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A short overview of vitamin C and selected cells of the immune system

Review Article

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Abstract: Vitamin C (ascorbic acid) is an essential water-soluble nutrient that primarily exerts its effect on a host defense mechanisms and immune homeostasis and is the most important physiological antioxidant. Stable intake of vitamin C is essential for life in humans because the body does not synthesize it. Even the numerous studies have demonstrated that vitamin C supplementation stimulates the immune system, prevents DNA damage and significantly decreases the risk of a wide range of pathologies; the potential protective mechanisms are still largely unknown. This review summarizes the recently known facts about the role of vitamin C on the selected cells of the immune system and potential molecular mechanisms involved. Further, in this review, many new data about the positive effects of vitamin C on the immune system, potential toxicological effects, vitamin C supplementation in disease development, as well as some proposed mechanisms of vitamin C activity, are discussed.

Keywords: *Vitamin C • Immune system • T-cells • Neutrophils • Dendritic cells*

1. A short overview of vitamin C and selected cells of the immune system

Different nutrients have been demonstrated to be required in adequate amount for an efficient and adequate immune response in animal and human studies. Nutrient deficiency affects the innate, adaptive and cellular immune responses and suppresses immune function, with resulting deregulation of normally coordinated host response to infection, and thereby enhancing the virulence of pathogens. Vitamin C, as an important nutritional antioxidant, is a major component of the body`s antioxidant system, which provides the protection from the toxic effects of reactive oxygen species (ROS). ROS are generated as by-products of normal aerobic respiration, during inflammation and after exposure to environmental toxins [1]. Excess generation of ROS in the cells is known to damage DNA (deoxyribonucleic acid), lipids and proteins resulting in several biological effects ranging from alterations in signal transduction to gene expression and apoptosis and oxidative stress development [2-5]. The pathophysiology of a number of important chronic diseases such as atherosclerosis, chronic inflammatory diseases and diabetes involves oxidative stress [6]. Ascorbic acid is a water-soluble antioxidant that quenches ROS in both the extra and intracellular compartments and protects DNA, proteins and lipids from oxidation by ROS [7]. This review summarizes the antioxidant effect of vitamin C on the selected cells of the immune system, including T-cells, neutrophils, DCs (dendritic cells), PBMCs (peripheral blood mononuclear cells), and potential molecular mechanisms involved. As discussed below, it considers effects of vitamin C supplementation in disease development as well as potential toxicological considerations.

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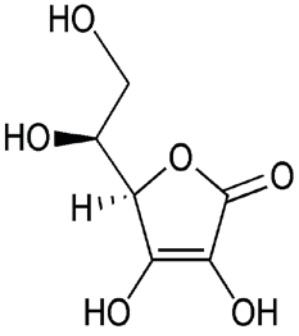
Table 1. The ascorbic acid distribution in human tissues (according to Rose et al. [98]).

Tissue	Tissue concentration (μM/l)
Eye	
Aqueous humor	0.9
Lens	1.1
Vitreous	2
Blood	
Platelets	1.9
Erythrocytes	0.034
Granulocytes	1.2
Leukocytes	3.8
Adrenal glands	1.9
Kidney	0.6
Brain	0.7

According to the Rose et al. [98], results were presented in units of μM/l, calculated as though all tissue weight in water.

Figure 1. Ascorbic acid (reduced form).

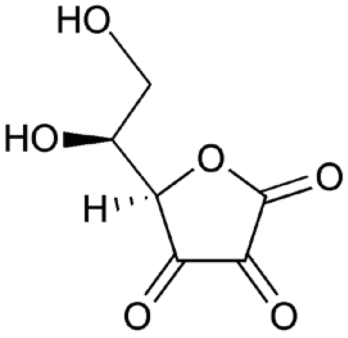


2. Ascorbic acid distribution

Vitamin C is present in plasma mainly in its reduced form, as ascorbic acid, at a concentration ranging from 10 to 160μM. The content of vitamin C in the cells and tissues may exceed the plasma concentrations by as much as 100- fold [8]. Therefore, vitamin C needs to be distributed in a regulated manner into organs at the appropriate concentration (Table 1) and this requires vitamin C specific transporters at the level of plasma membrane, which could concentrate the vitamin inside cells. Since there are two biological important forms of vitamin C, reduced (ascorbic acid; Figure 1) and the oxidized form (dehydroascorbic acid; Figure 2), and both chemical forms are transported intracellularly, cells express two different transporters for vitamin C.

The ascorbic acid transporters are members of the sodium-coupled ascorbic acid transporters or SVCTs. The SVCTs family is composed of two active members, SVCT1 (a high-capacity, low-affinity ascorbate transporter), and SVCT2 (a low-capacity, high-affinity

Figure 2. Dehydroascorbic acid (oxidized form).



transporter) [9]. Upon vitamin C transport via SVCTs, ascorbic acid is actively retained in the intracellular space of all tissues. Intracellular ascorbic acid concentration mainly depends of extracellular ascorbic acid concentration and the capacity of SVCTs. Having in mind fact that immune and inflammatory cells have intracellular concentration of ascorbic acid approximately 1mM (ten times higher than plasma concentrations) [10], it probably reflects the level of potential oxidative stress within these cells, since those cells are exposed to high ROS concentration during inflammatory reactions.

The dehydroascorbic acid transporters are members of the family of facilitative glucose transporters or GLUTs. Even GLUT family consists of 14 different members, named from GLUT1 to GLUT14, there is evidence indicating that at least three members (GLUT1, GLUT3, GLUT4) have the capacity of transporting dehydroascorbic acid [11,12]. After uptake into the cell, dehydroascorbic acid is rapidly reduced to ascorbic acid. The mechanism of this reduction differs depending on the cell type [13]. Presumably the cellular uptake of dehydroascorbic acid will only occur during inflammation and by increasing intracellular ascorbate, serves a protective function [14], which finally leads to extracellular reduction of ascorbic acid, as it can be observed in conditions of oxidative stress [15].

3. Vitamin C and some cells of the immune system

The immune system is highly dependent on adequate cell–cell communication, and any damage to the signaling systems (oxidative stress), will lead to an impaired immune responsiveness [16]. The intracellular antioxidant-oxidant balance is critical for immune cell function because it maintains the integrity of cell components and their function. Immune cells are particularly sensitive to oxidative stress because of the high content of polyunsaturated fatty acids in their

plasma membranes and a high production of ROS, which is part of their normal function [17].

Since the influence of antioxidant system on immune function is not well understood, general belief is that reduction of free radicals will prevent DNA damage to immune cells, thereby maintaining their functional and structural integrity. Indeed, ascorbic acid can reduce directly [18] or indirectly through the regeneration of vitamin E [19] damage to lymphocytes by ROS. It was suggested that ascorbic acid levels exert this effect by down-regulating ROS-dependent expression of proinflammatory IL (interleukin) genes via inhibition of transcription of NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells), which regulates the expression of proinflammatory cytokines, such as IL-1 and TNFα (tumor necrosis factor-alpha) [20]. These observations were confirmed in studies showing that ascorbic acid enhances antioxidant defenses of T-cells [21] and also increase T-cell responsiveness to antigens, suggesting a role in regulating immune function [22]. Therefore, a decrease in the intracellular content of these antioxidants may result in local increased apoptosis of immune cells and resulting immunosuppression [23]. These findings were confirmed in different studies which reported that increased ascorbic acid concentration successfully inhibited antigen-induced, withdrawal-induced, steroid induced and spontaneous T-cell apoptosis [24], Fas-induced apoptosis of monocytes [25] and increased cytotoxic activity of natural killer cells in humans [26], indicating that ascorbic acid could modulate the immune system by inhibiting T-cell apoptosis signaling pathways [27]. Similar results were shown in monocyte-derived DCs, where treatment with vitamin C and vitamin E resulted with no significant increase of apoptosis before and after receiving activation stimulus [28]. Also, the same study demonstrated that the levels of ROS were reduced while the NF-kB, protein kinase C and p38MAPK (P38 mitogen-activated protein kinase) pathways could not be activated, in DCs, following inflammatory agent stimulation. Allogenic T-cells (CD4+CD45RO, CD4+CD45RA and CD4+CD25- subsets) were anergized after exposure to vitamin-treated DCs, indicating that vitamin-treated DCs may be useful in tolerance induced protocols [28]. The observed property of vitamin C it is not only of interest in understanding the biology of DCs activation but, also, reveals the new therapeutic possibilities. Namely, vitamin C supplementation of 500mg orally, twice daily, can achieve serum level of 65μM [29]. This opens up the possibility of using vitamin-treated DCs for the induction of tolerance to autoantigens or alloantigens. In line with previous properties of vitamin C, clinical study has shown that the use of vitamin C and vitamin E resulted in a delay of chronic rejection of allogenic heart transplant [29], suggesting the effect of vitamins on DCs as a possible mechanism to explain the trial outcome.

Neutrophils are also known to accumulate millimolar concentrations of vitamin C, which is supposed to protect them from products of the oxidative burst [30]. However, how ascorbic acid affects neutrophil metabolism and function is still unclear, although it has been shown to affect chemotaxis and phagocytosis [31] and to influence the reactivity of microbicidal oxidant [32]. Neutrophils are released from bone marrow and are cleared from circulation within 10 hours [33]. This process ensures their safe disposal without release of the many cytotoxic and hydrolytic enzymes present in the cytoplasmic granules [34]. Delayed neutrophil apoptosis could be archived under conditions of active inflammation by certain cytokines and growth factors, such as TNFα and GM-CSF (granulocyte-macrophage colony-stimulating factor) [35]. Different studies have showed that neutrophil apoptosis could be inhibited under hypoxic conditions [36,37], which involves hypoxia inducible factor (HIF)-1 [37]. Recent study has shown that vitamin C deficiency could also delayed neutrophil apoptosis and that initially, there was increased survival, but eventually the cells became necrotic. The increased neutrophil survival was accompanied with elevated level of HIV-1 protein, indicating that vitamin C deficiency mimics the hypoxic response and prevents neutrophil apoptosis [38]. Further, vitamin C-deficient neutrophils failed to undergo morphological changes associated with apoptosis and therefore were not recognized or phagocytosed by macrophages. On the other hand, same cells reverted to apoptotic phenotype after vitamin C supplementation [38]. These findings support the hypothesis that neutrophil necrosis, as result of vitamin C deficiency, could be devastating to the tissues if it occurs in vivo. In particular, the release of highly active proteases, such as elastase, cathepsin G and collagenase, could degrade many tissues [39]. Whether this occurs in vitamin C-deficient organism is currently not clear, but it is interesting that extensive tissue injury is one of the major symptoms in scurvy, as main vitamin C deficiency. In a recent study, vitamin C supplementation in septic patients after abdominal surgery, for 6 consecutive days, showed the reduction in caspase-3 levels and activity, together with increased level of Bcl-2 (B-cell lymphoma 2) protein in neutrophils, suggesting the low level of apoptosis by caspase-3 and antiapoptotic effect of vitamin C in the mitochondria [40]. These results are in line with previous findings of an accumulation of high vitamin C levels by neutrophils, protecting them from products released during respiratory burst in an inflammatory setting

[41]. An increased number of neutrophils are observed in surgical patients, especially in those who develop sepsis [40,42]. It has been proposed that the presence of inflammatory mediators in septic patients produces an over-activation of neutrophils, which remain longer in peripheral blood circulation and exert lesional effects on tissues, thereby increasing the risk of organ failure [43]. Consequently, the antiapoptotic effect of vitamin C on neutrophils may be detrimental to the patient by increasing their presence in circulation [40]. Stimulation of human polymorphonuclear cells with vitamin C caused a significant increase in number of latex particles phagocytized by each individual cell, whereas the number of cells capable for phagocytosis remained unchanged [44]. The reference data concerning the effect of vitamin C on the capacity of phagocytosis is still controversial. On one hand, researchers have reported on an enhancing of vitamin C on phagocytic activity [45,46]. On the other hand, another study could not observe a stimulatory effect of vitamin C on the phagocytic activity, although treatment with vitamin C did induce a significant increase in bacterial killing [47]. Nevertheless, positive effect for vitamin C has been reported in inflammatory processes and adverse outcomes have been related to deficits in this vitamin, supporting its beneficial properties [48,49].

In a study of human PBMCs, vitamin C caused a marked increase in the number of apoptotic PBMC, when apoptosis was detected by using propidium iodide test, indicating a significant DNA fragmentation [44]. Simultaneously, when apoptosis was detected with caspase-3 method, the enzyme activity did not change, suggesting the existence of caspase-3 independent pathway for regulation of programmed cell death following incubation with vitamin C [44]. The observed effect of vitamin C on apoptosis of normal PBMC, suggests a suppressive effect of this antioxidants on DNA synthesis and it was shown in PBMC [44] and other cell types [50]. It was implied that the inhibition of cell division induced by S/G2 block in the cell cycle proceeds through generation of unidentified free radicals [51]. DNA damage caused in vivo by vitamin C may be due to its capacity to induce decomposition of lipid hydroperoxides which have been shown to generate genotoxins [52]. Stimulation of human PBMCs with vitamin C resulted with inhibited DNA synthesis and dose-dependent suppression of IL-10 secretion [44]. The existence of a relationship between DNA synthesis and IL-10 production has been established earlier in different cell types [53]. However, in study with human PBMC, results were obtained in vitro and although the vitamin dose was extrapolated from that applied in clinical practice the findings might be the outcome of dose dependence [44]. The modulatory role of vitamin C on the intracytoplasmatic production of pro-inflammatory cytokines has been shown in human mononuclear cells. The data demonstrate a dose-dependent inhibition of IL-6 and TNFα producing monocytes after LPS (lipopolysaccharide) stimulation and a selective decrease of IL-2 producing lymphocytes upon PMA (phorbol 12-myristate 13-acetate) stimulation in vitro [54]. Oxidative damage plays a key role in endotoxin (LPS)-induced sepsis, leading to an inappropriate activation of transcriptional factor NF-kB and to overexpression of inflammatory proteins [55]. Since most activation pathways of NF-kB are reliant on ROS, vitamin C may protect from dysregulation of the immune-inflammatory response by it antioxidant properties [56]. Further, after T lymphocyte activation, NF-kB signaling, which leads to IL-2 gene expression, was also found to be redox sensitive [57], which may explain the selective inhibition of IL-2 producing lymphocytes by vitamin C. However, other studies indicated that inhibition of NF-kB activation by vitamin C is not an antioxidant effect, because redox insensitive pathways are likely to be blocked [58]. These observations suggested that p38MAPK could be an intracellular target of vitamin C [59]. In line with those results, it has been shown that vitamin C can also function as a modulator of inflammatory responses by inhibiting GM-CSF, IL-3 and IL-5-induced signal transduction pathway [60]. Such observations of in vitro effect of vitamin C on pro-inflammatory cytokine production are likely to yield novel insight into the pathology of inflammation and could provide several implications for the clinical use of vitamin C as a potential anti-inflammatory drug. In human sepsis, circulating vitamin C levels are significantly depleted or even not detectable [56]. Since the IL-6 and TNF-α expression levels can predict the outcome of septic patients, intracytoplasmatic detection of cytokine production may become an additional valuable tool for monitoring the clinical efficiency of vitamin C [54].

4. Effect of vitamin C deficiency and supplementation in disease development

Vitamin C supplementation has been shown to have some clinical usefulness in the treatment of several autoimmune diseases, including asthma, allergy, phagocytic disfunction disorders and immunosuppressive disorders, as reviewed earlier [61]. Also, several different studies have shown that reduction in antioxidant capacity is major patophysiological factor in diseases, such as

Table 2. The ascorbic acid plasma concentrations in different disease states (according to McGregor et al. (1)).

	Vitamin C plasma concentrations (μMol/l)
Healthy	61.4-80
Diabetes	41.5
Gastritis	45.7
Pancreatitis	32.6
Pneumonia	30.6
Cancer	<24
Trauma, sepsis	10
Arthritis	27

rheumatoid arthritis [62], gastritis [49], critical illness [49], pancreatitis [63], diabetes [49] and cardiovascular disease [64]. Beside basic inflammatory response in these diseases, intensive oxidative stress represents additional pathophysiological burden. Consistent with these findings, there is clear evidence that restoring the oxidative capacity is a therapeutic strategy in treating chronic inflammation [65]. Decreased levels of vitamin C in serum have been reported in numerous diseases, as presented in Table 2. Topical application of vitamin C in patients with herpes simplex virus infections decreased the durations of the lesions and viral shedding [66]. Vitamin C was postulated to be effective in ameliorating symptoms of upper respiratory tract infections, especially the common cold, according to its immune-stimulating properties [67]. Concentration of vitamin C in plasma and leukocytes fall rapidly with the onset of the infection and return to normal concentrations with the ameliorating the symptoms, suggesting the dosage with vitamin C could be beneficial for the recovery process [68]. However, recent review of large number of studies, concluded that administration of more than 1g of vitamin C per day, had no consistent effect on the incidence of the common colds, but supported a moderate benefit on duration and severity of symptoms may also be of economic advantage [69].

5. Proposed mechanisms

Many different studies have been undertaken to evaluate the mechanisms by which ascorbic acid might influences the immune system. Up to now, there is no consistent consensus on ascorbic acid influence on the immune system. However, there are some proposed mechanisms of stimulating effect of ascorbic acid on immune system. Ability of ascorbic acid to reduce free radical production and resulting prevention of DNA damage to the cells of the immune system represents the most accepted immune system enhancing ascorbic acid activity, as reviewed earlier [70]. Further potential immunostimulatory activity was confirmed in vitro, where three different T-cell death pathways were inhibited, when T-cell were incubated with ascorbic acid, including activated and resting T-cells [27]. Furthermore, the same study confirmed that effector T-cells were more likely to enter S phase if treated with ascorbic acid. Other potential stimulatory mechanism of ascorbic acid suggested the increasing of intracellular nucleotide levels, modulation of pro-inflammatory cytokine synthesis and antagonism of immunosuppressive interaction between histamines and leukocytes [71]. Up-regulation of natural killer cell activity via stimulatory effect of ascorbic acid on protein kinase C activity represents another potential mechanism in ascorbic acid stimulation of immune system [26]. Finally, it has been shown that ascorbic acid stimulatory mechanism, in different cell types, prevents apoptosis by upregulating the Bcl-2 protein expression level, with resulting change in Bcl-2 and Bax (Bcl-2–associated X protein) protein ratio [72,21]. Intensive oxidative stress sensitizes T-cells to apoptosis, by decreasing the expression of Bcl-2 protein [73], which has been documented in different studies [74-79]. Protective role of ascorbic acid in Bcl-2 protein expression increasing and enhancing Bcl-2/Bax protein ratio, may allow T-cells to cope better with the effect of oxidative stress and resulting T-cell toxicity, as was reviewed earlier [80]. By detoxifying ROS, ascorbic acid and other antioxidants may therefore reverse the oxidative stress-induced decline in Bcl-2 and prevent cell death [73]. Further, vitamin C was shown to improve human immune response, such as antimicrobicidal, natural killer cell activities, lymphocyte proliferation and chemotaxis [81-85], indicating the important role of this vitamin in regulating the immune response.

6. Toxicological considerations

There is a long-standing debate about the potential adverse effects of high doses of vitamin C, especially with regards to increase of oxalate levels and kidney stone formation. Namely, earlier report has demonstrated that 8 g/day of ascorbic acid for 8 consecutive days, could cause harmful calcium oxalate crystalluria in persons who have a predisposition for increased crystal aggregation, indicating that these individual's response to ascorbic acid ingestion is probably rare and concluded that ingestion of these doses did not affect the principal risk factors associated with calcium oxalate kidney stone formation [86]. Another report observed a modest increase in urinary oxalate after administration 5 and 10 g/day ascorbic acid for 5 consecutive days [87]. These

results appear to be due to in vitro conversion of ascorbate to oxalate during the analytical procedure rather than in vivo conversion [88]. Later studies confirmed these observations, showing that large ascorbic acid doses did not produce kidney stones but, also, reduced the risk of kidney stone formation, concluding the ascorbic acid restriction, due to possibility of kidney stones formation, is unwarranted [86-88].

Pro-oxidative effects have been described in vitro for vitamin C in the presence of transition metal such as iron and copper [92]. Iron, through Fenton reaction, generates the highly reactive hydroxyl radical. However, in the body free transition metals do not normally exist and they are bound to proteins (transferrin, ceruloplasmin). In iron storage disease there are raised levels of free iron, and the use of high doses of ascorbic acid is contraindicated, as was confirmed with increased DNA damage in white blood cells [93]. With respect to protein and lipid oxidation caused by iron overload, ascorbic acid has been shown in vitro to be antioxidant [7]. Later studies, also, confirmed these results, showing that ascorbic acid is not pro-oxidant, in the presence of iron overload, in vitro [94] and with iron co-supplementation in vivo [95,96].

Gastrointestinal distress seems to be the most common adverse effect of high vitamin C intake. Usually these symptoms occur when ascorbic acid intake is more than 2g per day. The mentioned symptoms generally disappear within a week or two, with no further consequences, and may have been produced by other components such as sorbitol [97].

7. Conclusions

Adequate intake of ascorbic acid, as a potent antioxidant nutrient, is essential for proper health. This nutrient interacts with the human immune system, by supporting immune response and providing antioxidant protection to exogenously derived and endogenously generated ROS and help to avoid damaging effects to surrounding tissues at the site of inflammation. Vitamin C consumption decreases the risk of DNA damage in the cells of the immune system, enhances antioxidant defenses and increases T-cell responsiveness to antigens. Inadequate intake of this antioxidant may result in impaired function of immune cells and suppressed immunity, which predisposes to infections and aggravates malnutrition. Even vitamin C requirements vary greatly among the individuals, it is suggested that vitamin C supplementation is necessary to achieve optimal health. Therefore, we advise healthy people to consume vitamin C in order to ensure a proper function of immune system. On the other hand, we are far from being able to define the optimal levels of intake required to maintain an optimal immune response and to prevent or treat viral or other infectious diseases. Furthermore, additional studies that aim to determine the exact clinical benefit of high-dose ascorbic acid, as an antioxidant therapy, should be encouraged while making a rational assessment of its safety.

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