*BioMetals* **14:** 367–383, 2001.



*Review*

# Extracellular and immunological actions of zinc

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Received 27 December 2000; accepted 15 March 2001

*Key words:* cell biology, immunology, review, trace elements, zinc

**Abstract**

Zinc is an essential trace element for the immune system, but also very important in other organ systems. Every highly proliferating cell system is dependent on sufficient availability of zinc. During the last decades the influence of zinc on various cell systems have been investigated. Multiple effects of exogenously added zinc have been described in *in vitro* culture systems and in *in vivo* systems. However, most of these effects are so far poorly understood, and the dosages used in the *in vitro* systems are not comparable and sometimes unphysiologically high. Especially in the immune system a number of effects were described and over the last ten years we have come to understand some molecular mechanisms of zinc in this cell system. A zinc deficiency is accompanied by an immunodeficiency, resulting in an increased number of infections. However, the immune function is delicately regulated by zinc, since both increased and decreased zinc levels result in a disturbed immune function. Therefore, zinc supplementation must be accurately supervised. In this review, we discuss the activity of extracellular zinc in four sections. 1. The effect of zinc on different *in vitro* cell systems, including keratinocytes, osteocytes and leukocytes, and the concentrations of zinc needed for a specific cell response. 2. The modulation of the innate immune system *in vitro* and *in vivo*. 3. The role of zinc in the B cell response and antibody production. 4. Effects of zinc on the development and function of T cells.

*Abbreviations:* AIDS – acquired immune deficiency syndrome; BSA – bovine serum albumin; CD – cluster of differentiation; FCS – fetal calf serum; FIV – feline immune deficiency virus; HLA – human leukocyte antigen; IFN – interferon; Ig – immunoglobulin; IL – interleukin; IRAK – interleukin 1 receptor associated kinase; KIR – killer cell inhibitory receptor; LPS – lipopolysaccharide; MHC – major histocompatibility complex; MLC – mixed lymphocyte culture; MLR – mixed lymphocyte reaction; MT – metallothionein; NDV – Newcastle disease virus; NK – natural killer; PBMC – peripheral blood mononuclear cells; PMA – phorbol myristate acetate; PMN – polymorphonuclear neutrophils; ROS – reactive oxygen species; SF – serum free; SOD – superoxide dismutase; STZ – serum-treated zymosan; TCR – T cell receptor; TH – T helper; TNF – tumor necrosis factor; ZIP – zinc regulated metal transporter (ZRT) iron regulated metal transporter (IRT) like protein; ZnT – zinc transporter.

## Introduction

Zinc is an essential trace element for all organisms (Raulin 1869; Todd *et al.* 1934). In mammals, a zinc deficiency is primarily observed by its effects on highly proliferating cell systems like the skin and the immune system. Prasad *et al.* (1963) described a zinc deficiency syndrome in children from Persia practicing geophagia, which was characterized by anaemia, hypogonadism, hepatosplenomegaly, skin alterations, growth and mental retardation. With the discovery of acrodermatitis enteropathica (a rare autosomal recessive inheritable disease) it was clearly shown that these symptoms are dependent on zinc deficiency due to

a zinc-specific malabsorption syndrome (Neldner & Hambidge 1975). This disease shows a number of immunological alterations like thymic atrophy and a high frequency of bacterial, viral and fungal infections. Without treatment, this disease leads to death within a few years, whereas pharmacological zinc supplementation can reverse all symptoms (Neldner & Hambidge 1975). Since these early observations there is no doubt about the importance of zinc for the integrity of the immune system. During the last two decades, a number of reviews have reflected these issues. However, the groups focused on different topics other than the *in vivo* mouse model (King *et al.* 1995), *in vitro* systems (Bach 1981; Wellinghausen *et al.* 1997a; Wellinghausen & Rink 1998; Rink & Kirchner 2000), clinical trials (Prasad 2000) or nutritional aspects of zinc and immunology (Rink & Gabriel 2000).

The major problem in zinc biology is that there is no specialized zinc storage system in the body. Therefore we have to reach a steady state of zinc intake and excretion. The bioavailability of zinc depends on the composition of the diet and is influenced by a number of different factors, as reviewed elsewhere (Valberg *et al.* 1984; Favier & Favier 1990; Rink & Gabriel 2000). Besides the composition of the diet, the constitution (Weiss *et al.* 1998; Klainman *et al.* 1981; Yuzbasiyan-Gurkan *et al.* 1989) and age (Cakman *et al.* 1996; Rink *et al.* 1998) of the consumer is important for zinc resorption, leading to a number of contradictory recommendations according to the daily intake of zinc (Rink & Gabriel 2000).

Due to these problems, clinical trials are somewhat problematic. The total body content of zinc in humans is 2–4g, but zinc is called a trace element since its plasma concentration is only 12–16 $\mu$M (definetively normal) and with ranges from 10.1–16.8 $\mu$M in women and 10.6–17.9 $\mu$M in men. However, the plasma pool is the smallest zinc pool in the body, but a highly mobile and immunologically important one (Mills 1989; Favier & Favier 1990). In the serum, zinc is predominantly bound to albumin (60%, low-affinity), $\alpha_2$-macroglobulin (30%, high-affinity) and transferrin (10%) (Scott & Bradwell 1983). These distributions and affinities are also important for *in vitro* culture systems.

## Zinc supplementation in *in vitro* systems

The effect of extracellularly added zinc ions was investigated in different cell systems. However, the effective zinc dosages are difficult to compare due to the fact that different culture media and zinc sources were used. Generally, higher zinc dosages are needed, if the culture medium contains serum. Therefore, the percentage of serum as well as the source of the serum is important, since some zinc binding proteins are enhanced in fetal serum and the total protein content varies between different species. Furthermore, some zinc effects, like the IFN-$\gamma$ induction in T cells (Driessen *et al.* 1994; Wellinghausen *et al.* 1997b), is only observed in the presence of serum. This problem indicates that serum- or protein-free media are not an advantage every time, but the zinc effect is more clear and the amounts of zinc to be used are strongly reduced in comparison to media containing serum. However, the composition of serum-free media is not normally published by the manufacturer. Since most serum-free and all protein-free media (to the authors' knowledge) contain zinc themselves, the real zinc concentration is questionable for the investigator and the reader. The same is true for some conventional cell culture media, which vary in their zinc content from minute amounts up to 3 $\mu$M zinc, which is all below the stimulatory level. In serum-free media, albumin, transferrin and insulin are the normal protein supplements. All three have a zinc-binding capacity and influence the zinc-dependent response of the investigated cells (Wellinghausen *et al.* 1996b). Table 1 summarizes the effects of zinc in different *in vitro* cell systems like: keratinocytes, monocytes, T cells, thymocytes, neutrophils, neuroblastoma cells, pheochromocytoma cells, hepatocytes, fibroblasts, spermatozoa, astrocytes, osteocytes, osteoblasts, osteoclasts, epithelial cells and pancreatic islet cells.

Zinc has various effects on completely different cell systems, but the mechanism of zinc influx was controversial for a long period of time. Zinc added to a cell culture enters the cells within minutes (Wellinghausen *et al.* 1996b; Reyes 1996). Recently, Gaither & Eide (2000) described a human zinc transporter (hZIP) for zinc uptake from the environment, whereas so far only some zinc-specific transporters (ZnT) which avoid the efflux of zinc from intracellular pools were described. The ZnTs seem to be involved in intracellular redistribution of zinc and were first described in the nervous system (Palmiter & Findley 1995; Palmiter *et al.* 1996a,b; Tsuda *et al.* 1997). There is no report as to whether or not the ZnTs are associated with zinc uptake, whereas the transferrin receptor (CD71) and calcium ion channels were discussed in terms of unspecific transport of zinc in addition to facilitated

*Table 1.* Effect of extracellular zinc in *in vitro* cell systems. The table gives examples for *in vitro* effects of zinc on different cell types. Interestingly, the effective zinc content showed extreme variation. The examples are listed in increasing zinc amounts in the experimental system. Furthermore the culture conditions are indicated, since the protein amounts influences the free zinc content as discussed in the text.

| Zinc [$\mu$M] | Medium* | Cell type | Effect | Reference |
|---|---|---|---|---|
| $10^{-8}$–100 | BSA | rat osteoclasts | zinc is a highly potent inhibitor of osteoclastic bone resorption | Moonga & Dempster 1995 |
| 2–8 | SF | human keratinocytes | zinc gluconate induces the expression of V$\alpha$, $\alpha$3, $\alpha$2 & $\alpha$6 - integrins | Tenaud *et al.* 1999 |
| 2–20 | SF | human keratinocytes | zinc gluconate reduces the very late antigen(VLA)-3 expression induced by nickel gluconate | Sainte-Marie *et al.* 1998 |
| 25 | FCS | human neuro-blastoma BE(2)-cells | zinc sulfate decreases the level of apoptosis in neuronal cells exposed to toxin | Ho *et al.* 2000 |
| 50 | FCS | rat astrocytes | zinc chloride protects diethyldithiocarbamate-mediated toxicity associated with an increase of MT concentration | Wilson & Trombetta 1999 |
| 15–100 | SF | D10 N T-cell line | 100 $\mu$M zinc sulfate inhibits the IL-1 type I receptor-associated kinase (IRAK) | Wellinghausen *et al.* 1997 |
| 100 | FCS | HeLa human Hepa mouse | zinc sulfate facilitates activation of the DNA binding activity of recombinant MTF-1 | Bittel *et al.* 1998 |
| 10–100 | FCS | Osteoblast-like cells MC3T3-E1 | zinc sulfate inhibits mineralization during tissue formation | Togari *et al.* 1993 |
| 100 | FCS | PBMC of HIV positive patients | zinc chloride decreases the percentage of apoptotic cells compared with cells treated only with PHA | Neves *et al.* 1998 |
| 100 | FCS | mouse pancreatic islets | zinc sulfate induces MT to protect islets against toxicity mediated by reactive oxygen species | Ohly & Gleichmann 1995 |
| 100 | BSA | human monocytes | zinc aspartate moderately activates monocytes | Herold *et al.* 1995 |
| 50–150 | FCS | human keratinocytes | 100 $\mu$M zinc chloride induces cell proliferation | Parat *et al.* 1999 |
| 25–200 | BSA | chicken osteocytes | zinc sulfate alters bone resorptive rates | Chen *et al.* 1998 |
| 80–200 | FCS | mouse thymocytes | zinc sulfate induces apoptosis in CD4+CD8+$\alpha\beta$ $TCR^{lo}CD3\varepsilon^{lo}$ thymocytes | Telford & Fraker 1995 |
| 12–250 | FCS | intestinal epithelial cell line IEC-6 | zinc sulfate promotes intestinal epithelial wound healing by enhancement of epithelial cell restitution | Cario *et al.* 2000 |
| 30–250 | FCS | human PBMC | zinc sulfate induces IL-1, 6, TNF-$\gamma$, sIL-2R & IFN-$\gamma$ | Wellinghausen *et al.* 1996a,b, Driessen *et al.* 1994 |

| | | | | |
|---|---|---|---|---|
| 250 | FCS or SF | monocytes, Mono-Mac cell line | zinc sulfate induces IL-1$\beta$ in both systems and SF or FCS containing medium | Driessen *et al.* 1994 |
| 3–300 | FCS | rat pheo-chromocytoma PC12 cells | zinc potentiates the dopamine release evoked by ATP | Koizumi *et al.* 1995 |
| 30–300 | FCS | human PBMC | zinc chloride reduces the frequency of cell division and induces blast formation | Santra *et al.* 2000 |
| 100–300 | FCS | swiss 3T3 mouse fibroblasts | zinc chloride mimics the action of growth factors on intracellular MAP kinase activation and protein tyrosine phosphorylation | Hansson 1996 |
| 25–500 | FCS | mouse thymocytes | zinc sulfate inhibits glucocorticoid induced apoptosis in mouse thymocytes. Zinc concentrations lower than 25 $\mu$M had to be combined with ionophores. Concentrations between 80–200 $\mu$M induce apoptosis. | Telford & Fraker 1995 |
| 740 | FCS | neonatal mouse skin cells | zinc chloride protects against UV-induced genotoxicity | Record *et al.* 1996 |
| 500–1000 | BSA | human neutrophils | zinc chloride attracts leukocytes by inducing and promoting the chemotactic response | Hujanen *et al.* 1995 |
| 1000 | SF | human spermatozoa | zinc chloride elicits an inhibition of superoxide anion production and SOD-like activity | Gavella *et al.* 1999 |

*Culture conditions without serum (SF), with serum (FCS) or bovine serum albumin (BSA)

diffusion through amino acids and anionic exchange (Bentley 1992; Hogstrand *et al.* 1996). However, there are also contradictory reports for these mechanisms (Wellinghausen *et al.* 1996b). Since the exogenously added zinc increases the free intracellular zinc about 70% (measured by zinquin), but the total zinc uptake is about 300–600%, depending on the cell system (measured by atomic absorption spectroscopy), there must be a fast binding process to intracellular proteins (Wellinghausen *et al.* 1996b; Fischer *et al.*, manuscript in preparation). Both, the free and the total zinc uptake shows a fast increase within the first minutes and a saturation at the described maxima after 30–60 min (Wellinghausen *et al.* 1996b; Fischer *et al.*, manuscript in preparation). The new described human ZIP seem to be the main way of zinc influx into human cells (Gaither & Eide 2000), but their distribution in leukocyte subsets is so far not investigated.

## General effects on eukaryotic cells influencing immune functions

Zinc is a cofactor for more than three hundred enzymes out of all six classes of enzymes (Coleman 1992a,b; Vallee & Falchuk 1993) as it is important for the structural integrity or enzymatic activity of the enzymes (reviewed in Rink & Gabriel 2000). Furthermore, zinc modulates the activity of a number of enzymes. Factors interacting with DNA or RNA, like transcription and replication factors, contain a zinc finger motif (reviewed in Rink & Gabriel 2000). Therefore, a variety of general cell functions are influenced by the zinc concentration. For this reason, cell

proliferation is strictly zinc-dependent and, without zinc, highly proliferating cell systems, like the immune system, the skin and the reproductive system, show diverse dysfunctions. The dysfunction reflects two aspects, the ageing of the cells with functional deficits and the missing regeneration of the system by the production of new completely functional cells. Furthermore, different factors important for signal transduction need zinc for a regular function (reviewed by Beyersmann & Haase in this issue and Rink & Gabriel 2000).

Apoptosis, the physiological method of programmed cell death, is very important in the development and differentiation of complex organisms. The apoptosis is regulated by zinc (reviewed by Truong-Tran *et al.* in this issue). Especially in the immune system regulation and normal function are strictly dependent on apoptosis to exclude autoimmune T cells and B cells and to kill infected or tumorous cells by cytotoxic T cells or NK cells without side effects (reviewed by Wellinghausen & Rink 1998; Rink & Gabriel 2000).

These different zinc effects are very important but not restricted to any cell or organ system, as shown by the *in vitro* systems above. However, a slightly decreased zinc status may first influence the immune system, due to an increased number of infections. Despite these general consequences, there are also some direct effects of zinc on the immune system.

## Modulation of immunological functions by zinc

The immune system can be divided into different parts. The first line of defense is the innate immune system with granulocytes, monocytes and natural killer (NK) cells. These cells are completely differentiated in the peripheral blood and do not need further education for their function. Therefore, the response is very fast but lacks a memory. In contrast, the specific immune system, with the two parts, humoral (B cells) and cellular immunity (cytotoxic T cells) are produced as precursors and educated to recognize their specific antigen in the thymus (T cells) or bone marrow (B cells). The resulting naive (before antigen contact) lymphocytes differentiate after antigen contact into effector cells and memory cells. The memory cells are the basis for the immunological memory and the stronger reaction to a known antigen as a secondary response. Nowadays there is no doubt that zinc is an essential trace element for the immune system. The effects of zinc are multi-faceted and influence the innate as well as the specific part of the immune system. Furthermore, not only proliferation of the immune system depends on zinc but also the proliferation of the pathogens, thus decreasing zinc in the plasma is one acute phase response in infection. However, cellular and molecular mechanisms of zinc within the immune system were discovered only during the last 10 years.

### *Innate immunity*

The earliest step of an immune response is the recruitement of leukocytes from the blood stream to the infected tissue via chemotaxis, adhesion and diapedesis of the leukocytes. Zinc induces adhesion of myelomonocytic cells to the endothelium, whereas zinc chelation diminishes cell recruitment (Chavakis *et al.* 1999). The chemotaxis of neutrophils, the step before the adhesion, is decreased under zinc deficiency *in vivo*. *In vitro*, zinc itself showed a chemotactic activity on neutrophil granulocytes (PMN) (Hujanen *et al.* 1995). However, more important for the PMN is the general effect of zinc on cell proliferation, since PMN are produced and released by the bone marrow at a rate of 60 million cells per minute. Furthermore, the main functions of the cells of the innate immune system are impaired under zinc deficiency: natural killer (NK) cell activity, phagocytosis of macrophages and neutrophils, and generation of the oxidative burst (Keen & Gershwin 1990; Allen *et al.* 1983). Neutrophils do not respond with cytokine production to zinc, but seem to have an influence on the viability of these short-living cells (unpublished data). This may be due to the fact that PMN contains a high concentration of zinc binding proteins. Release of the S-100 $Ca^{2+}$ binding protein calprotectin during degradation of neutrophils inhibits reproduction of bacteria and *Candida albicans* by zinc chelation (Murthy *et al.* 1993; Clohessy & Golden 1995, Sohnle *et al.* 1991). Effects on neutrophil granulocytes are summarized in Figure 1.

The influence of zinc on NK cells could be partially explained on the molecular level, since zinc is required for the interaction of the p58 killer cell inhibitory receptor (KIR) on NK cells with MHC class I molecules (mainly HLA-C) on target cells (Rajagopalan *et al.* 1995). In contrast to the influence on the killer inhibitory signal, the positive signals did not require zinc (Rajagopalan *et al.* 1995). This may result in unspecific killing and functional loss of NK cells during zinc deficiency. In healthy elderly persons

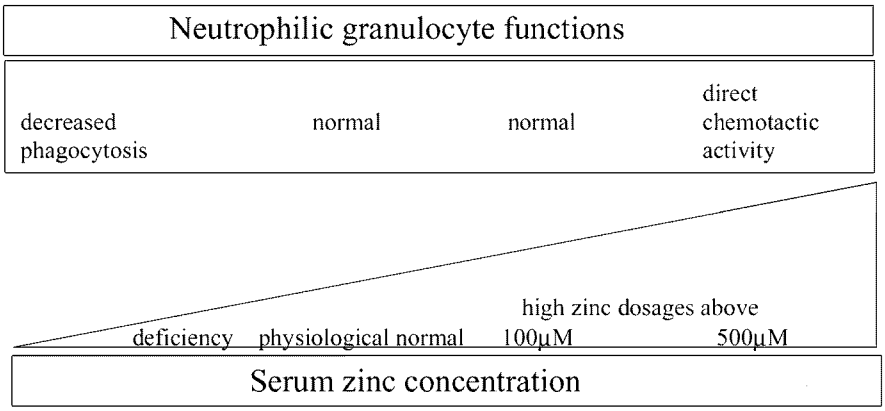


*Fig. 1.* Influence of zinc on the function of neutrophil granulocytes. Neutrophil increase their main immune functions with increasing zinc concentrations.

(SENIEUR-elderly), a group with decreased serum zinc without malnutrition, the number of NK cells is increased, but the killing activity is decreased (Rink *et al.* 1998; Rink & Seyfarth 1997). This effect on NK cells is also observed under experimental conditions of zinc deficiency *in vivo* and *in vitro* (Prasad 1998, 2000). However, *in vitro* zinc showed no effect on purified NK cells (Crea *et al.* 1990). Effects on NK cells are summarized in Figure 2.

A number of effects of zinc on monocytes were described *in vitro*. Zinc induced activation (Herold *et al.* 1993) and cytokine production in isolated monocytes as well as in monocytic cell lines (Driessen *et al.* 1994; Wellinghausen *et al.* 1997b). Furthermore, a number of cytokines induced in peripheral blood mononuclear cells (PBMC) could be related to being produced by the monocyte fraction, such as IL-1, IL-6 and TNF-$\alpha$ since these cytokines are produced in the absence of T cells as well (Driessen et al. 1994; Wellinghausen *et al.* 1996, 1997). At least for TNF-$\alpha$ it was shown that zinc induced a de novo synthesis of the mRNA (Wellinghausen 1996a). Monocyte activation by zinc is specifically enhanced by insulin and transferrin in the culture medium, whereas high serum content of the medium prevents the stimulation (Crea *et al.* 1990; Phillips & Azari 1974; Driessen *et al.* 1995; Wellinghausen *et al.* 1996b). This synergism is not mediated by the specific receptors (Wellinghausen *et al.* 1996b). Under serum-free conditions 50–100 $\mu$M zinc are sufficient for cytokine induction in monocytes, whereas under serum supplementation 250 $\mu$M are necessary (Driessen *et al.* 1994, 1995; Wellinghausen *et al.* 1996b). How monocytes are directly activated by zinc is unresolved, but protein tyrosine kinases as well as cAMP- and cGMP-dependent protein kinases are clearly involved (Wellinghausen *et al.* 1996). Monocytes showed a higher tolerance to exogenous zinc than lymphocytes, but there is no difference in the zinc uptake (Goode *et al.* 1989; Bulgarini *et al.* 1989; Wellinghausen *et al.* 1996b, 1997b). Interestingly, monocytes from zinc-deficient elderly persons showed a higher proinflammatory cytokine response to lipopolysaccharide and phorbol ester stimulation and have a preactivation of monocytes (Rink *et al.* 1998; Fagiolo *et al.* 1993, and unpublished data). On the other hand, *in vitro* zinc supplementation could restore the defective IFN-$\alpha$ production of PBMC (note that monocytes and dendritic cells are the main IFN-$\alpha$ producers) from zinc deficient elderly (Cakman *et al.* 1997). The effects on monocytes are summarized in Figure 3. In conclusion, the innate immune system needs zinc for the generation of the great number of cells and for the function on a molecular level. The effects on the innate immune system are summarized in Tables 2a–d.

*B cells*

Although B cells are the producers of antibodies and therefore the most important cells of the humoral immunity, there is little knowledge about these cells with regard to zinc. Zinc itself seems to have no direct influence on the activity of B cells (Crea *et al.* 1990). However, zinc deficient patients, like elderly and hemodialysis patients, showed a reduced response to vaccination (Fraker *et al.* 1986; Lighart *et al.* 1984; Bonomini *et al.* 1993; Sandstead *et al.* 1982; Cakman *et al.* 1996). For hemodialysis patients, at least we were able to correlate the response to the serum zinc concentration (Kreft *et al.* 2000). However, vari-

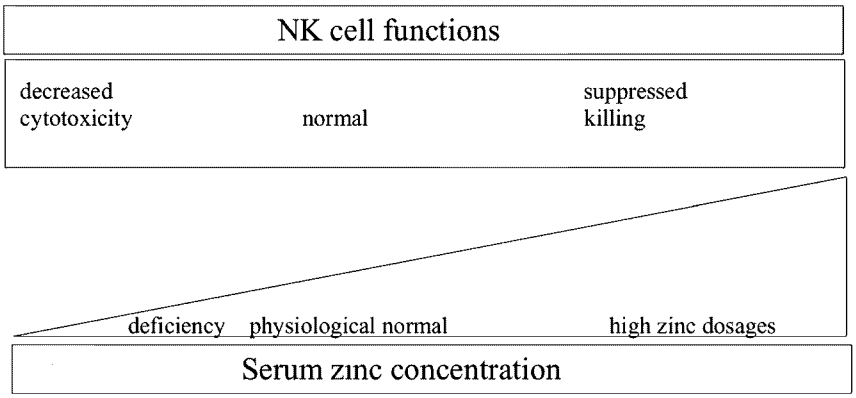


*Fig. 2.* Influence of zinc on the function of natural killer (NK) cells. Only zinc levels within the normal range can guarantee effective NK cell function.

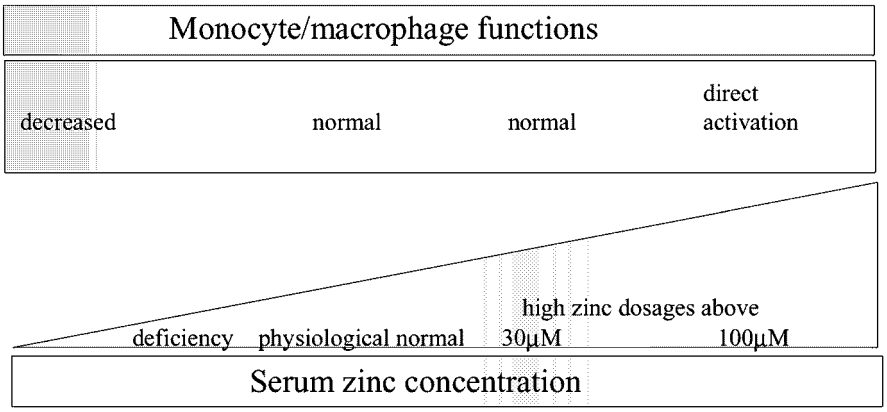


*Fig. 3.* Influence of zinc on the function of monocytes and macrophages. Monocytes are the only cell population that can be directly induced by zinc ions.

*Table 2a.* Innate immunity: zinc deficiency *in vivo*. Cells of the innate immune system showed impaired *in vitro* functions after *in vivo* zinc deficiency.

| Experimental system | Effect | Reference |
|---|---|---|
| NK cells | NK cell lytic activity decreases after 20 weeks of deficiency | Prasad 2000 |
| Elderly subjects | Reduced IFN-$\alpha$ production after stimulation with NDV | Cakman et al. 1997 |
| NK cells | NK cell lytic activity is decreased in zinc deficiency | Prasad 1998 |
| Zinc deficient diet for 3 weeks (Rat model) | Decreased NK and LPS activated NK cell activity is associated with zinc deficiency | Ozturk *et al.* 1994 |
| Human granulocytes | Zinc deficiency showed *in vivo* the reduction of the oxidative burst | Keen & Gershwin 1990<br>Allen *et al.*1983 |
| Human monocytes | Zinc deficiency impairs phagocytosis | Allen *et al.*1983<br>Keen & Gershwin 1990 |

*Table 2b.* Innate immunity: zinc supplementation *in vitro*. The *in vitro* supplementation of zinc can reverse or rarely improve immune functions with regard to cytoprotection or specific capabilities of immune cells.

| Experimental system | Effect | Reference |
|---|---|---|
| AK-5 cells, NK cells | Pretreatment of AK-5 with zinc sulfate resulted in complete inhibition of antibody-dependent NK-induced DNA fragmentation | Bright *et al.* 1995 |
| Human granulocytes: phagocytosis and killing of *S. aureus* and *S. epidermidis* | Cytoprotection of zinc against staphylococcal toxins | Sunzel *et al.* 1995 |
| Rat granulocytes isolated from peritoneal cavity | 1 mM zinc chloride attracts leukocytes by inducing and promoting the chemotactic response | Hujanen *et al.* 1995 |
| Isolated human monocytes | Zinc stimulates monocytes, no other isolated cell component of the human blood responds with stimulation | Wellinghausen *et al.* 1997 |
| Septic rat monocytes | Zinc inhibits the superoxide production after stimulation of both PMA and STZ | Srinivas *et al.* 1989 |
| NK cells, target clones HLA-Cw4 and 8 | Zinc is required for HLA-C mediated protection from lysis by NK cells | Rajagopalan 1995 |

*Table 2c.* Innate immunity: zinc supplementation *in vivo*. The *in vivo* zinc supplementation can modify and reverse immune dysfunctions caused by mild or severe zinc deficiency.

| Experimental system | Effect | Reference |
|---|---|---|
| Human monocytes from patients with leukemia | Orally administered zinc aspartate increases the capacity of monocytes to release of ROS after *in vitro* stimulation | Herold 1993 |
| Plasma of cervical carcinoma patients | Zinc supplementation increases IL-2 production of PBMC and restores thymulin production and NK cytotoxicity | Mocchegiani *et al.* 1999 |
| Septic rat monocytes | Increased superoxide production after PMA or STZ stimulation | Srinivas *et al.* 1989 |
| NK cells | NK cell lytic activity returns to normal range | Prasad 2000 |

*Table 2d.* Innate immunity: therapeutic zinc application. Four main examples regarding the therapeutic use of zinc as a modulator of the immune system. The positive effect of using orally applicated zinc solutions is supported by these examples.

| Disease | Possible effect of zinc | Reference |
|---|---|---|
| Common cold | Zinc gluconate stabilizes the cell membrane against viral penetration and increases IFN-$\alpha$ | Mossad *et al.* 1996 |
| Acrodermatitis enteropathica | Increases NK cell activity with regard to IL-2 induction and increases the phagocytic activity of phagocytes | Prasad *et al.* 1995 |
| Rheumatoid arthritis | impairment of PMN phagocytosis | Zoli *et al.* 1998 |
| Herpes simplex infection | Increased IFN-$\alpha$ production | Varadinova *et al.* 1993 |

*Table 3a. In vitro* effects of zinc deficiency on B cell functions (mouse model). Specific cell experiments support the hypothesis that B cell maturation depends on zinc.

| Cell type | Effect | Reference |
|---|---|---|
| precursor B cells ($CD45^+$ $CD43^-IgM^-$) | zinc deficiency induces apoptosis and reduces cell count 50–70% | Fraker *et al.* 2000 |
| Immature B cells ($CD45^+IgM^+IgD^-$) | zinc deficiency induces apoptosis and reduces cell count 50–70% | Fraker *et al.* 2000 |
| Pro-B cells (CD $45^+CD43^+6C3^+$) | high bcl-2 level protects against apoptosis caused by zinc deficiency | Fraker *et al.* 2000 |
| Mature B cells ($IgM^+IgD^+$) | high bcl-2 level protects against apoptosis caused by zinc deficiency | Fraker *et al.* 2000 |

*Table 3b. In vivo* effects of zinc deficiencies on B cells. *In vivo* zinc deficiency experiments support the findings made in *in vitro* models.

| Cell type | Effect | Reference |
|---|---|---|
| B cells | 91% decrease in severely deficient mice; 43% decrease in moderately deficient mice | Fraker *et al.* 1995 |
| $CD45^+$ $IgM^-$ | 56–96% decrease | Fraker *et al.* 1995 |
| Immature B cells $CD45^+$ $IgM^+$ | 35–80% decrease | Fraker *et al.* 1995 |
| Mature B cells $CD45^+IgM^+IgD^+$ | 5–70% decrease | Fraker *et al.* 1995 |
| B cells | IgM, IgG & IgA levels are increased | Rink & Seyfarth 1997 |

*Table 3c. In vitro* effects of zinc supplementation on B cells. *In vitro* supplementation with zinc reverses the dysfunctions induced by the zinc deficiency.

| Cell type | Effect | Reference |
|---|---|---|
| 70Z/3 murine pre-B leukemia cell line | zinc induces IL-4 associated CD5 downregulation | Jyonouchi *et al.*1991 |

*Table 3d. In vivo* effects of zinc supplementation on B cells. Zinc supplementation *in vivo* benefits the treated subjects according to the immune function.

| Subject | Effect | Reference |
|---|---|---|
| 6–35-month-old infants | lower respiratory infections were reduced by 10 mg/d | Fraker 2000 |
| Elderly | improved IgG antibody response to tetanus vaccine | Duchateau *et al.* 1981b |

ous vaccination studies were done with additional zinc supplementation, but in most cases, there was no increase of the antibody titer against the vaccine (Rawer *et al.* 1987; Grekas *et al.* 1992; Brodersen *et al.* 1995; Turk *et al.* 1998; Provinciali *et al.* 1998). The major problem in all these studies was, that the zinc uptake was not controlled and that the amount of zinc applied to the probands was not comparable and sometimes definitely too high (400 mg/day), since different groups reported a suppression of immune functions at high zinc dosages like 100 mg/day (Porter *et al.* 1977; Chandra 1984; Patterson *et al.* 1985; Provinciali *et al.* 1998; Rheinhold *et al.* 1999; Rink & Kirchner 1999). But if zinc supplementation is done in the right way, IgG response to vaccination could be improved (Duchateau *et al.* 1981b). This may be related to the induction of apoptosis in immature B cells and B cell precursors by zinc deficiency (Fraker *et al.* 2000). Since mature B cells due to a high Bcl-2 level are more resistant to zinc deficiency, B cell memory is less affected than a primary response, like initial vaccination (Fraker *et al.* 2000). Other possible mechanisms are the increase of IFN-$\alpha$ production by zinc (Cakman *et al.* 1997) or the restoration of impaired T cell help (Sandstead *et al.* 1982; Mocchegiani *et al.* 1995a). Both these explanations could also explain the failure of studies with high zinc dosages, since these inhibit IFN-$\alpha$ production as well as T cell functions (Cakman *et al.* 1997; Wellinghausen *et al.* 1997b). Effects of zinc on B cells and B cell functions are summarized in Figure 4 and Tables 3a–d.

*T cells*

One of the first *in vivo* observations regarding zinc was thymic atrophy, which resulted in an impaired T cell development and decreased T cell counts (Osati-ashtiani *et al.* 1998; Fraker *et al.* 1995). Essential steps in thymic function are dependent on the thymic hormone thymulin (a nonapeptide), which is only active after binding of zinc as a cofactor (Bach 1981, 1983). Thymulin is secreted by thymic epithelial cells and induces markers of differentiation in immature T cells (Saha *et al.* 1995). Besides these intrathymic functions on thymocytes and immature T cells, thymulin also acts on mature peripheral T cells. It modulates the cytokine release by PBMC and induces proliferation of CD8 T cells in combination with IL-2 (Coto *et al.* 1992; Safie-Garabedian *et al.* 1993). Therefore, zinc influences immature and mature T cells through the activation of thymulin. As expected, substitution of zinc can reverse the zinc deficiency-induced changes in the thymus and on peripheral cells (Mocchegiani *et al.* 1995). This effect is also observed in AIDS patients (Mocchegiani *et al.* 1995). In contrast to other lymphocyte populations, a direct effect of zinc on T cells was observed. Thirty years ago it was first described that zinc induced blast transformation in human lymphocytes (Berger & Skinner 1974; Sood *et al.* 1999; Kirchner & Rühl 1970; Rühl *et al.* 1971). Furthermore, zinc induced the expression of the high affinity receptor for IL-2 (Tanaka *et al.* 1989), one effect resulting in decreased proliferation of T cells in zinc deficiency (Crea *et al.* 1990; Dowd *et al.*

*Table 4a. In vitro* effects of zinc deficiency on T cell function. Zinc is essential for the effectiveness of T cells according to their immune functions.

| Cell type | Effect | Reference |
|---|---|---|
| HUT-78 ($Th_0$ T cell line) precultured in zinc deficient medium | IL-2 gene expression, IL-2R and NF-$\kappa$B is reduced | Prasad 2000 |

*Table 4b. In vivo* effects of zinc deficiency on T cells. *In vivo* experiments point out the predominant role of zinc according to T cell maturation.

| Experimental system | Effect | Reference |
|---|---|---|
| Dietary induction of zinc deficiency | within 8 weeks reduced lymphocyte, granulocyte, and platelet counts | Prasad 2000 |
| Dietary induction of zinc deficiency | reduced thymulin activity in serum. Imbalance of $TH_1$ and $TH_2$. Decrease in the percentage of $CD_8^+CD_{73}^+$ T cells (cytotoxic T cell precursor) | Prasad 1998 |
| Elderly subjects | Reduced T cell counts | Cakman *et al.* 1997 |
| $Th_1$-T cells of zinc deficient subjects | Lower IL-2 and IFN-$\gamma$ production | Prasad 2000 |

1986). IL-2 itself, as well as the soluble IL-2 receptor (sIL-2R) and interferon (IFN)-$\gamma$ (all mainly T cell products) are induced by zinc in human PBMC (Salas & Kirchner 1987; Scuderi 1990; Driessen *et al.* 1994). However, at least the induction of IFN-$\gamma$ is dependent on the presence of monocytes (Salas & Kirchner 1987; Driessen *et al.* 1994; Rühl & Kirchner 1978; Wellinghausen *et al.* 1997b). The secretion of IFN-$\gamma$ by T cells depends on the induction of IL-1 in monocytes, since anti-IL-1 could inhibit the T cell activation (Driessen *et al.* 1994). However, zinc concentrations over 100 $\mu$M in serum-free culture medium stimulate monocytes but inhibit T cell functions, since T cells have a lower intracellular zinc concentration and are more susceptible to increasing zinc levels than monocytes (Goode *et al.* 1989; Bulgarini *et al.* 1989; Wellinghausen *et al.* 1997b). Since the increase of intracellular free zinc in monocytes and T cells is equal after exogenous addition of zinc (Wellinghausen *et al.* 1996b, 1997b), the lower tolerance leads to a T cell blockade. Therefore, stimulation of monocytes and T cells by zinc is dependent on the amount of free zinc ions as a counterpart to the protein composition of culture media, as discussed above.

While the zinc-induced activation of T cells is IL-1-dependent, the molecular mechanism is the inhibition of the IL-1 type I receptor associated kinase IRAK by zinc (Driessen *et al.* 1994; Wellinghausen *et al.* 1997b). This mechanism is also the basis for the inhibition of the IL-1-dependent growth of the murine IL-1 indicator cell line D10 (Wellinghausen *et al.* 1997b). Whereas for the IL-1 blockade amounts of 100 $\mu$M are necessary, the alloreactivity of T cells in the mixed lymphocyte reaction/culture (MLR or MLC) could already be inhibited by amounts over 50 $\mu$M (Campo *et al.* 2001).

In contrast to T cell stimulation, T cell inhibition by an excess of zinc could also be observed *in vivo* (Chandra 1984; Duchateau *et al.* 1981a), but these effects are similar to those observed in zinc deficiency. This means, that the T cell activity is critically regulated by the zinc concentration. This may be the reason why some autoimmune diseases with a T cell pathology, like rheumatoid arthritis, are associated with moderate zinc deficiency (Simkin 1976). In some clinical trials, at least a zinc supplementation reduced the pain score in rheumatoid arthritis (Simkin 1976). This led to the presumption that zinc deficiency increased allo- or autoreactivity, whereas it is inhibited by high zinc dosages. The observation that decreased plasma zinc levels in preganancy are associated with an increased risk of preterm delivery and abortion fits in

*Table 4c. In vivo* effects of zinc supplementation on T cells. Zinc supplementation can reverse the T cell dysfunctions caused by zinc deficiency.

| Zinc supplementation | Effect | Reference |
|---|---|---|
| 25 mg zinc sulfate for the treatment of residents | Zinc increases the number of $CD_4^+DR^+$T cells and cytotoxic T-lymphocytes | Fortes *et al.* 1998 |
| 2 mg/kg/d zinc acetate | Weight gain and recovery from marasmus | Castillo-Duran *et al.* 1987 |
| Zinc supplementation | Zinc restores the decreased thymulin activity | Prasad 1998 |
| Oral zinc supplementation in old mice for 1 month | Full recovery of thymic functions after zinc supplementation | Mocchegiani *et al.* 1995 |
| Elderly subjects | Restores T-cell help | Cakman *et al.* 1996 |
| Low weight infants | Doubled responders to DTH after zinc supplementation of 2 mg/kg/d | Fraker *et al.* 2000 |

*Table 4d. In vitro* effects of zinc on T cells. Zinc effects on T cells seem to be contradictory because they stimulate T cell functions and decrease alloreactivity and apoptosis.

| Cell type | Effect | Reference |
|---|---|---|
| PBMC with regard to T cells | Zinc induces an increased IFN-$\gamma$ production | Driessen *et al.* 1995 |
| Enriched human T cells and D10 mouse T cells | Higher zinc concentrations directly inhibit the IL-1$\beta$ dependent T cell stimulation | Wellinghausen *et al.* 1997 |
| Mixed lymphocyte culture (MLC) | Zinc suppresses alloreactivity in the MLC | Campo *et al.* 2001 |
| Interleukin-2-dependent feline T-lymphocyte cell line inoculated with NCSU-1 (FIV) were supplemented with 1 mM zinc chloride | Zinc decreases the percentage of cells undergoing apoptosis and prevented the loss of $CD_4^+$ lymphocytes | Johnson *et al.* 1996 |

*Table 4e.* Zinc therapy with regard to T cells. The predominant effects caused by zinc deficiency in regard to T cells are based on a zinc dependent T cell maturation. So these defects can be reversed by zinc supplementation.

| Disease | Possible zinc effect | Reference |
|---|---|---|
| Sickle cell disease | Reconstitution of thymocyte function decreases hospitalization and vaseoocclusive pain | Prasad *et al.* 1999 |
| Acrodermatitis enteropathica | Zinc reconstitutes thymocyte functions and reverses all skin and systemic symptoms | Prasad 1995 |
| AIDS | Zinc inhibits T cell apoptosis and increases thymocyte proliferation | Mocchegiani *et al.* 1995 |
| Rheumatoid arthritis | T cell suppression and blocking IL-1 signal transduction | Zoli *et al.* 1998 |
| Down syndrome | Zinc directly influences leukocytes and thymus hormones and reverses the haematological symptoms | Trubiani *et al.* 1996 |
| Crohn's disease | Zinc suppresses T-cells and reconstitutes the thymus function. This results in an alleviation of skin lesions and improvement of visual acuity | Brignola *et al.* 1993 |

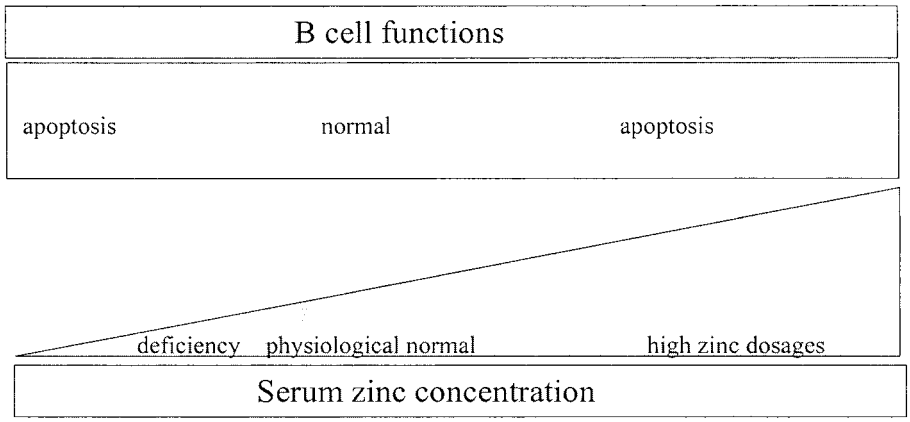


*Fig. 4.* Influence of zinc on the function of B cells. The normal range of zinc concentration is obligatory for the correct B cell function. B cells sensitively respond to zinc level changes.

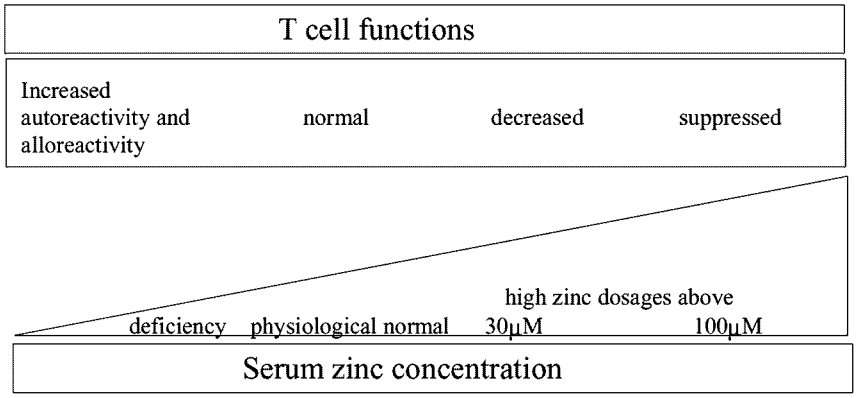


*Fig. 5.* Influence of zinc on the function of T cells. T cell functions are delicatly regulated by the serum zinc level. Low zinc increases abnormal functions whereas high zinc amounts unspecifically suppresses T cells.

with this model (Bedwal & Bahuguna 1994; Jameson 1993; Favier 1992).

In conclusion, the T cell activity is regulated by zinc and the normal physiological value seems to be slightly below the optimal concentration of T cell functions. A further reduction of this value leads to a dysfunction with autoreactivity, whereas at concentrations above 30 $\mu$M the T cell inhibitory effects of zinc take place. These inhibitory effects of zinc might be a new tool for zinc therapies. The influence of zinc on T cells *in vivo* and *in vitro* is summarized in Figure 5 and Tables 4a–e.

## Perspective

The reviewed data clearly indicate that zinc is essential for an intact immune system. However, we need more information on a molecular basis to understand the role of zinc on the different cell subsets. Furthermore, investigations regarding the dose response of zinc have to be done in detail, since we still do not know the best effective dose of zinc *in vitro* and, more importantly, *in vivo*. Controlled clinical studies, with zinc supplementation and longitudinal measurement of the zinc content in the serum and cell compartments are missing. Since all experiments investigating the immune system need specific stimulants for the leukocyte subsets to be investigated, we also need further information regarding the influence of zinc on the stimulants themselves (reviewed by Wellinghausen & Rink 1998; Rink & Gabriel 2000). Since cytokine functions and detection are also influenced by zinc, i.e., through zinc-activated $\alpha_2$-macroglobulin, some results have to be reevaluated or the experimental design has to be changed (James 1990). In conclusion, it is difficult to find an immunostimulant and test system

completely independent of zinc in its function *in vivo* or *in vitro*.

However, the perspective of zinc in the immune system seems to be very powerful. On the one hand, zinc may be a new immunosuppressant to be used without massive side effects, a role of zinc which so far has not been investigated in detail. More important seems to be the use of zinc as a supplement for different patient groups and especially to increase the immune response in elderly persons. With this perspective, zinc may be the most important trace element in public health.

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