1,25(OH)\(_2\)D\(_3\).
Involved in cell proliferation and differentiation
Most cells of the immune system except B cells express vitamin D receptors
Enhances innate immunity by increasing the differentiation of monocytes to macrophage

Vitamin D Deficiency correlates with higher susceptibility to infections due to an impaired localized innate immunity and defects in antigen-specific cellular immune response (diminished DTH).
1,25(OH)\(_2\)D\(_3\) inhibits maturation of dendritic cells (downregulation of IL-12, upregulation of IL-10, inhibition of antigen-presenting capacity) reducing capacity to induce T-cell proliferation and cytokine production, supporting a Th2 response.
Supplementation of individuals with autoimmune disorders with 1,25(OH)\(_2\)D\(_3\) together with a high calcium diet exerts an inhibitory effect on the progression of the disease (suppression of Th1 response, promoting Th2 response).

Vitamin E Most important fat-soluble antioxidant; protection of membrane lipids from oxidative damage
Reduced production of immune suppressive factors such as PGE\(_2\) in macrophages
Optimizes and enhances immune response (Th1 response)

Vitamin E In rare cases of vitamin E deficiency in humans, impaired T-cell function and DTH test were reported.
Supplementation in healthy adults significantly increased T-cell proliferation, improved the CD4+/CD8+ ratio, and decreased parameters of oxidative stress.
Supplementation of elderly individuals improved overall immune function by altering the age-associated anti-inflammatory Th2 response (impaired IL-2, DTH, T-cell proliferation; increased IL-4 and IL-6) to a proinflammatory Th1 response (increased IL-2, decreased expression of IL-4, shift to greater proportion of antigen-experienced memory T cells).
Dysregulation of immune response in the aged is associated with an increased susceptibility to infections and possibly neoplastic diseases.

DTH = Delayed-type hypersensitivity.
(<1.2 μM) were administered a selenium supplement (50 or 100 μg/day selenium as sodium selenite or placebo over 15 weeks), and given an oral live attenuated poliovirus vaccine after 6 weeks. Selenium supplementation improved the cellular immune response through an increased production of IFN-γ and IL-10, an earlier peak T-cell proliferation, and an increase in CD4+ Th cells, whereas humoral immune responses were not altered. Selenium-supplemented subjects showed a more rapid clearance of the poliovirus and a reduction in specific mutations observed in the placebo group. Overall the data suggest that an additional daily intake of 100 μg of selenium appears to optimize immune function [121].

Supplementation with selenium stimulated immune functions also in those with normal selenium status. In humans, daily supplementation with 200 μg of selenium, given over 8 weeks, induced high affinity IL-2 receptors and enhanced proliferation and differentiation of cytotoxic effector cells, and increased NK cell activity. This effect seems not to be related to the antioxidant capacity of selenium (GSPX), since it is seen at plasma selenium concentrations above those required for saturation of GSPX, but is related to the potential of selenium to upregulate the expression of receptors for the growth-regulating cytokine IL-2R on the surface of activated lymphocytes and NK cells [121–123]. In an earlier study, 11 adult men were fed foods naturally high or low in selenium for 120 days. Intake of selenium was stabilized at 47 μg/day for 21 days, and then changed to either 13 or 297 μg/day for 99 days. Serum immunoglobulins, complement components, and primary antibody responses to influenza virus were unaltered, but antibody titers against diphtheria vaccines were 2.5-fold increased in the high selenium group. Cytotoxic T lymphocytes and activated T cells were significantly increased. The immune-enhancing properties of selenium were suggested to be the result of improved activation and proliferation of B lymphocytes and of enhanced T-cell function [124]. The relation between blood micronutrient concentrations and NK cell variables was evaluated in 62 healthy, free-living Italian subjects (aged 90–106 years). A strong association between the relative number of peripheral lymphocytes expressing markers of NK cell activity was found with the selenium concentration (r = 0.409; p = 0.018), but not between the NK cell cytolytic activity and the blood concentration of selenium [34].

The age-related decline in immune response [15–17] was shown to be improved in 22 institutionalized elderly patients, supplemented with 100 μg/day, as selenium-enriched yeast, or placebo for 6 months. Lymphocyte proliferation in response to mitogens was enhanced by 138% in the selenium group and restored to the level of healthy young individuals [125]. In a randomized, double-blind, placebo-controlled study in 725 elderly patients (>60 years), low-dose supplementation of a combination of selenium and zinc (100 μg and 20 mg/day, respectively, for 2 years) provided significant improvement by increasing the humoral response after vaccination. The authors suggest that their observation could have considerable public health implications by reducing morbidity from respiratory tract infections [126]. Selenium supplementation (960 μg/day for 3 months) was shown to decrease NF-κB activity in peripheral blood mononuclear cells from type-2 diabetic patients. Since oxidative stress and the subsequent activation of NF-κB have been linked to the development of vascular complications and NF-κB has been identified in atherosclerotic plaques, the observed 80% reduction in the highly increased NF-κB activity in diabetic patients by selenium supplementation may be an efficient pharmacologic tool to prevent or delay the onset of morbid consequences of type-2 diabetes [127].

Zinc

The immune-related functions of zinc have recently been reviewed [42, 128–130]. Zinc has been shown to have antioxidant activity in vitro and in vivo (cofactor of SOD; binding and stabilization of protein thiols) and is involved in the cytosolic defense against oxidative stress caused by ROS produced and released by activated macrophages [119, 131]. However, high levels of zinc might also act as a pro-oxidant by causing a reduction in SOD activity [132]. Zinc deficiency in human lung fibroblasts was shown to induce oxidative stress and increase DNA damage and p53 protein expression, resulting in impaired antioxidant defense, compromised DNA repair mechanisms, and thus rendering the cell more susceptible to oxidative DNA damage [133]. Lowered zinc status, such as in subclinical zinc deficiency and zinc deficiency, impairs macrophage functions (phagocytosis, intracellular killing activity), neutrophil functions (chemotaxis, generation of the oxidative burst), NK cell activity, and complement activity [129].

In humans, zinc deficiency has been reported in patients with acrodermatitis enteropathica, a genetic disorder of zinc malabsorption, and in patients receiving total parenteral nutrition without zinc. These patients showed thymic atrophy, impaired lymphocyte proliferation response to mitogens, a deficient thymic hormone activity (thymulin), decreased ratios of CD4+/CD8+ cells, decreased NK cell activity and monocyte cytotoxic-
ity. These alterations could be corrected by sufficient zinc supplementation [134]. Studies in an experimental human model and in patients with sickle cell disease demonstrated that zinc deficiency caused an imbalance between the cell-mediated Th1 and humoral Th2 immune functions. Whereas production of IL-4, IL-6, and IL-10 (Th2 response) were not affected during zinc deficiency, the production of IFN-γ, IL-2, and TNF-α (Th1 response) was decreased. Zinc deficiency also decreased serum thymulin activity, recruitment of T-naïve cells, NK cell activity, and the percentage precursors of cytolytic T cells. Zinc is an essential cofactor for the thymic hormone thymulin, which induces several T-cell markers, and promotes T-cell function, including allogenic cytotoxicity, suppressor functions, and IL-2 production. It also modulates cytokine release by peripheral blood mononuclear cells and induces the proliferation of CD8+ T cells which function as cytotoxic cells able to recognize and kill pathogens [128]. On repletion with zinc, these parameters returned to the normal range. Studies in a human malignant T lymphoblastoid cell line (HuT-78), which is highly responsive to zinc status, showed that the effect of zinc was on the gene expression of IL-2 and IL-2Rα, and through the reduced nuclear binding of NF-κB to DNA. Prolonged zinc supplementation (50–75 mg elemental zinc/day as zinc acetate for up to 3 years) increased IL-2 production, significantly decreased the incidence of microbial respiratory and urinary tract infections, and the number of hospitalizations [134].

The alterations in the immune response with an inadequate zinc status or zinc-deficient status are considered to be an important contributor to the increased susceptibility to infections, especially during childhood. Indeed, zinc supplementation of young children with inadequate zinc status reduced the duration of diarrhea and pneumonia [134–137]. A recent randomized controlled trial in Bangladeshi children younger than 2 years showed significantly fewer incidents of pneumonia in the zinc-supplemented group (70 mg weekly over 12 months) than in the placebo group (relative risk 0.83; 95% CI 0.73–0.95), a small but significant effect on incidence of diarrhea (relative risk 0.94; 95% CI 0.88–0.99), and a reduced mortality secondary to pneumonia [138]. These findings confirmed earlier studies with comparable outcome [135, 136], but a recent study in India showed no overall effect on the duration of hospitalization or clinical signs associated with severe pneumonia infection in children aged 2–23 months following treatment with 10 mg zinc sulfate twice daily (together with standard therapy) during hospitalization [139]. Therefore, further research is required. Further, results of zinc supplementation trials in children with acute lower respiratory infections were not consistent [140, 141]. In a recent short-term randomized, controlled, 2-by-2 factorial trial in 187 Indigenous Australian children (aged <11 years), supplementation with zinc (20 mg daily in infants under 12 months, others 40 mg for 5 days) and/or vitamin A did not show any benefit regarding time to clinical recovery from fever and tachypnea, duration of hospitalization, or readmission within 120 days. Children given zinc had an increased risk of readmission (relative risk 2.4; 95% CI 1.00–6.1) [142].

On the basis of the antiviral interaction of zinc with rhinovirus [143] several randomized and placebo-controlled clinical trials, mainly with lozenges of zinc gluconate, were conducted. The meta-analysis by the Cochrane Collaboration concluded that there was no convincing and consistent evidence supporting zinc to be effective in the treatment of the common cold [144]. More recent trials with zinc gluconate and zinc acetate did not resolve this issue [145, 146]. The positive trial with zinc acetate [147], demonstrating a significantly shorter overall duration of cold symptoms (4.5 vs. 8.1 days; p < 0.005), cough (3.1 vs. 6.3 days), nasal discharge (4.1 vs. 5.8 days) and a significantly decreased total severity score for all symptoms (p < 0.002), suggested that zinc acetate instead of zinc gluconate lozenges should be used in these trials, since zinc acetate would be completely available as Zn2+ ions at physiological pH [148]. Thus, further carefully conducted clinical trials are necessary to prove or disprove the efficacy of zinc acetate lozenges against the common cold.

The age-related decline in immunity in the elderly might be partly related to a marginal zinc status or zinc-deficiency response [15–17], since inadequate zinc intake is prevalent in this population group [149, 150]. Altered immune functions in the elderly mainly concern T-cell-mediated immune functions such as diminished T-cell count, dysfunction of Th cell subpopulations, defects in T-cell proliferative response to mitogens, impaired antibody response, impaired DTH activity, and lowered IL-2 and IFN-γ production after stimulation. Further increased production of anti-inflammatory cytokines IL-4 and IL-6 indicates a shift from a proinflammatory Th1 cell response to an anti-inflammatory Th2 response with aging [151]. Several studies have shown that the impaired immune status in the elderly can be reversed by adequate supplementation with zinc [152]. In a cross-over design, zinc-deficient women and men (50–80 years of age) received placebo for 3 months and thereafter 30 mg/day of zinc for 6 months. Supplementation improved IL-1 pro-

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**Vitamins and Trace Elements and Immune Response**

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Supplementation may enhance NK cell immune function and zinc concentration indicating that providing zinc supplementation alone improved the cell-mediated immune response significantly in an older population (increased number of CD4+ T cells and cytotoxic T lymphocytes) [155]. An Italian study in healthy free-living subjects aged >90 years found a strong association between the number of lymphocytes expressing markers of NK cell activity and zinc concentration indicating that providing zinc supplementation may enhance NK cell immune function in elderly subjects [34]. Clinical benefit was shown in a study in 725 institutionalized elderly patients who received an oral daily supplement of trace elements (20 mg zinc and 100 μg selenium) or a vitamin supplement for 2 years. Whereas no effect could be seen on DTH skin response, antibody titers after influenza vaccine were higher in the group on zinc and selenium, and the number of patients without respiratory tract infections during the study was higher in the group receiving trace elements indicating that supplementation improved the humoral response after vaccination. Therefore, zinc supplementation may restore thymulin activity, which induces an enhanced anti-influenza response [126]. A study in 384 subjects (mean age 82 years) showed no effect of prolonged supplementation with zinc (400 mg/day) or zinc/arginine (4 g/day), for 60 days and starting 15 days before influenza vaccination, on antibody titer against influenza and on viral antigens in comparison with subjects receiving vaccine alone, indicating no improvement of zinc on the response of elderly subjects to influenza vaccination [156]. Overall, evidence indicates that an adequate intake of zinc is essential for optimal immune function and protection from infections, especially in the elderly. However, the dosage of intake should be carefully chosen. In vitro studies showed that normal physiological serum concentrations of zinc (2–15 μM) had no effect on immunologic functions of peripheral blood mononuclear cells from healthy subjects, but pharmacological concentrations of 100 μM induced apoptosis and increased expression of caspase-3 and pro-apoptotic genes, whereas at even higher concentrations of about 300 μM zinc decreased the expression of anti-apoptotic factors [157]. The studies indicate that moderate zinc supplementation is most likely of benefit on immunity in an inadequate zinc status (children, elderly), and that routine supplementation with high doses might have adverse effects.

**Copper**

Copper has been shown to play a role in the development and maintenance of the immune system, and a large number of experimental studies have demonstrated that copper status alters several aspects of neutrophil, monocyte, and T-cell function in the immune system [158–160]. The copper-containing enzyme SOD, working together with catalase and GSPX in the cytosolic antioxidant defense against ROS, is essential in the dismutation of superoxide anion to oxygen and H2O2, and diminishes damage to lipids, proteins, and DNA [119, 161, 162].

Human data showing the effects of copper on the immune response are limited, also in the elderly population, mainly due to the inability to precisely assess marginal and moderate copper deficiency status due to the lack of sensitive and specific biomarkers and the fact that copper homeostasis is maintained over a wide range of intakes, mainly through changes in endogenous excretion, especially at lower intakes [163, 164]. The effect of changing the amount of copper intake on the immune response of healthy adults (21–32 years of age) was studied during a 90-day metabolically controlled study with copper intake of 0.66 mg/day for the first 24 days, 0.38 mg/day for the next 42 days, and 2.49 mg/day for the last 24 days [165]. Intake of 0.38 mg/day significantly decreased the in vitro responsiveness of T lymphocytes to mitogenic activation and increased the percentage of circulating B cells, but had no effect on serum IL-2 receptor concentration, the percentage of peripheral monocytes, neutrophils, total T cells, Th cells (CD4+ and CD8+), and NK cell or neutrophil phagocytic activity. The dosage of 2.49 mg copper/day did not restore these parameters to pre-study levels, even though serum copper and ceruloplasmin concentrations were restored to normal. During the lower copper intake there was no increased susceptibility to infections, as observed in copper-deficient animals or in children with Menke’s syndrome, a congenital disease resulting in copper deficiency [166], but participants in the study were not copper-deficient. These findings of impaired T-cell function (proliferation, IL-2 receptor) with low copper intakes are consistent with data reported in animal studies. In the human Jurkat T-cell line, made copper-deficient by incubation with a chelator, IL-2 synthesis after mitogens stimulation was decreased by 75% and mRNA for IL-2 by 50%, indicating that copper plays a specific role in the transcription of the IL-2 gene [167].

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In a recent study [168], the long-term effect of a high intake of copper was investigated on immune function parameters. Nine men received for 18 days an average of 1.6 mg copper/day with the diet, thereafter supplemented their usual diet to a total intake of 7 mg/day for 129 days, and finally had an intake of 7.8 mg/day for another 18 days. High intakes of copper significantly reduced the percentage of circulating neutrophils, serum IL-2 receptor, and the antibody titer against the Beijing strain of influenza. The average inflammatory response as measured by IL-6 almost doubled during supplementation, but did not reach significance. Immunization caused a 47-fold increase in antibody titer against the Beijing influenza strain in the control subjects, whereas in the supplemented subjects this increase was only 14-fold. The results from this study and previous observations [165] indicate that both copper deficiency and high copper intake over longer periods modulate the immune response. These observations are in agreement with data showing dietary copper deficiency to impair innate and acquired immunity and that copper deprivation exerts a shift to an anti-inflammatory Th2 cytokine-mediated immune response [169]. The physiological significance is to be evaluated, but the results on the immune response may suggest an adverse effect of high copper intake [168]. However, a double-blind supplementation study in middle-aged healthy volunteers on the effect of copper (3–6 mg/day over 6 weeks; mean dietary intake 1–1.7 mg/day) on red blood cell oxidizability showed no pro-oxidant effects in red blood cells. Copper supplementation protected red blood cells against in vitro-induced peroxidation, with no changes in SOD activity [170].

Iron

The immune-related functions of iron have been subject to several recent reviews [171–174]. Iron is essential for electron transfer reactions, gene regulation, binding and transport of oxygen, and regulation of cell differentiation and cell growth. Iron is a critical component of peroxide and nitrous oxide-generating enzymes. It is involved in the regulation of cytokine production and mechanism of action, and in the activation of protein kinase C, which is essential for phosphorylation of factors regulating cell proliferation. In addition, iron is necessary for myeloperoxidase activity which is involved in the killing process of bacteria by neutrophils through the formation of highly toxic hydroxyl radicals. Therefore, any alteration in cellular iron homeostasis to either deficiency or overload has unfavorable functional consequences on the immune system. Since pathogens such as infectious microorganisms and viruses require iron and other micronutrients for replication and survival as well, it seems essential to restrict access of the infecting microorganism to iron, but to maintain a suitable concentration of iron that the host can mount an optimum immune response and avoid the possibility of excess amounts of iron which may induce free radical-mediated damage [174].

Macrophages acquire iron from transferrin via endocytosis of transferrin receptor (TFR)-bound iron regulated by iron-regulatory protein, but also through erythocyte phagocytosis which seems not to be regulated by cellular iron status. In iron deficiency, neutrophils, being dependent on surface expression of TFRs, have a reduced activity of myeloperoxidase, and a reduction in migration to inflammation sites. As a consequence intracellular killing of bacteria is significantly impaired in iron deficiency. Also NK cells are sensitive to iron imbalance and show a lower killing activity in iron deficiency, since they require a sufficient amount of iron for differentiation and proliferation [175, 176].

The proliferative phase of T cells is dependent on iron. Activation of T cells stimulates production of TFR on the cell surface by an IL-2-dependent pathway which then facilitates iron uptake. Cellular iron availability modulates the differentiation and proliferation of Th cell subsets, with Th1 cells to be more sensitive than Th2 cells to restriction in iron status, resulting in inhibition of DNA synthesis in Th1 cells with no effect on Th2 cells [177]. Iron deficiency impaired immune functions regulated by IFN-γ such as NK cell activity, DTH, and T-cell proliferation, and functions which are downregulated by IL-10 (respiratory burst). Also the ratio of T lymphocytes (CD4+ to CD8+ cells) in blood was reduced in iron deficiency whereas the number of cells was unchanged [171]. Resting B cells can continuously take up iron, as they express low levels of TFR on the cell surface, and are therefore less sensitive to changes in iron homeostasis. Parameters of humoral immunity were unchanged in iron-deficient subjects indicating that B-cell-mediated immunity is not significantly affected by iron deficiency, with antibody production in response to immunization with antigens to remain unaltered [178].

In a recent study in 142 hospitalized Malawian children the observed cytokine pattern associated with iron deficiency indicated a more favorable condition in iron deficiency than in iron-replete children to combat infection (higher percentage of CD8+ cells producing IL-6; increased formation of IFN-γ, higher percentage of lymphocytes producing TNF-α) [179], whereas in a study in
72 homebound older women iron deficiency was associated with impairments in cell-mediated and innate immunity. In the iron-deficient women T-cell proliferation upon stimulation with concanavalin A and PHA was only 40–50% of that in iron-adequate women. Whereas phagocytosis did not differ, the respiratory burst was significantly reduced by 28%, suggesting that iron-deficient women may be more vulnerable to infections than women with an iron-sufficient status [180]. Overall, iron deficiency promotes a shift from a proinflammatory Th1 cytokine-mediated immune response to an anti-inflammatory Th2 response, a decline in an IFN-γ-stimulated innate immune response with an increased susceptibility to intracellular infections.

International guidelines recommend supplementation with iron and folic acid in children <2 years in regions with a high prevalence of anemia [181], but this recommendation has turned out to be controversial, especially in regions with malaria, since some study findings in children suggest that iron deficiency is protective against malaria [174, 182], whereas other findings indicate that iron supplementation causes an increased rate of malaria, pneumonia, and diarrhea [174, 183]. A meta-analysis of 28 randomized controlled trials examining iron intake showed no increase in the pooled estimate of the incidence rate ratio for all infectious illnesses (respiratory tract infections, diarrhea, malaria, and other infections) in iron-supplemented children, but a higher risk of diarrhea (p = 0.04) and a non-significant increase in malaria in the iron-supplemented group [183]. A trial in preschool children (n = 24,067) in Tanzania showed a 12% higher rate of serious adverse events due to malaria associated with iron (12.5 mg) and folic acid (50 μg) supplementation. In addition, in a sub-study, children (n = 2,413) with iron-deficiency anemia and treated for malaria and other infections had a significantly lower risk of adverse events (hospitalization or death; p = 0.006) associated with concomitant iron/folic acid supplementation, whereas iron-replete children showed a non-significant trend toward an increased risk of adverse events when supplemented with iron [184]. On the contrary, a comparable trial in a region without malaria in southern Nepal, equally well designed and controlled, showed that supplementation with iron and folic acid (with and without zinc) had no deleterious effect on their risk of death [185]. A recent review found in 5 of the 7 identified trials no significantly increased risk in the iron-supplemented groups associated with malaria infection and iron supplementation, and 2 trials indicated a greater risk of adverse events due to malaria [186]. These findings indicate a potential risk of routine supplementation with iron and folic acid in preschool children in areas with high rates of malaria and other infections, whereas in areas with iron deficiency and absent malaria routine iron/folic acid supplementation is without harm and is likely to have long-term benefits [187].

Iron overload, determined by increased serum iron concentrations and transferrin saturation, is a less frequent condition than iron deficiency, but high contents of tissue iron resulting from transfusional iron overload (sickle cell anemia, thalassemia, renal diseases) or from hereditary hemochromatosis have been associated with effects on the immune system. Iron overload decreased proliferation capacity, numbers and activity of helper T cell (CD4+) with increases in the CD8/CD4 ratio, impaired generation of cytotoxic T cells, altered immunoglobulin secretion, and increased levels of the cytokines IL-4, IL-6, and IL-10. Iron interferes with cytokine activities by directly inhibiting IFN-γ activity and IFN-γ-mediated pathways in macrophages, such as formation of the proinflammatory cytokine TNF-α, and the impaired production of NO due to inhibition of the transcription of inducible NO synthase by iron. Consequently, defects in macrophage phagocytosis were seen consistently in subjects with iron overload [176]. It was therefore suggested that long-term iron supplementation and a status of iron overload may result in increased susceptibility to infections by modulation of cellular immunity (weaker Th1 cytokine-mediated response, increased Th2 response), an impaired killing of intracellular pathogens by macrophages, and a growth-promoting effect of iron to the pathogen. In addition, iron is directly involved in cytotoxic immune defense by production of highly toxic hydroxyl radicals in neutrophils and macrophages [171].

A summary of the role of these trace elements on the immune system is given in table 3.

Conclusions

Inadequate intake and status of vitamins and trace elements may lead to suppressed immunity which predisposes to infections and aggravates malnutrition. Evidence has accumulated over the recent decade that in humans these specific nutrients selectively influence the immune response, induce dysregulation of a coordinated host response to infections in case of deficiency and oversupply, and that deficiency may impact virulence of otherwise harmless pathogens. Thus, vitamins and trace elements are required at appropriate intakes for the immune system
Table 3. Role of selected trace elements on the immune system and effects of deficiency and supplementation

<table>
<thead>
<tr>
<th>Trace Element</th>
<th>Role in the immune system</th>
<th>Effects of deficiency and supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium</td>
<td>Key role in redox regulation and antioxidant function mediated through enzymes (GPX) and other selenoproteins (e.g. thioredoxin reductase, involved in the regulation of DNA biosynthesis, activity of transcription factors, and gene expression) Essential for optimal immune response, influencing both the innate and acquired immunity. Supplementation normalizes age-related decline in immune response.</td>
<td>Deficiency and selenium undernutrition cause viruses to undergo mutations to more virulent forms in the host. In adults with marginal status a low supplementation enhanced the cellular immune response (increased IFN-γ production, Th1 response), and had a more rapid clearance of an orally given poliovirus, and lower numbers of mutations indicating that subjects had a functional selenium deficit with suboptimal immune response and a deficit in viral handling. In healthy subjects supplementation augmented T-lymphocyte-mediated immune response, enhanced proliferation, increased the response to antigen stimulation, increased cytotoxic and NK cell activity, and increased IFN-γ (Th1 response). Supplementation of elderly restores the age-related defect in cell proliferation (NK cell and cytotoxic activity) preventing an increased susceptibility to inflammatory and malignant disease. For optimum immune response intakes greater than the current RDA may be necessary.</td>
</tr>
<tr>
<td>Zinc</td>
<td>Is essential for highly proliferating cells, especially in the immune system. Influences both innate and acquired immune functions. Is involved in the cytosolic defense against oxidative stress (SOD activity) and is an essential cofactor for thymulin which modulates cytokine release and induces proliferation. Adequate intake supports a Th1 response. Helps to maintain skin and mucosal membrane integrity. Unbound zinc ions exert a direct antiviral effect on rhinovirus replication.</td>
<td>Deficiency causes increased oxidative stress with higher susceptibility to oxidative DNA damage. Deficiency induces imbalance by suppression of Th1 response (decreased IFN-γ and IL-2, impaired NK cell activity, reduction in macrophage functions (phagocytosis, intracellular killing, generation of oxidative burst, chemotaxis), reduction in cytolytic T-cell activity, decreased DTH) with unaffected Th2 response leading to increased susceptibility to infections, especially during childhood. Deficient status induces acceleration of pre-T- and B-cell apoptosis causing depletion of peripheral lymphocytes (lymphopenia) and thymic atrophy. Supplementation increases cellular mediators of innate immunity (e.g. phagocytosis of macrophages and neutrophils, NK cell activity, generation of oxidative burst, DTH activity), antibody response, and increased numbers of cytotoxic CD8+ T cells (Th1 response). Supplementation of elderly individuals improves impaired immune function by reversing the age-associated decrease in NK cell activity (impaired killing of virus-infected cells and tumor cells) supporting a Th1 response providing protection against infections. Supplementation in high dosages (&gt;100 mg/day) and leading to concentrations of &gt;100 μM were shown to suppress IFN-γ production and T-cell functions, and decrease expression of anti-apoptotic factors, stimulating apoptosis.</td>
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<td>Copper</td>
<td>Cu/Zn-SOD is a key enzyme in the defense against ROS, and in maintaining intracellular antioxidant balance, suggesting an important role in inflammatory response. Adequate intake supports a Th1 response and both deficiency and excessive intake modulate the immune response.</td>
<td>Data on an effect of deficiency in humans are limited due to its efficient homeostatic regulation and lack of appropriate parameters to determine status. Available data in subjects with marginally adequate intake showed a decrease in T-cell proliferation and an increase in circulating B cells, but no effect on serum IL-2 receptor concentration, on neutrophil phagocytic activity, NK cell activity, and no increase in the incidence of infections during low intake. Long-term high intake (7 mg/day) in healthy men significantly reduced the percentage of circulating neutrophils, serum IL-2 receptor, and antibody titer against the Beijing strain of influenza, and enhanced the average inflammatory response (IL-6); no pro-oxidant effect was observed, and this intake protected red blood cells against in vitro-induced peroxidation.</td>
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<td>Iron</td>
<td>Essential for cell differentiation and growth, component of enzymes critical for functioning of immune cells (e.g. ribonucleotide reductase involved in DNA synthesis; myeloperoxidase involved in killing bacteria by neutrophils) Involved in the regulation of cytokine production and action.</td>
<td>Deficiency impairs secretion of cytokines (IFN-γ, TNF-α, IL-2) and reduces NK cell activity, T-cell proliferation, DTH response, impairs bactericidal macrophage activity, causes a reduction in the ratio of CD4+/CD8+ cells, and a small decrease in IL-10, indicating that deficiency affects both innate and cell-mediated immunity (suppression of a Th1 response, limited decline in Th2 response). Th1 cell subsets are more sensitive to deficiency than Th2 cell subsets due to the lower expression of surface transferrin receptors and a smaller iron pool. Deficiency presents more favorable conditions to combat infections and providing iron may be harmful when given during infections or malignant disease. There is little evidence that oral iron supplementation to deficient subjects inhibits immune response or increases susceptibility to most infections, possibly with the exception of HIV, malaria-related diseases, and pneumonia. Deficiency does not affect B-cell-mediated immunity and antibody production is maintained. Iron overload has been shown to suppress immune function (Th1 response) by diminishing the activity of regulatory cytokines (IFN-γ, IL-2, IL-12), to cause a shift in the ratio of CD4+/CD8+ with expansion of CD8+ cells, a decrease in NK cell activity leading to an increased susceptibility to infectious diseases, and to promote microbial growth. Iron overload does not affect B lymphocyte activity. Withdrawal of iron by chelators in iron overload patients strengthens a Th1 response.</td>
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GPX = Glutathione reductase; RDA = recommended dietary allowance; DTH = delayed-type hypersensitivity; ROS = reactive oxygen species; SOD = superoxide dismutase.
Micronutrients impact the immune response by exerting a regulatory mechanism on the differentiation of precursor T cells to a T-cell population with either a Th1 proinflammatory cytokine profile or a Th2 anti-inflammatory cytokine profile. Th1 cells produce IFN-γ to stimulate immunity to intracellular pathogens and IL-2 to promote Th1 cell growth. Th2 cells produce IL-4 that stimulates immunity to extracellular pathogens and promote Th2 cell growth. Human data demonstrate that an adequate intake or status of vitamins B₆, folate, B₁₂, C, E, selenium, zinc, copper, and iron supports a Th1 response with sufficient production of proinflammatory cytokines contributing to the maintenance of an effective immune response and to counteract infections. The antioxidant nutrients vitamins C and E, and the trace elements selenium and zinc contribute to the scavenging of ROS and help to avoid damaging effects to surrounding tissues at the site of inflammation. Selenium, copper, and zinc are involved in the antioxidant defense as cofactors of enzymes such as GSPX and SOD. Vitamins C and E prevent overproduction of PGE₂ by macrophages avoiding suppressive effects on cellular and humoral immunity, and participate in the regulation of intracellular signaling through NF-κB. Selenium deficiency allows viruses to undergo mutations to more virulent forms in the host.

An inadequate status or deficiency of these micronutrients induces an imbalance by either suppression or impairment of the Th1 cytokine response with a shift to an anti-inflammatory Th2 cytokine response, supporting an enhanced susceptibility to infections. Zinc deficiency induces an imbalance by suppression of a Th1 immune response, but without affecting humoral immunity. Data on an effect of copper deficiency or marginal status in humans are limited due to a lack of appropriate parameters to determine status and the rather efficient homeostatic regulation of copper status. Iron deficiency affects both innate and cell-mediated immunity (suppression of Th1 response and decline in Th2 response), and deficiency may therefore be more favorable in counteracting infections, and, thus, supplementation with iron may be harmful when administered during infections and malignant diseases.

In general, supplementation or repletion of an inadequate or deficient status reversed the immune response to a dominant Th1 cytokine-mediated response. Supplementation with vitamin C improved anti-microbial and NK cell activity, lymphocyte proliferation and DTH response. Vitamin E supplementation decreased parameters of oxidative stress, proliferation of lymphocytes, and improved the CD4+/CD8+ ratio. Zinc, selenium, or iron supplementation increased cellular mediators of innate immunity mediated by Th1 cytokines with limited effects on B cells. On the other hand, there may also be harmful effects of supplementation. In patients with iron overload Th1 cytokine-mediated immune response was suppressed with an increased susceptibility to infections. High intakes of folic acid (>400 μg/day) together with a folate-rich diet (>233 μg/day) were related to an impairment in NK cytotoxicity, and supplementation with high doses of zinc (>100 mg/day) was shown to suppress IFN-γ production and to stimulate anti-apoptotic factors. Long-term intake of high copper-containing supplements (7 mg/day) reduced antibody titers against an influenza strain and enhanced the average inflammatory response, and iron supplementation may promote microbial growth.

Consistent evidence shows that supplementation of elderly subjects with vitamin B₆, folate, vitamin E, selenium, or zinc improved the overall immune function and reversed the age-associated alteration of an anti-inflammatory Th2 response back to a proinflammatory Th1 cytokine-mediated immune response providing higher protection against intracellular infections, whereby several micronutrients showed substantial immune response only with intakes higher than the current recommended dietary allowances (vitamins B₆, E, and selenium).

Vitamin A plays an important role in both cell-mediated and humoral antibody response and acts as an anti-inflammatory substance (Th2 response). Deficiency impairs both innate immunity (mucosal epithelial regeneration) with increased inflammation mediated by cytokines from macrophages, as well as the adaptive immune response to infection (diminished antibody-mediated response directed by Th2 cytokines) with an impaired ability to counteract extracellular pathogens. Likewise, vitamin D (1,25(OH)₂D₃) supports a Th2 response, and deficiency is correlated with a higher susceptibility to infections due to impaired localized innate immunity and defects in antigen-specific cellular immune response.
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