T-Lymphocytes: A Target for Stimulatory and Inhibitory Effects of Zinc Ions

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Abstract: The trace element zinc is a crucial cofactor for many proteins involved in cellular processes like differentiation, proliferation and apoptosis. Zinc homeostasis is tightly regulated and disturbance of this homeostasis due to genetic defects, zinc deficiency, or supplementation influences the development and the progression of various infectious and autoimmune diseases. The immune system is strongly impaired during zinc deficiency, predominantly the cell-mediated response by T-lymphocytes. During zinc deprivation T-lymphocyte development, polarization into effector cells, and function are impaired. This leads to reduced T-cell numbers, a decreased ratio of type 1 to type 2 T-helper cells with reduced production of T-helper type 1 cytokines like interferon-gamma, and compromised T-cell mediated immune defense. Accordingly, disturbed zinc homeostasis increases the risk for infections, and zinc supplementation restores normal immune function. Furthermore, several disorders, like mycobacterial infections, asthma, diabetes, and rheumatoid arthritis are accompanied by decreased zinc levels and in some cases disease progression can be affected by zinc supplementation. On the molecular level, apoptosis of T-cell precursors is influenced by zinc *via* the Bcl-2/Bax ratio, and zinc ions inhibit caspases-3, -6, -7, and -8. In mature T-cells, zinc interacts with kinases involved in T-cell activation, like protein kinase C and the lymphocyte protein tyrosine kinase (Lck), while higher zinc concentrations are inhibitory, reducing the activities of the interleukin-1 receptor-associated kinase (IRAK) and calcineurin. Taken together, zinc homeostasis influences T-lymphocytes *via* several molecular targets, leading to a modulation of T-cell-dependent immune responses.

Key Words: T-lymphocytes, zinc, zinc deficiency, signal transduction, immune system.

INTRODUCTION

Zinc is an essential trace element with a variety of cellular functions in all organ systems [1]. It is a cofactor of more than 300 enzymes; it is crucial for the structural integrity of transcription and replication factors, e.g. in zinc finger motifs; and *in silico* analysis indicates the existence of potential zinc binding motifs in up to 10% of the genes of the human genome [1, 2]. Consequently, changes in the intracellular zinc concentration have a strong influence on processes like proliferation, differentiation, and apoptosis [3-5].

The human body has no zinc storage system, hence, a daily nutritional zinc uptake is necessary [6]. To supply the cells with the required amount of zinc, it is distributed *via* the serum, where the total concentration of 12-16 μM predominantly exists bound to several proteins like albumin, α-microglobulin, and transferrin [7]. Zinc uptake into the cells and its intracellular homeostasis are mediated by 15 proteins of the Zip (Zrt- and Irt-like protein) family, transporting zinc into the cytosol, 10 proteins of the zinc transporter (ZnT) family, transporting this ion out of the cytosol, and by zinc binding proteins, primarily metallothionein [8-10].

Disturbance of the cellular zinc availability, either by zinc deficiency or a dysfunction of zinc metabolism, leads to multiple disorders and disturbed cellular functions, and in severe cases even death [11, 12]. The symptoms of zinc deficiency include a compromised function of the immune system, which leads to an enhanced susceptibility to infectious diseases. Predominantly the cell-mediated immune response is influenced by zinc deprivation, and here in particular T-lymphocyte mediated functions [13, 14].

In this review, we will discuss the role of zinc in T-cell development and function, investigate its role in several infectious and autoimmune diseases, and the potential benefit of pharmacological zinc supplementation. Finally, we will review the evidence for a role of zinc ions in signal transduction processes of T-lymphocytes, and summarize the potential molecular targets for zinc in T-cell signaling.

EFFECTS OF ZINC DEFICIENCY ON T-LYMPHOCYTES

T-Lymphocyte Development

T-lymphocytes are the central part of the adaptive immune system, involved in the cell-mediated defense against pathogens and regulation of all immune responses. They emerge in the thymus and pass through different stages of development. Initially, they are so-called double negative cells, expressing no co-receptors on their surface ($CD4^-CD8^-$, pro-T-cells). After a double positive stage ($CD4^+CD8^+$, pre-T-cells), they become single positive T-lymphocytes ($CD4^+$ $CD8^-$ or $CD8^+CD4^-$), and leave the thymus as naïve T-cells.

Zinc deficiency leads to thymic atrophy and affects T-lymphocyte development [15]. Fraker and her group demonstrated impaired T-cell lymphopoiesis in chronically zinc-deficient mice, with substantial losses in the double positive $CD4^+CD8^+$ pre-T-cells [16]. Under the same conditions,

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myelopoiesis and B-cell lymphopoiesis were still intact. The T-cell deficits were due to increased apoptosis among pre-T-cells, and it was suggested that the reduced expression of anti-apoptotic proteins like Bcl-2 and Bcl-X_L causes an increased susceptibility of this cell type to apoptosis, compared to pro-T-cells, T-helper (Th), and cytotoxic T-lymphocytes (CTLs) [17-19]. In contrast, in an experimental model of human zinc deficiency the recruitment of naïve T-helper cells and the percentage of precursor cells of CTLs were both decreased during mild zinc deficiency, although these cells express relatively high levels of Bcl-2 [18, 20]. One possible explanation for this contradiction is the existence of multiple pathways for apoptotic cell death in T-cell development, in which Bcl-2 represents not the only modulator. Yet, it should also be kept in mind that these results were observed in different species.

An alternative hypothesis is based on the observation that the activity of the hormone thymulin, which is important for T-lymphocyte differentiation in the thymus, is zinc-dependent. Hence, zinc deprivation could also impair T-cell maturation by reduced thymulin activity [21, 22]. However, all mechanisms are not mutually exclusive and can jointly be the explanation for reduced numbers of T-cells during zinc deficiency.

T-Lymphocyte Function

Mature T-cells can be divided into different subgroups. The $CD8^+$ CTLs mediate cell death by direct interaction with infected cells. The $CD4^+$ Th-cells are further distinguished into Th1-cells, Th2-cells, Th17, and regulatory T-cells (T_{reg}). Th1-produced cytokines like interferon (IFN)-γ activate macrophages (cell-mediated responses), while Th2-cells promote antibody production by activating B-cells (humoral responses) [23]. The T_{reg}-cells control the activity of autoreactive and allogeneic T-cells and are classified as naturally occurring regulatory T-cells, which need cell-cell contact for suppression, and inducible regulatory T-cells, which act through cytokine-dependent signaling cascades [24, 25].

In general, the proliferative response after mitogen stimulation is diminished during zinc deficiency [26-28]. Another aspect is an influence on the polarization of naïve T-helper-cells into Th1- or Th2-lymphocytes. During zinc deprivation the Th1/Th2 balance is disturbed, leading to reduced production of the Th1 cytokines IFN-γ and interleukin (IL)-2. Accordingly, the cell-mediated immune response is impaired, while the Th2-dependent humoral immune responses remain less affected [26, 29, 30]. In addition, the delayed type hypersensitivity (DTH), a mainly Th1-mediated hypersensitivity reaction, fails during zinc deficiency [31, 32]. Zinc supplementation causes reconstitution of Th1-mediated responses, leads to normal cytokine profiles, and results in improved DTH reaction and lymphocyte proliferation [26, 33-38].

A connection between zinc and the function of T_{reg}-cells has not been reported, so far. Research in our own laboratory demonstrates that zinc can increase the number of T_{regs} in the mixed lymphocyte culture [unpublished observation]. Given the profound impact of zinc on T-lymphocyte development, polarization into effector cells, and function, this certainly is a worthwhile target for future investigation.

ZINC STATUS AND DISEASE

Zinc Deficiency and Disease

Zinc deficiency can be caused by inadequate intake, poor gastrointestinal absorption or augmented excretion [39]. The disturbance of zinc metabolism can either cause diseases or promote disease progression (Table (**1**)). Among other effects, the functional impairment of T-cell mediated responses can alter the susceptibility to infection, and influence the development of autoimmune diseases and allergies.

Table 1. Zinc Status and Disease

Disorder	Zinc Deficiency	Zinc Supplementation Benefit	References
Acrodermatitis Enteropathica	+	health reconstitution	[43-45]
Ageing	+	reconstituted immune response, reduced infections	[34,36,56]
Common Cold	-	potentially beneficial, requires confirmation	[68-70]
Leprosy/Tuberculosis	+	beneficial effects	[62-65]
HIV/AIDS	+	questionable, potentially harmful	[71-77]
Diabetes Type 1	+	may prevent disease initiation and diabetic complications; no beneficial effect on disease progression	[94]
Rheumatoid Arthritis	+	probably not effective	[105-107,110-112]
Allergic Asthma	+	beneficial effects	[116]

Clinical symptoms associated with human zinc deficiency include thymic atrophy and increased susceptibility to bacterial and viral infections, which clearly demonstrates an effect of zinc on immunity, in particular on T-lymphocytes, *in vivo*. Additional symptoms comprise dermatitis, alopecia, anorexia, and diarrhea [40-42]. These observations are characteristic for patients with the autosomal recessive inheritable disease Acrodermatitis enteropathica (AE). It is associated with severe zinc deficiency, based on a mutation in SLC39A4, the gene encoding Zip4, a transport protein responsible for intestinal absorption of zinc [43, 44]. Without treatment, patients succumb to infections, whereas zinc supplementation reconstitutes normal immune function and reverses all other symptoms [45].

A milder degree of zinc deficiency is a common observation in the elderly, and this marginal zinc deficiency is suspected to have clinical relevance. Ageing is often associated with a decline in immune function, so-called immunosenescence, defined as changes occurring in all parts of the immune system with increasing age [46, 47]. This leads to an augmented frequency of infections and autoimmune diseases, and several lines of evidence link marginal zinc deficiency to immunosenescence [48, 49]. Zinc deprivation can result from malnutrition, lower intestinal absorption, or increased renal excretion. As a consequence, elderly people exhibit dysfunctions of the cell-mediated immunity, manifesting in reduced T-cell amounts and disturbed functions, and an imbalance between the T-helper subtypes [29, 50-53]. Different studies show that zinc supplementation can reconstitute immune responses and reduce the frequency of infections [34, 36, 54-56]. Therefore, well-balanced nutritional zinc uptake is able to counteract the age-dependent deterioration of the immune system.

Zinc deficiency, either due to diminished zinc uptake in AE or age-dependent disturbed zinc metabolism, causes impaired immune function. In addition to the two examples above, other persons who have insufficient nutritional supply of zinc are also at risk. A severe problem is malnourishment in developing countries, where zinc deficiency leads to substantial morbidity and mortality from infections [57]. For example, zinc supplementation can reduce the severity and duration of acute and persistent childhood diarrhea [58].

Mycobacterial Infections

In addition to zinc deficiency caused by metabolic defects or malnutrition, several diseases are associated with zinc deficiency, and zinc supplementation has been tested as a pharmacological intervention to promote the cell-mediated response of the immune system.

Among the T-cell subsets, Th1-induced activation of infected macrophages *via* cytokines constitutes the central defense mechanism against intracellular pathogens like mycobacteria and certain parasites. Several granulomatous diseases like syphilis, leishmaniasis, tuberculosis, and leprosy are characterized by the disruption of the effective clearance of intracellular pathogens, which infect and multiply within macrophages. Mycobacterial infections like leprosy and tuberculosis are also associated with lower serum zinc levels [59, 60], and a reduced Th1/Th2 balance leads to disturbed killing due to diminished activation of infected macrophages [61]. Hence, zinc supplementation should be an effective pharmacological intervention against intracellular pathogens and different zinc supplementation studies demonstrate beneficial effects on severity and duration of disease after oral zinc administration [62-65]. However, it has not yet been confirmed that zinc supplementation acts *via* an activation of the Th1-mediated immune response in these diseases. Therefore, additional investigations should clarify the mechanism of zinc supplementation in the treatment of leprosy and tuberculosis.

Viral Infections

Clearance of viral infections involves cytotoxic T-lymphocytes. As zinc deficiency reduces the development of cytotoxic precursor T-cells and the function of peripheral cytotoxic T-cells, it should aggravate viral infections, which are probably resolved less efficiently by the immune system [66]. Additional antiviral defense is provided by interferons, and an induction of the production of IFN-α and IFN-γ by zinc in leukocytes *in vitro* indicates an additional way in which it affects the immune response to viruses [54, 67]. Taken together, this indicates that zinc supplementation could be effective for the treatment of viral infections.

It has been suggested that oral zinc supplementation is an useful treatment for the common cold, but while several single studies reported significant improvements, a meta-analysis of the data did not provide sufficient statistical confirmation of effectiveness for zinc supplementation as a therapeutic intervention [68-70].

Disease progression in patients with acquired immune deficiency syndrome (AIDS) is associated with a decline in the $CD4^+$-T-cell population, deregulated immune function, and increased opportunistic infections. Mechanistic similarities like the loss of T-helper-cell numbers and function, and clinical parallels like an increased susceptibility to infections, show resemblances between the effects of the human immune deficiency virus (HIV) and zinc deficiency. Taken together with the observation of a prevalence of reduced concentrations of zinc in HIV-1-infected male and female drug users and other HIV-1-infected cohorts [71-73], zinc supplementation seems to be ideally suited to counteract disease progression in AIDS patients.

While a single report described an increase in the number of $CD4^+$-cells after zinc supplementation [33], several recent papers did not confirm a zinc effect on lymphocyte subsets or $CD4^+$-, $CD8^+$- and $CD3^+$-cell counts [74, 75]. On the contrary, zinc intake may even be a risk factor for increased disease progression and mortality in AIDS patients [76, 77]. This corresponds well to the observation that increased availability of zinc may be advantageous for HIV-1, because it enhances the enzymatic activity of viral integrase and is a component of HIV-1 nucleocapsid proteins. Moreover, the HIV-1 transactivating protein has a unique cysteine-rich region with zinc binding properties [78-82]. Therefore, a mild zinc deficiency could be beneficial regarding the progression of disease and zinc supplementation may be counterproductive.

Autoimmune and Allergic Diseases

Autoimmunity and allergies are an uncontrolled and harmful immune response against self antigens or external allergens, respectively. The involvement of T-cells, and in particular T-helper cells, suggests that a modulation of zinc homeostasis could have an impact on disease onset or progression.

Diabetes Type 1

Type 1 diabetes is characterized by a continuous destruction of insulin producing beta cells of the pancreas and requires treatment with exogenous insulin *via* injections. It is a mainly Th1-mediated disorder, because T-cell invasion into the pancreas is a critical step in the pathogenesis of type 1 diabetes. Furthermore, isolated lymphocytes from non-obese diabetic mice produced large amounts of IFN-γ and less IL-4, pointing at Th1-cells as contributors to the destruction of islet beta cells [83-85].

Zinc is important for the structural conformation of insulin, its storage and secretion from the pancreas, and it exerts an insulin-like (insulinomimetic) effect by activating insulin/IGF-1-receptor dependent signaling cascades [86-89]. Abnormal homeostasis of trace elements has been implied as one mechanism involved in the onset of diabetes and the development of diabetic complications [86, 90], and recently single nucleotide polymorphisms in proteins involved in zinc homeostasis, like ZnT-8, have been identified as indicators for an increased risk for type 1 diabetes [91]. Furthermore, different animal models demonstrated that the use of zinc chelators induces diabetes by β-cell destruction [92, 93]. Notably, type 1 and type 2 diabetes are both accompanied by a loss of zinc, which was attributed to increased urinary excretion [94]. Then again, low zinc concentrations in drinking water were associated with an increased risk for the onset of type 1 diabetes [95, 96]. Therefore, zinc deficiency could also contribute to disease onset rather than being its consequence.

Zinc deficiency contributes to diabetic complications like retino-, nephro- and neuropathy [94] and the improvement of diabetic complications in response to zinc supplementation was explained by a reduction of oxidative stress [97-100].

Conversely, considering the effect of zinc deficiency on the Th-balance and a proposed Th1-mediated course of disease, it is also possible that reduced zinc levels slow down disease progression. While zinc deprivation contributes to the onset of diabetes type 1 and aggravates related complications, Th1-mediated β-cell destruction could be attenuated by zinc deficiency. This hypothesis is supported by the observation that zinc supplementation has no beneficial effect on the disease itself, but leads to an increase in HbA1c in the serum, a parameter for the deterioration of metabolic control [101, 102].

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic disease, affecting tissues throughout the body. Although a Th2 cytokine production has been described in an early phase [103], RA seems to be mediated by pro-inflammatory Th1-cells, accompanied by an impaired differentiation of Th2-cells [104].

Patients with RA have reduced serum zinc levels, which may be due to malabsorption [105-107]. Alternatively, pro-inflammatory cytokines are known to induce the expression of the zinc importer Zip14 and metallothionein in hepatocytes, leading to enhanced zinc uptake and storage into liver cells [108,109]. Hence, the chronic inflammation during RA could lead to zinc translocation from the plasma into the liver.

As in the case of diabetes, supplementation studies were performed to evaluate the therapeutic benefit of zinc administration. While a single report describes improvements, zinc was found to be ineffective in the majority of cases [110-112].

It has been suggested that several of the current anti-RA drugs work by altering the Th1/Th2 balance [113]. As in the case of type 1 diabetes, zinc supplementation is an ineffective treatment, and zinc is not helpful for regulating the pathological Th1-mediated responses.

Type 1 Hypersensitivity Disorders

Allergen-reactive Th2-cells are involved in type 1 hypersensitivity disorders like immediate allergic reactions, asthma, and urticaria. In atopic individuals, genes controlling IL-4 expression are up-regulated and there also might be decreased control of Th2-cell activity, leading to increased Th2-responses to environmental allergens [114]. Therefore, substances like zinc, which promote a Th1 reaction, leading to increased secretion of IFN-γ with concomitant suppression of Th2, could be an appropriate immunological therapy for Th2 mediated allergies.

The level of intracellular free zinc decreases in the inflamed airway epithelium of a mouse model of allergic inflammation [115]. On the other hand, zinc supplementation reduces the levels of eosinophils and lymphocytes in bronchoalveolar lavage of acute and chronic inflammation models of sensitized mice [116].

In humans, several studies link the risk of atopy, bronchial reactivity, and allergic-type symptoms to low zinc intake [117,118]. Reduction of zinc levels in serum, plasma, and hair have been reported in asthmatic individuals [119]. Childhood asthma is influenced by maternal diet during pregnancy and by nutrition during early childhood and is associated with deficiencies of zinc and the vitamins E and D [120]. Additionally, reduced zinc levels in infants are correlated to the risk of wheezing [121].

In contrast to diabetes and RA, type 1 hypersensitivity disorders show a clear Th2-prevalence. In accordance with the effect of zinc on the Th-balance, zinc deficient individuals seem to be more susceptible to generate allergic disorders. While the etiology of diseases like allergic asthma includes genetic predisposition and environmental exposure, there is also evidence for a link to the zinc status.

MOLECULAR BASIS OF THE INTERACTION BETWEEN ZINC AND T-LYMPHOCYTES

Zinc deficiency and supplementation affect the immune response, in particular T-cells, in several ways. Zinc is essential for T-cell development, due to its impact on apoptosis.

Furthermore, higher zinc concentrations were shown to inhibit T-cell functions *in vitro* [13] and supplementation of volunteers has lead to an inhibition of the mixed lymphocyte reaction *ex vivo* [122]. On the other hand, sufficient supply with zinc is essential for T-cell function. Although it is far from being a complete picture, several interactions between zinc and intracellular signaling cascades have been described that can explain these immunomodulatory effect of zinc on the molecular level.

Within the cell, there are two major zinc pools: on the one hand zinc is tightly bound to several proteins [1], and on the other hand there is a pool of free zinc, which is biologically active by binding to regulatory sites of several target proteins. The concentration of free zinc ions has been assessed to be in the picomolar to low nanomolar range and is maintained by the zinc binding protein metallothionein and zinc transport proteins [123,124]. This free zinc interferes with signaling pathways that regulate apoptosis (Fig. (**1**)), and the inhibition or activation of T-cells (Fig. (**2**)), respectively.

Zinc and Apoptosis

Zinc deficiency induces apoptosis in different cell types, including T-cells. As already mentioned, Fraker and her group explain the loss of precursor T-cell subsets in the thymus of zinc deficient mice with apoptosis that depends on a zinc-mediated glucocorticoid increase, resulting in reduced Bcl-2 expression [125,126]. In addition, a direct effect of *in vitro* zinc supplementation on a monocytic cell line alters the ratio of mitochondrial-associated proteins in control of apoptosis. An increase of the ratio of the apoptotic modulators Bcl-2/Bax in response to zinc supplementation enhances the resistance of the investigated cells to apoptosis [127]. Nevertheless, the use of millimolar zinc in this study certainly exceeds physiological levels and the resultant effect on Bcl-2 and Bax could also be explained by the induced stress, which would not be encountered *in vivo*.

There have been reports of interactions of zinc with other proteins involved in apoptosis, explaining the observation that cytotoxic precursor T-cells with normal Bcl-2 expression also undergo programmed cell death as a result of zinc deficiency [18, 20]. The calcium-dependent endonuclease that mediates DNA fragmentation is inhibited by zinc [128]. However, this target is beyond the "point of no return" for programmed cell death and an inhibition could explain a suppression of DNA fragmentation during apoptosis, but not the effect on cellular survival.

The observation that caspase-3 is directly inhibited by zinc [129] identified a more likely target for the anti-apoptotic effect of zinc. Caspase inhibition is not limited to caspase-3, an inhibition of caspases -3, -6, -7, and -8 by low micromolecular zinc was shown, with caspase-6 being the most sensitive, inhibited at 0.3 μM (apparent binding constant) [130]. These measurements were performed in the presence of β-mercaptoethanol, a thiol-based reducing agent that binds to zinc and leads to an overestimation of the re-

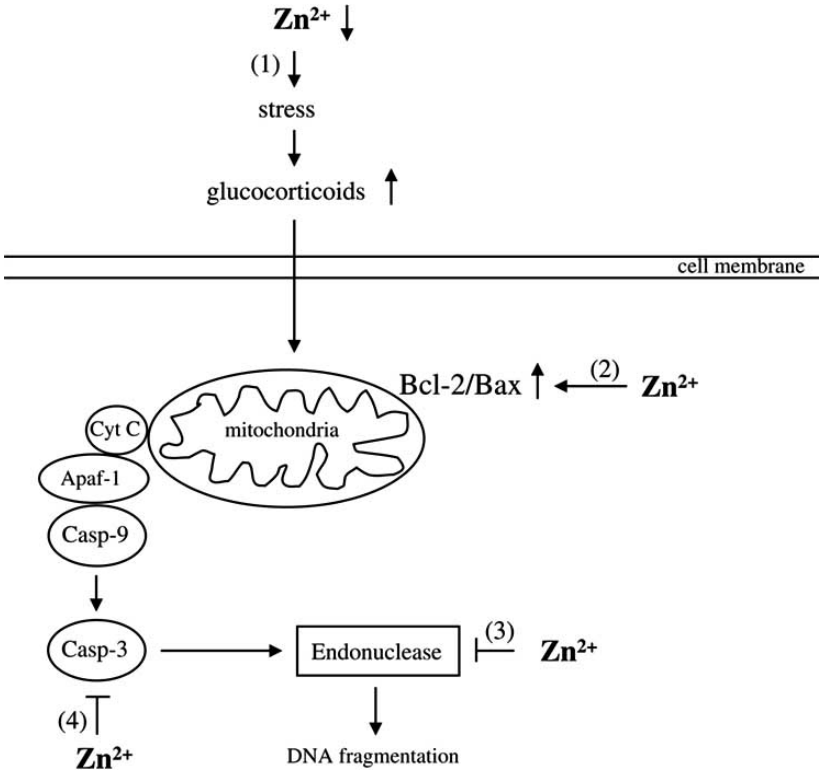

Fig. (1). Effects of zinc on apoptosis. Zinc modulates the susceptibility of T-cells to apoptosis by influencing exogenous and endogenous modulators, resulting in an anti-apoptotic effect of zinc. (**1**) Zinc deficiency induces stress that leads to apoptosis by increased glucocorticoid levels. (**2**) Zinc supplementation raises the Bcl-2/Bax ratio, thereby acting anti-apoptotic. (**3**) Zinc inhibits the endonuclease that mediates apoptotic DNA fragmentation. (**4**) Zinc inhibits several initiator and effector caspases that are essential for the proteolytic cascade in apoptosis.

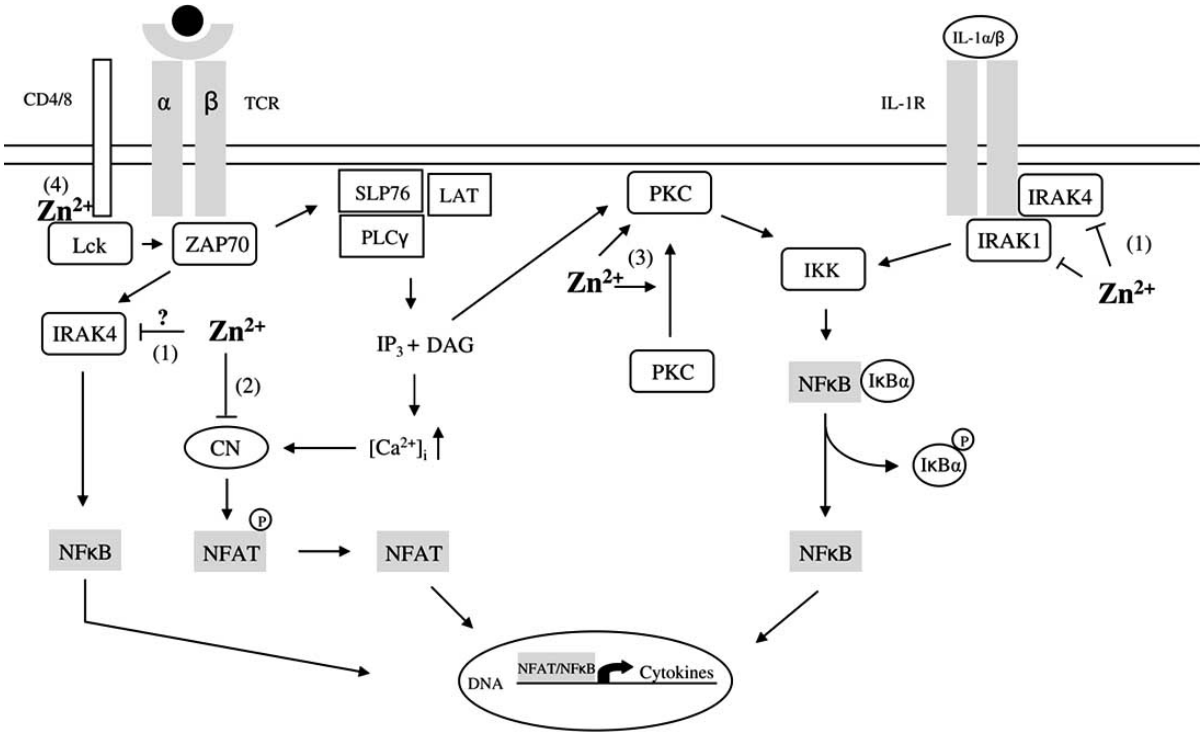

Fig. (2). Inhibitory and stimulatory effects of zinc on T-cell signal transduction. Depending on its concentration, zinc exerts inhibitory or stimulatory effects on T-cell signal transduction. So far, effects have been described on Interleukin 1 receptor (IL-1R) and T cell receptor (TCR) signaling. (**1**) Zinc inhibits IRAK activity in response to IL-1R stimulation. In T-cells, IRAK4 mediates NF B signaling in response to TCR activation, therefore, IRAK inhibition by zinc might also influence TCR initiated signals. (**2**) Zinc inhibits the phosphatase calcineurin (CN), reducing IL-2 expression in response to T-cell stimulation. (**3**) Zinc regulates multiple aspects of PKC activation, including the affinity to phorbol esters and translocation to the plasma membrane. (**4**) Zinc connects protein interface sites of Lck and the co-receptors CD4 and CD8, leading to recruitment of Lck to the TCR signaling complex and its activation through transphosphorylation.

quired concentration of the metal. Accordingly, an effect of the β-mercaptoethanol concentration on the IC_{50} of zinc was shown for caspase-3 [130]. When measurements were performed in the absence of reducing agents and chelators, and using HEPES, a buffer substance with minimal interaction with zinc, an IC_{50} below 10 nM was found [131]. This value is in the physiological range of free intracellular zinc, suggesting that endogenous cellular zinc can inhibit caspase-3. A model for a mechanism of caspase-3 inhibition has been suggested, based on reversible binding of zinc to cysteine 163 in the catalytic site [132].

Zinc homeostasis modulates the susceptibility to apoptosis *via* different mechanisms, which are shown in Fig. (**1**). While zinc deficiency stimulates apoptosis by inducing exogenous stress in the form of increased glucocorticoid production, on the cellular level there are endogenous anti-apoptotic effects of zinc on caspases and the Bcl-2/Bax ratio.

INHIBITORY SIGNALING IN T-LYMPHOCYTES

IRAK

Interleukin-1 receptor-associated kinases (IRAK) form a unique family of serine/threonine kinases, which are involved in the signaling cascades of two kinds of receptors, Toll-like receptors (TLRs) and the IL-1 receptor (IL-1R).

While TLRs are only of minor importance for T-cells, IL-1R is involved in T-cell activation by monocyte-secreted IL-1β. It was shown that high zinc concentrations (0.1 mM) inhibit IL-1 β-stimulated IFN-γ production in primary human T-cells and IL-1 dependent proliferation of a murine T cell line. In the same study, a direct inhibition of immunoprecipitated IRAK by zinc was demonstrated [13,133].

Among the four mammalian members of the IRAK family, only IRAK1 and IRAK4 exhibit kinase activity [134, 135]. Recent data point towards another signaling pathway involving IRAKs. While IRAK1 does not seem to participate in T-cell receptor (TCR) signaling, IRAK4 is involved in TCR-mediated nuclear factor kappa B (NF B) activation, because NF B signaling in response to TCR stimulation is diminished in $IRAK4^{-/-}$ mice [136,137].

By inhibiting IRAK kinase activity, zinc can inhibit two central signaling pathways in the activation of T-cells, TCR and IL1-R, thereby acting as a negative regulator in T-cell activation.

Calcineurin

Calcineurin (CN) is a Ca^{2+}/calmodulin-dependent serine/threonine phosphatase, which dephosphorylates the nuclear factor of activated T-cells (NFAT). In resting cells the different NFAT proteins, with NFAT1-4 being Ca^{2+}/Calcineurin-dependent, are phosphorylated and reside in the cytoplasm until they are dephosphorylated by CN following TCR stimulation and Ca^{2+}-influx, translocate to the nucleus, and

become transcriptionally active [138]. NFAT activates transcription of various genes during an effective immune response, especially IL-2 [139,140].

While CN activity is regulated by Ca^{2+} and Calmodulin *in vivo*, the two divalent cations nickel and manganese can stimulate CN *in vitro* [141-143]. Several reports describe an inhibition of purified CN by zinc, although in most cases the activation was induced by nickel instead of a physiological stimulus [141,144,145]. Kubohara and his group analyzed the zinc effect on CN activity *in vitro*, using bovine brain CN and purified calmodulin. They showed that zinc at physiological concentrations (10 nM-10 μM) directly inhibits the activity of CN by competing with Ni^{2+} [146]. Interestingly, zinc is essential for the catalytic domain of CN, which contains a Fe^{2+}-Zn^{2+} binuclear centre [147]. The inhibition seems to be specific for zinc, as Fe^{2+} fails to inhibit CN [146].

Cyclosporin A (CsA) and FK506 inhibit CN *via* immunophilins and serve as immunosuppressants for the treatment of transplant recipients [148,149]. In Jurkat cells, a human T-cell line, zinc specifically blocks NFAT-induced IL-2 mRNA-expression and protein production in the same manner as the CN inhibitor FK506 [149]. In addition, all three agents act as inhibitors of the mixed lymphocyte culture (MLC), an *in vitro* model for graft rejection (alloreactivity) [122,150,151]. The *in vivo* treatment with CsA and FK506 exhibits severe side effects like superinfections, because they suppress alloreactivity as well as the recognition of antigens in the context of normal immune responses, while zinc supplementation suppresses allogeneic reactions without affecting the antigenic response [122]. Therefore, there seem to be differences in the mechanism of action between zinc and FK506 and CsA and the effect of zinc may result from a combination of CN inhibition with other cellular targets, including IRAK. In this respect, it should also be considered that the number of T_{reg}-cells, suppressors of effector T-cells in alloreactivity and autoimmunity, is increased in response to zinc supplementation in the MLC [our unpublished observations]. Hence, the zinc effect on generation or function of T_{regs} could be another mechanism to control the scale of an immune response.

STIMULATORY SIGNALING IN T-LYMPHOCYTES

PKC

The protein kinase C (PKC) family of serine/threonine kinases consists of 12 different isoforms, each PKC isoform having unique roles in the regulation of several cellular functions [152]. The PKCs serve as intracellular receptors for mitogenic phorbol esters and diacylglycerol and are important for activation, differentiation, and cellular survival of T-cells [153,154].

Phorbol ester treatment results in the release of zinc ions from the nucleus and mitochondria to the cytosol, making PKC activation a mechanism by which the intracellular zinc distribution is modulated. Based on these findings, a role for zinc in T-cell signal transduction was proposed [155]. The PKCs contain a regulatory domain, which includes two cysteine-rich motifs. These belong to zinc fingers that depend on zinc for accurate folding [156,157]. A later study has shown a release of zinc ions from the intramolecular zinc fingers in the regulatory domain of the PKC in response to lipid second messengers like diacylglycerol, or PKC activation by reactive oxygen species. Here, PKC is the origin for an increase of free intracellular zinc that can be measured with fluorescent probes [158].

The interaction between PKC and zinc is not limited to an effect of PKC on the intracellular free zinc concentration and distribution, in return, zinc also influences PKC activity. Routtenberg and his group described a Ca^{2+}-dependent effect of zinc on PKC activity, with low Ca^{2+}-concentrations leading to zinc-dependent PKC activation, while in the presence of high Ca^{2+}-concentrations PKC is inhibited by zinc [159]. Other groups demonstrated that zinc treatment stimulates multiple steps during PKC activation, resulting in augmented PKC activity, increased affinity to phorbol esters, and enhanced binding to the plasma membrane [155,160,161]. The increase in cytoskeletal compared to cytosolic PKC was only observed with zinc, but not calcium, and was inhibited by a zinc chelator [160]. The authors therefore suggested that zinc regulates PKC translocation to the cytoskeleton in contrast to calcium, which controls binding of PKC to the lipid components of cell membranes. These data are supported by the fact that zinc deficiency reduces PKC translocation to the plasma membrane in murine T-lymphocytes after mitogen stimulation [162].

Lck

The lymphocyte protein tyrosine kinase (Lck) belongs to the family of Src protein tyrosine kinases and is expressed in T-cells, natural killer (NK)-cells and in the brain. The kinase contains six functional regions: the Src homology (SH) 4 domain, a unique region, the SH3 domain, the SH2 domain, the catalytic domain, and a short negative regulatory tail [163]. Src kinase activity is regulated by differential tissue expression, intracellular localization, lipid modifications at the N-terminus, interactions of the SH2- and SH3-domains, and by phosphorylation [164].

Lck plays an important role in signal transduction in response to T-cell stimulation, because as one of the first kinases being activated, it phosphorylates the T-cell antigen receptor complex and several other kinases and adaptor proteins (Fig. (**2**)) [165].

Zinc can promote recruitment of Lck to the TCR signaling complex and its activation by promoting the binding to two distinct protein interface sites (Fig. (**3**)). The unique domain of Lck and the tails of both co-receptors CD4 and CD8 exhibit conserved cysteine motifs. The association between Lck and each co-receptor is coordinated by zinc ions, based on high affinity binding to the cysteine motifs, connecting both proteins [166-169].

In addition to recruitment to the TCR signaling complex, another zinc dependent interface site mediates dimerization of the SH3 domains of two Lck molecules. The activation of Lck involves trans-autophosphorylation on the activating tyrosine residue 394, and dimerization is likely to facilitate this process [170]. The authors concluded that this kind of zinc-induced activation mechanism is Lck specific, because the residues that form the zinc binding site are not found in other Src kinase family members. Such a mechanism would

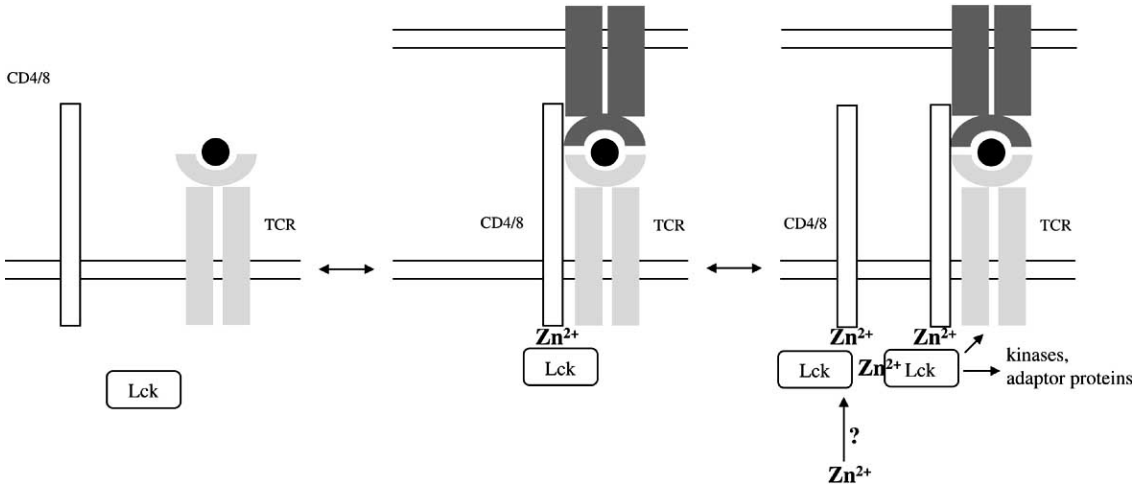

Fig. (3). Zinc-induced activation of Lck. Two zinc dependent steps are involved in the activation and recruitment of the protein tyrosine kinase Lck to the TCR signaling complex that forms in response to antigenic T-cell stimulation. Lck is recruited to the TCR signaling complex by its association with the co-receptor molecules CD4 or CD8. This interaction depends on a protein interface stabilized by a zinc binding site with ligands from both proteins. In addition, another zinc interface site of the SH3 domains of Lck leads to dimerization and activation by promoting trans-autophosphorylation.

also be an explanation for a direct activation of Lck, which has been observed as an increased kinase activity of the immunoprecipitated kinase at micromolar zinc concentrations [171].

In other cell types, zinc activates signaling pathways by inhibition of protein tyrosine phosphatases, hereby preserving the tyrosine phosphorylation of receptors and upstream kinases [87-89,172]. Phosphatases are also potent regulators of TCR signaling [173], hence, further study is needed to analyze the contribution of phosphatase inhibition by zinc to its influence on the activation of TCR signaling.

The observation that zinc can act as a positive regulator of TCR signaling confirms former observations, where zinc acts co-mitogenic in response to phytohemagglutinin stimulation [34,174].

CONCLUSION

Zinc homeostasis has a strong impact on T-lymphocytes. During zinc deprivation, their development, polarization into effector cells, and functional effectiveness is impaired. Several molecular targets exist, including proteins involved in the regulation of apoptosis, like Bcl-2/Bax and caspases. Additionally, signal transduction involving PKC and Lck is promoted by zinc, and the inhibition of IRAK and CN leads to suppression of T-cell activity in response to zinc supplementation.

Although zinc deprivation does not affect lymphopoiesis of B-cells and myelopoiesis [16], T-cells are not the only type of immune cells influenced in their functionality. Cells of the innate immune system like macrophages, neutrophils, NK-cells, and granulocytes are functionally impaired by zinc deficiency, and show functional restoration after zinc supplementation [4]. Hence, in addition to a direct influence on T-cells, there are indirect effects, e.g. by an altered profile of secreted monokines [13], and T-cell unrelated effects of zinc like a role in the activation of monocytes [175] and mast cells [176,177].

Still, many open questions remain. Are there any additional signaling pathways affected by zinc in T-cells that have not been identified so far? In other cell types, cyclic nucleotide phosphodiesterases [178,179], mitogen activated protein kinases [180,181], and protein tyrosine phosphatases [89,182] were regulated by zinc, and all of these proteins are important for signal transduction in T-cells. Furthermore, no mechanistic explanation exists for the selective effect of zinc on different T-cell subtypes, especially the balance between Th-cells.

Zinc supplementation is the obvious treatment option for immune deficiency resulting from a lack of zinc, but there also is potential for cases where an improvement of Th1-mediated immune response or a shift in the Th-balance from Th2 towards Th1 is desired. It may even be possible to utilize the inhibitory effects for a targeted suppression of unwanted T-cell reactions. However, this will require further research into the effects of zinc on signaling pathways, different immune cells, and the resulting long term consequences.

ABBREVIATIONS

AE	=	Acrodermatitis enteropathica
AIDS	=	Acquired immune deficiency syndrome
CN	=	Calcineurin
CsA	=	Cyclosporin A
CTL	=	Cytotoxic T-lymphocytes
DTH	=	Delayed type hypersensitivity
HIV	=	Human immune deficiency virus
IFN	=	Interferon
IL	=	Interleukin
IRAK	=	Interleukin-1 receptor-associated kinase

IL-1R	=	Interleukin-1 receptor
Lck	=	Lymphocyte protein tyrosine kinase
MLC	=	Mixed lymphocyte culture
NFAT	=	Nuclear factor of activated T-cells
NFκB	=	Nuclear factor kappa B
NK	=	Natural killer
PKC	=	Protein kinase C
RA	=	Rheumatoid arthritis
SH	=	Src homology
TCR	=	T-cell receptor
Th	=	T-helper
T_{reg}	=	Regulatory T-cell
TLR	=	TOLL-like receptor
Zip	=	Zrt- and Irt-like protein
ZnT	=	Zinc transporter

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