Zinc and Health: Current Status and Future Directions

Zinc-Altered Immune Function and Cytokine Production[1]

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ABSTRACT Although the intriguing role of zinc as an essential trace element for immune function is well established, particular progress in determining the molecular principles of action of this ion was made recently. Leukocyte responsiveness is delicately regulated by zinc concentration. Zinc deficiency as well as supraphysiologic levels impair immune function. Furthermore, the activities of many immunostimulants frequently used in immunologic studies are influenced by zinc concentration. Therefore, our knowledge from in vitro studies is widely dependent on the zinc concentration, and when not in physiologic range, immunologic responses are artificially low. Decreased production of TH1 cytokines and interferon-α by leukocytes in the healthy elderly person is correlated with low zinc serum level. The defect in interferon-α production is reconstituted by the addition of physiologic amounts of zinc in vitro. Interestingly, zinc induces cytokine production by isolated leukocytes. Zinc induces monocytes to produce interleukin-1, interleukin-6 and tumor necrosis factor-α in peripheral blood mononuclear cells and separated monocytes. This effect is higher in serum-free medium. However, only in the presence of serum does zinc also induce T cells to produce lymphokines. This effect on T cells is mediated by cytokines produced by monocytes. Stimulation also requires cell-to-cell contact of monocytes and T cells. Information is presented to illustrate the concepts that the zinc concentration must be taken into account whenever in vitro studies are made or complex alterations of immune functions are observed in vivo. J. Nutr. 130: 1407S—1411S, 2000.

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Zinc is a cofactor of >300 enzymes. It is involved in a variety of general cellular functions, including signal transduction, transcription and replication (Coleman 1992, Vallee and Falchuk 1993). The immune system is strongly influenced by zinc, because it is one of the most highly proliferative organs. Furthermore, zinc specifically interacts with components of the immune system. The important role of zinc as an essential trace element for immune function has already been well established (Bach 1981, Crea et al. 1990, Cunningham-Rundles et al. 1980, Good 1981, Keen and Gershwin 1990, Kruse-Jarres 1989, Wellinghausen et al. 1997, Wellinghausen and Rink 1998). However, progress in determining the molecular principles of action of this ion was made recently. In this review, advances in the field of zinc immunology are focused on the interaction of zinc with human leukocytes on a cellular and molecular basis, the influence on immunostimulants and the therapeutic use of zinc. This review critically reflects the importance of knowledge about the zinc status for the interpretation of in vivo observations and in vitro experiments with leukocytes.

Zinc deficiency

The physiologic plasma zinc concentration is low (12–16 μM) and reflects a minor pool of total body zinc (Bettger and O'Dell 1993). However, it represents a very mobile and immunologically important pool. Zinc is transported to cells bound to proteins, predominantly albumin, α_2-macroglobulin and transferrin, but only free zinc ions seem to be biologically active (Borth and Luger 1989, Gless et al. 1992, Phillips 1976, Vallee and Falchuk 1993). The function of α_2-macroglobulin is regulated by zinc itself. Zinc alters the structure of α_2-macroglobulin and enhances its interaction with cytokines and proteases, and in this way, it indirectly influences immune function (Borth and Luger 1989, James 1990).

Various diseases associated with an impaired immune response are characterized by low plasma zinc levels or a noticeable zinc deficiency. Zinc absorption and nutritional aspects of zinc status are beyond the scope of this article and are reviewed in detail by others (Prasad 1995, Vallee and Falchuk 1993). Zinc deficiency can be studied in the zinc-specific malabsorp-

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tion syndrome, acrodermatitis enteropathica, a rare autosomal recessive inheritable disease (Neldner and Hambidge 1975). This extreme form of zinc deficiency shows thymic atrophy and a high frequency of bacterial, viral and fungal infections. Untreated, this disease is lethal within a few years, but pharmacologic zinc supplementation can reverse all symptoms (Neldner and Hambidge 1975). Impairment of immune function has been attributed to zinc deficiency in other conditions like malnutrition and in certain malignancies as well (Good 1981, Prasad et al. 1997, Schloen et al. 1979). Furthermore, a decreased serum zinc level is observed in chronic inflammatory or infectious diseases. This often reflects a redistribution of serum zinc into the liver within the acute phase reaction, caused by increased production of proinflammatory cytokines, mainly interleukin (IL)[3]-1 and IL-6, and the subsequent induction of zinc-binding metallothionein in hepatocytes (Kushner 1982, Singh et al. 1991).

Zinc therapy

In acrodermatitis enteropathica as well as in unspecific malabsorption syndromes, zinc has been successfully used to restore impaired immune functions (Cunningham-Rundles et al. 1980, Neldner and Hambidge 1975). A major and unresolved question is the optimal therapeutic dosage of zinc. The dosage to reverse zinc deficiency should be adapted to the actual requirements to avoid negative effects on the immune system. Therefore, the control of plasma zinc concentration, not exceeding 30 μmol/L, may be a limiting factor, as discussed later. However, the immunosuppressive effect of zinc may be a completely new therapeutic tool for the selective suppression of lymphocyte functions. In comparison to conventional immunosuppressive drugs, zinc has the advantage of being extremely nontoxic, even in dosages well exceeding the recommended dietary intake (Fosmire 1990). Interestingly, in rheumatoid arthritis, an autoreactive T-cell disease, diminished plasma zinc levels have been reported (Naveh et al. 1997). Oral application of zinc sulfate over a 12-wk period has shown a clear clinical benefit with regard to joint swelling, morning stiffness and estimation of overall disease activity in rheumatoid arthritis patients (Simkin 1976). This effect might be due to the T-cell inhibitory influence of zinc.

Low serum zinc levels and impaired immunologic functions are reported in patients undergoing hemodialysis and in elderly individuals, selected according to the immunogerontologic SENIEUR (Lighart et al. 1984) protocol (Bonomini et al. 1993, Cakman et al. 1996, Fraker et al. 1986, Sandstaed et al. 1982). Significantly, decreased zinc values (Bonomini et al. 1993) or concentrations at the lower limits of the normal range (perhaps due to zinc supplementation via the dialysate) are described in hemodialysis patients. These low zinc level have clinical relevance because they are related to an impaired immune response to diphtheria vaccination (Kreft et al. 2000). The immune defects in elderly individuals mainly concern cell-mediated immunity, including a diminished T-cell count, dysfunction of T helper cell subpopulations and a decreased secretion of interferon (IFN)-α after virus stimulation in vitro (Cakman et al. 1996, Sandstaed et al. 1982). Interestingly, the plasma zinc levels are significantly lower compared with the control groups but still within the normal range (Cakman et al. 1996), a situation similar to that seen in hemodialysis patients. The diminished IFN-α secretion by elderly individuals has been fully reconstituted by the in vitro addition of zinc. However, high dose zinc supplementation, achieving 7–8 times the physiologic value, blocks IFN-α induction in elderly individuals (Cakman et al. 1997). Zinc has also been shown to be effective in the treatment of the common cold (Al-Nakib et al. 1987, Eby et al. 1984, Mossad et al. 1996), but there are several possible explanations for this effect (Bashford et al. 1986, Korant and Butterworth 1976, Pasternak 1986, Ratka et al. 1989), which are discussed in detail by P. Fraker and J. L. Jackson in this supplement. There are limited data about zinc status in human immunodeficiency virus–infected patients. A decreased serum zinc level is repeatedly reported for human immunodeficiency virus–infected patients, but the relationship between zinc status and disease progression is conflicting (Baum et al. 1995, Beach et al. 1992, Koch et al. 1996). Independent of disease progression, hypozincemia has been associated with a higher incidence of opportunistic bacterial infections (Koch et al. 1996), and oral zinc supplementation leads to an increase in the CD4 count and to a reduced incidence of opportunistic infections (Isa et al. 1992, Mocchegiani et al. 1995b). These reports clearly show that zinc supplementation has some clinical benefit by restoring impaired immune function. However, the molecular basis of these effects is largely unknown (Rice et al. 1995). Furthermore, zinc influences the in vitro systems to investigate the immune response. Some of the molecular mechanisms of zinc treatment and the interference with immunostimulants are discussed next.

Zinc in innate immunity

Natural killer (NK) cell activity, phagocytosis of macrophages and neutrophils and certain functions like chemotaxis and generation of the oxidative burst are impaired by decreased zinc concentrations in vivo (Allen et al. 1983, Keen and Gershwin 1990). The p58 killer cell inhibitory receptor (KIR) on NK cells requires zinc for the recognition of major histocompatibility complex (MHC) class I molecules on target T cells (Rajagopalan et al. 1995). Zinc may not only influence NK cell–mediated killing but also could modulate cytolytic T-cell activity (Mingari et al. 1998). Besides reduced NK- and T-cell functions, a reduced capability of mononuclear phagocytes to kill intracellular *Trypanosoma cruzi* has been reported in zinc-deficiency states (Cook-Mills et al. 1990, Wirth et al. 1989). The deprivation of essential nutrients such as iron and zinc represents a simple but effective mechanism to fight foreign pathogens. The S-100 Ca^{2+} binding protein calprotectin (formerly known as L1 protein) chelates zinc when released by the degradation of neutrophils. Zinc chelation within abscesses inhibits the reproduction of bacteria and *Candida albicans* (Clohessy and Golden 1995, Murthy et al. 1993, Sohnle et al. 1991).

Zinc and T cells

Initial evidence for the essential role of zinc in the immune system was related to its importance for the development of T cells. Zinc deficiency causes thymic atrophy, and the thymus changes are reversible by zinc supplementation (Mocchegiani et al. 1995a), confirming that zinc interferes with the earliest steps of T-cell maturation. This effect depends in part on the regulation of thymulin, an important thymic hormone secreted by thymic epithelial cells (Dardenne et al. 1982, Hadden 1992). For thymulin, zinc is an essential cofactor. Differentiation of immature T cells in the thymus is induced by thymulin. Furthermore, thymulin regulates the functions of

[3] Abbreviations used: IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NK, natural killer; PBMC, peripheral blood mononuclear cells; PKC, protein kinase C; TNF, tumor necrosis factor.

mature T cells in the periphery. Modulation of cytokine secretion by peripheral blood mononuclear cells (PBMC) and proliferative effects on CD8 T cells in combination with IL-2 were reported for thymulin (Coto et al. 1992, Safie-Garabedian et al. 1993). The expression of the high affinity receptor for IL-2 on mature T cells (Tanaka et al. 1989) is induced by thymulin. This is in agreement with the observation that zinc deficiency is associated with decreased T-cell proliferation after mitogen stimulation (Crea et al. 1990, Dowd et al. 1986). Zinc regulates lymphocyte homeostasis not only by maintaining proliferation but also by suppressing death, namely through the inhibition of apoptosis as discussed by Peter Zalewski in the present supplement (Sundermann 1995, Zalewski and Forbes 1993). However, cell activation by zinc could also prevent the cells from undergoing apoptosis.

Direct effects of zinc on mononuclear cells

One of the first observations regarding the interaction of zinc with leukocytes was that zinc ions induce blast transformation in human lymphocytes (Berger and Skinner 1974, Kirchner and Rühl 1970, Rühl et al. 1971). More recently, it was discovered that zinc induces a specific cytokine response in human PBMC. Zinc stimulated PBMC in a dose-dependent manner to release IL-1, IL-6, tumor necrosis factor (TNF)-α and IFN-γ ((Driessen et al. 1994, Salas and Kirchner 1987, Scuderi 1990). IL-1, IL-6 and TNF-α are directly induced in monocytes by zinc. This effect is independent of the presence of lymphocytes, because separated monocytes and monocytic cell lines respond to zinc (Driessen et al. 1994). At least for TNF-α it has been shown that cytokine release after zinc stimulation is caused by the induction of mRNA transcription rather than by the enhanced translation or stabilization of already expressed mRNA (Wellinghausen et al. 1996a).

In contrast to the direct stimulation of monocytes, the stimulative effect on T cells represents an indirect effect that is dependent on monocytes in the culture (Driessen et al. 1994, Rühl and Kirchner 1978, Wellinghausen et al. 1997). IFN-γ and sIL-2 receptor release by T cells is mediated by monocyte-released IL-1 and IL-6 and a cell-to-cell contact between monocytes and T cells (Driessen et al. 1994, Wellinghausen et al. 1997). Zinc fails to induce cytokine production in isolated and monocyte-depleted T cells (Hadden 1995, Wellinghausen et al. 1997), B cells (Crea et al. 1990), NK cells (Crea et al. 1990) or neutrophils (Rink et al. unpublished results). Despite the different response of these leukocyte subsets, the zinc-mediated activation of monocytes and T cells is strongly regulated by the protein composition of the culture medium. Insulin and transferrin, common supplements in serum-free cell culture media, specifically enhance zinc-induced monocyte activation by a non–receptor-dependent mechanism (Crea et al. 1990, Driessen et al. 1995c, Phillips and Azari 1974, Wellinghausen et al. 1996b). However, complete fetal calf serum in the culture medium prevents monocyte stimulation by low zinc concentrations due to binding of free zinc ions. In serum-free culture medium, higher zinc concentration (~100 μM) stimulates monocytes but inhibits T-cell functions. This may depend on the cellular tolerance of zinc in these leukocyte subsets. T cells have a lower intracellular zinc concentration than monocytes. Furthermore, T cells are more susceptible to increasing zinc levels than monocytes (Bulgarini et al. 1989, Goode et al. 1989). In conclusion, indirect T-cell stimulation by zinc takes place only in concentrations high enough for monokine induction but not exceeding the critical concentration for T-cell suppression. The physiologic zinc level obviously represents a concentration that ensures optimally balanced T-cell function.

We recently discovered a zinc-dependent mechanism of T-cell inhibition. IL-1–dependent proliferation of the T cell line D10 is inhibited by high zinc concentration. The molecular basis of this effect is the zinc-specific inhibition of the IL-1 type I receptor–associated kinase at a concentration that represents ~8 times the physiologic serum level (Wellinghausen et al. 1997). This in vitro observation correlates with the T cells' inhibitory effect after high dose zinc supplementation in vivo as observed in clinical studies (Chandra 1984, Duchateau et al. 1981). Interestingly, much lower concentrations of zinc, representing 3–4 times the physiologic zinc level, inhibit alloreactivity in the mixed lymphocyte culture model (aCampo C., Wellinghausen, N., Faber, C., Fischer, A. & Rink, L. unpublished results).

As described, high zinc concentrations are inhibitory for T-cell functions, but sometimes T-cell functions also are dysregulated in moderate zinc deficiency. For example, rheumatoid arthritis and other diseases with autoreactive T-cell pathology are often associated with low serum zinc levels (Simkin 1976). Low zinc intake during pregnancy and decreased plasma zinc levels correlate with an increased risk of preterm delivery and abortion (Bedwal and Bahuguna 1994, Favier 1992, Jameson 1993), which might reflect the activation of normally suppressed alloreactive T cells. In conclusion, T-cell function is delicately regulated by the concentration of zinc in the cell or plasma.

Molecular basis of zinc-mediated effects

As described earlier, zinc has a number of effects on leukocytes in vivo and in vitro. However, we still do not know how zinc mediates its effects and how it enters the cells. Here we describe some recent insights into zinc signaling. The transferrin receptor (CD71) was considered to be a particular candidate for specific zinc uptake, because some of the serum zinc is bound to transferrin (Cunningham-Rundles et al. 1980, Mathe et al. 1985, Phillips 1976). However, data clearly indicating CD71 as a zinc receptor are not available, and thus no specific receptor that facilitates zinc uptake or triggers intracellular messages has yet been confirmed. Zinc enters the cell within minutes and its uptake increases a little during the first hours (Naber et al. 1994, Wellinghausen et al. 1996b). Surprisingly, in contrast to its divergent effect on monocytes and T cells, zinc uptake does not differ significantly in either cell population (Wellinghausen et al. 1997). The majority of zinc that is taken up by PBMC is bound to proteins, because the total intracellular zinc concentrations exceed the amount of free zinc (Rink et al. unpublished results).

So far we have fragmentary information that zinc signaling does occur. Protein tyrosine kinases, as well as cAMP- and cGMP-dependent protein kinases, are clearly involved in zinc-mediated stimulation (Wellinghausen et al. 1996b). Furthermore, zinc increases the activity of protein kinase C (PKC) and regulates its intracellular translocation, but zinc is not part of the active center (Csermely et al. 1988, Zalewski et al. 1990). Structural stabilization of PKC depends on a unique zinc cluster motif (Vallee and Falchuk 1993). However, an involvement of PKC in zinc-induced signal transduction in PBMC has not been confirmed (Wellinghausen et al. 1996b). Zinc is also integrated in the active center of phospholipase C, but its effect on cell activation is questionable (Coleman 1992). Immunologically more important than a specific interaction of zinc with certain molecules is a general influence of zinc on the fluidity of lipids and thus also of biological mem-

branes as discussed by others in the present supplement (Bettger and O'Dell 1993, Chvapil 1976, Kruse-Jarres 1989).

Zinc-altered activity of immunostimulants

Experimental immunology mainly depends on experimental subsets where leukocytes are stimulated by immunostimulants. Therefore, current knowledge about different leukocyte subsets is based on stimulation of the cells with specific or unspecific stimulants. The independence of the immunostimulant from the changes in the experimental system to be evaluated is a prerequisite. Here we summarize that zinc alters the function of different immunologically relevant mitogens and bacterial stimulants. Interestingly, one of the earliest reports about the immunobiology of zinc concerned its comitogenic activity with phytohemagglutinin (Duchateau et al. 1981, Fraker et al. 1986, Warner and Lawrence 1986). Whether this effect depends on an alteration of phytohemagglutinin or whether zinc is really comitogenic is still unresolved. Another explanation is that zinc enhances the activity of contaminating lipopolysaccharide (LPS). Zinc, even in substimulatory concentrations, acts synergistically with LPS with respect to cytokine induction in leukocytes (Driessen et al. 1995a and 1995b). This synergism is based on a specific zinc-induced structural alteration of LPS. Through the addition of zinc, LPS is transformed in its biologically more active, less-fluid form (Wellinghausen et al. 1996). Due to the described synergism in even minimal, substimulating concentrations of both LPS and zinc (Driessen et al. 1995c), exceptional care must be exercised in cell culture experiments to exclude unwanted effects.

In contrast to its synergism with LPS, zinc inhibits the function of some bacterial superantigens (Driessen et al. 1995a and 1995b). Zinc inhibits only superantigens binding to the MHC class-II β-chain, like *Staphylococcus aureus* enterotoxins (SE) A, D and E and the *Mycoplasma arthritidis* superantigen (Bernatchez et al. 1997, Fraser et al. 1992, Kim et al. 1994, Sundström et al. 1997). The interaction between these superantigens and the MHC-II β-chain is mediated by a zinc cluster involving amino acids from the superantigen and histidine-81 of the MHC-II β-chain. High-dose zinc might saturate both sites independently, thus preventing complex formation. The absence of a zinc-binding motif in superantigens that bind only to the MHC-II α-chain explains the lack of inhibition by excess zinc. Interestingly, zinc influences superantigens in a second way. SED forms homodimers due to zinc bridges, which have the capacity for T-cell–independent interaction with MHC-II molecules, resulting in direct monocyte activation (Sundström et al. 1996).

Zinc is important for leukocyte functions both in vivo and in vitro. The interaction within the immune system is complex and delicately regulated by zinc. Zinc deficiency leads to dysfunction of the immune system, but in addition, high doses of zinc have negative effects on leukocyte functions. Although knowledge about the molecular mechanisms of zinc has increased during the past years, we still do not know the most effective therapeutic dosage. From in vitro studies we learned that zinc levels of >30 μM were found to have more inhibiting than stimulating effects to the immune system. However, these inhibiting effects might be useful as a new therapeutic tool. Because most experimental systems in immunologic research depends on the stimulation of leukocytes in vivo or in vitro, the modulation of immunostimulants by zinc is a trap. Zinc-specific alteration of the activity of stimulants might mimic effects on the immune system. As a consequence, the zinc concentration should be considered whenever complex alterations of immune functions are observed in vivo or in vitro.

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