Allergy and Autoimmunity **Edited by: U. Sack/K. Conrad**

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The influence of the trace element zinc on the immune system

DOI 10.1515/labmed-2015-0060
Received February 6, 2015; accepted February 10, 2015

Abstract: Clinical and experimental examinations showed a close relationship between zinc as an essential trace element and the immune system. Thus, cellular and humoral components from both the innate and the adaptive immune system are affected by zinc. Human zinc deficiencies are frequently connected with disturbed immune functions. Controlled zinc substitution results in a normalization of zinc serum levels, zinc homeostasis, and the immunological parameters. As shown in in vitro experiments, low zinc concentrations stimulate functional parameters of immune cells, but high zinc concentrations are suppressive or cytotoxic for these cells. Recently, the immunosuppressive effect of zinc was demonstrated in animal models of T-cell-dependent autoimmune diseases, like experimental autoimmune encephalomyelitis. Moreover, decreased serum/plasma zinc concentrations have been detected in patients with different autoimmune diseases. Prospective studies should verify the possibility of controlled immunosuppressive zinc therapies for these diseases.

Keywords: autoimmunity; immunodeficiency; zinc; zinc status; zinc supplementation.

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Introduction

The essential trace element zinc is indispensable for many biological processes in humans, animals and plants. Zinc acts as a regulator or coenzyme of more than 300 enzymes, is a component of transcription factors, acts as an antioxidant, affects the stability of biological membranes and the assembly of multi-protein complexes, such as the T-cell receptor. In addition, zinc is involved in the synthesis of DNA and RNA as well as protein synthesis, regulates the expression of hormones and hormone receptors, and is important for the metabolism of neurotransmitters, growth, sex and thyroid hormones, as well as for the storage of insulin in the pancreas. This trace element is also ascribed a crucial role in the maintenance of sensory functions and immune homeostasis [1–4].

Zinc is a regulatory element or cofactor of many enzymes, including various dehydrogenases, carboanhydrases, matrix metalloproteinases, the angiotensin-converting enzyme, alkaline phosphatase, as well as DNA and RNA polymerases. It serves catalytic, co-catalytic and enzyme-structure-supporting functions [1, 2, 5]. By participating in cellular phosphorylation and dephosphorylation processes, zinc is able to act as an intracellular signaling molecule and thus modify the action of growth factors, hormones and cytokines [6, 7]. Various research groups have also discussed a direct role of zinc, as an intracellular “second messenger” [8]. Overall, these characteristics underline the essential role of zinc in growth, as well as in the development and maintenance of biological functions.

The average total zinc content of an adult is about 2–3 g [1]. The bones and muscles contain about 85% of the total body zinc, liver and skin about 11%, with the remaining 4% being found in other organs and tissues, especially in the prostate, testes, eyes, brain, heart, and pancreas. Human serum or plasma accounts only for approximately 0.1% of the total zinc. Approximately 99% of the trace element is located in the body cells. Fifty percent of this can be detected in the cytosol, and 30%–40% in

the nucleus; the remainder is membrane-bound [1, 9]. The intracellular zinc homeostasis is closely connected to the survival and development of cells, i.e. to the regulation of the cell cycle, the proliferation, differentiation and apoptosis [10, 11].

The regulation of the free and thus biologically active zinc ions in the cell plays a critical role in the development and progression of disease. This zinc homeostasis is controlled by numerous proteins. Unlike iron, which is mainly bound to heme, zinc maintains interactions with approximately 3000 human proteins [12]. Metallothionein and zinc transporters are the main components behind the maintenance of cellular zinc homeostasis. Metallothioneins are important for the resorption and storage of zinc. They, therefore, protect against metallotoxicity and oxidative stress. Zinc transporters ensure, in particular, the influx (ZIP transporters) and efflux (ZnT transporters) through cellular membranes [13]. Disorders of the zinc metabolism also affect mitochondrial function and can cause DNA damage.

To achieve a zinc homeostasis, the German Society for Nutrition, taking into account an average absorption rate of 30% as well as mandatory and optional zinc losses, has recommended a daily oral intake of 7 mg of zinc for women and 10 mg for men [14]. In particular, animal-based foods like poultry, beef, pork or dairy products are good sources of zinc and have a good bioavailability. Plant-based foods usually contain only small amounts of zinc and, due to a high content of phytate (phytic acid), can reduce zinc absorption significantly [1–3].

According to the World Health Organization (WHO), zinc deficiency is the fifth most common cause of high morbidity and mortality in developing countries [15–17]. But in industrialized countries of the West, too, it is assumed that approximately 40% of the population suffer from marginal, latent zinc deficiency [1]. In addition, in those countries, zinc deficiency is considered responsible for the pathogenesis of chronic inflammatory diseases such as diabetes mellitus, atherosclerosis, chronic liver, kidney, pancreatic and intestinal diseases, as well as rheumatic diseases.

A rapid increase in knowledge about the role of zinc in the physiological processes in various tissues and organs was achieved in the last two decades through the application of modern immunological, pharmacological, molecular-biological and physicochemical methods and procedures. Thus, close links between the trace element zinc and the immune system have been shown through clinical observations and experimental studies in the past [18–21]. Zinc, through its functional diversity, i.e. the regulation of many enzymes, transcription factors and zinc-binding proteins, influences components of both cellular and humoral immunity. The trace element affects the function and activity of cells of the innate and the adaptive immune system, such as neutrophils, granulocytes, monocytes/macrophages, NK-cells, dendritic cells as well as T- and B-lymphocytes. In addition, zinc acts on cytokine production, the activity of the complement system and antibody production [18–21].

Severe zinc deficiency in humans and zinc deficiency induced in animal experiments are associated with impaired immune cell function and consequently with secondary immunodeficiency. A number of clinical studies have shown that in those deficiency situations controlled zinc substitution results in a normalization of immunological parameters.

Depending on the cell-culture system used, low in vitro concentrations of zinc can have a stimulating effect on different functional parameters of immune cells of healthy test subjects; higher zinc concentrations, however, are suppressive or cytotoxic to these cells [2, 22, 23].

As human serum or plasma contains only approximately 0.1% of the total zinc content, the significance of an analysis of the respective zinc concentration is not optimal. Clearly diminished serum/plasma zinc concentrations have been detected in connection with a number of autoimmune diseases. Furthermore, data from animal studies suggest that in such diseases activated autoreactive T-cells might represent the target structure of a controlled zinc therapy. These aspects and issues will be discussed in more detail in the following sections.

Impact of zinc deficiency on the innate and adaptive immune system in vivo

Zinc deficiency can have alimentary (general malnutrition, phytate-rich diet or parenteral nutrition), iatrogenic (e.g. glucocorticoid therapy, contraceptives or penicillamine) or genetic (sickle cell anemia, acrodermatitis enteropathica) causes, as well as be brought on by absorption disorders (chronic inflammatory bowel disease, pancreatic insufficiency), increased zinc demand (during pregnancy, strong growth, chronic diseases, competitive sports) or increased zinc excretion (renal failure, liver cirrhosis) [1–3, 24]. Clinical symptoms of zinc deficiency can include diarrhea, increased susceptibility to infection, impaired wound healing, hair loss, dermatitis, decreased appetite, loss of taste, as well as impaired growth and reproductive capacity [2, 3].

Zinc homeostasis disorders affect both the innate and the adaptive immune system [17, 25]. Thus, zinc deficiency situations lead to decreased chemotaxis of *neutrophils* [26, 27]. Impaired or reduced activities and functions of these immune cells have been described in connection with zinc deficiency after parenteral nutrition and patients with Down syndrome [28–30]. In addition, it has been shown that zinc deficiency reduces the activity of NADPH oxidase of the neutrophils, resulting in a reduced formation of reactive oxygen species and reduced killing ability [31, 32]. Interestingly, the involvement of the trace element zinc in the regulation of the release of net-like extracellular structures, so-called "neutrophil extracellular traps" (NETs), has been described recently [33].

Furthermore, it has been demonstrated that zinc deficiency in vivo induces a reduced adhesion and chemotaxis of *monocytes* as well as a disturbance of macrophage maturation and activity [24–26]. It is interesting to note that an experimentally induced zinc deficiency in human monocytes produced both an increased elimination of bacterial pathogens by means of phagocytosis and respiratory burst and an inhibition of IL-6 and TNF-α production [34]. In contrast, studies on the monocyte/macrophage cell line HL60 have shown that zinc deficiency can induce a stimulation of the mRNA of the cytokines TNF-α, IL-1β and IL-8-mRNA [35].

Zinc deficiency also affects *NK-cells*. This leads to a decreased NK-cell count in peripheral blood and impaired functional activity of NK-cells. Thus, a reduced chemotaxis and decreased lysis of virus-infected cells or tumor cells have been detected [36, 37]. Prasad et al. [38] have been able to show that both tumor patients with zinc deficiency and healthy people with zinc deficiency exhibit diminished NK-cell activity due to eating habits, or in healthy people, due to experimentally-induced zinc deficiency (diet). Reduced NK-cell counts and NK-cell function have also been reported for elderly subjects with zinc deficiency [39]. A decreased number *of dendritic cells* (including Langerhans cells of the skin) has been detected both in patients with zinc deficiency and in an experimental murine system [40].

Deviations of zinc homeostasis, within the framework of the adaptive immune system, affect particularly strongly the formation, maturation and function of *T-cells* [41]. In the thymus, thymocytes (pre-T-lymphocytes) mature to T-lymphocytes, facilitated by the peptide hormone thymulin. This peptide hormone formed by thymic epithelial cells plays a key role in T-cell maturation in the thymus and requires zinc as an important structural element in order to be biologically active [42]. Consequently, zinc deficiency has a negative effect on the T-cell maturation in the thymus. As has been shown in mouse models, zinc deficiency causes thymic atrophy with an approximately 50% reduction in thymocyte counts [41, 43]. Under these deficient conditions, there is also an increased rate of apoptosis in the thymus, as has been demonstrated [44].

Furthermore, due to zinc deficiency situations in vivo, a decreased ability of mononuclear cells (MNC) from peripheral blood for mitogen-induced T-cell proliferation (lymphocyte transformation test) or cytokine production (for example, IL-2, IFNγ) has been observed. This has been confirmed for deficient conditions of the trace element after parenteral nutrition [28], in patients with liver cirrhosis [45, 46], in hemodialysis or peritoneal dialysis patients [47], in individuals with Down syndrome [30], in tumor patients and in subjects with zinc deficiency due to dietary habits (e.g. vegetarians) or after going on a diet [38].

Moreover, it has been shown that zinc also influences T-cell differentiation processes. As has been reported, children with diarrhea-caused zinc deficiency exhibit a reduced number of naive T-cells [48]. In addition, the $CD4^+/CD8^+$-T-cell ratio is reduced, in particular, by a decrease in the number of $CD4^+$ T-cells if there is a deficit of this biometal [49]. Likewise, there is a Th1/Th2 imbalance with a reduced number of Th1-T-cells and decreased Th1 cytokine production (IL-2, IFN-γ, TNF-α), whereas the Th2 cells are affected to a minor degree [49, 50].

Apart from this, zinc deficiency causes *B-cell* maturation and T-cell-dependent antibody production to be reduced [25].

In the synopsis of the data one can see that zinc deficiency conditions with different causes impair immune cell functions and thus produce secondary immune deficiency (refer to Figure 1). In a series of clinical studies and animal-experiment studies, it has been shown that, in connection with zinc deficiency, a controlled zinc substitution results in a normalization of the body's zinc homeostasis and immunological parameters described (see also "Substitution of zinc deficiency in vivo").

Influence of zinc on the function of immune cells in healthy test subjects in vitro

The influence of zinc compounds or commercial zinc preparations on the functions of the immune cells of healthy test subjects was studied intensively in different cell-culture systems in the past (especially MNC, isolated monocytes and isolated T-cells) [1, 18–21].

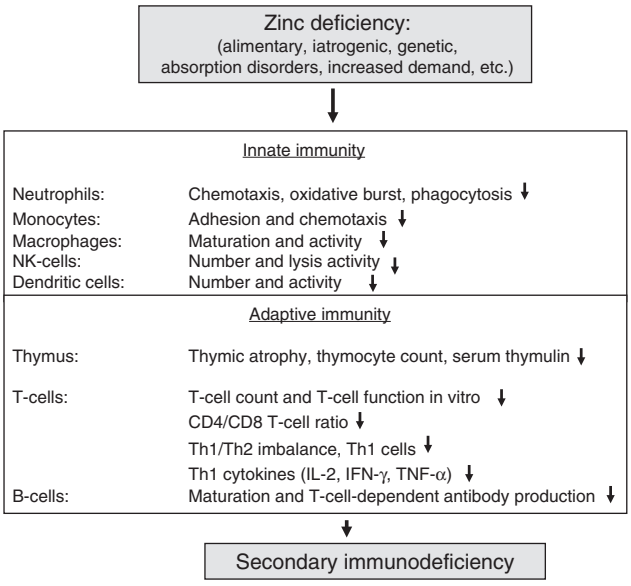

Figure 1: Relationship between zinc deficiency, the dysfunction of the immune system and associated secondary immunodeficiency.

Preincubation of human monocytes with $ZnCl_2$ concentrations (20–40 μM), non-toxic for these cells, reduced the lipopolysaccharide (LPS)-induced monocyte activation (adherence, formation of reactive oxygen metabolites, IL-1β mRNA expression) [51]. Incubation of MNC from peripheral blood, that is, a cell mixture of monocytes, T-cells, B-cells and NK0 cells, with $ZnSO_4$ alone, however, led to an increased release of the cytokines IL-1β, IL-6, TNF-α and IFN-γ [52, 53]. In combination with LPS, $ZnSO_4$ (10–100 μM) induced in MNC also increased IL-1β- and IFN-γ production [54, 55]. The mechanism for this seems to lie in the influence on the mobility of the LPS chains by means of zinc [56]. Incubation of MNC with suboptimal concentrations of LPS and zinc also stimulated the IL-1β release [57]. Interestingly, low levels of zinc have no stimulatory effect on the mitogen-stimulated cytokine release of human MNC. In contrast, when stimulated with superantigens, cytokine production was impaired by $ZnSO_4$ [55].

Hayashi et al. [58] used Jurkat cells, a human T-cell line, to observe a zinc-dependent inhibition of IFN-γ production in the presence of elevated zinc concentrations.

Our research group has been able to document that $ZnCl_2$, ZnO or $ZnSO_4$, in a concentration range from 100 μM to 200 μM, impairs DNA synthesis (proliferation) and the production of different T-cell cytokines (IL-2, IL-6, IL-10) of mitogen-stimulated MNC and isolated T-lymphocytes in a dose-dependent manner [22]. Prasad et al. [59] and Sprietsma [60] first discussed an effect of zinc on the regulation of T-cell differentiation into Th1 and Th2 cells, in which, in particular, a decrease in Th1 cells occurs. Campo et al. [61] demonstrated that $ZnSO_4$ impairs the production of the Th1 cytokine IFN-γ of human MNC, stimulated by a mixed lymphocyte culture, in a dose-dependent manner. A biphasic effect of zinc on the IFN-γ production of human activated T-cells with a maximum stimulation of 3 μM and incipient inhibition from 25 μM zinc has been reported by Aydemir et al. [62].

Mouse models have shown further that higher zinc concentrations, by affecting the IL-6/STAT3 signaling cascade, also impair the development of Th17 cells, which play a key role in the pathogenesis of autoimmune diseases. In the process, preventive zinc additions via direct STAT3 binding inhibited the maturation of naive $CD4^+$ T-cells into Th17 cells. This led to the loss of the α-helical structure and development of STAT3 with concomitant loss of activity [63].

Our own studies on the effect of zinc hydrogen aspartate (Zink-HA, Unizink 50), a commercially available zinc preparation, on the proliferation and cytokine release of isolated human T-cells confirmed for the first time that Zink-HA inhibited the proliferation (DNA synthesis) of human anti-CD3/CD28 antibodies and mitogen-stimulated T-cells significantly in non-cytotoxic concentrations of up to 150 μM. Furthermore, Zink-HA suppressed the production of various Th1 (IL-2, IFN-γ, TNF-α), Th2 (IL5) and Th17 cytokines (IL-17, GM-CSF) in a dose-dependent manner. These results were confirmed on anti-CD3 antibody or mitogen-stimulated mouse splenocytes [64, 65].

We observed, moreover, that depending on the cell culture system, very high zinc concentrations (200–500 μM) were cytotoxic to pokeweed-mitogen (PWM)-stimulated human MNC and T-cells [22, 64, 65]. Other authors have also reported the toxic effect of high concentrations of zinc on lymphocytes and monocytes in vitro [66].

One possible explanation for the sometimes conflicting effects on MNC, monocytes and T-cells could be a concentration-dependent zinc effect on various signaling pathways of these cells and thus, *inter alia*, on cytokine production [20].

Consequently, stimulating and inhibiting effects can be created by different stimulations (LPS, mitogens, antibodies). The use of different zinc compounds (ZnO, $ZnCl_2$, $ZnSO_4$, Zink-HA) does not seem to have any influence.

In considering these results, it should be noted that the respective cell-culture systems and, in particular, the cell-culture media used have a decisive influence on the concentration of "free zinc ions", which act on immune cells and induce the effects variously observed. Haase et al. demonstrated recently that components of cell-culture media, such as albumin or human or calf serum,

significantly influenced the effect of zinc compounds in cell-culture experiments [67].

Zinc status in patients with autoimmune diseases

The quantification of zinc concentration in serum, whole blood, plasma (lithium heparinate) or 24 h urine should be carried out in approved clinical-chemistry laboratories and by flame atomic absorption spectroscopy, plasma emission spectroscopy or direct electro-thermal atomic absorption spectroscopy. Photometric methods are insufficient as a method of determination [68, 69].

Non-metal collection systems should be used for collecting blood samples. Hemolysis and the associated release of zinc from erythrocytes, for example, due to excessive congestion or aspiration during the collection of the blood sample must be avoided at all cost. Since the zinc concentration in the blood decreases after food intake, the blood should be collected on an empty stomach.

The reference range for adults is 9–18 μmol/L (serum), and 9–22 μmol/L for women and 12–26 μmol/L for men (plasma) [1, 68].

In recent years, attempts have been made by several research groups to find more suitable parameters for reliable statements about the human "zinc status" by analyzing the concentrations of zinc in cells (especially in erythrocytes, leukocytes and lymphocytes). Unfortunately, these efforts have not been successful so far. Attempts to infer from the expression of individual zinc-transporter proteins the "zinc status" of a patient have not been successful either [1, 69].

Due to the very small proportion of the total zinc content that human serum or plasma represents (only about 0.1%), the validity of an analysis of serum or plasma zinc concentrations is still a point of discussion and/or criticism. Still, serum zinc is currently considered the best biochemical indicator of the "zinc status" [70].

Thus, for the aforementioned zinc-deficiency conditions with alimentary, iatrogenic and genetic causes, it was possible to detect clearly reduced zinc serum/plasma concentrations for a number of chronic diseases, such as various autoimmune diseases [1, 25].

Autoimmune diseases affect approximately 5%–7% of the population and are, therefore, in terms of frequency, the third most common after cardiovascular diseases and cancer. In addition to a genetic predisposition, environmental variables and physical or psychological stress, other factors such as hormonal status, age and immune status appear to play a crucial role in the pathogenesis of autoimmune diseases.

The most common autoimmune diseases include autoimmune thyroid disease, rheumatoid arthritis (RA), celiac disease, type-I diabetes mellitus, systemic lupus erythematosus (SLE), autoimmune liver disease and multiple sclerosis (MS).

Various authors have observed reduced zinc concentrations in the serum or plasma of patients with RA compared with healthy subjects [71–75]. Yazar et al. [76], however, found no differences in the plasma zinc concentrations of patients with RA. Patients with celiac disease [77–80], type-I diabetes mellitus [81, 82], SLE [83, 84], autoimmune hepatitis [85, 86], primary biliary cirrhosis [87, 88] and pemphigus vulgaris [89] also exhibited decreased serum zinc concentrations.

Several authors have described decreased zinc concentrations in the plasma [90–92] or serum [93] of MS patients compared to healthy subjects. These findings are in contrast to observations made by Dore-Duffy et al. [94], who reported slightly elevated plasma zinc levels in a study of 68 MS patients compared to a healthy control group. Recently, Ghazavi et al. [95] analyzed the zinc concentrations in the serum of 60 MS patients. They also found significantly reduced zinc levels in the sera of the patients, as compared to those of a corresponding control group.

In conclusion, despite the existing problems related to the analysis/diagnosis of a zinc deficit in a variety of autoimmune diseases, there is a clear reduction of serum or plasma zinc levels. These results point to a potential regulatory role of zinc in the pathogenesis of autoimmune diseases. Immunosuppressive zinc therapy could potentially represent a novel therapeutic approach for such diseases (see also "Immunosuppressive zinc therapy in connection with autoimmune diseases in vivo").

Substitution of zinc deficiency in vivo

Upon occurrence of clinical zinc deficiency symptoms, the serum or plasma concentrations of the trace element should be analyzed. Zinc substitution is indicated in principle for all forms of laboratory-proven zinc deficiency. This therapy should always be conducted under the control of "serum zinc" in intervals of 6–8 weeks [96].

Zinc substitution is absolutely indicated in patients with acrodermatitis enteropathica. This disease is based on a gene mutation that encodes the intestinal zinc

importer ZIP 4. This leads to a severe zinc deficiency syndrome, whose immunological cardinal symptoms are characterized by thymic atrophy, quantitative and functional impairment of lymphocytes, and increased susceptibility to infection. Moynahan already wrote in 1974 [97] that zinc substitution in patients with acrodermatitis enteropathica can be a life-saving measure.

In internal medicine, chronic liver and intestinal diseases, as well as parenteral nutrition, are the main indications for zinc substitution. Accordingly, 70%–75% of patients with decompensated liver cirrhosis exhibit a more or less pronounced zinc deficiency. In various studies, an improvement of liver function, nutritional status and hepatic encephalopathy, as well as a decrease of ascites and an improvement of immunological parameters have been demonstrated in these patients following controlled zinc substitution [20, 96, 98–101].

A general dosage indication of a zinc preparation that is reliable in individual cases is hardly possible. Kästner [102] recommended a dose of ten times the regular daily intake of zinc with food (up to 10 mg per day for adults), by way of a zinc salt, for the optimal treatment of acrodermatitis enteropathica. According to Prasad [103], oral zinc doses of up to 45 mg of elemental zinc per day are considered non-toxic. Various authors have pointed out that at very high zinc doses (100–300 mg of zinc per day), disorders of the immune functions can be observed [16, 104, 105]. Our research group has found that zinc concentrations higher than 500 µmol (roughly equivalent to a daily dose of 45 mg elemental zinc) induce toxic effects on immune cells, while inhibiting DNA synthesis and cytokine production [22]. Overall, clinical and animal studies have shown that controlled zinc substitution in connection with zinc deficiency results in a normalization of the body's zinc homeostasis and immunological parameters (see Figure 2) [25].

Immunosuppressive zinc therapy in connection with autoimmune diseases in vivo

In recent years, the use of *immunosuppressive zinc therapy* has been considered as well, particularly in the context of T-cell-facilitated autoimmune diseases and transplant rejection reactions [16, 64, 65, 106]. This additional form of controlled zinc therapy is shown in the right section of Figure 2.

It must be noted at this point that so far no clinically applied, immunosuppressive high-dose zinc therapy has been established for any autoimmune disease.

The immunosuppressive effect of zinc has been confirmed via various autoimmune animal models in recent years. Tran et al. [107] and Ohkawara et al. [108] described a therapeutic effect of zinc compounds in connection with experimental colitis induced by dextran sulfate sodium (DSS).

Kitabayashi et al. [63] have shown a preventive effect of zinc in collagen-induced arthritis.

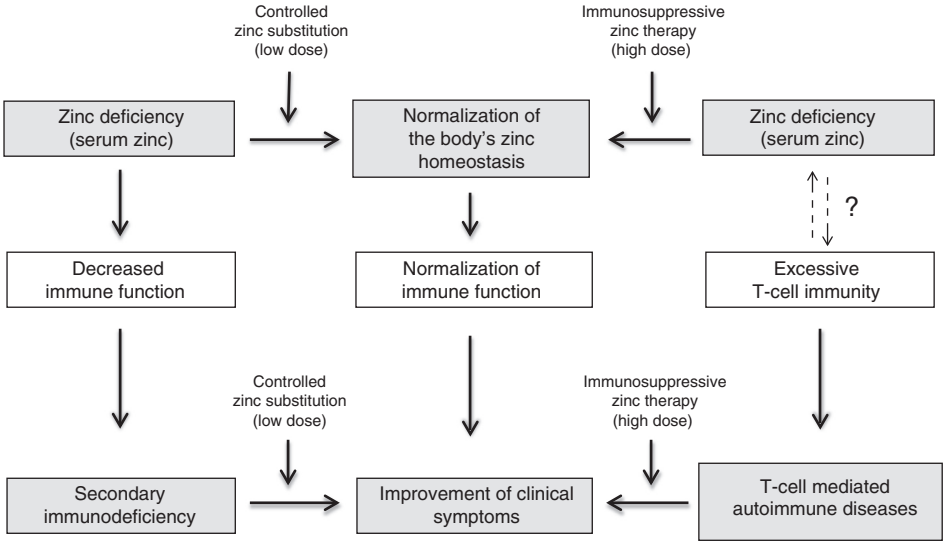

Figure 2: Representation of the relationship between zinc deficiency and secondary immunodeficiency with controlled zinc supplementation as well as in the case of a T-cell-mediated autoimmune disease with existing zinc deficiency and controlled immunosuppressive zinc therapy.

In an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), Penkowa et al. [109] and Kitabayashi et al. [63] observed a preventive effect on the severity of the disease. Our group also documented that Zink-HA (Unizink), a commercially available zinc preparation with excellent bioavailability, can reduce, preventively and therapeutically by both intraperitoneal and oral administration, the clinical symptoms of active EAE in SJL/J-mice significantly [64, 65]. In such T-cell-facilitated autoimmune diseases, thus, activated autoreactive T-cells could represent the target structure of an *immunosuppressive zinc therapy*. This form of therapy, too, should be conducted under close control of the "serum zinc level".

Outlook

Overall, it can be said that zinc is an important trace element for the homeostasis of the immune system. A large number of clinical symptoms are associated with a deficiency of this biometal. Currently, the validity of an analysis of the serum or plasma zinc levels is not deemed optimal in this context. For this reason, further studies are needed to arrive at standardized statements about the "zinc status" of a person.

Furthermore, one should examine the extent by which the application spectrum of controlled zinc substitution could be expanded. In addition, future clinical studies should investigate the possible use of controlled immunosuppressive zinc therapy in connection with T-cell-facilitated autoimmune diseases, such as MS.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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