Micronutrients at the Interface Between Inflammation and Infection – Ascorbic Acid and Calciferol. Part 1: General Overview with a Focus on Ascorbic Acid

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Abstract: As elements of the antioxidant system, cofactors of enzymes, components of transcription factors, and epigenetic modulators, micronutrients, such as vitamins and trace elements, influence various metabolic processes that are directly associated with immune functions. Specifically, the vitamins C and D have been shown to have significance immune function. Therefore, the objective of this review is to elucidate interactions between micronutrients and the immune system. In the initial section of this review, we present a general overview of interactions between the immune system and micronutrients, with a focus on the immunobiologically relevant functions of vitamin C. Immune competent cells accumulate vitamin C against a concentration gradient, with a close relationship between vitamin C supply and immune cell activity, especially phagocytosis activity and T-cell function. Accordingly, one of the consequences of vitamin C deficiency is impaired resistance to various pathogens, while an enhanced supply increases antibody activity and infection resistance.

Keywords: Micronutrients, vitamin C, immune system, infections, phagocytosis.

1. INTRODUCTION

The immune system is a diffusely structured network of organs distributed throughout the body that consists of cellular and humoral factors. These immune factors mediate the nonspecific (innate) and the antigen-specific (acquired) immune responses of the organism (Fig. **1**).

Just as with other organs, the immune system requires appropriate energy and nutrient supplies. Energy and nutrient supplies are especially important for the immune system because its individual components are characterized by high turnover rates, leading to a higher substrate requirement compared to most other body systems. Only if all essential nutrients are supplied in appropriate amounts can the biochemical and cellular mechanisms that provide the basis of the immune system work optimally [2]. Therefore, it is not surprising that the immunocompetence of an organism is significantly influenced by its intake of micronutrients (vitamins, minerals, and certain fatty acids) [3-9]. In contrast, the activity of the immune system can affect nutrient status, as can be observed in immune diseases, including acquired immunodeficiency syndrome (AIDS) [10-12] and rheumatoid arthritis [13-15]. Accordingly, dietary factors have become increasingly important in the potential treatment of immunological inflammatory diseases [16-20].

The objective of this overview is to elucidate interactions between the immune system and micronutrients, with a special focus on the immunobiologically relevant vitamins ascorbic acid (vitamin C) and – in part two of the review – calciferol (vitamin D).

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2. MICRONUTRIENTS AND IMMUNE FUNCTION

As outlined above, active immune cells are characterized by a high proliferation rate that results in a high nutrient requirement. Accordingly, an appropriate supply of all micronutrients is mandatory for the function of all of the biochemical processes forming the basis of the immune system [21, 22]. In the immune system, vitamins and trace elements primarily serve as the following [23-25]:

- Cofactors of enzymes involved in DNA replication, transcription, translation, and in cellular signal transduction pathways involved in the differentiation and proliferation of immune cells (e.g., B vitamins, zinc),
- Elements of the antioxidant system, which are needed for the integrity of immune cells because they are subject to a high burden of reactive oxygen species (ROS) (e.g., vitamins E, C, selenium),
- Parts of transcription factors (e.g., vitamins A, D) that act as co-determinants in gene expression levels of immunologically relevant proteins, and
- Epigenetic modulators that are involved in DNA methylation (e.g., folic acid) and posttranslational modification of histones (biotinylation *via* biotin and poly(ADP-ribosylation) *via* niacin).

Table **1** provides an overview of the immunological significance of selected vitamins and trace elements [1, 2, 23, 26-29].

3. ASCORBIC ACID

In an immunobiological context, vitamin C has been the most well-studied micronutrient. Since Linus Pauling (1901-1994) published his *locus classicus* "vitamin C and the

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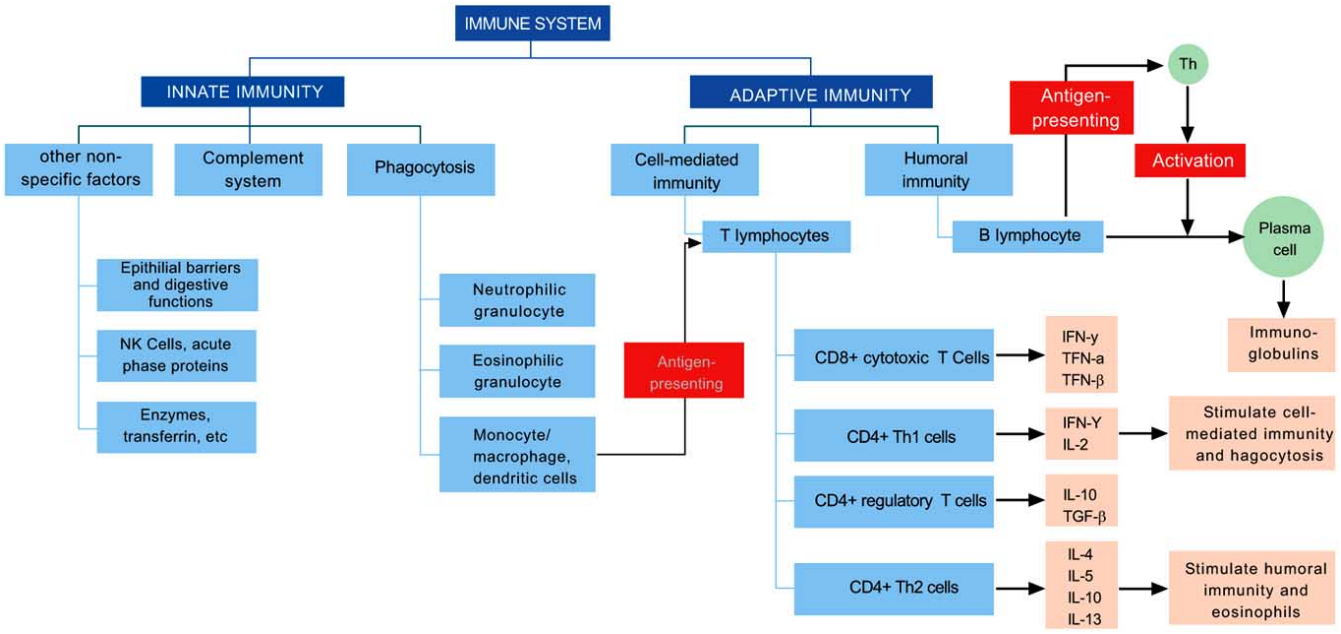

Fig. (1). Overview of the functional compartmentation of the immune system [1]. Th: T-helper cells; IFN: Interferon; IL: Interleukin; CD: Cluster of differentiation.

Common Cold" in 1970 [30], the significance of this vitamin in immune function has been constantly discussed. Research has particularly focused on the question of whether vitamin C is relevant to the prevention and treatment of the common cold; a question that has yielded controversial [31-42].

3.1. Vitamin C – Structure and Biochemistry

Vitamin C is the generic term for L-threo-Hex-2-enono-1,4-lactone and its derivatives with L-ascorbic acid activity. Chemically, vitamin C is a gluconic acid lactone derived from glucuronic acid. Among four possible stereoisomers, the L-enantiomer is the physiologically active form.

The characteristic property of ascorbic acid is its ability to reduce various substances while being reversibly oxidized to dehydroascorbic acid. Dehydroascorbic acid can be enzymatically reduced back to ascorbic acid, and therefore has full vitamin activity.

Due to its strong reducing potential, ascorbic acid is involved in numerous metabolic processes. Of particular importance is its role in hydroxylation reactions catalyzed by mono- and dioxygenases (prolyl 4-, prolyl 3- and lysyl hydroxylase; trimethyl lysine and γ-butyrobetaine hydroxylase, dopamine β-hydroxylase, 4-hydroxyphenylpyruvate dioxygenase and HIF prolyl 4-hydroxylase). The mono- and dioxygenases catalyzed hydroxylation reactions include, among others [43-48],

- the cotranslational hydroxylation of residual proline and lysine in the procollagen;
- the hydroxylation of tryptophan in serotonin biosynthesis;
- the synthesis of catecholamines;
- the α-amidation in the biosynthesis of numerous peptide hormones such as gastrin, cholecystokinin, calcitonin, vasopressin and oxytocin;
- the hydroxylation of β-hydroxy-ε-N-trimethyllysine and γ-butyrobetaine in endogenous carnitine synthesis.

Further physiological activities of ascorbic acid include the following:

- A role in iron transfer from the iron transporting protein transferrin to the iron storage protein ferritin,
- detoxification of numerous substances in the liver by stimulating the synthesis of cytochrome P450,
- promotion of intestinal iron absorption by reducing nearly non-absorbable Fe^{3+} to more easily absorbable Fe^{2+} and by inhibiting the production of insoluble iron-tannin and iron-phytate complexes, and
- participation in the antioxidant defense localized in the hydrophilic compartment of cells.

The immunobiological relevance of some of these ascorbic acid-dependent functions will be discussed in further detail in section 3.2.3 ("Molecular mechanisms of vitamin C for the immunocompetence").

3.2. Vitamin C – Immunobiological Significance

An immunological function of vitamin C is indicated simply because the vitamin C concentration of 1-4mM in immunocompetent cells (lymphocytes, neutrophils, and monocytes) is 10- to 100-fold higher than the concentration in plasma [49-51]. This immunological function of vitamin C is confirmed by the observation that immune cells accumulate vitamin C against a concentration gradient [52-54]. In general, mononuclear leucocytes exhibit 2- to 3-fold higher vitamin C concentrations than polymorphonuclear cells [55-57]. Moreover, the vitamin C concentration in polymorphonuclear cells is more strongly associated with serum concentrations of vitamin C than the concentration in mononuclear leucocytes is [56-58].

Table 1. Immunological Significance of Selected Vitamins and Trace Elements [1, 2, 23, 26-29]. ↓: Decreased; (↓): Possibly Decreased; ↑: Increased; (↑): Possibly Increased; →: No Influence; n.a.: Not Applicable; C: Controversial Result

Micronutrient	Biochemical Functions	Immunological Functions	Influence on Immunocompetence
Vitamin A	• As retinoids, regulate transcription of genes with corresponding proteins involved in control of cell growth and differentiation. • As retinyl esters, carriers of mannose and thus involved in the glycoprotein synthesis.	• Essential factor for the integrity of skin and mucosa, which function as antigen barriers. • Essential for the humoral and cellular immune responses.	*Deficiency:* • Weakening of the skin and mucosal barriers and increased infection risk; • Proliferation and cytotoxicity of T cells ↓; • Antigen specific response ↓ • Proinflammatory effect (TNF-α synthesis ↑); • Modification of the Th1:Th2 relation in favor of Th2. *Supplementation (physiological doses):* • Phagocytosis ↑ • Synthesis of Interferon-γ, TNF-α ↓ • Antibody production ↑ *Supplementation (non-physiological doses):* • Antigen-specific response ↓
Vitamin E	• Antioxidant defense. • Signal transduction.	• Reduces the immunosup-pressive effects of free radicals in respiratory burst. • Modulation of eicosanoid synthesis *via* inhibition of the cyclooxygenase and lipoxygenase; thus increased anti-inflammatory activity. • Essential for the humoral and cellular immune responses.	*Deficiency:* • Antigen-specific response ↓ • Proliferation and cytotoxicity of T lymphocytes ↓ • Phagocytosis ↓ *Supplementation (physiological doses):* • T-cell proliferation ↑ • Beneficial effect on $CD4^+$:$CD8^+$ ratio • Oxidative stress ↓ • Th1 activity ↑ *Supplementation (non-physiological doses):* • Antigen-specific response ↓
Vitamin B_6	• Coenzyme in porphyrin and amino acid metabolism, thereby involved in nucleic acid and protein synthesis. • Modulation of the effects of steroid hormones.	• Essential for antibody and cytokine synthesis.	*Deficiency:* • Maturation and proliferation of lymphocytes ↓ • Activity of T cells ↓ • Antibody production ↓ • Interleukin-2 synthesis of T-helper cells ↓ *Supplementation (physiological doses):* • Lymphocyte proliferation ↑ • Interleukin-2 synthesis of T-helper cells ↓
Folic acid	• Coenzyme in metabolism of amino acids, purines, pyrimidines and choline; thus involved in nucleic acid and protein synthesis. • Epigenetic modification of DNA *via* methylation.	• Essential factor for the growth of fast proliferating immune cells. • Essential factor for antibody and cytokine synthesis.	*Deficiency:* • Thymus weight ↓ • Activity of neutrophils ↓ • Cytotoxicity of T cells ↓ • Antibody production ↓ • Lymphocyte proliferation ↓ • Activity of natural killer cells ↓ *Supplementation (physiological doses):* • Lymphocyte proliferation ↑ • Cytotoxicity of natural killer cells ↑
Vitamin B_{12}	• Coenzyme in the metabolism of homocysteine, odd-numbered fatty acids and branched-chain amino acids.	• Close relation with folic acid metabolism, thus essential for the growth of immune cells, and antibody and cytokine synthesis.	*Deficiency:* • Activity of neutrophils ↓ • Activity of natural killer cells ↓

(Table 1) contd…..

Micronutrient	Biochemical Functions	Immunological Functions	Influence on Immunocompetence
Iron	• An integral part of hemoglobin and myoglobin, involved in erythrocyte oxygen transport and oxygen storage in muscle. • Part of metalloenzymes like di- and monooxygenases, thus involved in the eicosanoid, carnitine, collagen, and neurotransmitter synthesis. • Part of heme containing enzymes including guanylate cyclase, NO synthase, peroxidase and catalase, thus involved in the signal transduction and antioxidant defense.	• Essential factor for lymphocyte maturation, differentiation and proliferation. • Essential factor for cytokine synthesis • As part of NADPH oxyreductase and myeloperoxidase, involved in the synthesis of antimicrobial hypochlorite.	*Deficiency:* • Secretion of interferon γ, TNF-α and interleukin 2 ↓ • Activity of natural killer cells ↓ • Proliferation of T cells ↓ • Bactericidal activity of macrophages ↓ *Iron overload:* • Activity of natural killer cells ↓ • $CD4^+$:$CD8^+$ ratio ↓
Zinc	• Integral part, or effecter, of more than 150 enzymes of all six enzyme classes, thus involved in carbohydrate, lipid, amino acid and nucleic acid metabolism. • "site-specific antioxidant". • As part of transcription factors involved in the regulation of gene expression.	• Essential factor of thymulin synthesis, thus required for T cell activity (cytotoxicity etc.). • Essential factor for cytokine synthesis. • Essential factor for the growth of all fast proliferating immune cells. • Protection of immune cells against oxidative damage. • Essential factor for the integrity of skin and mucosa, both of which are antigen barriers.	*Deficiency:* • Synthesis of interferon γ and interleukin 2 ↓ • Activity of natural killer cells ↓ • Activity of macrophages ↓ (phagocytosis ↓; bactericidal effect ↓; chemotaxis ↓) • T-cell activity ↓ • Thymus atrophy *Supplementation (physiological doses):* • Phagocytosis ↑ • Activity of natural killer cells ↑ • Antibody synthesis ↑ *Supplementation (non-physiological doses):* • Lymphocyte proliferation ↑ • Interferon γ synthesis ↓ • T-cell activity↓
Selenium	• Part of glutathione peroxidase, thus involved in the degradation of peroxides, e.g., hydrogen peroxide. • Part of deiodinases, thus involved in the conversion of thyroxine (T4) to triiodothyronine (T3). • Part of thioreductases, thus involved in redox metabolism.	• Essential factor for lymphocyte activity. • Protection of immune cells against oxidative damage.	*Deficiency:* • Synthesis of proinflammatory eicosanoids ↑ • Antibody production ↓ • Lymphocyte proliferation ↓ • Cytotoxicity of immunocompetent cells ↓ *Supplementation (physiological doses):* • Interferon γ synthesis ↑ • Th1 response ↑ • T lymphocyte proliferation ↑ • Cytotoxicity of natural killer cells ↑

The effect of a single oral dose of vitamin C on the intracellular ascorbate concentration of immunocompetent cells, like monocytes and neutrophils, is characterized by a sigmoidal response curve (Fig. **2**). In adults, a vitamin C intake of approximately 100 mg/d results in complete intracellular saturation [50].

The biokinetic association between vitamin C dose and immune cell concentration underlines the specific function of vitamin C in the cellular immune response [59]. Indeed, experimentally induced vitamin C deficiency impairs cellular [60-66], but not humoral, immune defense [62, 67-71]. Accordingly, a study in healthy men showed that reduction of vitamin C intake for 60 days, from 250 to 5, 10, or 20 mg per day, leads to a lower immune response as shown by a delayed-type skin reaction which is considered as an appropriate marker for immune response. Even after increasing the vitamin C intake to 60 mg/d or 250 mg/d for three weeks, the immune responses in these subjects were still lower than prior to the start of the study [72]. Also important to note is that vitamin C is quickly consumed in infectious diseases [73]. During infections, the concentration of the micronutrient decreases in leucocytes [31, 74-78]. The reduction of ascorbate during infections is associated with an increase in dehydroascorbate. While in healthy subjects, only 5-10% of vitamin C is dehydroascorbate, the percentage increases in patients with meningococcal meningitis or pneumonia to 65-85% [79]. This finding indicates that in patients with infections ascorbate is oxidized due to

increased production of ROS [35] and that endogenous enzymatic reduction has reached its functional limit.

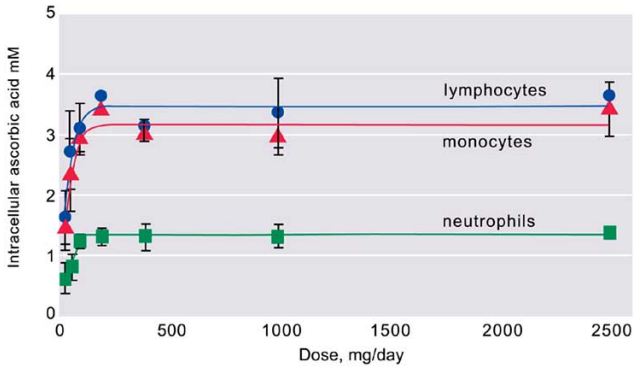

Fig. (2). Dose-dependent concentration of intracellular vitamin C in immunocompetent cells following single oral dose of vitamin C [59 on the basis of 50].

3.2.1. Significance of Vitamin C for the Functional Integrity of Phagocytes and T Cells

While vitamin C does not affect the number of phagocytes, the activity of these immune cells seems to be influenced by intra- and extracellular vitamin C concentration. The following findings are relevant in this context [35, 59, 80]:

- Phagocytosis activity is reduced by suboptimal vitamin C intake, as shown in the majority of animal studies that have investigated it [63-66, 81], although the results are not consistent [78, 82];
- Phagocyte chemotaxis is influenced by vitamin C concentration [63, 66, 82-91]. While *in vitro* an ascorbate-induced increase in chemotaxis seems to require non-physiologically high vitamin dosages [68, 92], vitamin C can affect phagocyte chemotaxis *in vivo* by reducing blood histamine, as observed in subjects after vitamin C intake [92, 93];
- A decline of the intracellular vitamin C concentration in leucocytes is associated with impaired immune activity [94];
- One uncontrolled [68] and two placebo-controlled interventional studies [95, 96] in healthy subjects indicated that vitamin C supplementation of 1500-3000 mg/d (intervention period of weeks to months) can increase lymphocyte proliferation. However, in the study by Nieman *et al.* [96], an effect of vitamin C supplementation with a trend towards statistical significance (P=0.07) could only be shown after adjustment of the changes in the T cell number;
- There may be a dose-related association between extracellular vitamin C concentration and/or vitamin C intake and the proliferation of lymphocytes: A physiological vitamin C concentration of 5-50 µg/ml increases lymphocyte proliferation [97, 98], whereas supraphysiological concentrations show inhibiting effects on proliferation [97-101];
- In diseases associated with phagocyte dysfunction, such as Chediak-Higashi syndrome, phagocyte activity can be improved by vitamin C supplementation [100, 102-109];
- *In-vitro* studies [99, 100, 110-116] and animal studies [62, 69, 117, 118] confirm an enhancing effect of vitamin C on T-cell proliferation, whereas vitamin C supplementation in humans leads to inconsistent results [68, 95, 99, 110, 113, 119-125].

3.2.2. Functional Significance of Vitamin C in Resistance to Infections

A number of results from studies in humans and other species underline the functional significance of vitamin C in infection resistance. The following observations are relevant in the context of infection resistance [35, 59]:

- Under experimental conditions, vitamin C deficiency reduces resistance to various microbial agents, such as bacteria including *Mycobacterium tuberculosis* and *Rickettsia*, as well as fungal infections such as *Candida albicans*.
- An improved vitamin C supply increases the antibody reaction, the activity of neutrophils, and resistance to infections caused by rabies, poliomyelitis, parainfluenza viruses, *Salmonella gallinarum* and *E. coli*. Moreover, animal studies have shown lower mortality from infectious and parasitic diseases after vitamin C supplementation [126-128]. Consistent with these findings is the observation of virustatic activity of vitamin C against Herpes simplex [129-132], Rhino [76, 133] and Human immunodeficiency viruses [134-137]. The results of an interventional (though uncontrolled) study in HIV-positive subjects indicate that megadoses of vitamin C (50-200 g/d) may reduce the risk of opportunistic infections [130].

3.2.3. Molecular Mechanisms by which Vitamin C Provides Immunocompetence

Based on the biochemical functions of vitamin C described in Section 3.1, the immunomodulating effects of this micronutrient probably result from the following factors:

- Its antioxidant potential [138-140];
- its role in the *de-novo* synthesis of carnitine [141];
- its relevance to the synthesis of tetrahydrobiopterin [142];
- its iron metabolism modulating effects [143].

These aspects will be described in more detail in the following subsections.

3.2.3.1. Immunobiological Relevance of the Antioxidant Capacity of Vitamin C

Activated phagocytes synthesize high amounts of reactive superoxide anion radicals ($O_2^{\cdot-}$) in a process catalyzed by NADPH oxidase (EC 1.6.3.1). These radicals are then reduced by superoxide dismutase (SOD; EC 1.15.1.1) to hydrogen peroxide (H_2O_2) and/or react with already available hydrogen peroxide, producing highly reactive hydroxyl radicals ($OH^{\cdot}$). H_2O_2 can be oxidized to hydrochloride ions if chloride anions (Cl^-) are available, this reaction is catalyzed by the enzyme myeloperoxidase (EC 1.11.1.7) [Overview by 144-148]. Biologically, reactive oxygen compounds function in the elimination of bacteria

[149-152] (Fig. **3**). However, although they are essential for the immune system, reactive oxygen compounds also damage phagocytes, impairing their function [153, 154]. Therefore, vitamin C plays a pivotal role in the integrity of phagocytes because it acts as an effective water-soluble antioxidant, trapping excess oxygen radicals and thus protecting immune cells against damage [155]. Macrophages especially seem to require an adequate vitamin C supply: during phagocytosis they consume vitamin C [49, 156], which is one of the most important antioxidants localized in the hydrophilic phase of biosystems [157, 158].

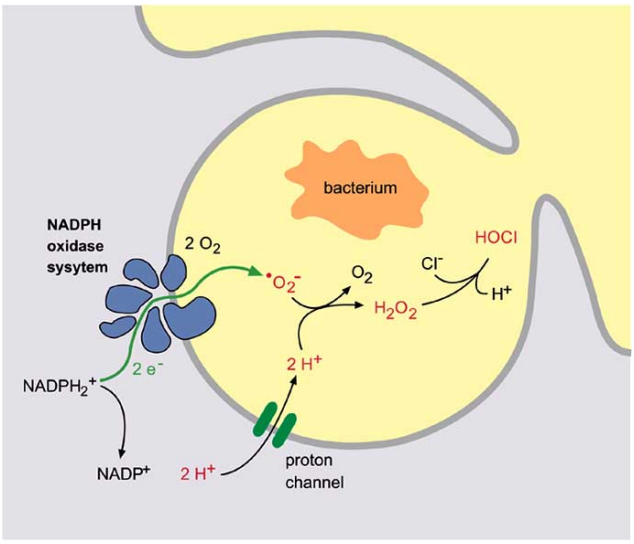

Fig. (3). Functions of the NADPH oxidase system in the plasma membrane of phagocyting cells [59, modified from 193]. MPO: myeloperoxidase.

Biophysically, the central significance of vitamin C for the antioxidant defense of phagocytes can be explained by its properties, which include the following [44]:

- The low one-electron redox potential of ascorbate (282 mV) allows the vitamin to interact with, and trap, almost all reactive oxygen and nitrogen species relevant to biotic systems. In this way vitamin C acts as an effective scavenger molecule for superoxide anion radicals ($O_2^{-\cdot}$), hydroxyl ($OH^{\cdot}$), peroxide radicals ($ROO^{\cdot}$), hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO), among others [159, 160].
- The interaction of ascorbic acid with the previously mentioned reactive molecules is characterized by a high reaction constant (> 10^5 mol/ l*s^{-1}). Therefore, the radicals are effectively, i.e., fast and permanently, neutralized [161-164].
- The oxidized form of ascorbic acid can be regenerated efficiently in the organism. This recycling process ensures that sufficient amounts of biologically active vitamin C are available [165].

3.2.3.2. Immunobiological Relevance of Vitamin C by its Involvement in the Carnitine Synthesis

The hydroxycarbonic acid carnitine (β-hydroxy-γ-trimethyl aminobutyric acid) is a quaternary ammonium compound. Carnitine is endogenously synthesized from the amino acids lysine and methionine in a multi-stage process. In the synthesis process, L-methionine is the methyl group donator, whereas protein-bound L-lysine provides the remaining C-skeleton. In addition to iron, niacin, and vitamin B_6, vitamin C is also required for carnitine synthesis. Vitamin C serves as a cofactor for two enzymes involved in carnitine synthesis: β-hydroxy ε-N-trimethyl lysine hydroxylase (EC 1.14.11.8) and γ-butyro betaine hydroxylase (EC 1.14.11.1). The first enzyme catalyzes the mitochondrial hydroxylation of ε-N-trimethyl lysine, which is released by cellular proteins, whereas the second enzyme introduces the hydroxylation of γ-butyrobetaine localized in the cytosol [166].

In addition to its function as a carrier of long-chain fatty acids, carnitine has various immunological effects [167]. These effects are;

- Stimulation of phagocytosis and chemotaxis in granulocytes and macrophages [168-170],
- stimulation of immunoglobulin synthesis (IgG) [171];
- stimulation of the acute phase response [172].

3.2.3.3. Immunobiological Relevance of Vitamin C – Influence on the Synthesis of Tetrahydrobiopterin and Nitric Oxide (NO)

Tetrahydrobiopterin (BH_4) acts as a single-electron donor, and it is a cofactor of inducible NO synthase (NOS; EC 1.14.13.39) that oxidizes arginine *via* multiple steps generating citrulline and NO [173, 174]. BH_4 easily reacts with ROS, especially peroxynitrite. Being oxidized to a trihydrobiopterin radical in this process, BH_4 is inactivated and thus removed from NO synthesis [175]. Ascorbate increases NO synthesis by reducing trihydrobiopterin radicals to active BH_4 and/or protecting BH_4 against oxidation [176-182]. Therefore, vitamin C plays an important role for the NO supply of the cell. The immunological relevance of this fact becomes clear when the role of NO as a key substance for cellular immunity is considered. Cytokine-activated macrophages produce increasing amounts of NO [183, 184], which exhibit microbicidal effects [185] and modulate the function of T lymphocytes: inhibition of T lymphocyte mitogenesis [186-191] and inducting transformation of Th1 to Th2 helper cells [192].

The second part of this article will focus on the role of vitamin D in the immune system, and the significance of an adequate micronutrient supply for immune competence will be discussed.

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