Immunity Enhanced by Trace Elements

Zinc-Altered Immune Function[1]

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ABSTRACT   Zinc is known to be essential for all highly proliferating cells in the human body, especially the immune system. A variety of in vivo and in vitro effects of zinc on immune cells mainly depend on the zinc concentration. All kinds of immune cells show decreased function after zinc depletion. In monocytes, all functions are impaired, whereas in natural killer cells, cytotoxicity is decreased, and in neutrophil granulocytes, phagocytosis is reduced. The normal functions of T cells are impaired, but autoreactivity and alloreactivity are increased. B cells undergo apoptosis. Impaired immune functions due to zinc deficiency are shown to be reversed by an adequate zinc supplementation, which must be adapted to the actual requirements of the patient. High dosages of zinc evoke negative effects on immune cells and show alterations that are similar to those observed with zinc deficiency. Furthermore, when peripheral blood mononuclear cells are incubated with zinc in vitro, the release of cytokines such as interleukins (IL)-1 and -6, tumor necrosis factor-α, soluble IL-2R and interferon-γ is induced. In a concentration of 100 μmol/L, zinc suppresses natural killer cell killing and T-cell functions whereas monocytes are activated directly, and in a concentration of 500 μmol/L, zinc evokes a direct chemotactic activation of neutrophil granulocytes. All of these effects are discussed in this short overview.   J. Nutr. 133: 1452S–1456S, 2003.

KEY WORDS: • *zinc* • *immunology* • *cytokines* • *leukocytes*

Zinc is known to be essential for growth and development of all organisms (1–4). It is important for enzymes of all six classes as well as transcription and replication factors (5,6). The human body contains 2–4 g of zinc, but in the plasma, zinc only occurs in a concentration of 12–16 μmol/L. Although the zinc plasma pool is very small, it is highly mobile and immunologically very important (7,8). In the serum, zinc is predominantly bound to proteins (9). There are different factors that influence zinc metabolism as well as homeostasis (10). Recently, hints were found to suggest that free intracellular zinc concentrations are in the femtomol-per-liter range, which suggests a high intracellular zinc-binding capacity (11).

Zinc is necessary for the normal function of the immune system (6,12). Even mild zinc deficiency, which is widely spread in contrast to severe zinc deficiency, depresses immunity of humans (13). There are some groups that are at high risk of zinc deficiency such as elderly people, vegetarians and patients with renal insufficiency.

Influence of zinc depletion on immune functions

Innate immunity. The innate immunity as the first line of defense represents a natural protection against infections. It is not highly specific and responds to different antigens in the same way. It is not able to produce memory cells.

The functions of the innate immunity are disturbed by altered zinc levels. In vitro, not only the recruitment of neutrophil granulocytes (14) is concerned but also the chemotaxis, because zinc concentrations of ~500 μmol/L induce chemotactic activity in polymorphonuclear leukocytes directly (15). In vivo, natural killer (NK)[3] cell activity, phagocytosis of macrophages and neutrophils and generation of the oxidative burst are impaired by decreased zinc levels (16,17). The number of granulocytes is shown to be decreased during zinc deficiency (18).

On the other hand, zinc is required by human beings and pathogens for proliferation. Thus, decreasing plasma zinc levels during an acute phase in infection is a defense mechanism of the human organism. It was shown that the S-100 Ca^{2+}-binding protein calprotectin is released during degradation of neutrophils, chelation of zinc and inhibition of reproduction of bacteria and *Candida albicans* by this way (19–21).

[3] Abbreviations used: IFN, interferon; IL, interleukin; NK, natural killer; PBMC, peripheral blood mononuclear cells; sIL, soluble interleukin; TH, T-helper; TNF, tumor necrosis factor.

TABLE 1

Correlation between zinc concentration and cells of innate immunity

Cell type	Zinc deficiency	Physiologic normal zinc level	High zinc dosage
Monocytes/macrophages	Decreased functions	Normal	> 30 μmol/L: normal > 100 μmol/L: direct activation
Neutrophil granulocytes	Decreased phagocytosis	Normal	> 100 μmol/L: normal > 500 μmol/L: direct chemotactic activity
Natural killer cells	Decreased cytotoxicity	Normal	Suppressed killing

NK cells are important for immunity against infections and tumors. The NK cell number and activity are dependent on the serum zinc level (22), and it was shown that with zinc deficiency, the NK cell activity and the relative number of precursors of cytolytic cells are decreased (23). Zinc is needed by NK cells for the recognition of major histocompatibility cell complex class I molecules by the p58 killer cell inhibitory receptors on the NK cells to inhibit the killing activity (24). However, only the inhibitory signals are zinc dependent in contrast to the positive ones. Thus, zinc deficiency might evoke nonspecific killing. The correlation between zinc concentration and the function of cells of innate immunity is shown in **Table 1**.

Specific immunity. B and T cells of the specific immune system have a great variety of specific receptors (antibodies and T-cell receptors) and can produce memory cells that respond quickly and powerfully to antigens to which they have been primed.

B cells. B cells represent the main cells of humoral immunity. After stimulation, B cells differentiate to antibody-producing plasma cells. B cells were shown to be less dependent on zinc for proliferation than T cells (25,26); therefore, the influence of zinc deficiency on B-cell development is not comparable to the situation of T cells (27–29). B lymphocytes and their precursors (especially pre-B and immature B cells) are reduced in absolute number during zinc deficiency, whereas changes among mature B lymphocytes are only slight. This might be due to the induction of apoptosis in those cells (30). However, low zinc levels have no influence on the cell-cycle status of precursor B cells and only modest influence on cycling pro-B cells (31). Thus, there are fewer naive B cells during zinc deficiency that can react on neoantigens. Taking into account that the number of T cells is also reduced during zinc deficiency and that the most antigens are T-cell dependent, it is probable that with zinc deficiency, the body is unable to respond with antibody production in response to neoantigens. This assumption is consistent with findings that show that B-lymphocyte antibody production is disturbed during zinc depletion (32). Furthermore, studies reveal that antibody production as a response to T-cell–dependent antigens is more sensitive to zinc deficiency than antibody production in response to T-cell–independent antigens (33). Zinc-deficient mice show reduced antibody recall responses to antigens for which they were immunized. This effect is observed in T-cell–independent and –dependent systems. Thus, immunologic memory is also influenced by zinc (32,34), but because mature B cells are more resistant to zinc deficiency due to a high Bcl-2 level, B-cell memory is less affected than the primary response (30). The relationship between zinc level and B-cell function is shown in **Table 2**.

T cells. T cells are effector cells as well as important regulating cells of the specific immune system. Zinc influences not only NK cell-mediated killing as mentioned above; it also affects the activity of cytolytic T cells (35). The relative amount of CD8$^+$CD73$^+$ T lymphocytes is found to decrease during zinc deficiency (23). These cells are predominantly precursors of cytotoxic T lymphocytes (CD8$^+$), and CD73 is known to be needed on these cells for antigen recognition and proliferation as well as cytolytic process generation (36). Furthermore, it was shown that zinc is involved in the development of T cells, because zinc deficiency is responsible for thymic atrophy (37). Thymulin is a hormone that is produced by the thymus and released by thymic epithelial cells (38,39). Zinc is an essential cofactor for thymulin. It regulates not only the differentiation of immature T cells in the thymus and the function of mature T cells in the periphery; it also modulates cytokine release by peripheral blood mononuclear cells (PBMC), induces proliferation of CD8$^+$ T cells in combination with interleukin (IL)-2 (40,41) and ensures the expression of the high-affinity receptor for IL-2 on mature T cells (42). Studies reveal that as a consequence of zinc deficiency, T-cell proliferation decreases after mitogen stimulation (43,44). Zinc supplementation is able to reverse the zinc deficiency-induced changes in the thymus and on peripheral cells (37), which was also observed in patients with acquired immune deficiency syndrome (45).

Besides cytotoxic T cells, T-helper (TH) cells (CD4$^+$) are affected by zinc and zinc deficiency, which causes an imbalance between TH1 and TH2 functions. This was observed in altered secretion of the typical TH1 and TH2 cytokines during zinc depletion: the TH2 products IL-4, -6 and -10 remain unchanged during zinc deficiency, whereas the TH1 cell products interferon (IFN)-γ and IL-2 are decreased. Production of both IL-2 and IFN-γ is corrected by zinc supplementation (23). The relationship between zinc level and T-cell function is shown in Table 2.

In vitro and in vivo zinc supplementation

In vitro, many effects of zinc on immune cells are found by assessing the cytokine concentration in the samples after zinc

TABLE 2

Correlation between zinc concentration and cells of specific immunity

Cell type	Zinc deficiency	Physiologic normal zinc level	High zinc dosage
T cells	Decreased normal functions, increased autoreactivity and alloreactivity	Normal	> 30 μmol/L: functions decreased > 100 μmol/L: functions suppressed
B cells	Apoptosis	Normal	Apoptosis

stimulation. Cytokines are modulators within the immune system, and zinc is able to influence this complicated network. When PBMC are stimulated with zinc, IL-1 and -6, tumor necrosis factor (TNF)-α, soluble (s)IL-2 receptor and IFN-γ are released (46–48). The secretion of IL-1 and -6 and TNF-α is induced in monocytes directly and is not dependent on the presence of lymphocytes (48). It was shown that TNF-α release after PBMC stimulation with zinc is caused by a de novo transcription of mRNA and not by enhanced translation of already-expressed mRNA (49).

In vitro, zinc supplementation of purified T cells shows no cytokine release, which leads to the assumption that T-cell stimulation with zinc is dependent on the presence of monocytes (48,50,51). Indeed, investigations show that IL-1 and -6 and cell-to-cell contact between monocytes and T cells are necessary for T cells to release IFN-γ and sIL-2R (48,51). Zinc is incapable of inducing cytokine production in isolated and monocyte-depleted T cells (51,52), B cells (44), NK cells (44) or neutrophils (Fischer, Gabriel & Rink, unpublished results). Moreover, activation of T cells and monocytes is dependent on the amount of free zinc ions and the protein composition in the culture medium. It was shown that transferrin and insulin specifically enhance zinc-induced monocyte stimulation by means of a nonreceptor-dependent mechanism (44,49,53,54). High levels of serum proteins in the culture medium inhibit monocyte activation, because proteins lower the available free zinc by binding it. In serum-free culture medium, concentrations $> 100 \ \mu$mol of zinc/L stimulate monocytes but prevent T cells from activating. This occurs because T cells have a lower intracellular zinc concentration and are more susceptible to increasing zinc levels than monocytes (51,55,56). Experiments show that inhibition of the IL-1 type I receptor-associated kinase by zinc is responsible for this finding, because T cells are activated by IL-1, which is secreted by monocytes after zinc stimulation (48,49,51). Thus, T-cell activation by zinc takes place when zinc concentrations are high enough for monokine induction but do not exceed the critical concentrations for T-cell suppression.

Zinc concentrations at three to four times the physiologic level do not decrease T-cell proliferation in vitro nor show immunosuppressive effects in vivo but do suppress alloreactivity in the mixed lymphocyte culture (57), which is a common in vitro model in transplantation medicine. This suppression of alloreactivity is observed in vitro as well as in vivo (Faber et al., unpublished results). Thus, this could be a new pathway for the selective modulation of T-lymphocyte functions, because conventional immunosuppressive pharmaceuticals used on patients after transplantation to prevent graft rejections show many severe side effects. Furthermore, zinc might be used for the therapeutic treatment of other T-cell–mediated reactions such as rheumatoid arthritis.

Studies reveal that supplementation and optimal intake of zinc restore impaired immune response and decrease infection incidence in vivo (58). Zinc supplementation results in increased numbers of T and NK cells and elevated production of IL-2 and sIL-2R. Furthermore, lymphocyte response to phytohemagglutinin stimulation as well as NK cell activity improves significantly compared to the placebo group (58). Numbers of CD4$^+$ T cells and cytotoxic T lymphocytes increase significantly after zinc supplementation, and cell-mediated immune response improves (59).

However, the optimal therapeutic dosage that is required to reverse symptoms of zinc deficiency is still unclear, and the pharmacologic zinc dose should be adapted to the actual requirements to avoid negative side effects on immune functions. Therefore, zinc plasma levels should not exceed 30 μmol of zinc/L. On the other hand, zinc is almost nontoxic, even in dosages that exceed the recommended daily intake (60). However, zinc supplementation in high dosages changes the benefits into negative effects, and alterations are recognized that are similar to those of zinc deficiency. T-cell functions are inhibited after high zinc dosages (51), and zinc in concentrations that represent seven to eight times the physiologic zinc level block IFN-α production (61). Different groups report suppression of immune functions when the oral zinc intake is 100 mg of zinc/d (62–65).

Response to vaccination is rather low in zinc-deficient persons such as elderly or hemodialysis patients (66,67). At least in hemodialysis patients, it is possible to find a relationship between serum zinc concentration and the vaccination response (70). However, many trials do not confirm a correlation between humoral response and zinc supplementation when zinc is provided as an adjuvant in vaccination (62,69,70). The different outcomes of these studies might be due to the different zinc dosages used, which sometimes were up to 400 mg of zinc/d. As mentioned above, a zinc intake of 100 mg/d is enough to suppress immune responses. In conclusion, low-dose supplementation of zinc yields an improvement in the humoral response after vaccination (71), whereas supplementation with high dosages does not improve antibody production (62). Improvements might be due to restored B-cell functions after zinc supplementation, because these are reduced during zinc deficiency. Other mechanisms might include the increase of IFN-α production by zinc (61) or the restoration of impaired T-cell help (66,68). On the other hand, both mechanisms could explain the negative effects of high zinc dosages in these cases, because IFN-α production and T-cell functions are inhibited by high dosages (51,61).

The importance of zinc for an intact immune system has been known for a long time, but still there are many areas in which the role of zinc is unclear such as on molecular and intracellular bases. Specific stimulants are needed to investigate the different leukocyte subsets of the immune system. Because it is known that zinc influences these stimulants (72–75), it is important to get further information about them.

In conclusion, we show that zinc supplementation has beneficial effects for patients who suffer from zinc deficiency, e.g., elderly individuals who often show a high rate of infections. It helps these patients restore their immune systems, but as yet there is no standard therapeutic dosage. Zinc administration must be adjusted to the patient's actual requirements, because high dosages show negative effects on the immune system. Furthermore, zinc might be used in immunosuppressive therapy in the future, because it has immunosuppressive properties in concentrations where no severe side effects were recognized. Thus, zinc is a very promising trace element toward public health.

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