ANNUAL REVIEWS

*Annual Review of Nutrition*

# Dietary and Physiological Effects of Zinc on the Immune System

Inga Wessels, Henrike Josephine Fischer, and Lothar Rink

Institute of Immunology, Faculty of Medicine, RWTH Aachen University, 52074 Aachen, Germany; email: Lrink@ukaachen.de

Annu. Rev. Nutr. 2021. 41:133–75

First published as a Review in Advance on July 13, 2021

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

https://doi.org/10.1146/annurev-nutr-122019-120635





## Keywords

zinc biology, immune response, inflammatory diseases, zinc, zinc deficiency

## Abstract

Evidence for the importance of zinc for all immune cells and for mounting an efficient and balanced immune response to various environmental stressors has been accumulating in recent years. This article describes the role of zinc in fundamental biological processes and summarizes our current knowledge of zinc's effect on hematopoiesis, including differentiation into immune cell subtypes. In addition, the important role of zinc during activation and function of immune cells is detailed and associated with the specific immune responses to bacteria, parasites, and viruses. The association of zinc with autoimmune reactions and cancers as diseases with increased or decreased immune responses is also discussed. This article provides a broad overview of the manifold roles that zinc, or its deficiency, plays in physiology and during various diseases. Consequently, we discuss why zinc supplementation should be considered, especially for people at risk of deficiency.

## Contents



## 1. INTRODUCTION

Zinc plays key structural and catalytic roles in more than 300 enzymes and transcription factors (261). It impacts virtually all cells of the body and is indispensable for the development and function of especially the immune system (81, 261). Chronic zinc deficiency results in a number of symptoms, including growth retardation, thymic atrophy, and disturbed immune response, resulting in higher susceptibility to infections as well as decreased wound healing and regeneration from tissue injury. Most of those symptoms are explained by zinc's impact on proliferation, differentiation, apoptosis, gene transcription, and signaling, including the regulation of mitogenic signaling pathways (19, 75, 123, 133, 197). Clear clinical symptoms are observed in severely zinc-deficient humans and animals. However, mild zinc deficiency in the elderly and during obesity was associated with disturbances in hematopoiesis; with low-grade but constantly elevated inflammatory and oxidative stress markers; and consequently with an inefficient immune response against bacterial, viral, and fungal infections. Such unphysiological responses are easily overcome by zinc supplementation (116, 264).

## 1.1. Zinc Homeostasis

After being absorbed in the gastrointestinal tract, zinc is transported to the tissues via the bloodstream. Within cells, zinc is further redistributed between organelles (118). Intra- and extracellularly, zinc is largely bound to zinc binding proteins such as albumin or α2 macroglobulin (in the serum) and metallothionein (MT) or calprotectin (in the cytoplasm). Since the effects of altered intracellular zinc concentrations largely depend on the magnitude and the duration of extracellular zinc supply, zinc homeostasis needs to be tightly regulated to prevent adverse effects. So far, 24 zinc transporting proteins have been identified in humans. One subgroup of these proteins includes 14 so-called Zrt-like and Irt-like proteins (Zips), also known as solute carrier family 39 (SLC39) proteins, SLC39A1–14, that increase cytoplasmic zinc by importing zinc from the extracellular space or from intracellular organelles [including the endoplasmic reticulum (ER), the Golgi apparatus, and vesicles]. The other subgroup consists of 10 zinc transporters, SLC30A1–10, that decrease cytosolic zinc levels (33, 118). Disturbance of even 1 of those 24 transport proteins may result in disease. In case of functional loss of Zip4, the most important zinc importer in the intestine, severe zinc deficiency, known as acrodermatitis enteropathica, occurs; this state can be lethal if not treated (118, 261). Some zinc transporting proteins transport other metal ions. Zip14, for example, may transport zinc as well as iron, manganese, and perhaps cadmium (18). Furthermore, transporters known to transport calcium were recently found to conduct zinc and may be involved in the generation of a zinc wave (245). Export of zinc from the cytoplasm via secretory vesicles was described as well (99).

Of the intracellular zinc binding proteins, MT-1 through MT-4 appear to be the most important. MT-1 can bind up to seven zinc ions, which can be released and subsequently alter cellular activity and metabolism. The expression of MT-1 is sensitive to intracellular zinc conditions (168, 169, 261). Zinc can be excreted from the body through both the pancreas and the small intestine. The fecal excretion of zinc is sensitive to zinc status, while additional zinc is lost via urine, semen, and sweat (59, 135).

Zinc is transferred to the tissues during the acute phase response (APR) and other inflammatory reactions, resulting in transient serum hypozincemia, which normalizes during the resolution of inflammation (156, 259). Whether the described transfer provides zinc to the tissues to prevent apoptosis, to support their recovery from injury, or both is debated. Transient serum hypozincemia may be a systemic danger signal that is essential for the activation of immune cells, but this possibility remains to be proven (259, 261). Zinc deficiency can be genetically acquired or occurs due to malnutrition, pregnancy, lactation, aging, or a vegetarian or vegan lifestyle (116, 242, 261, 264). Furthermore, during various inflammatory, autoimmune, and cancerous diseases, zinc deficiency was observed (81, 263). The World Health Organization (WHO) estimates that approximately one-third of the world population is affected by zinc deficiency (270). Due to the lack of a reliable biomarker to assess zinc status and to uncover mild zinc deficiencies, this percentage may be even higher (98, 242).

## 1.2. Lack of a Valid Biomarker for an Individual's Zinc Status

Our knowledge of the multiple roles that zinc plays in human health and disease has increased during recent years. With new and more sensitive tools for analyzing a cell's function and metabolism as well as for measuring zinc concentrations in various fluids and intracellular zinc, the molecular mechanisms underlying zinc's effects are becoming clearer. Despite all those improvements and developments, we are still not able to reliably assess the zinc status of an individual, since we still lack a valid biomarker (162, 223). Current studies largely rely on measuring serum or plasma zinc levels. A clear indication of zinc deficiency is a drop in serum levels to less than 642.5 μg/L (96).

However, since serum zinc levels are affected by recent food intake as well as by the degree of hydration/dehydration of an individual, those numbers only estimate current zinc status. Moreover, circadian variations of serum zinc levels were observed, and certain drugs and hormones may also affect serum zinc levels. Another problem when determining zinc status is that serum and plasma zinc levels require adjustment for inflammation (if present), since zinc is transferred from serum to tissue during the APR (18, 151, 175). Thus, disturbances in zinc-dependent immunological processes may become apparent, even though serum zinc levels appear to be normal. Early symptoms of especially mild deficiencies in zinc are quite general and can easily be attributed to various diseases. Consequently, zinc deficiency is hard to diagnose and can be hidden. Currently, zinc-specific questionnaires in combination with serum zinc levels appear to estimate an individual's zinc status well (242). Height-to-age ratio was suggested as an additional parameter in the assessment of the zinc status of growing infants and children younger than 5 years (122). Alternatively, assessing MT expression or free intracellular zinc levels of circulating leukocytes was suggested (98, 101), but research is ongoing.

The recommended daily allowance for zinc is 11 mg for men and 8 mg for women (WHO) (263). A summary of the zinc content of various foods and factors that alter zinc's bioavailability can be found elsewhere (165, 233). Zinc uptake is, for example, negatively affected by fiber components, substances formed during (deep) frying, phytic acid, and polyphenols; polyphenols are found in tea leaves and coffee beans (233). All these factors should be considered when one is addressing zinc deficiency.

## 1.3. Zinc Supplementation in Humans and Cell Culture Experiments

As indicated above, the global prevalence of zinc deficiency is high. Thus, zinc supplementation can generally be recommended, especially for individuals at risk of zinc deficiency. Mild zinc deficiency is hard to diagnose. However, long-term daily zinc intake via supplements meeting the recommended daily allowance (8–15 mg/day, depending on the country and organization) and not exceeding 20 mg/day can be regarded as safe (89). In clinical supplementation studies, the chosen doses are often higher but are usually used for a limited time, and in most cases individuals with mild to severe zinc deficiency are treated. The upper limits for daily intake are 25 mg (European Food Safety Authority) and 40 mg (WHO) (263). Thus, supplementing between 20 and 40 mg/day can be regarded as high dose supplementation. Some studies use zinc supplements exceeding 40 mg/day, which can be regarded as very high dose supplementation, also known as pharmacological doses (225). High doses should be recommended only if clear symptoms of zinc deficiency are observed (72).

Before starting clinical studies, the effects of zinc supplementation are regularly investigated using cell cultures. In this regard, physiological conditions are difficult to define. Although we know that the physiological concentration of zinc in plasma is between approximately 10 and 18 μM, modeling plasma conditions in cell cultures is complex, as recently discussed in detail (90). In medium, zinc is, for example, largely buffered by fetal calf serum (FCS) since serum contains various zinc binding factors such as albumin. Hence, the concentration of free, available zinc is reduced. Moreover, the binding of zinc by protein depends on the zinc compound (190). Empirical data suggest that, in medium with 10% FCS, the addition of 30 μM zinc sulfate induced effects similar to those observed when physiological concentrations of zinc were supplemented in humans. Adding concentrations of up to 50 μM of zinc to cell cultures can be regarded as high, and adding concentrations exceeding 50 μM are very high. The latter may result in the induction of apoptosis or necrosis in the cultured cells (247). Thus, vitality tests are required to test the effects of a certain zinc compound and concentration.

### 1.4. Consequences of Excess Zinc Exposure

Although zinc is a nutritional supplement with little to no side effects, it is no exception from the rule that "the dose makes the poison." Intoxication with zinc is rare but has sometimes been reported. Zinc uses for which concentrations above the recommended values were reported include galvanization (to prevent metals from rusting), die casting, cosmetics, and dental adhesives (72).

To prevent high intake of zinc, for example, from metal fumes, legal limits have been defined. The Occupational Safety and Health Administration enforces a limit of 1 mg/m$^3$ of zinc chloride fumes and 5 mg/m$^3$ of zinc oxide fumes in workspace air over an 8-h workday. The National Institute for Occupational Safety and Health limits zinc chloride in workspace air to 1 mg/m$^3$ as well, but over a 10-h workday (3). The upper recommended limit for zinc in drinking water is 5 mg/L, as recommended by the US Environmental Protection Agency (188).

Individuals exposed to high zinc concentrations, such as mine workers, show symptoms of the gastrointestinal tract (such as nausea, vomiting, and stomach cramps) and pancreatic complications. Additionally, anemia, fatigue, decreased high-density lipoprotein cholesterol, neutropenia, and impaired immune functions were reported, while carcinogenic effects of excessive intake are not likely (39, 72, 195). However, chronic zinc toxicity is rarely achieved in vivo since excessive zinc can be excreted (135).

Since zinc and copper share some transport mechanisms, zinc intoxications can result in copper deficiency. High zinc doses are therefore actually used to treat Wilson's disease, a genetically based disease in which patients suffer from elevated copper levels in blood and tissues (4).

## 2. BIOLOGICAL FUNCTIONS OF ZINC

The immune system is the most highly proliferating cellular system of the human body, and especially for the adaptive immune response, directed proliferation and apoptosis are key features. In this section, we briefly discuss zinc's role in proliferation, apoptosis, and intracellular signaling, which are central to regulation of the immune response.

### 2.1. Zinc Coordination Within Protein Structures

Approximately 10% of the entire proteome in eukaryotes belong to the zinc proteome (130). This percentage is exceeded only by calcium and magnesium. Zinc binding motifs are found within proteins as well as in intermolecular binding sites. Generally, 90% of the zinc proteome can be classified into two major groups: enzymes and transcription factors. The widespread dependency of proteins on zinc underlines zinc's importance for cellular functions (261). The function of proteins depends on their structure as well as their state of charge, and zinc can affect both factors, even at very low concentrations. Four types of zinc binding sites can be distinguished: structural, catalytic/cocatalytic, regulatory, and protein interface sites (16) (**Figure 1*a***). While the first three types were identified in the 1990s, the last variant was discovered in the early 2000s (15). The binding sites differ in their amino acid ligands. Most frequently, zinc is bound to histidine (His), glutamate (Glu), aspartate (Asp), and cysteine (Cys) (17).

Structural sites (**Figure 1*a,i***) consist of four amino acids, with Cys being the most frequent one (147). However, combinations of Cys with His, Glu, or Asp are possible. In this type of binding site, zinc is required to maintain the structure of the protein as well as quaternary structures of protein complexes. In zinc finger proteins, such as DNA/RNA binding proteins, zinc is required for the interaction of amino acids with nucleotides. Within the polypeptide sequence, zinc coordinates to a combination of Cys thiol and His imidazole residues, resulting in the classical zinc finger structure composed of an α-helix and an antiparallel β-sheet with a stoichiometry of one zinc

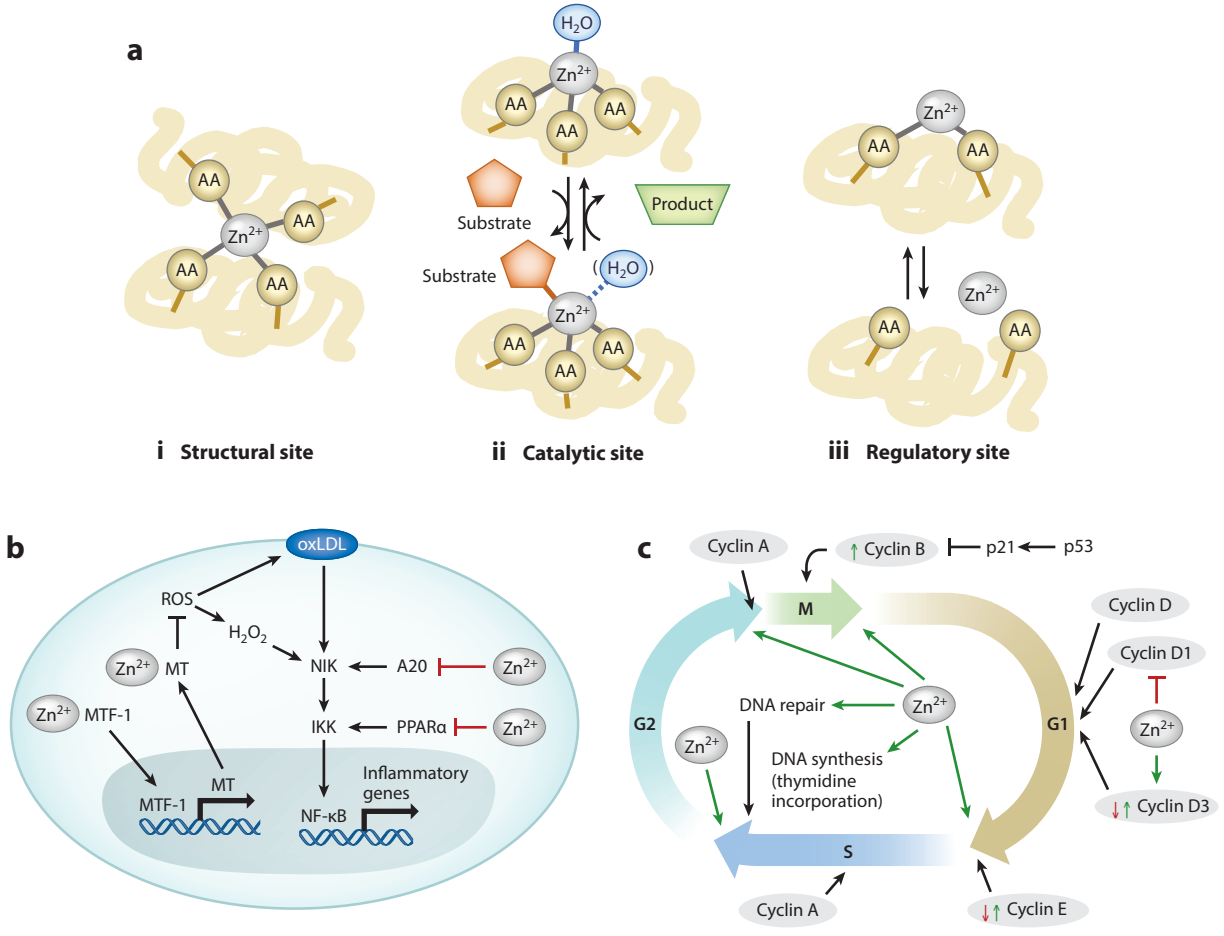



**Figure 1**

Functions of zinc in protein structure, redox balance, and the cell cycle. (*a*) Zinc binding motifs differ with regard to their function. (*i*) In structural sites, zinc is bound to four AA ligands. These AAs are mostly cysteine, glutamate, aspartate, and histidine. (*ii*) In catalytic sites, zinc binds to three AA ligands and either a water molecule ($H_2O$) or (not shown) a hydroxyl ion ($OH^-$). During catalysis, the substrate binds to zinc and is converted to a product. Depending on the enzyme, $H_2O/OH^-$ is released or stays attached to zinc. (*iii*) In regulatory sites, zinc is reversibly bound to two AA ligands and impacts protein structure. (*b*) Zinc regulates the redox balance of cells. The major pathways of oxidative stress are regulated by zinc (indicated by red blunted arrows). Zinc inhibits the formation of ROS and blocks the NF-κB signaling pathway on different levels. Furthermore, zinc can induce MT expression via MTF-1, and MT can neutralize ROS. (*c*) Zinc's impacts on the cell cycle. Two checkpoints, namely the progression from G1 to S phase and the transition from S to G2 phase, depend on zinc. The effects of zinc on the different steps of the cell cycle are shown with green arrows (where zinc is required) and a red blunted arrow (where zinc inhibits function). For the various cyclins, the impact of zinc deficiency on cyclin expression is indicated by small red arrows, and the impact of zinc supplementation is depicted with small green arrows. For panels *b* and *c*, black arrows indicate zinc-independent enhancing effects, and black blunted arrows denote zinc-independent inhibitory effects. Abbreviations: A20, tumor necrosis factor alpha–induced protein 3; AA, amino acid; G1/G2 phase, gap 1/2 phase; IKK, IκB kinase; M, mitosis; MT, metallothionein; MTF-1, metal-responsive transcription factor 1; NF-κB, nuclear factor kappa light-chain enhancer of activated B cells; NIK, NF-κB-inducing kinase; oxLDL, oxidized low-density glycoprotein; PPARα, peroxisome proliferator–activated receptor α; ROS, reactive oxygen species; S phase, synthesis phase.

ion per protein. This zinc finger structure can bind to DNA and RNA targets and is found in, for example, transcription factors (138). Recently, other zinc domains have been described on the basis of the stoichiometry of zinc-to-protein binding: the so-called LIM domain (two zinc ions per protein), the zinc hook (one zinc ion per two proteins), and the zinc clasp (one zinc ion per three proteins) (131). Collectively, zinc is an indispensable part of protein structures and is essential for protein-protein or protein–amino acid interactions. Thus, zinc is involved in the regulation of fundamental cellular processes and is indispensable for the development and functions of all immune cells.

## 2.2. Zinc as a Cofactor in Enzymatic Functions

Many of the more than 300 enzymes that require zinc for their catalytic actions are involved in metabolic processes such as protein and nucleic acid synthesis as well as lipid and carbohydrate metabolism (130). Zinc's properties explain its presence at catalytic sites: As a Lewis acid (i.e., an electron-pair acceptor) and due to having amphoteric character, zinc can participate in enzymatic redox reactions, even though it is redox stable and therefore a reliable partner. Interestingly, zinc is the only metal found to be a cofactor of enzymes from all six enzyme classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (249). The function of all zinc enzymes is based on the binding of zinc, and even in cases of zinc deficiency, most protein-bound zinc is retained in the catalytic site. If intracellular zinc levels would fall below a certain threshold, zinc could be removed from its structural protein binding sites or catalytic sites. However, the cell would already be programmed for apoptosis in those highly zinc-deficient conditions.

Three-dimensional modeling of proteins has helped to identify a high, still-increasing number of zinc-containing enzymes. The structure of zinc-containing catalytic sites consists of three amino acids and either a water molecule or a hydroxyl molecule ($OH^-$) (**Figure 1*a,ii***) (147). $H_2O$ or $OH^-$ is always a ligand of zinc and can participate in the catalytic reaction. The zinc-bound water can be ionized or polarized and can thus function as a nucleophile or an acid/base (16). Additionally, the rapid ligand exchange potential of zinc facilitates high turnover numbers, as seen for zinc alcohol dehydrogenases (114).

Cocatalytic sites, in contrast, are often found in enzymes with more than one binding site for zinc or transition metals. The cocatalytic sites most frequently contain Asp and His. Moreover, a water molecule can be involved in the interaction of both sites. Since catalytic and cocatalytic sites are often found at opposing ends of the protein, their zinc-dependent interaction is also important for the three-dimensional structure of the enzyme (248).

In addition to having a role in catalytic function and stabilization of the three-dimensional structures of proteins, zinc can also act in a regulatory manner (132). In this case, binding of zinc induces structural changes that modulate the function of a protein (**Figure 1*a,iii***). These conformational changes are based on the protein's physicochemical properties and structure, on the protein's affinity for zinc, on the stoichiometry of the interaction between the protein and zinc, and on the amino acids involved. In contrast to structural and catalytic sites, which generally have a high affinity for zinc, regulatory sites show a lower affinity for zinc to allow for its reversible binding (57). This low affinity for zinc enables responses to fluctuations in intracellular zinc concentration, i.e., zinc flux after activating signals. Some enzymes, for example, protein tyrosine phosphatases (PTPs), can be inhibited by transient fluctuations of intracellular free zinc, even in the picomolar range (266). More examples, especially of zinc's role in key enzymatic reactions regarding immune cells, can be found below, underlining that zinc is indispensable for an effective immune reaction.

## 2.3. Zinc's Role as a Proantioxidant

Zinc's proantioxidative character has long been known. It is involved in both the production and neutralization of reactive oxygen species (ROS) (127).

Under physiological conditions, ROS are produced during cellular respiration or to eliminate phagocytosed pathogens and are neutralized without harming cells. Inflammation and other stressors can increase ROS production or decrease antioxidative processes. Excessive intracellular ROS levels, also known as oxidative stress, can result in tissue damage. Although zinc is redox inert, it acts as a proantioxidant and affects cellular redox balance on several levels (127) (**Figure 1*b***). Consequently, zinc deficiency is often associated with increased oxidative stress, while zinc supplementation is correlated with reduced ROS production. However, chelation of zinc in human neutrophil cultures had no impact on ROS production (93), indicating that the effects of zinc on redox metabolism seem to be complex. Zinc activates antioxidant molecules and proteins, such as glutathione and copper/zinc superoxide dismutase, and reduces the activity of oxidant-promoting enzymes, such as inducible nitric acid synthase (127). Furthermore, zinc can inhibit the superoxide-producing enzyme NADPH (nicotinamide adenine dinucleotide phosphate) oxidase (199). This action was first described for neutrophil cytoplasts that were cultured in vitro in medium supplemented with 1 mM zinc (97). Zinc also protects free sulfhydryl groups in proteins from oxidation and is, as stated above, also required for the structural integrity of proteins, including those involved in redox balance (127). Indirect mechanisms of zinc's antioxidative function include the induction of MTs, which can act as radical-scavenging proteins (28). The expression of MTs is induced by metal-responsive transcription factor 1 (MTF-1), which is sensitive to intracellular free zinc concentrations (11). During zinc deficiency, MT expression is decreased, resulting in limited elimination of free radicals. In addition, zinc bound to the sulfur clusters of MTs can be released by oxidation, suggesting that alterations in redox balance can feed back on zinc homeostasis.

Taken together, these findings show that zinc is central for intracellular redox balance. In zinc deficiency, several antioxidative mechanisms are lost, resulting in excessive intracellular ROS levels, which are associated with damage to all kinds of cells, including immune cells.

## 2.4. Zinc's Effects on Cell Cycle and Cell Death

Zinc's important role in cell growth and proliferation is widely accepted. The cell cycle in eukaryotic cells is characterized by a series of coordinated phases: gap (G)1 phase, DNA replication or synthesis (S) phase, G2 phase, and mitosis (M) phase, each characterized by the expression of phase-specific cyclins (26) (**Figure 1*c***). Each phase entry is tightly regulated to ensure completion of one phase before the next one is initiated.

In rats fed a zinc-deficient diet for 5 days, DNA synthesis was reduced in the liver, kidney, and spleen relative to rats fed a zinc-adequate diet for this time frame (265). Furthermore, cells cultured in zinc-deficient medium revealed defects in DNA synthesis, explained by the requirement of zinc for thymidine incorporation. Moreover, increased DNA damage was observed in the cultured cells, although such damage was not associated with zinc deficiency–induced arrest in the cell cycle (48). Furthermore, within G1 phase before the start of S phase, zinc-dependent gene expression is required for successful progression to S phase, as determined in cells cultured in zinc-deficient and zinc-reconstituted medium (49, 160). Zinc is also important during S phase and is indispensable for progression to G2 and M phases (47) (**Figure 1*c***). In contrast, another study suggested that zinc may be required only until a certain point during G1 phase and for the transition from M phase back to G1 phase (269). A different study showed that both zinc deficiency and zinc excess cause arrest at the G2/M checkpoint (47). Thus, zinc may not directly affect DNA synthesis in

S phase. Instead, zinc-dependent expression of p53, and consequently upregulation of p21 and p21-mediated inhibition of Cdc2/cyclin B, may play a role; this possibility was investigated in zinc-supplemented human bronchial epithelial cells (269). p53 is a central regulator of the cell cycle and apoptosis. Interestingly, zinc does not affect the expression of p53, but both the stability and the activity of p53 decreased in cells treated with a zinc chelator (176).

Zinc also impacts the expression of cyclins, suggesting that both DNA replication and transcriptional regulation of cell cycle proteins depend on zinc (47, 194). Zinc chelation reversibly inhibited the cell cycle–induced upregulation of cyclins B, D3, and E in vitro. In the case of cyclin B, zinc reconstitution even increased mRNA expression relative to controls. However, high zinc concentrations ($\geq$400 $\mu$M) were used to resupplement the zinc-deficient cells (47). Similarly, a more recent study revealed that zinc chelation reduced cyclin D2 and cyclin E mRNA expression in the murine T cell line CTLL-2 (194). Consequently, zinc chelation significantly decreased interleukin (IL)-2-driven cell cycle entry into S and G2/M phases. However, stimulation of the CTLL-2 cells with zinc (20 $\mu$M) and pyrithione (10 $\mu$M) did not affect cyclin D2 or cyclin E mRNA expression or cell proliferation. Thus, zinc is required for IL-2 receptor–induced signaling but does not replace IL-2 as a key stimulus of T cell proliferation (194). Furthermore, zinc deficiency augmented the cyclin D1–dependent proliferation of tumor cells, overexpressing cyclin D1. The role of zinc in cyclin D1 expression in nontumor cells remains to be investigated (71).

A long-term blockade of the cell cycle and subsequent apoptosis when zinc is limited make up only one of several mechanistic explanations for the association of zinc deficiency with cell death. In addition to this indirect effect, zinc directly impacts cell death, including apoptosis, pyroptosis, autophagy, and presumably necrosis. Apoptosis—or programmed cell death, which is required during organ development and tissue homeostasis—is characterized by DNA fragmentation, chromatin condensation, apoptotic body formation, and caspase cascade activation. Zinc can have antiapoptotic or proapoptotic effects (77). Furthermore, intracellular zinc homeostasis is altered during apoptosis (71). Excellent detailed reviews can be found elsewhere (e.g., 76). A causal link between apoptosis and zinc deficiency was discovered in tissues of zinc-deficient rodents (64). Furthermore, caspase-3, which cleaves the cell cycle regulator p21, is activated in cell cultures treated with a zinc chelator. Subsequently, p21 accumulates and causes a dramatic increase in CDK2 expression, resulting in premature entry into S phase and thus apoptosis (44, 66). In contrast, zinc directly inhibits the catalytic activity of caspase-3 and procaspase-3 (6). The activity of other caspases, including caspase-6 and caspase-9, is also affected by changes in zinc homeostasis (108, 234). All of the apoptotic caspases, except caspase-7, are inhibited by zinc binding at available zinc concentrations of 1–10 nM (65). However, zinc can suppress apoptotic endonucleases (63, 243). Since zinc is antiapoptotic even at concentrations that have no impact on apoptotic endonucleases, additional mechanisms probably exist. Interestingly, zinc deficiency had the most profound impact on proliferating cells in tissues with high turnover, suggesting that dividing cells are especially prone to zinc deficiency–induced apoptosis. This scenario shows once more the importance of zinc for the immune response since the interplay of the cell cycle and apoptosis are vital for the generation of sufficient numbers of immune cells and the depletion of self-reactive immune cells, respectively.

Necrosis is characterized by swelling of the cell and its organelles, followed by plasma membrane rupture and the release of cytoplasmic content. While the impact of zinc on apoptosis is well characterized, the involvement of zinc in necrosis is less studied. Some studies found that cells in zinc-deficient medium underwent necrotic cell death (134, 170). However, it remains to be clarified whether zinc has antinecrotic properties or whether the observed effects were due to a failure to induce apoptosis (213).

Autophagy, also known as autophagic cell death (ACD), is accompanied by large-scale autophagic vacuolization of the cytoplasm. The impact of zinc on ACD was discovered in cells of the central nervous system. When intracellular zinc in astrocytes was reduced by chelation, autophagy and cell death were suppressed (145). In line with this finding, zinc supplementation promoted autophagy in cell culture, which was shown for various cell types (155, 158). However, in some cells severe zinc deficiency seems to activate ACD, presumably by a mechanism that involves Krüppel-like factor 4 (152, 154). Other mechanisms that may generally play a role in the zinc-mediated regulation of ACD seem to involve the ERK1/2 (extracellular signal–regulated kinase 1/2)-mTOR (mechanistic target of rapamycin) pathway (109, 159).

Furthermore, MTs play an important role in the zinc-mediated regulation of ACD: Loss of MT-3 suppresses autophagy in glioma cells (146). The interaction of MTs with transcription factors (for example, the interaction of MT-2A with the transcription factor homeobox containing 1) can promote autophagy (163). Lack of the zinc transporter Zip13 inhibits autophagy via a mechanism that involves increased DNA methyltransferase activity (144), and loss of Zip1 function was associated with inhibition of hyperglycemia-induced ACD (50). Lastly, the concentration of labile zinc increases during ACD, and this increase is blocked when autophagy is inhibited (145). Thus, whether the rise in intracellular free zinc is a cause or consequence of ACD should be investigated. It has also not been clarified to what extent nutritional zinc impacts ACD in vivo. In mice fed a low-zinc (1-ppm) diet for 28 days, ethanol-induced ACD was inhibited, while zinc supplementation (150-ppm diet) increased ACD (157). In studies in yellow catfish, zinc supplementation (146.65-ppm diet) augmented ACD, and MTF-1 was involved in this process (257). In light of an immune response, autophagy suppresses inflammation through degradation of the inflammasome.

Pyroptosis occurs during inflammatory responses. The regulatory mechanisms of pyroptosis share some similarities with the apoptosome and involve zinc-suppressed caspase-3. Thus, an impact of zinc on pyroptosis seems likely. Adenosine triphosphate (ATP) induces the inflammasome and subsequent pyroptosis (276). ATP is released by bacteria and host cells and activates AMP-activated protein kinase, IL-1β production, and the release of active caspase-1. In murine macrophages, zinc chelation reduced ATP-induced IL-1β release and blocked caspase-1 activation (41). In a cell-free model, zinc chelation had no direct effect on caspase-1 activation. Intracellularly, zinc chelation is likely to indirectly act on the inflammasome via blocking of the ATP-channel pannexin-1 and to thus prevent pyroptosis (41). Since pyroptosis is a part of the antimicrobial immune response to allow rapid pathogen clearance by removing intracellular replication niches, this is another example of the role of zinc in the immune response.

## 2.5. Zinc's Importance in Metabolism

The regulation of cellular metabolism is a central event during immune cell activation. There are many hints for a role of zinc in metabolism. For example, disturbances of zinc homeostasis have been linked with metabolic diseases such as diabetes mellitus (111). This disease is characterized by increased urine volume, which may increase the loss of zinc. A meta-analysis of several studies revealed that zinc supplementation (ranging from 15 to 660 mg/day in varying formulations) balanced glycemic control in models of both type 1 and type 2 diabetes, indicating a role of zinc in glucose metabolism (111, 112). For example, zinc was discovered to be part of the insulin complex (227). Furthermore, insulin-responsive aminopeptidase, an enzyme that is required for the maintenance of the glucose transporter GLUT4 and thus for insulin-mediated glucose uptake from the blood, is zinc dependent (120). Zinc has insulino-mimetic effects, directly impacts signaling pathways, and thus also modulates the insulin receptor (INSR) signaling pathway (1, 221). Since this pathway is involved in the activation of T cells (70), zinc likely modulates their metabolic

activation. Zinc inhibits PTP1B (29), the enzyme that regulates the phosphorylation of the INSR. This inhibition leads to increased phosphorylation of the INSR and in turn to increased signaling that causes upregulation of glucose transporters (111). This result suggests that zinc is involved in insulin-mediated glucose uptake and in the decrease of blood sugar levels and may have beneficial effects in diabetes patients. Additionally, due to having anti-inflammatory capacities, zinc has an ameliorating impact on the autoimmune response toward pancreatic cells in type 1 diabetes mellitus (T1DM) (see Section 6).

Furthermore, zinc is involved in other metabolic pathways like carbohydrate and lipid metabolism. The link between zinc and lipid metabolism was discovered in the 1980s. A strong negative association between (*a*) dietary zinc intake and (*b*) heart disease, hypertension, and hypertriglyceridemia has been described. In humans, zinc supplementation impacts serum cholesterol levels: Both total cholesterol and LDL cholesterol were significantly decreased in various zinc supplementation studies, which included type 2 diabetes patients, obese individuals, and healthy subjects. The most significant effects were observed in diabetic and obese individuals (208). Murine models of atherosclerosis showed that zinc deficiency impacts fatty acid metabolism and increases plasma cholesterol levels as well as total fatty acid concentration (211, 232). Mechanistically, fatty acid translocase expression in the aorta was upregulated in zinc-deficient mice, whereas lipoprotein lipase was found to be increased only in zinc-adequate mice, and both effects were linked to PPARγ (peroxisome proliferator–activated receptor γ) activity (211).

Zinc also impacts glycation as well as glycosylation, the most common posttranslational modification of proteins. During glycation, advanced glycation end products (AGEs) are produced; AGEs impact every cell type and are thought to be involved in aging and some chronic diseases, including diabetes (179, 237). Endogenous AGEs are formed during normal metabolism, but excess levels promote oxidative stress, inflammation, and apoptosis. Zinc shows antiglycation properties in vitro (supplementation with 125, 250, and 500 μM zinc) as well as in rats supplemented with 100 mg/kg per day via gavage (219, 244). Furthermore, zinc supplementation of cell cultures with 1, 10, or 100 μM zinc chloride downregulated the RAGE (receptor for AGE) channel that is involved in AGE-induced oxidative stress. Zinc may thereby limit cellular damage in vitro (279). Zinc also regulates glycosylation: In fungi, zinc deprivation led to the downregulation of glycosylation as well as of ER proteins that are involved in this process (54). In mice, zinc deprivation modified *O*-glycosylation and sialylation of secreted mucins in the gut (166). Goblet cells produced significantly higher amounts of short *O*-glycans relative to zinc-adequate controls, due to zinc's effects on the initial glycosyltransferases that are responsible for *O*-glycan biosynthesis (166).

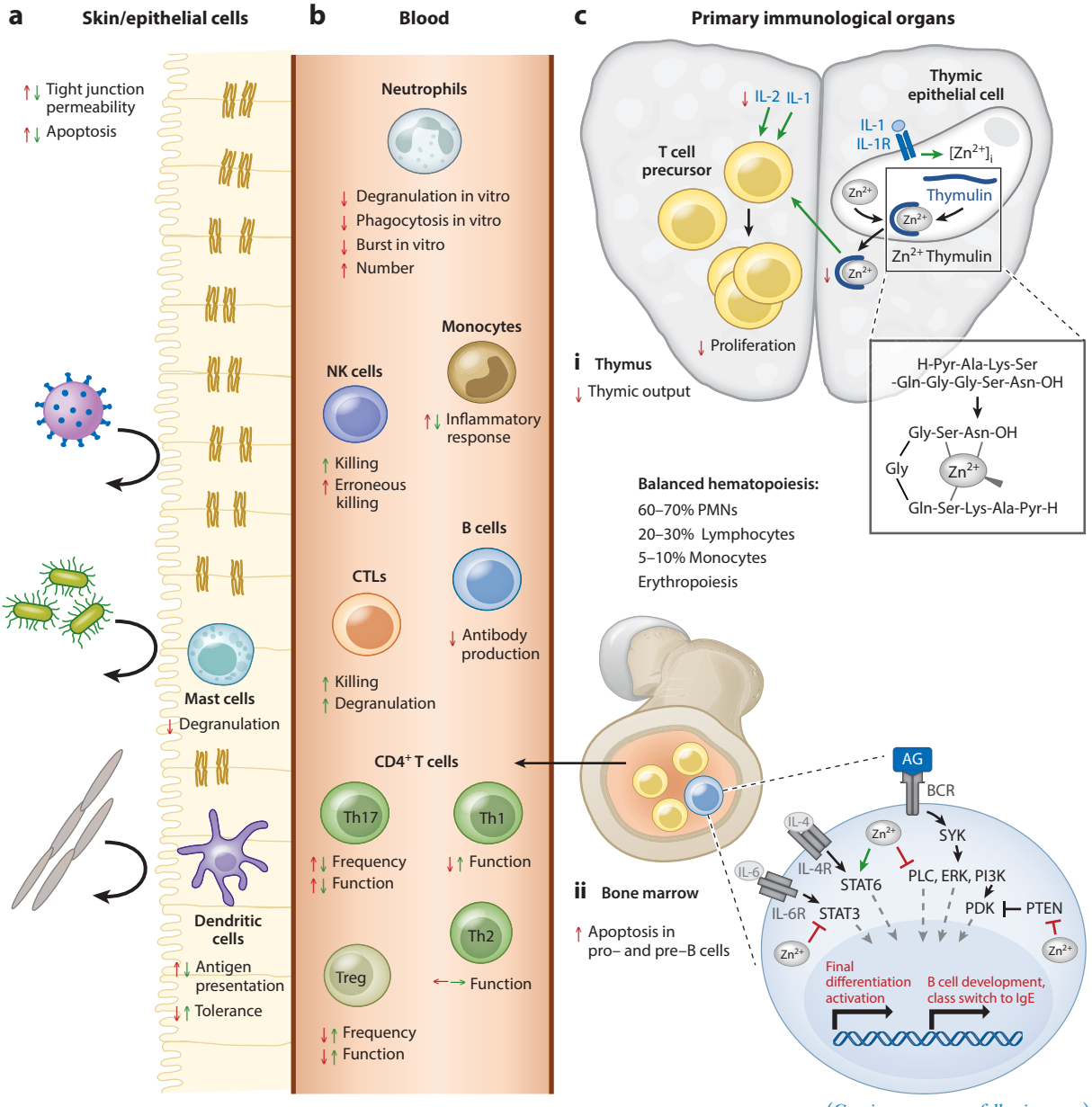
Taken together, these findings show that zinc impacts different levels of cellular metabolism, altering the response to systemic metabolites as well as their levels and modifications.

## 2.6. Role of Zinc in Intracellular Signaling

Extracellular changes in zinc homeostasis are observed during, for example, the APR, oxidative stress, neuronal activation, and mast cell degranulation, affecting zinc levels in serum, the synaptic cleft, the brain, and other tissues. Even the smallest changes in free zinc levels may induce changes in the activity of immune cells. Those extracellular alterations in zinc homeostasis may be detected by a recently discovered zinc-sensing receptor (ZnR), classified as G protein–coupled receptor 39 (99, 184). ZnR ligation activates inositol triphosphate 3 signaling and calcium release, and this activation subsequently results in phosphorylation of signaling molecules in the ERK and AKT pathways.

In addition to alterations in extracellular zinc, changes in intracellular free zinc were observed after activation of various cell types. Here, one can distinguish zinc signals according to the

duration of the increase or decrease in intracellular free zinc: Alterations may last for only seconds to minutes (termed zinc flux or, in some models, zinc sparks) or for minutes to hours (termed zinc wave) (168). Finally, significant and constant changes in extracellular zinc may result in alterations of the expression and activity of zinc transporters and in subsequent changes in intracellular zinc. Alterations in intracellular zinc levels, which are stable for hours to days, are termed homeostatic zinc signals (91, 92, 168, 174, 261). The immune-related effects of zinc signals have been described in detail (91, 92, 174), and examples can be found in **Figures 2** and **3** and Section 3.




(*Caption appears on following page*)

**Figure 2** (*Figure appears on preceding page*)

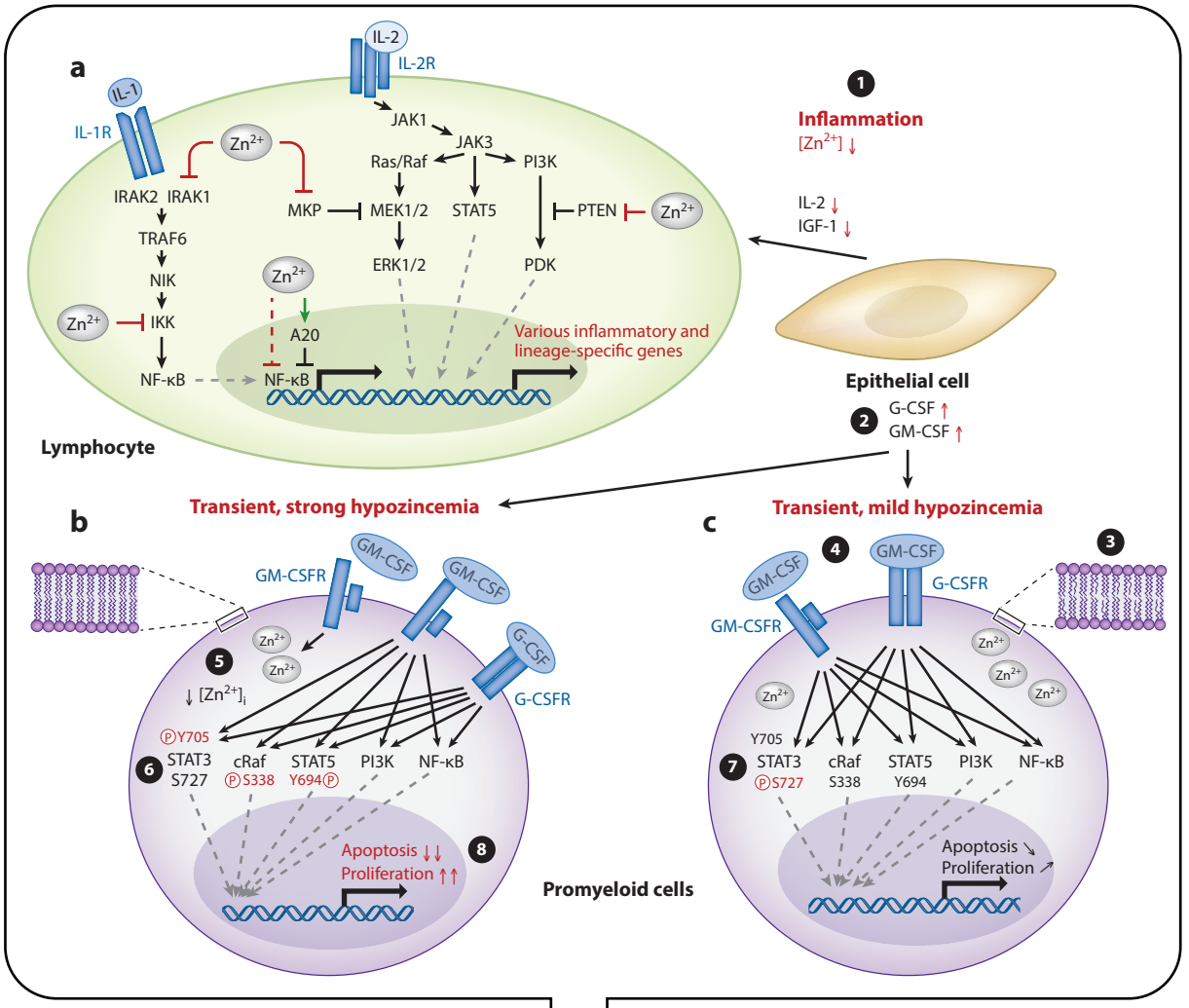
Zinc-modulated processes in preventing infectious diseases. Zinc modulates mechanisms of pathogen clearance for the different types of immune cells, their development, and the barrier functions of skin and epithelial cells. Green arrows illustrate mechanisms that are modulated by zinc supplementation, and red arrows indicate altered functions and frequencies in zinc deficiency. (*a*) Zinc regulates the barrier function of skin and epithelial cells as well as the function of cells residing at this barrier, such as mast cells and dendritic cells. (*b*) Zinc modulates different subsets of immune cells in the bloodstream. Neutrophils, the most abundant type of immune cells and the first cells to be recruited to infections, increase in function and frequency during zinc deficiency. Monocytes and NK cells are more active during zinc deficiency. Cells of the adaptive immune system include B cells, which provide protective antibodies; CTLs, which can kill infected target cells; and Th cells. Th cells can be subdivided into Th17 cells, which are involved in the killing of extracellular pathogens; Th1 cells, which fight intracellular infections; Th2 cells, which are important for the elimination of parasites; and Tregs, which are required for tolerance and tissue homeostasis. Zinc regulates adaptive immune cells, as indicated for each cell type. (*c*) The development of immune cells depends on zinc as well. The two primary immunological organs, thymus and bone marrow, require zinc for their function. (*i*) In the thymus, zinc is required for the production of functional thymulin and is involved in the response of T cells to IL-1 and IL-2. The signaling pathways of these cytokines are depicted in **Figure 3**. (*ii*) In the bone marrow, cell maturation shifts during zinc deficiency, favoring the myeloid lineage. Increased apoptosis is observed in lymphoid progenitors, whereas myeloid cell differentiation is augmented (see **Figure 3**). Zinc also modulates the development of B cells in the bone marrow: Key signaling events are inhibited (*red blunted arrows*) or enhanced (*green arrow*) by zinc. Abbreviations: AG, antigen; BCR, B cell receptor; CTL, cytotoxic T lymphocyte; ERK, extracellular signal–regulated kinase; IL-(R), interleukin (receptor); NK cell, natural killer cell; PDK, phosphoinositide-dependent kinase 1; PI3K, phosphoinositide 3-kinase; PLC, phospholipase C; PMN, polymorphonuclear neutrophil; PTEN, phosphatase and tensin homolog; STAT, signal transducer and activator of transcription; Th cell, T helper cell; Treg, regulatory T cell.

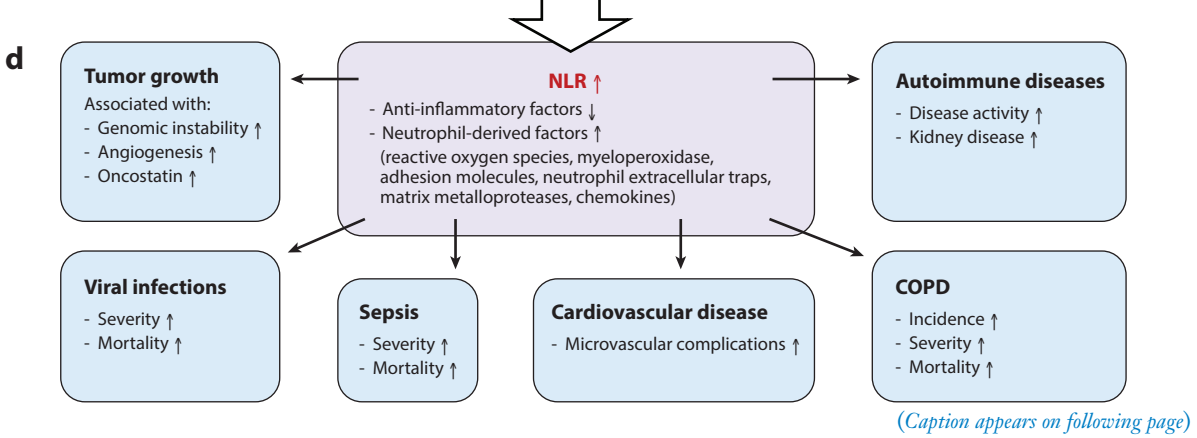
The total cellular zinc concentration is in the range of hundreds of micromolar. However, the free zinc ion concentration, available for intracellular signaling, is in the picomolar range. Zinc transients are predominantly explained by the quick and reversible release of zinc from proteins, especially MTs, or intracellular organelles, including vesicles with high zinc content (termed zincosomes), and strongly impact intracellular signaling (168).

The zinc ions that are released during a zinc wave or zinc flux are also termed zinc transients. The transients were observed quickly after activation of mast cells by FcεRI, lipopolysaccharide (LPS), or IL-33 and after ligation of the T cell receptor (TCR) and B cell receptor (BCR), which is discussed in more detail below. Interestingly, calcium signals can also be upstream of a zinc signal. The interaction of calcium and zinc signals is attracting increasing attention (148, 193).

Zinc was suggested as the main player regarding the phosphatase/kinase balance. Zinc inhibits the activity of human PTPs, which are key enzymes in the regulation of phosphorylation and are thus central to the control of cellular signaling and metabolism, in a picomolar to nanomolar range (174, 266). In general, phosphatase activity exceeds kinase activity in a cell. Thus, the reversible inhibition of phosphatases by zinc ions may be the key to permitting or preserving phosphorylation of signaling molecules (92). In addition to being activated by changes in phosphorylation, signaling molecules can be activated by caspases, another group of enzymes that are sensitive to alterations in zinc homeostasis. The association between zinc, caspase activation, and apoptosis is well described (65). The association between zinc and nonapoptotic roles of caspases, including proliferation, tumor suppression, differentiation, neuronal development, and aging, needs to be further explored (231, 278).

Phosphodiesterases (PDEs) require a catalytic zinc ion for their activity (52). They are largely involved in controlling cAMP and cGMP signaling. However, the inhibition of PDEs by zinc has also been described (32, 251), which is still a matter of discussion, since the median inhibition concentration (IC50) found is in the micromolar range (174). Most analyses were performed with cell lysates and purified enzymes in buffer, which may overestimate the IC50 for zinc. Thus, the inhibition of PDEs in cells should be further investigated (52, 251).




(*Caption appears on following page*)

**Figure 3** (*Figure appears on preceding page*)

Consequences of transient zinc deficiency for lymphocytes and neutrophils. During inflammation, transient serum hypozincemia occurs (①), which impacts the expression of cytokines and growth factors and their signaling pathways in lymphocytes (*a*) as well as promyeloid cells (*b*,*c*). (*a*) In lymphocytes, zinc modulates the signaling pathways of the key cytokines IL-1 and IL-2. (*b*,*c*) Preexisting zinc deficiency augments a decrease in serum zinc, while smaller changes are expected in a zinc-adequate or a zinc-supplemented individual. In turn, epithelial cells are activated and secrete GM-CSF and G-CSF. Zinc deficiency is associated with increased G-CSF and GM-CSF expression (*b*), which is attenuated by zinc supplementation (②). High intracellular zinc levels are associated with increased membrane fluidity in promyeloid cells (③). Increased intracellular zinc levels result in decreased surface levels of the GM-CSFR in promyeloid cells and mature neutrophils (④). Ligation of the GM-CSFR transiently decreases free intracellular zinc in promyeloid precursors and primary neutrophils (⑤). Under zinc-supplemented conditions, no changes in intracellular zinc are observed after GM-CSFR ligation (*c*). Ligation of G-CSFR and GM-CSFR activates several intracellular signaling pathways. High zinc conditions attenuate the phosphorylation (P) of STAT5 (tyrosine 694), cRaf (serine 338), and STAT3 (tyrosine 705) (⑥). G-CSF-induced phosphorylation of STAT3 at serine 727 (⑦) is augmented by zinc supplementation. GM-CSF and G-CSF increase proliferation and survival of myeloid cells (⑧). Attenuation of G-CSF and GM-CSF-induced signaling in high zinc conditions may explain the less pronounced increase in the NLR relative to preexisting zinc deficiency. (*d*) As a result, the NLR increases during zinc deficiency. An elevated NLR is observed in a plethora of diseases, including infections, autoimmunity, and cancer. Abbreviations: A20, tumor necrosis factor alpha–induced protein 3; COPD, chronic obstructive pulmonary disease; ERK, extracellular signal–regulated kinase; G(M)-CSF, granulocyte(-macrophage) colony–stimulating factor; G(M)-CSFR, G(M)-CSF receptor; IGF-1, insulin-like growth factor 1; IKK, IκB kinase; IL, interleukin; IL-R, interleukin receptor; IRAK, receptor-associated kinase 1; JAK, Janus kinase; MEK, mitogen-activated protein kinase kinase; MKP, mitogen-activated protein kinase phosphatase; NF-κB, nuclear factor kappa light-chain enhancer of activated B cells; NIK, NF-κB-inducing kinase; NLR, neutrophil-to-lymphocyte ratio; PDK, phosphoinositide-dependent kinase; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; STAT, signal transducer and activator of transcription; TRAF, tumor necrosis factor receptor–associated factor.

Alterations in the activity of zinc-dependent proteins such as transcription factors (e.g., MTF-1) represent another mechanism for how zinc participates in intracellular signaling. Zinc transients can be directly sensed by MTF-1, which subsequently induces gene transcription (87).

In addition to the above-described temporary zinc transients (flux and wave), the long-lasting homeostatic changes in intracellular zinc were associated with changes in signaling, although in a broader context. Zinc-dependent, epigenetically active enzymes play a dominant role in this regard, as summarized in detail (40, 196, 258, 268). One well-studied example is the group of histone deacetylases (HDACs). Deacetylation of histone and nonhistone proteins—including transcription factors such as Foxp3, by zinc-dependent and non-zinc-dependent, but zinc-regulated, HDACs—affects chromatin structure and thus promoter accessibility or transcription factor stability, respectively (128, 196, 217, 260).

## 2.7. Zinc in the Regulation of Ion Channels

The intracellular concentration and fluctuation of metal ions are involved in numerous intracellular signaling pathways. Divalent ions such as calcium and magnesium act as second messengers, whereas monovalent ions like sodium or potassium mostly regulate membrane potential.

Zinc affects the conformation of ion channels by directly binding to His, Cys, Asp, or Glu residues. Consequently, ion flow is either activated or inhibited (104). Ion channels are subtyped into voltage-gated, ligand-gated, second messenger–gated, or mechanosensitive channels (187). Potassium ion channels are one such example of zinc-modulated ion channels. Some potassium channels are prone to conformational changes caused by binding to extracellular zinc, while other potassium channels, namely the potassium transporter transient receptor potential channel A1 (TRPA1) (10) and large-conductance voltage–activated and calcium-activated Slo1 $K^+$ (BK) channel (104), are activated by increasing intracellular zinc levels (105).

Calcium channels are involved in signaling pathways, regulating a variety of intracellular processes, including motility, transcription, and apoptosis, and thus strongly impact immune cell functions. Zinc replaces calcium in mitochondrial calcium transporters and in calcium channels

located in excitable membranes. Moreover, calcium channels are also permeable for zinc (8). However, for L-type calcium channels, zinc can be transported only when calcium levels are low, since the affinity of the receptor for zinc is low (31). Besides being transported through calcium channels, zinc can also modulate the activity of calcium channels. Zinc can suppress voltage-dependent calcium channels by binding to the α1 pore region. Store-operated calcium channels, which play a central role in calcium entry in activated lymphocytes following antigen-receptor stimulation, can also be regulated by zinc binding because zinc acts as a competitive inhibitor for calcium transport. In this case, zinc can bind to a Cys residue and thus block calcium flux (83). The important role of ion channels in signal transduction in various immune cells, especially T and B cells, explains how zinc indirectly affects intracellular signaling and the immune response.

## 3. ZINC IN HEMATOPOIESIS

Bones are important reservoirs of zinc containing approximately 30% of total body zinc, which can be adjusted in cases of mild zinc excess or deficiency (204). However, impaired skeletal development, bone growth, and fracture healing; low bone mineral density; and disturbed bone marrow metabolism were found in humans and rodents during zinc deficiency (167, 178, 204, 218, 239). Low serum zinc levels revealed predictive value regarding altered bone mineral density in humans, and dietary-induced zinc deficiency caused a reduction in bone density in rats (178, 239). In line with these findings, zinc supplementation increased bone health, as monitored by improved bone mineral density and the prevention of bone loss, which was described for diabetic rats supplemented with 0.25 mg/kg daily by oral gavage (9, 107, 204). Among the underlying mechanisms, zinc's importance in the activation of alkaline phosphatase, as well as its impact on intracellular signaling, transcription factor binding, and thus collagen synthesis was suggested (43, 229). With regard to collagen, zinc's importance in protein stability may play a role as well (12, 229).

In light of the importance of zinc for bone health (9), alterations in hematopoiesis, which primarily takes place in the bone marrow, can be expected during changes in zinc homeostasis, especially when zinc is limited (**Figure 2**). In fact, various research groups found serum zinc deficiency in anemic patients (46, 197, 204). A very recent study on chronic kidney disease–related anemia found that bone and serum zinc can be redistributed to the human bone marrow to support erythrocyte development. Those newly produced erythrocytes were high in zinc while serum zinc levels were lower in anemic patients than in nonanemic control patients (46, 218). Those data are in line with the hypotheses that zinc homeostasis is well balanced and that zinc transporters and zinc binding proteins efficiently transfer zinc to tissues and cells requiring zinc (107).

However, the possible causality between zinc deficiency and anemia is not entirely clear. Zinc supplementation (34 mg/day for 3 weeks) attenuated anemia symptoms in patients with initially low serum zinc levels (<80 mg/dl), who suffered from chronic renal failure (80). Whether only anemic patients with lower serum zinc and kidney disease benefit from zinc supplementation remains to be investigated in future clinical studies. Deeper insight into the fundamental interactions between zinc and iron (202, 247)—especially with regard to erythrocyte development, maintenance, and recycling—might form the basis to better understand zinc's role in the etiology of anemia and the use of zinc in its therapy.

Regarding leukocytes, there are several studies describing altered development during zinc deficiency, implying that zinc may be a major player during physiological hematopoiesis (74, 103, 124). Early and recent studies in zinc-deficient rodents suggest that the development of myeloid cells is prioritized at the cost of lymphoid cells, resulting in an increased neutrophil-to-lymphocyte ratio (NLR) (124, 180). An increase in the NLR was observed during the APR in humans, during

which zinc is transiently transferred to the tissues, causing serum hypozincemia. Although needing to be experimentally proven, a direct link between zinc homeostasis and hematopoiesis is likely. Thus, an increase in the NLR may also be expected in chronically zinc-deficient humans. Monitoring zinc status during differentiation of myeloid precursors into mature monocytes or granulocytes may be a first step toward testing this hypothesis. Subsequent investigations of the impact of zinc homeostasis on maturation of myeloid cells combined with retrospective studies on patients with disturbed NLR would be a fundamental prerequisite before clinical studies could be planned.

Neutrophils are highly reactive cells that are equipped with their complete antimicrobial weaponry when they leave the bone marrow. In addition to antimicrobial peptides such as lysozyme, they can form ROS and reactive nitrogen species (RNS) and inflammatory mediators such as calprotectin (94, 262). Elevated numbers of those cells, which are associated with chronic serum zinc deficiency (74), increase the risk for overshooting immune responses (56).

### 3.1. T and B Cell Development

The major explanations for the decrease in T cell numbers observed during zinc deficiency are thymic atrophy, low thymulin production and activity, decreased IL-2 levels, altered IL-2-induced signaling, and thus an increase in lymphocyte apoptosis paralleling decreased proliferation (61, 75, 117). Thus, all the fundamental roles of zinc described in Section 2 seem to play a role during the regulation of thymic development and the maturation of thymus-derived cells. However, not all lymphocyte subtypes and developmental stages are affected in the same magnitude, as detailed in **Figure 2**.

The highest loss is observed in immature CD4/CD8 double-positive T cells that are the precursors of mature T cells. They are prone to apoptosis due to generally low expression of anti-apoptotic Bcl-2 (B cell lymphoma 2), which is augmented during zinc deficiency, as well as due to the activation of caspases (103, 169). Pre–T cell development is also strongly affected during zinc deficiency; here, the lower production and zinc-dependent activity of thymulin are largely involved (182). Metalloenzymes such as thymulin often require zinc binding and a subsequent conformational change to maintain full activity (58). Furthermore, data from a recent study suggest that PP2A (protein phosphatase 2A)-mediated increase in expression of the transcription factor cAMP-responsive element modulator α (CREMα) is responsible for deacetylation of the IL-2 promoter and for the subsequently reduced IL-2 expression and secretion observed during zinc deficiency (128). This study underscores that cellular functions such as IL-2 expression can be affected by alterations in zinc homeostasis on multiple levels. In this example, zinc affected gene expression (of CREMα) and enzymatic activity (of HDAC1). Fortunately, most of the above-described lymphopoiesis-related symptoms can be reversed when zinc is supplemented and adequate serum zinc levels are reestablished (116, 182, 261).

The impaired B cell development observed during zinc deficiency, which especially affects pro– and pre–B cells, is also largely explained by increased apoptosis (180). B cells are critically dependent on the expression of Zip7 and Zip10. The latter is expressed on the surface of B cells, and its deletion leads to splenic atrophy and to a reduction in B cell numbers (123, 180). Although mature B cells are generally more resistant to zinc deficiency, their survival requires tonic signals from their antigen receptor, the BCR. BCR signaling and subsequent proliferation, activation, and antibody production seem to depend on Zip10 as well (102). Zip7-deficient mice showed lower intracellular free zinc levels, an increase in phosphatase activity, and thus decreased phosphorylation of signaling molecules downstream of the pre-BCR and BCRs (13). Strikingly, *Zip7* mutations in humans occurred with absent B cells, agammaglobulinemia, and early-onset

infections (13). Furthermore, Zip7 is upregulated during B cell activation, and intracellular zinc levels are increased, which may be important for their proliferation (189).

Both T and B cells are required for an efficient and pathogen-specific adaptive immune response and for the formation of an immunological memory. Low levels of either or both cell types may increase susceptibility to infectious diseases, to the development of cancer, and to the failure of vaccination and may be a prerequisite to autoimmune diseases (AIDs) due to a lack of regulatory cells (210, 263, 264).

### 3.2. Natural Killer Cell Development and Function

While natural killer (NK) cell numbers remained stable, zinc deficiency was associated with impaired functions both in vitro and in zinc-deficient human patients (7, 183). In contrast, zinc supplementation of initially zinc-deprived subjects (12 mg/day via supplement in humans; 2,500 ppm of zinc oxide for 14 days via diet in pigs) increased NK cell activity in vivo (126, 183). Similarly, zinc supplementation significantly elevated the activity and the percentage of NK cells within cultured leukocytes in vitro (177). In addition, zinc seems to be especially important for the differentiation of $CD34^+$ progenitor cells via GATA3 and, in line with the findings above, induced higher levels of cytotoxic activity and a higher number of perforin-producing and of CD94-bearing $CD56^+$ cells relative to unsupplemented $CD34^+$ cultures (185). Inefficient killing activity of NK cells may result in recurrent viral infections and cancer.

NK cell activation is inhibited by the detection of major histocompatibility complex (MHC)-I molecules on the surfaces of other cells via killer inhibitory receptors (KIRs). Since zinc is required for the multimerization of KIR domains and interaction with MHC-I, KIR-induced signaling is disturbed during zinc deficiency (207, 246). This missing MHC-I binding may also result in unspecific killing, which remains to be investigated in detail. In contrast, zinc supplementation enhances interferon (IFN)$\gamma$ secretion by phytohemagglutinin-activated peripheral blood mononuclear cells (PBMCs) in vitro, which is attributed to NK cells and may support the activation of other immune cells, thus supporting the antiviral response (177). Finally, zinc also directly increases the killing activity of both a NK cell line and primary human NK cells. Rapid zinc influx after zinc deficiency boosts NK cell cytotoxicity in vitro and may support an efficient antiviral and anticancer response (214).

### 3.3. Myelopoiesis

In severely zinc-deficient rodents, myelopoiesis seems to be conserved. Relative to zinc-adequate mice and rats, higher numbers of polymorphonuclear neutrophils (PMNs), monocytes, and their products were found in zinc-deficient animals (74, 75, 220), suggesting that the limited amounts of zinc are devoted to sustaining primarily the innate immune response. Similarly, data regarding the in vitro differentiation of promyeloid HL-60 cells into monocytes revealed a decrease in free intracellular zinc during maturation as well as increased differentiation into monocytes in zinc-deficient cultures (62). These findings are in line with the augmented increase in myelopoiesis and the resulting higher NLR paralleling acute serum hypozincemia during the APR (156, 259). A transient increase in the NLR during the early phase of an infection may be beneficial since high numbers of macrophages and PMNs may efficiently clear the infection. However, if elevated numbers of highly reactive PMNs persist, detrimental effects can be expected, as summarized in Section 6.

Zinc may be involved in fine-tuning of polarization into macrophage subtypes. Zinc deficiency supported polarization into proinflammatory, M1-like macrophages in a murine colitis

model (100). Knocking down Zip7 expression and thus zinc uptake in human myeloid THP-1 cells favored the generation of M2-like cells, which have a rather anti-inflammatory phenotype and are typically involved in tissue remodeling and repair. The Zip7 knockdown additionally decreased the ability of THP-1 cells to express IL-1β and tumor necrosis factor (TNF)α as well as phagocytosis (272). Furthermore, in vitro zinc supplementation (5 μM zinc, 0.5 μM pyrithione) of Zip7 knockout cells inhibited activation-induced polarization into M2-like cells, whereas zinc did not affect differentiation into M1-like cells under the same conditions (272). In another study, zinc (50 μM) decreased in vitro M2-like polarization of THP-1 cells, while differentiation into proinflammatory M1-like macrophages remained unaltered (60). Assuming that zinc deficiency augments the polarization of M1-like macrophages into a proinflammatory phenotype, the high number of inflammatory cells may result in an overshooting inflammatory response. Major products of macrophages are IL-6, TNFα, and IL-1β, and thus chronically zinc-deficient individuals are at risk of chronic inflammatory processes, as observed in AIDs, or cytokine storm and subsequent tissue damage if they get infected (264).

Unfortunately, little is known about the role of zinc in the regulation of differentiation-related genes important during myelopoiesis, such as transcription factors. Thus, direct effects are difficult to estimate but are likely due to the strong impacts of zinc on signaling, transcription factor activity, epigenetics, protein stability, and thus gene expression (92, 258). However, recent data suggest that zinc can alter growth factor–induced signaling not only in lymphocytes (117) but also in myeloid cells: In vitro studies in promyeloid cell lines revealed that zinc supplementation (100 or 20 μM zinc, 10 μM pyrithione) decreases granulocyte-macrophage colony–stimulating factor (GM-CSF)-induced MAPK signaling and G-CSF-induced signal transducer and activator of transcription (STAT)3 activation (14, 262). Our own unpublished data for mature human granulocytes, in contrast, reveal an increase in GM-CSF receptor expression and subsequent MAPK signaling after zinc supplementation (50 μM zinc, 1 μM pyrithione) and contrasting data for promyeloid cells, suggesting that effects on precursors and mature cells may differ (M. van der Laan, A. Büttgenbach, L. Rink & I. Wessels). As G-CSF and GM-CSF effects depend on the developmental stage of myeloid cells, inducing either differentiation in precursors or activation and chemotaxis in mature granulocytes, zinc's effects may also differ with differentiation status (73). Interestingly, the zinc status of precursors and mature myeloid cells may differ and decrease with maturation, supporting this assumption (62). Furthermore, the addition of zinc (2–100 μM) promotes long-term self-renewal of murine embryonic stem cells in culture and inhibits expression of differentiation markers (106, 181).

Increased granulopoiesis and monopoiesis may also be explained by altered expression of myeloid growth factors. In this regard, zinc supplementation of mice (30 mg/kg, 3 days) after spinal cord injury increased G-CSF release of microglia and macrophages, while in vitro incubation of human PBMCs with zinc-loaded γ-globulin decreased the release of G-CSF and GM-CSF relative to incubation with untreated γ-globulin (45, 149), providing no clear answer but suggesting that zinc's effect on growth factor expression probably depends on the cell type and stimulus.

Consequently, multiple questions regarding the role of zinc in myelopoiesis remain to be answered, and in vivo studies in acute but also chronically zinc-deficient human subjects are warranted to find the missing pieces of the puzzle.

### 3.4. Differentiation of Dendritic Cells and Mast Cells

In addition to having a role in monopoiesis and granulopoiesis, zinc is important in dendritic cell (DC) development. In this context, maturation induced by LPS resulted in decreased intracellular free zinc levels, as described below (181). Also, A20 (TNFα-induced protein 3) can inhibit DC

maturation (55), supporting a role of altered zinc homeostasis during DC differentiation, as zinc activates A20 expression, thus suppressing gene expression (55). As DC maturation is a prerequisite for antigen presentation and thus activation of adaptive immune cells, disturbed DC maturation may consequently disturb the adaptive immune response against pathogens. More studies on the effects of zinc deficiency are required in this regard.

Data revealing elevated differentiation of mast cells within the bone marrow and other tissues in zinc-deficient rats date back to the late 1970s and early 1980s and are very limited (27, 235). In addition to showing increased cell numbers, mast cells of zinc-deficient rats were bigger in size and contained proportionally increased numbers of mitochondria and specific granules, which may partly explain the epithelial hyperplasia found in the mucosa of the rat's cheek during zinc deficiency (235).

## 4. ZINC IN INFECTIOUS DISEASES

Zinc's antimicrobial properties begin with preventing pathogens from entering the human body, as adequate zinc is well known to protect the integrity of tissue barriers and to support the regeneration of damaged tissues as well as wound healing (133). This property was shown for organs such as the intestine, liver, and lungs in humans and mice and has also been suggested for the blood-brain barrier (19, 24, 165, 262). As underlying mechanisms, induction of the expression of tight junction proteins such as occludins, claudins, and zonula occludens by zinc via effects on gene expression is discussed. Moreover, the inhibition of apoptosis in vascular and tissue cells and their increased proliferation may play a role (65, 165, 215). However, zinc deficiency is characterized by increased barrier permeability, for example, a leaky gut (165, 215). Here, not only are important trace elements and minerals lost from the body, resulting in a vicious circle of aggravated nutritional deficiencies, but also pathogens can more easily enter the body.

As discussed above, an increased NLR is regularly observed in humans and animals with zinc deficiency, which can be rebalanced with zinc supplementation. A mildly increased NLR is a well-accepted marker of inflammation and oxidative stress; a strongly elevated NLR is a risk factor for severe disease progression, complications, and mortality (113, 253, 256), as explained below. Reducing the NLR may therefore positively influence disease progression, as illustrated in **Figure 3** (113). In patients with preexisting zinc deficiency, an augmented inflammation-induced NLR and thus a risk of disease complications are likely.

One might expect that supporting the development and function of innate immune cells might be beneficial during the antimicrobial response. However, preexisting zinc deficiency is often associated with an overshooting and prolonged inflammatory response and elevated oxidative stress (129, 198, 267). Moreover, preexisting zinc deficiency augments the recruitment of highly reactive innate immune cells to infected tissues, as observed in acute respiratory distress syndrome and sepsis (36, 129, 262). Here, cell numbers and disease severity were inversely correlated with serum zinc status (101, 259). Among others, high serum levels of TNFα, IL-1β, IL-6, IL-17, G-CSF, IL-1RA, C reactive protein, and calprotectin were correlated with zinc deficiency in mice and during in vitro experiments using human cells (22, 23, 25, 150, 260).

The mechanisms underlying the zinc deficiency–induced increased expression of inflammatory genes—namely zinc's importance during intracellular signaling and its effects on the epigenome, on balancing redox metabolism, and on posttranslational modifications—are discussed elsewhere (22, 127, 258). The observation that cell polarization is skewed to M1-like cells, T helper (Th)2 cells, and Th17 cells during zinc deficiency adds evidence for the overshooting inflammatory response and explains high levels of IL-6 and IL-17 (140). There are many uncovered mechanisms, and thus we refrain from going into detail here (79). As a result of hyperinflammation

and overproduction of ROS and RNS, host tissue such as the lungs and intestine is damaged, facilitating entry of additional pathogens and increasing the risk for secondary infections.

The detrimental consequences of a high NLR and thus elevated amounts of PMN products such as ROS, RNS, and matrix-degrading peptides that may cause tissue destruction, combined with the lack of T and B cells and thus weak specific pathogen clearance by individuals with a preexisting zinc deficiency, are obvious.

Although the anti-inflammatory effects of zinc supplementation are described in numerous studies, surprisingly little research has focused on zinc's role during expression of anti-inflammatory mediators, which are important for the resolution of the inflammatory response as well as for preventing hyperinflammation and tissue damage. Existing data, however, show that serum zinc levels normalize in parallel with the decrease in the proinflammatory response at the end of the APR, when expression of anti-inflammatory factors is starting (259). Thus, zinc deficiency may suppress the anti-inflammatory response, and inducing the compensatory anti-inflammatory reaction, for example, by zinc supplementation at later stages of the APR, may counterbalance the prolonged inflammatory reaction induced by longer-lasting serum hypozincemia. This hypothesis is supported by findings of decreased secretion of IL-8 and IL-1RA in cultures of LPS-stimulated primary human neutrophils in which zinc was chelated before activation (94). In contrast, polarization of Th2 cells and their ability to express IL-4 and IL-10 appeared to be unaltered in zinc deficiency (198). Thus, this question remains to be addressed by further research. The effects of zinc on gene expression via altering intracellular signaling and transcription are well investigated, but only for inflammatory and antimicrobial genes. Whether there is a general difference in the regulation of pro- versus anti-inflammatory genes is a fundamental question that needs to be investigated.

### 4.1. Extracellular Bacterial and Fungal Infections

Phagocytes, including predominantly macrophages and neutrophils, are major players during the response against extracellular pathogens. In vitro investigations of human granulocytes revealed that the acute chelation of zinc as an in vitro model for zinc deficiency results in lower formation of neutrophil intracellular traps (NETs), chemotaxis, phagocytosis, and degranulation, indicating reduced antimicrobial activity (93, 94). However, release of zinc binding calprotectin by myeloid cells, which can inhibit the growth of pathogens such as *Staphylococcus aureus* by depriving it of zinc, increased when zinc was chelated in vitro (150). Furthermore, the prioritization of myelopoiesis over lymphopoiesis and the increased recruitment of neutrophils to infected tissues during zinc deficiency observed in vivo indicate that the in vivo setting is more complex and difficult to model in vitro (124, 129). In mice, preexisting zinc deficiency caused overactivation of myeloid cells, a hyperinflammatory response, subsequent tissue damage, and increased mortality from sepsis (129). In line with this finding, activation-induced NET formation and neutrophil-specific products, oxidative stress, inflammatory cytokine levels, and tissue damage decreased with zinc supplementation before inflammation was induced (22, 25, 259, 262). Here, zinc supplementation schemes ranged between 24 h and weeks prior to induction of infection. Zinc supplementation was achieved by giving an oral dose of 45 mg of zinc daily (in humans), by feeding a high-zinc diet (100–180 ppm in mice), or by injecting 15 μg/kg of elemental zinc (in mice). Regarding macrophages, research on murine macrophage cultures in vitro revealed that zinc chelation increases the production of nitric oxide, which is a marker of proinflammatory M1-like macrophages (38). Upregulated expression of inflammatory cytokines and ROS in zinc-deficient monocytes is also regularly observed. The underlying mechanisms, which largely involve the effects of zinc deficiency on Toll-like receptor (TLR)-induced signaling, are well described (38, 252). Furthermore, LPS stimulation leads

to upregulation of Zip8 expression, which is accompanied by an increase in intracellular zinc, underscoring the importance of zinc for macrophage functions (153).

DCs, the most important antigen-presenting cells, initiate the T cell response. DCs react to LPS stimulation via TLR-4 with downregulation of Zip6 and upregulation of zinc exporters, leading to a decrease in intracellular zinc concentration accompanied by increased maturation of DCs. This reduction of intracellular zinc is important for key functions of DCs such as surface expression of MHC-II (125) and thus activation of adaptive immune cells, including T cells.

Zinc deficiency strongly impacts polarization of Th cells (140, 172, 198). Generally, polarization into Th1 cells is impaired during zinc deficiency, while Th2 cell products like IL-4, IL-6, and IL-10 remain unaffected, leading to an imbalance in the Th1/Th2 ratio toward Th2 cells and to unbalanced cell-mediated immune responses (198). In the context of extracellular pathogens, polarization toward Th17 cells is most important. In PBMCs that were cultured in zinc-deficient medium, the balance between Treg and Th17 cells was shifted toward Th17 cells (140). When zinc (50 μM) was added to mixed lymphoid cultures, Treg cell polarization was induced (172). An increased polarization toward Treg cells was also observed when pigs, subjected to weaning stress, were fed a high zinc (2,500-ppm) diet (126). Short-term zinc deficiency as a regular event during the APR thus helps to activate Th17 cells and to mount a proper immune response. However, chronic zinc deficiency disturbs immune balance and can be associated with autoimmunity, as discussed in Section 6 (263).

Cytokine stimulation of Th cells induces transient upregulation of MT expression. As MT-deficient T cells generally produce less proinflammatory cytokines but show an increase in anti-inflammatory cytokine secretion, a decisive role of zinc during activation of pro- versus anti-inflammatory genes can be assumed (212). Multiple stimulants transiently increase intracellular zinc levels, presumably as part of the signaling process, as found largely in cell culture experiments (51, 92, 117, 173). When zinc influx was reduced in T cells via siRNA-mediated knockdown or knockout of Zip6, T cell activation was blunted and cytokine production impaired (51). Moreover, T cell activation via the TCR decreased EVER expression, which was paralleled by the accumulation of intracellular free zinc. As EVER proteins are positive regulators of ZnT1, which sequesters zinc in organelles, an association between both events is likely (142). High intracellular zinc concentrations directly impact the TCR signaling pathway (see Section 6). Here, zinc binds to Lck and interferes with Zap70 phosphorylation as one of the underlying mechanisms. Increased intracellular zinc concentrations were achieved by adding zinc to cell cultures (103, 275).

As for T cells, activation of B cells results in increased intracellular zinc levels (189), which may involve zinc transport via Zip10 (102). Interestingly, zinc deficiency augments IL-6-induced signaling in a B cell line in vitro, as monitored via increased STAT3 phosphorylation and higher proliferation (86). However, due to the reduced numbers of B and T cells during zinc deficiency, zinc's relevance in vivo, especially regarding the overall adaptive immune response against extracellular pathogens, needs to be addressed.

### 4.2. Intracellular Pathogens

Tuberculosis, lepromatous leprosy, and leishmaniasis are examples of diseases caused by intracellular pathogens. Th1 cells, whose function is impaired during zinc deficiency, play an important role during the response against intracellular pathogens (198). Th1 cells induce ROS production in phagocytes to kill intracellular pathogens. Zinc-dependent antioxidant enzymes neutralize ROS, preventing damage to the phagocyte, as described above (95). When zinc-deficient individuals were supplemented with zinc, ROS production also decreased (21). Zinc supplementation (45 mg/day for 12 months) of elderly subjects with initially decreased serum zinc restored adequate serum zinc levels and decreased oxidative stress markers (201). In zinc-adequate

individuals, additional supplementation with 45 mg elemental zinc daily for 8 weeks significantly increased serum zinc and significantly lowered the in vivo generation of oxidation by-products (200). Hence, zinc deprivation may lead to an accumulation of ROS. Consequently, damage of activated phagocytes by ROS as well as ROS-associated tissue injury, which is reversible by zinc supplementation, can be expected in zinc-deficient individuals.

Macrophages can kill organisms such as *Histoplasma capsulatum* by reducing zinc content in the phagosome and starving the pathogen (238). Alternatively, macrophages can kill intracellular microbes by intoxication with excess amounts of zinc and copper, as shown for *Mycobacterium tuberculosis* (35). Whether those macrophage functions are altered in zinc-deficient or zinc-(re)supplemented individuals remains to be explored.

### 4.3. Extracellular Parasites

Several studies have addressed the link between zinc status and parasite infection. As discussed above, zinc-adequate conditions are essential for preserving tissue barriers (**Figure 2**) and thus important for preventing parasites from entering through the intestine (228). Moreover, a study found that plasma zinc concentrations were negatively correlated with the numbers of the helminth *Trichuris trichiura* in Jamaican children (42). However, this study did not address whether reduced zinc levels were the cause or the consequence, and zinc supplementation had no impact on the effects of conventional therapy, as monitored by the reinfection rate (84). In rodents fed a zinc-deficient diet, the nematodes *Trichinella spiralis* (rats) and *Strongyloides ratti* (rats) and the helminth *Heligmosomoides polygyrus* (mice) displayed higher burdens and better survival of the parasites (69, 84).

Th2 cells are central players during the response to parasites. IL-4, secreted by activated Th2 cells, activates B cells to produce IgE. IL-4-induced signaling and subsequent STAT6 phosphorylation are disturbed in T cells cultured in zinc-deficient medium, offering one explanation for the inefficient antiparasite response (86). The disturbance of IL-4-induced signaling may result in an impaired switch to IgE in zinc-deficient patients. IgE-mediated stimulation of mast cells is a central mechanism in the antiparasite response. The requirement of zinc for FcεRI-induced degranulation and cytokine expression by mast cells was extrapolated from the disturbance of both of these developments in models of zinc deficiency (115). Moreover, human mast cells release zinc via ZnT1 following FcεRI aggregation, which may be another antiparasite mechanism (186).

In this regard, zinc's role during Th2-mediated allergic responses should be mentioned. The pathogenesis of allergic diseases such as atopic dermatitis, asthma, allergic rhinitis, and allergic conjunctivitis implies a type 1 hypersensitivity response that is mediated by allergen-specific IgE bound to mast cells. A study carried out in humans suffering from allergies revealed a significant association of total and allergen-specific IgE levels with low serum zinc levels (230). In this study, serum hypozincemia was defined as $<120$ μg/dl, which is, compared to other studies, in a normal to higher range (230). Although the cause and consequence are not clear here, these data suggest that zinc supplementation of patients with allergies and low serum zinc levels may be beneficial during treatment of allergic immune responses. This possibility is supported by the results of some supplementation studies, in which, for example, eczema severity in cases of atopic dermatitis was reduced when patients were zinc supplemented (12 mg/day) for 8 weeks (121). Most of the dermatitis patients were zinc deficient when supplementation was started but zinc adequate after 8 weeks of oral treatment, as monitored by measuring hair zinc (121). In children suffering from asthma, zinc supplementation (50 mg/day for 8 weeks) caused significant clinical improvement (82). In all subjects, low initial serum zinc concentrations were found, and zinc concentrations were significantly increased in the zinc-supplemented group reaching adequate levels.

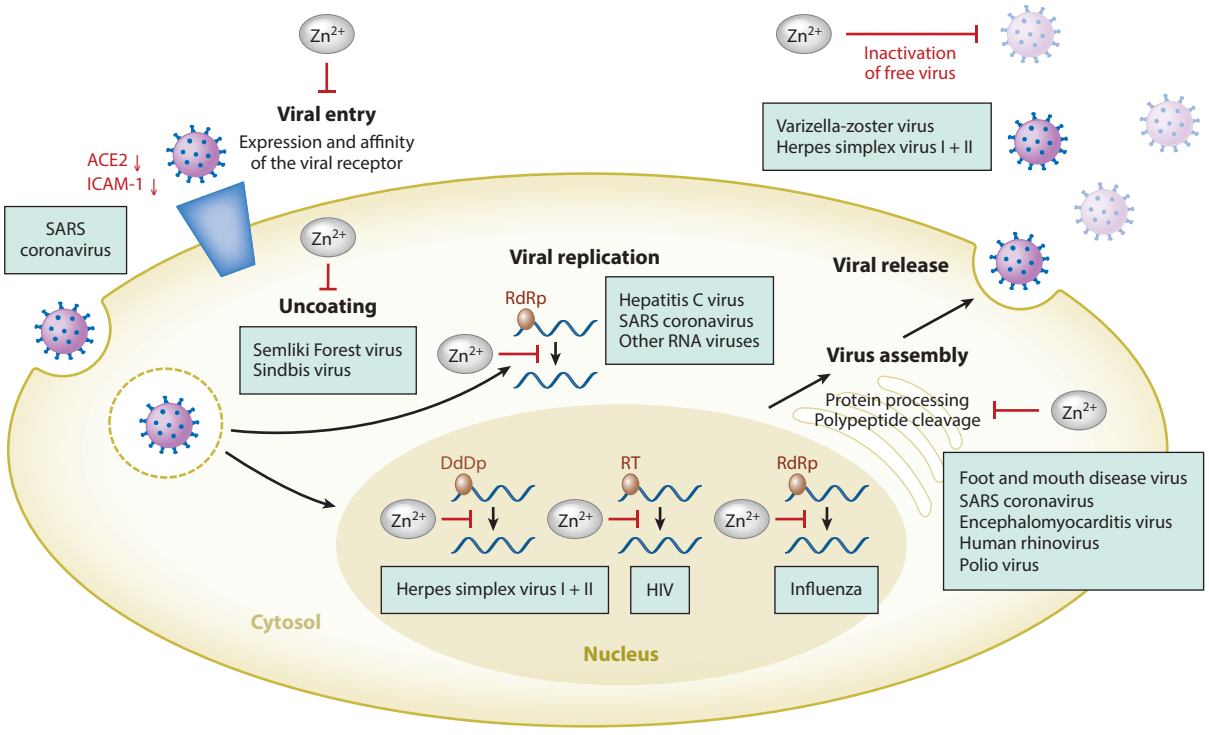



**Figure 4**

Zinc-dependent modulation of viral infections. Zinc ameliorates the progression of viral infections on different levels, including both the inactivation of free virus and viral replication. The inhibitory effects of zinc on the different steps of viral replication are denoted by red blunted arrows. The green boxes give information on the virus type for which these effects were observed. Abbreviations: ACE, angiotensin-converting enzyme; DdDp, DNA-dependent DNA polymerase; ICAM-1, intercellular adhesion molecule 1; RdRp, RNA-dependent RNA polymerase; RT, reverse transcriptase; SARS, severe acute respiratory syndrome.

Thus, zinc appears to be an important modulator of Th2-mediated immune responses elicited by parasitic infections and allergies. Such findings, together with increased mast cell numbers and a higher number of mitochondria and specific granules in mast cells found in zinc-deficient rats (235), suggest an association of zinc deficiency with the development of allergies.

## 4.4. Antiviral Immune Response and Vaccination

Zinc's antiviral capacity has been described for various steps throughout the life cycle of a virus, as shown in **Figure 4**. The importance of zinc becomes extensively clear during zinc deficiency, in which susceptibility to viral infections and severe disease progression is significantly increased, as recently discussed in relation to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (250, 264). Moreover, zinc deficiency is common in patients chronically infected with human papilloma virus (HPV), HIV, and hepatitis C virus (HCV) (210).

Direct antiviral effects of zinc were reported for respiratory syncytial virus, HIV, coronaviridae, picornaviridae (rhinovirus, coxsackievirus, cardio virus A, foot and mouth disease virus), HPV, equine arteritis virus (EAV), varicella-zoster virus, metapneumovirus, herpes simplex virus (HSV1 and HSV2), and HCV (110, 137, 210, 241). Zinc supplementation was studied by adding zinc extracellularly to cultures of virus-infected cells or in clinical zinc supplementation studies. As effective doses of zinc alone or in combination with an ionophore significantly vary between

studies and depending on the virus, we refer to a recent review by Read et al. (210), which provides a detailed overview of the various studies and treatment regimens, and we describe only some examples here. Regarding the underlying mechanisms, zinc destabilizes the viral envelope, prevents fusion of the virus with the host membrane, lowers the function of the viral polymerase, inhibits the translation and subsequent processing of viral proteins, and impairs the release of viral particles (210, 241). When zinc was used together with an ionophore such as hinokitiol (HK) (which transports various metals) or pyrithione (zinc specific), low zinc doses (2 μM) effectively attenuated RNA synthesis via inhibition of the viral RNA-dependent RNA polymerase. This attenuation was observed in cases of SARS-CoV-2, EAV, poliovirus, influenza, and various picornaviridae (110, 136, 241). Similarly, zinc inhibited DNA-dependent DNA polymerase of HSV and reverse transcriptase of HIV (68, 88). However, unphysiologically high zinc concentrations (200 μM) were used here, and the vitality of the infected cells was not assessed (88). Moreover, the cleavage of large viral polypeptides into smaller peptides, which is necessary for the formation of the various viral proteins and thus new viral particles, was significantly inhibited by zinc (100 μM), as observed for various picornaviridae in vitro (136). In line with this finding, treatment with HK (45–125 μM) or pyrithione (5–10 μM) decreased the activity of rhinoviral 2A protease and subsequent cleavage of eIF4GI (eukaryotic translation initiation factor 4γ1), which is essential for initiation of translation as well as the proper processing of viral polyproteins (137). The zinc ionophores did not affect the vitality of the infected cells (137). In another study focusing on HSV, pyrithione treatment (10 μM) and thus increased intracellular zinc suppressed the expression of glycoprotein D and infected cell polypeptide 4. The involvement of those proteins in transcriptional activation of other viral genes explains the strong impact of zinc on viral replication. Virus-induced alterations in the ubiquitin-proteasome system result in decreased expression of host genes, including expression of antiviral mediators, and thus viral replication, capsid translocation, viral gene expression, replication, and immune evasion are promoted. Various tested concentrations of zinc inhibited hijacking of the cellular ubiquitin-proteasome system (20, 205). A third antiviral effect of pyrithione (10 μM), found in HSV-infected cells, was a reduction of virus-induced IκB-α degradation, which resulted in lower activation of NF-κB (205). 26S proteasome activity was not affected, but promyelocytic leukemia protein (PML) in the nucleus showed enhanced stability when pyrithione was added to cells (205). Investigations on coxsackievirus B3 revealed that zinc may hamper the processing of viral polyprotein via interfering with the viral protease $3CD^{pro}$. PDTC1 (pyrrolidine dithiocarbamate 1) was used to transport zinc into the cell (141). Interestingly, HPV has developed a mechanism to alter zinc homeostasis of its host cells. It can interact with ZnT1 in complex with EVER-2, blocking the export of zinc from organelles, increasing nuclear zinc, and subsequently activating AP1, which is essential during expression of viral genes (143). Influenza, HCV, measles virus, and coxsackie virus upregulated MT expression. The consequence of MT upregulation and the resulting benefits for the virus are controversial (210).

As discussed above, alteration of the immune response during zinc deficiency is largely involved in increased susceptibility to severe viral infections. Here, the decreased production of antiviral IFNα and the lack of virus-specific antibodies in particular can be detrimental, while zinc supplementation (70–200 μM, nontoxic) can reestablish IFNα levels and augment its activity (30). IFN primarily induces JAK (Janus kinase)-STAT signaling cascades, which are affected by altered zinc, and zinc in physiological concentrations inhibits a variety of pro- and antiviral phosphatases, which awaits further analyses, especially regarding the net effects in vivo. Also, TLR-induced signaling, which is required for activating innate immune cells during viral infections, was disturbed when zinc was depleted in cell culture experiments (38). Moreover, altered zinc homeostasis due to, for example, alcohol abuse can disturb the activity of alveolar macrophages; such disturbance is associated with elevated susceptibility to respiratory syncytial virus and with an increased

risk for subsequent pneumonia. Zinc supplementation using various treatment regimens and zinc concentrations, in contrast, was beneficial regarding the prevention and treatment of viral infections, including viral warts (summarized in References 81 and 210). Interestingly, early research dating back to the 1970s suggests that the impact of zinc on viral replication may be independent of the onset of supplementation; this hypothesis was investigated by adding 100 μm of zinc to cell cultures, probably exceeding the physiological concentration that can be achieved in human serum in vivo (136). More details on the underlying mechanisms have been discussed in detail (81). As the current SARS-CoV-2 pandemic has made apparent, efficient viral clearance has enormous economic potential and underscores the need for new antiviral substances to support global health. However, the above-described effects of zinc are often derived from experiments, in which intracellular zinc of the virus-infected cell was strongly elevated, for example, using zinc ionophores, and have frequently been investigated in vitro. More research is necessary to ideally increase zinc in infected cells without intoxicating the cells. Moreover, future investigations should be limited to physiological zinc concentrations that can be reached in human serum. Alternatively, indirect ways to increase intracellular zinc could be investigated. One approach would be increasing IFN levels, as MT expression is increased by IFNs, and zinc influx may subsequently occur (78). Altogether, zinc's antiviral effects seem to be specific for the different known viruses.

Due to the strong effects of zinc deficiency on immune cell functions, including those of B cells, various studies investigated whether a preexisting zinc deficiency would affect the efficiency of vaccination. Studies using zinc-deficient mice indicate a lower response to vaccination with pneumococcal surface proteins and lower pathogen clearance during subsequent infection with *Streptococcus pneumoniae*, as well as a lower response to vaccination against hepatitis B (236, 277). Although these findings implicate zinc supplementation, at least of zinc-deficient individuals, in increasing the vaccination-induced production of antibodies, the outcomes of supplementation studies have varied considerably. Since various outcomes were recently discussed in great detail (164), we describe only a few examples here. Zinc treatment (20 mg/day for 42 days) of zinc-deficient children (6–9 months old) before giving two cholera vaccine shots improved vaccination efficiency (5). Although zinc supplementation increased the number of children (2–5 years old) responding to vaccination, zinc reduced the amount of vaccination-induced antibodies, which is in line with results from a Norwegian research group, who applied much higher zinc doses (135 mg/day) (119, 203). However, supplementation was started only 2 days before the first vaccination, and subjects were between 20 and 29 years old (119, 203). Nevertheless, studies generally show that early zinc supplementation may be beneficial regarding vaccination relative to the application of zinc together with the vaccine, which had no or negative effects, suggesting that further research may uncover ways to use zinc for boosting the immune response to vaccination (37). Unfortunately, no currently registered trials on this topic are found, although data are available, suggesting that zinc-deficient individuals may have a lower vaccination response due to impaired immune functions, especially affecting adaptive immune cells (165); this area should be investigated in more detail.

## 4.5. Zinc in the Therapy of Infectious Diseases

There are many studies investigating possible benefits of zinc regarding inflammatory diseases, and excellent articles are available summarizing the results from recent supplementation studies, including the various treatment strategies tested (22, 81, 222, 264). The optimal concentration and duration of zinc supplementation vary depending on the disease and on the estimated zinc status. However, it is important to indicate that, according to our current knowledge, preventive zinc supplementation, especially of zinc-deficient individuals, is favorable, as preventive zinc

supplementation decreases disease severity, progression, and mortality. In contrast, nontreated preexisting zinc deficiency can be regarded as a risk factor of detrimental disease outcomes. Zinc supplementation during the acute phase of an inflammatory disease may be detrimental as well, although fewer studies have addressed this point (129, 139, 262). The effects of zinc supplementation during the recovery phase need to be investigated in the future.

In general, zinc supplementation of groups at risk for either zinc deficiency or infections is highly recommended. The latter includes medical personnel and individuals who are exposed to elevated pathogen loads. Patients with scheduled major surgery might also benefit from zinc supplementation during the period before the intervention (2). An example in which zinc supplementation has already been successfully applied in line with WHO recommendations is the prevention of diarrhea and pneumonia in children, especially in developing countries; this intervention has resulted in significantly decreased mortality (274).

## 5. ZINC IN LEUKEMIA AND CANCER

Globally, cancer is the second-leading cause of death; it accounts for approximately one in six deaths (271). Disturbed hematopoiesis, which occurs during oncogenesis, is associated with altered zinc homeostasis: Low serum zinc and increased intracellular zinc in cancer cells were observed. The altered expression of numerous zinc transporters and subsequent changes in intracellular signaling were linked to leukemia progression and are currently investigated as targets for therapeutic intervention, as illustrated in **Figure 5** and discussed in detail elsewhere (191, 254). Both excessive

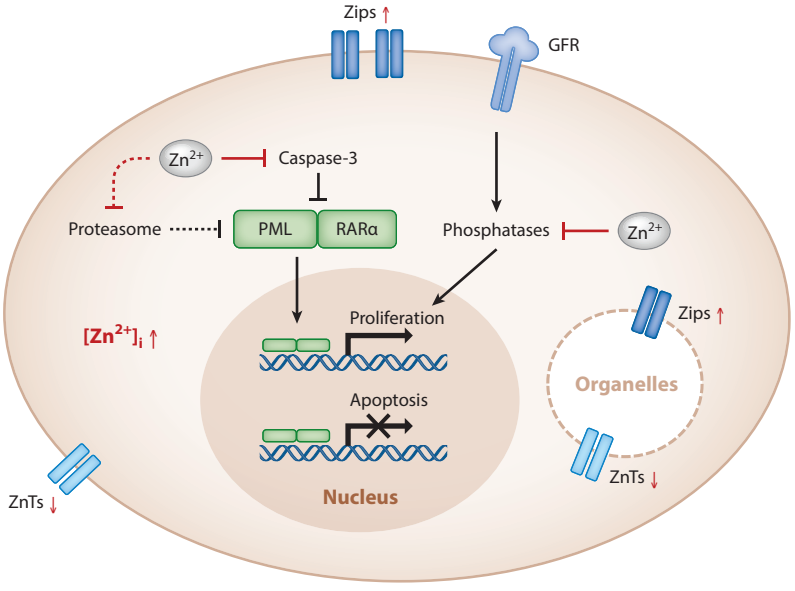



**Figure 5**

Zinc-mediated alterations of cell survival during cellular transformation. During leukemia, elevated intracellular free zinc concentrations are regularly observed due to altered expression of the Zips (zinc importers) and ZnTs (zinc exporters). In acute promyelocytic leukemia, this elevated intracellular zinc concentration may stabilize the oncofusion protein PML-RARα by inhibiting its caspase-3-mediated protein degradation. Furthermore, zinc can inhibit proteasomal protein degradation. The signaling pathway of GFR is also regulated by zinc because it inhibits the function of phosphatases, thus increasing cell proliferation. Abbreviations: GFR, growth factor receptor; PML, promyelocytic leukemia protein; RARα, retinoic acid receptor alpha; Zip, Zrt- and Irt-like protein; ZnT, zinc transporter.

zinc and insufficient zinc result in misfolding of the p53 tumor suppressor and in subsequent loss of its functions (85, 161). Whether reestablishing zinc homeostasis restores or intensifies parts of the functions of p53 needs to be tested for each cancer type, as discussed in detail elsewhere (34, 191, 254).

Recently, Ollig et al. (189) systematically investigated whether immortalization of B cells resulted in alterations in free intracellular zinc concentration. Epstein-Barr virus transformation was used for immortalization and caused the generation of lymphoblastoid cells. In those cells, overexpression of Zip7, its activation by phosphorylation, and an increase in intracellular free zinc levels were detected (189, 240). Another important observation is that key players during initiation and development of various types of leukemia are zinc binding enzymes and zinc finger proteins, whose function may be affected by changes in zinc homeostasis (206).

Degeneration of cancer cells often involves mutations and chromosome rearrangements that cause the formation of fusion proteins and nonphysiological protein complexes. Zinc's importance in complex formation and protein complex stability may play a role here, as underscored by the finding that zinc deficiency results in destabilization of the PML–retinoid acid receptor alpha (RARα) fusion protein in a human PML cell line (278). Caspase-3 is one of the suggested mechanisms underlying the destabilization of the oncofusion protein during zinc deficiency (170). Our own recent data for tumor cell lines suggest that PML-RARα is not the only oncofusion protein destabilized when cells are deprived by zinc (R. Goerg, L. Rink & I. Wessels); this possibility needs further investigation and verification for leukemia patients in vivo.

## 6. ZINC IN AUTOIMMUNE DISEASES

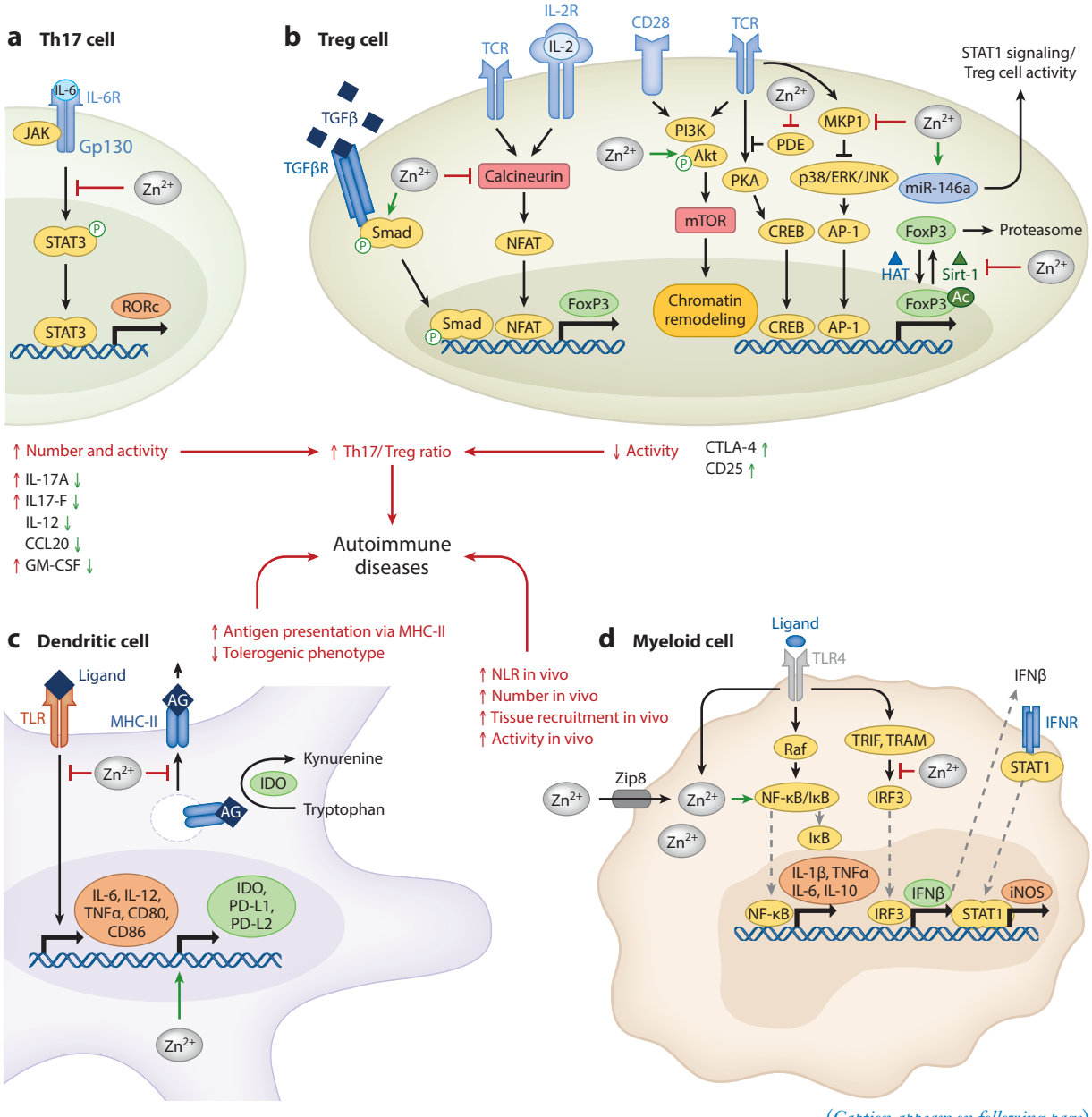
The prevalence of AIDs, in which host cells and tissues are attacked specifically by their own immune cells, is estimated to be approximately 3.2–9.4% (53, 255). Although the incidence of AIDs is probably lower than the incidences of obesity, cardiac diseases, and cancers, a considerable number of patients are affected by AIDs, and as therapeutic options are limited and expensive, the search for new approaches is ongoing. Well-known examples of AIDs and affected tissues or molecules include T1DM (pancreas), Crohn's disease (CD) (intestine), rheumatoid arthritis (RA) (joints), multiple sclerosis (MS) (neurons), and Sjögren's syndrome (SjS) (mitochondria or centromeres). The key elements in organ-specific AIDs are overshooting Th1 and Th17 responses, while in systemic AIDs, Th2 cells and macrophages are key players. Hyperproduction and activity of neutrophils are being uncovered in more and more AIDs (224, 263). Autoreactive T and B cells as well as malfunctioning DCs and Treg cells are regularly found in AIDs underlying the disturbed tolerance (255).

As recently summarized (224, 263) and illustrated in **Figure 6**, evidence of the association of zinc deficiency in the etiology of AIDs is accumulating, although the question of whether zinc deficiency is a trigger or a consequence of disease has not been clearly answered. Decreased serum, plasma, or hair zinc levels are often found in patients with AIDs or corresponding animal models, as has been well studied in MS, T1DM, and RA (192, 263). However, in RA, tissue zinc was elevated despite low serum zinc; further studies are needed to uncover this paradox in RA and other AIDs regarding a possible redistribution of zinc to the tissues. The mechanisms underlying the association between zinc homeostasis and the disturbed immune response found in patients with AIDs are in many cases suggested (**Figure 6**) but are not entirely clear, as the mechanisms underlying AIDs are poorly understood.

An elevated NLR was found in several AIDs and was associated with disease activity and onset in relapsing-remitting MS patients (56). An increased NLR is also a hallmark of zinc deficiency, as shown in mice and suspected in humans, and thus benefits of zinc supplementation to rebalance

immune cell ratios can be expected. Some AIDs are characterized by the increased recruitment of highly reactive neutrophils to the tissue, by an overshooting inflammatory response, and by altered redox metabolism. A multitude of those symptoms were suggested to be related to altered zinc homeostasis during AID and are classic consequences of zinc deficiency (127, 224, 260, 267).

In line with the hypothesis that zinc deficiency negatively impacts AIDs, zinc supplementation reduced disease severity in cases of T1DM, MS-related depression, and RA (263). Supplementing 50 mg of elemental zinc for 12 weeks reduced depression. However, no neurological effects




(Caption appears on following page)

**Figure 6** (*Figure appears on preceding page*)

The role of zinc homeostasis in the development of AIDs. Zinc deficiency is frequently observed during AIDs. Both zinc supplementation and zinc deficiency impact the secretion of cytokines by immune cells and influence many factors that are involved in AIDs. These effects are indicated by small green arrows (zinc supplementation) and small red arrows (zinc deficiency) next to the cytokines and features. The impact of zinc on the underlying signaling pathways is indicated within the cells. Positive regulation is indicated by green arrows, and inhibition is depicted as red blunted arrows. (*a*,*b*) Zinc modulates signaling pathways in (*a*) Th17 cells and (*b*) Treg cells and thus impacts the Th17/Treg ratio as well as the NLR. (*c*) Zinc modulates the activation of DCs, the expression of cytokines, and their antigen-presenting capacity. During zinc deficiency, antigen presentation is enhanced, whereas the tolerogenic phenotype is reduced, supporting the pathogenesis of AIDs. (*d*) Zinc deficiency increases the number and reactivity of myeloid cells. Intracellular zinc impacts central signaling pathways, and zinc deficiency induces increased cytokine production. Furthermore, the recruitment of myeloid cells to tissues is enhanced. All of these factors can promote the development of AIDs. Abbreviations: Ac, acetylation; AG, antigen; AID, autoimmune disease; AP-1, activator protein 1; CCL20, chemokine (C-C motif) ligand 20; CD, cluster of differentiation; CREB, cAMP response element binding protein; CTLA-4, cytotoxic T lymphocyte–associated protein 4; DC, dendritic cell; ERK, extracellular signal–regulated kinase; FoxP3, forkheadbox P3; GM-CSF, granulocyte-macrophage colony–stimulating factor; HAT, histone acetyltransferase; IDO, indoleamine-2,3-dioxygenase; IFNβ, interferon beta; IFNR, IFN receptor; IL, interleukin; IL-2R, IL-2 receptor; iNOS, inducible nitric oxide synthase; IRF3, interferon regulatory factor 3; IκB, inhibitor of κB; JAK, Janus kinase; JNK, c-JUN N-terminal kinase; MHC, major histocompatibility complex; MKP, mitogen-activated protein kinase phosphatase; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa light-chain enhancer of activated B cells; NFAT, nuclear factor of activated T cells; NLR, neutrophil-to-lymphocyte ratio; P, phosphorylation; PD-L, programmed death ligand; PDE, phosphodiesterase; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; RORc, RAR-related orphan receptor C; Sirt-1, Sirtuin-1; STAT, signal transducer and activator of transcription; TCR, T cell receptor; TGF, transforming growth factor; Th, T helper cell; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAM, TRIF-related adaptor molecule; Treg, regulatory T cell; TRIF, TIR domain–containing adaptor–inducing IFNβ; Zip, Zrt- and Irt-like protein.

were observed. Other studies did not reveal any effects of zinc supplementation regarding AIDs (171, 209, 222). In view of the usage of different zinc concentrations, different zinc compounds, and different durations during supplementation and the varying results, the optimal dose of supplementation needs to be defined. In a very recent study investigating the effects of zinc supplementation (0.25 mg/kg, oral gavage, 8 weeks) on T1DM in rats, zinc prevented diabetic osteoporosis; this result probably involves zinc's hypoglycemic effects, the inhibition of lipogenesis, and improved bone marrow metabolism (204). Decreased serum zinc and elevated cerebrospinal fluid zinc levels were found in MS patients. Results from zinc-deficient PBMC cultures suggest that zinc deficiency augments Th17 polarization while Treg cell functions are lost, which may relate to MS symptoms (140). In a murine model of MS, daily zinc supplementation (0.3 mg/kg body weight) starting 2 days before disease induction attenuated disease symptoms and reestablished self-tolerance by decreasing the activation of effector T cells while increasing the number and function of Treg cells (216, 226, 263). In vitro analyses revealed that adding zinc (150 μM zinc aspartate for 3 days) to stimulated human T cells reduced expression of a variety of AID-related genes, including those encoding activation-induced IL-2, IFNγ, TNFα (Th1), IL-4, IL-5, IL-13 (Th2), IL-17, and GM-CSF (Th17) (226).

## 7. CAUSALITY BETWEEN ZINC DEFICIENCY AND DISEASES: WHAT WE NEED TO ADDRESS

As discussed above, zinc deficiency has been observed in association with many diseases and inflammatory reactions (175, 263). However, in multiple cases, including anemia, allergies, and AIDs, whether zinc deficiency is involved in disease etiology or a consequence of the disease is not clear.

An urgently needed prerequisite to advance our knowledge regarding the causality of zinc deficiency and other diseases is a valid biomarker to assess a person's zinc status. One major obstacle in this regard is the strong, but not well-defined, effect of inflammation on serum zinc levels. It has been suggested that serum zinc levels should be normalized to the inflammatory state of an

individual; however, how this should be accomplished has not been defined. Different methods and calculations have been suggested (151, 175), but the lack of agreement between the adjustment methods suggests that further investigation is needed. Furthermore, it needs to be defined which of the various inflammatory markers is best suited for normalization of serum zinc levels. The main inflammatory markers for allergy differ from those defining sepsis or chronic inflammatory autoimmune diseases. Finally, a vicious circle may form since zinc deficiency may augment the proinflammatory response. Thus, disease-specific adjustment methods may be needed.

For certain diseases, assessing serum zinc levels may even be strongly misleading. Leukemia and tumor cells were often found to accumulate zinc, leaving the patient with serum hypozincemia (254). Tissue zinc derived from RA patients was higher whereas serum zinc was lower relative to healthy controls (263). In addition, data on changes in the zinc content of zinc-rich organs (e.g., brain and bone marrow) in cases of altered zinc supply are only beginning to emerge (43, 46, 273). A better understanding of local versus systemic changes in zinc homeostasis may form the basis for deeper investigations into the causal relation between zinc deficiency and disease.

In animal models, specific diseases can be modeled and are actively induced. This approach has the advantage that the effects of preexisting zinc deficiency and of zinc reconstitution can be clearly investigated. In most cases, preexisting zinc deficiency augments inflammatory disease, while prophylactic zinc supplementation reduces disease severity or even prevents the development of disease. Clinical studies on zinc supplementation, for example, before planned major surgery, reveal similar benefits for humans but are rare (2, 67). Since clinical human studies on the effects of preexisting zinc deficiency are hardly feasible, so far our knowledge and hypotheses have been based on the results of animal models and on data from cell culture studies. One way to address this issue may be retrospective analyses of large quantities of serum zinc data. Large numbers may overcome the problem of the inaccuracy of serum zinc as a marker for zinc status. However, serum zinc measures are not yet included in routine laboratory tests for humans. Thus, physicians need to regularly assess the zinc status of their patients to enable retrospective studies. Such knowledge should help relate preexisting zinc deficiencies with the development and severity of certain diseases.

## 8. SUMMARY AND CONCLUSION

Alterations in the immune response due to preexisting zinc deficiency increase susceptibility to infections, and zinc deficiency has been found in all kinds of diseases, although cause and consequences need to be more clearly defined. While a transient decrease in serum zinc during the APR appears to be necessary to activate proinflammatory immune cells and to mount an efficient immune response, normalization of zinc homeostasis seems to be essential for anti-inflammatory and antiredox reactions as well as for the recovery of immune cell numbers, including an increase in Treg cells. If serum hypozincemia is augmented or prolonged, severe consequences, including tissue damage and severe progression of inflammatory diseases, can be expected. Thus, even mild zinc deficiencies should be addressed, and supplementation should be considered for patients at risk of zinc deficiencies. This advice is strongly supported by positive results from supplementation studies, revealing great benefits regarding various infectious diseases, certain autoimmune conditions, allergies, and certain cancers.

Although we have seen great progress in understanding zinc's role in the immune system, there are many open questions. Future research should focus on the development of a reliable biomarker for zinc status. Significant future challenges are to define zinc's interaction more closely with other nutritional elements and to include that information in the development of efficient treatment strategies.

## DISCLOSURE STATEMENT

L.R. is a consultant for Zinpro and Köhler Pharma and received research grants from Zinpro and Wörwag Pharma. L.R. is president of the International Society for Zinc Biology, vice president of the German Society for Minerals and Trace Elements, and parent committee member of the Society for Trace Elements in Man and Animals. I.W. and H.J.F. are not aware of any biases that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

L.R. is a member of Zinc-Net.

## LITERATURE CITED

1. Adachi Y, Yoshida J, Kodera Y, Kato A, Yoshikawa Y, et al. 2004. A new insulin-mimetic bis(allixinato)zinc(II) complex: structure-activity relationship of zinc(II) complexes. *J. Biol. Inorg. Chem.* 9(7):885–93
2. Adjepong D, Jahangir S, Malik BH. 2020. The effect of zinc on post-neurosurgical wound healing: a review. *Cureus* 12(1):e6770
3. Agency for Toxic Substances and Disease Registry (ATSDR). 2005. *Toxicological profile for zinc.* **https://www.atsdr.cdc.gov/ToxProfiles/tp60.pdf**
4. Agnew UM, Slesinger TL. 2021. Zinc toxicity. In *StatPearls*. Treasure Island, FL: StatPearls. **https://www.ncbi.nlm.nih.gov/books/NBK554548/**
5. Ahmed T, Svennerholm A-M, Al Tarique A, Sultana GNN, Qadri F. 2009. Enhanced immunogenicity of an oral inactivated cholera vaccine in infants in Bangladesh obtained by zinc supplementation and by temporary withholding breast-feeding. *Vaccine* 27(9):1433–39
6. Aiuchi T, Mihara S, Nakaya M, Masuda Y, Nakajo S, Nakaya K. 1998. Zinc ions prevent processing of caspase-3 during apoptosis induced by geranylgeraniol in HL-60 cells. *J. Biochem.* 124(2):300–3
7. Allen JI, Perri RT, McClain CJ, Kay NE. 1983. Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. *J. Lab. Clin. Med.* 102(4):577–89
8. Alvarez-Collazo J, Díaz-García CM, López-Medina AI, Vassort G, Alvarez JL. 2012. Zinc modulation of basal and β-adrenergically stimulated L-type $Ca^{2+}$ current in rat ventricular cardiomyocytes: consequences in cardiac diseases. *Pflüg. Arch.* 464(5):459–70
9. Amin N, Clark CCT, Taghizadeh M, Djafarnejad S. 2020. Zinc supplements and bone health: the role of the RANKL-RANK axis as a therapeutic target. *J. Trace Elem. Med. Biol.* 57:126417
10. Andersson DA, Gentry C, Moss S, Bevan S. 2009. Clioquinol and pyrithione activate TRPA1 by increasing intracellular $Zn^{2+}$. *PNAS* 106(20):8374–79
11. Andrews GK. 2001. Cellular zinc sensors: MTF-1 regulation of gene expression. *Biometals* 14(3–4):223–37
12. Andrulewicz-Botulińska E, Wiśniewska R, Brzóska MM, Rogalska J, Galicka A. 2018. Beneficial impact of zinc supplementation on the collagen in the bone tissue of cadmium-exposed rats. *J. Appl. Toxicol.* 38(7):996–1007
13. Anzilotti C, Swan DJ, Boisson B, Deobagkar-Lele M, Oliveira C, et al. 2019. An essential role for the $Zn^{2+}$ transporter ZIP7 in B cell development. *Nat. Immunol.* 20(3):350–61
14. Aster I, Barth L-M, Rink L, Wessels I. 2019. Alterations in membrane fluidity are involved in inhibition of GM-CSF-induced signaling in myeloid cells by zinc. *J. Trace Elem. Med. Biol.* 54:214–20
15. Auld DS. 2001. Zinc coordination sphere in biochemical zinc sites. *Biometals* 14(3–4):271–313
16. Auld DS. 2013. Zinc-binding sites in proteins. In *Encyclopedia of Metalloproteins*, ed. RH Kretsinger, VN Uversky, EA Permyakov, pp. 2554–59. New York: Springer
17. Auld DS, Falchuk KH, Zhang K, Montorzi M, Vallee BL. 1996. X-ray absorption fine structure as a monitor of zinc coordination sites during oogenesis of *Xenopus laevis*. *PNAS* 93(8):3227–31
18. Aydemir TB, Cousins RJ. 2018. The multiple faces of the metal transporter ZIP14 (SLC39A14). *J. Nutr.* 148(2):174–84

19. Aydemir TB, Sitren HS, Cousins RJ. 2012. The zinc transporter Zip14 influences c-Met phosphorylation and hepatocyte proliferation during liver regeneration in mice. *Gastroenterology* 142(7):1536–46.e5
20. Banks L, Pim D, Thomas M. 2003. Viruses and the 26S proteasome: hacking into destruction. *Trends Biochem. Sci.* 28(8):452–59
21. Bao B, Ahmad A, Azmi A, Li Y, Prasad A, Sarkar FH. 2014. The biological significance of zinc in inflammation and aging. In *Inflammation, Advancing Age and Nutrition*, ed. I Rahman, D Bagchi, pp. 15–27. Amsterdam: Elsevier
22. Bao B, Prasad AS, Beck FWJ, Fitzgerald JT, Snell D, et al. 2010. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. *Am. J. Clin. Nutr.* 91(6):1634–41
23. Bao B, Prasad AS, Beck FWJ, Godmere M. 2003. Zinc modulates mRNA levels of cytokines. *Am. J. Physiol. Endocrinol. Metab.* 285(5):E1095–102
24. Bao S, Knoell DL. 2006. Zinc modulates cytokine-induced lung epithelial cell barrier permeability. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 291(6):L1132–41
25. Bao S, Liu M-J, Lee B, Besecker B, Lai J-P, et al. 2010. Zinc modulates the innate immune response in vivo to polymicrobial sepsis through regulation of NF-κB. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 298(6):L744–54
26. Barnum KJ, O'Connell MJ. 2014. Cell cycle regulation by checkpoints. *Methods Mol. Biol.* 1170:29–40
27. Bélanger LF. 1978. The influence of zinc-deprivation on the mast cell population of the bone marrow and other tissues. *J. Nutr.* 108(8):1315–21
28. Bell SG, Vallee BL. 2009. The metallothionein/thionein system: an oxidoreductive metabolic zinc link. *ChemBioChem* 10(1):55–62
29. Bellomo E, Massarotti A, Hogstrand C, Maret W. 2014. Zinc ions modulate protein tyrosine phosphatase 1B activity. *Metallomics* 6(7):1229–39
30. Berg K, Bolt G, Andersen H, Owen TC. 2001. Zinc potentiates the antiviral action of human IFN-α tenfold. *J. Interferon Cytokine Res.* 21(7):471–74
31. Bertolo RFP, Bettger WJ, Atkinson SA. 2001. Calcium competes with zinc for a channel mechanism on the brush border membrane of piglet intestine. *J. Nutr. Biochem.* 12(2):66–72
32. Beyersmann D, Haase H. 2001. Functions of zinc in signaling, proliferation and differentiation of mammalian cells. *Biometals* 14(3–4):331–41
33. Bin B-H, Seo J, Kim ST. 2018. Function, structure, and transport aspects of ZIP and ZnT zinc transporters in immune cells. *J. Immunol. Res.* 2018:9365747
34. Blanden AR, Yu X, Loh SN, Levine AJ, Carpizo DR. 2015. Reactivating mutant p53 using small molecules as zinc metallochaperones: awakening a sleeping giant in cancer. *Drug Discov. Today* 20(11):1391–97
35. Botella H, Stadthagen G, Lugo-Villarino G, de Chastellier C, Neyrolles O. 2012. Metallobiology of host-pathogen interactions: an intoxicating new insight. *Trends Microbiol.* 20(3):106–12
36. Boudreault F, Pinilla-Vera M, Englert JA, Kho AT, Isabelle C, et al. 2017. Zinc deficiency primes the lung for ventilator-induced injury. *JCI Insight* 2(11):e86507
37. Braga CBM, Santos IKFM, Palmeira P, Peria FM, Ribeiro SMF, et al. 2015. Effect of zinc supplementation on serological response to vaccination against *Streptococcus pneumoniae* in patients undergoing chemotherapy for colorectal cancer. *Nutr. Cancer* 67(6):926–32
38. Brieger A, Rink L, Haase H. 2013. Differential regulation of TLR-dependent MyD88 and TRIF signaling pathways by free zinc ions. *J. Immunol.* 191(4):1808–17
39. Briffa J, Sinagra E, Blundell R. 2020. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon* 6(9):e04691
40. Brito S, Lee M-G, Bin B-H, Lee J-S. 2020. Zinc and its transporters in epigenetics. *Molecules Cells* 43(4):323–30
41. Brough D, Pelegrin P, Rothwell NJ. 2009. Pannexin-1-dependent caspase-1 activation and secretion of IL-1β is regulated by zinc. *Eur. J. Immunol.* 39(2):352–58
42. Bundy DAP, Golden MHN. 1987. The impact of host nutrition on gastrointestinal helminth populations. *Parasitology* 95(3):623–35

43. Ceylan MN, Akdas S, Yazihan N. 2021. Is zinc an important trace element on bone-related diseases and complications? A meta-analysis and systematic review from serum level, dietary intake, and supplementation aspects. *Biol. Trace Elem. Res.* 199:535–49
44. Chai F, Truong-Tran AQ, Evdokiou A, Young GP, Zalewski PD. 2000. Intracellular zinc depletion induces caspase activation and p21 Waf1/Cip1 cleavage in human epithelial cell lines. *J. Infect. Dis.* 182(Suppl. 1):85–92
45. Cheknev SB, Apresova MA, Moryakova NA, Efremova IE, Mezdrokhina AS, et al. 2014. Production of the growth factors GM-CSF, G-CSF, and VEGF by human peripheral blood cells induced with metal complexes of human serum γ-globulin formed with copper or zinc ions. *Mediat. Inflamm.* 2014:518265
46. Chen Y-H, Jeng S-S, Hsu Y-C, Liao Y-M, Wang Y-X, et al. 2020. In anemia zinc is recruited from bone and plasma to produce new red blood cells. *J. Inorg. Biochem.* 210:111172
47. Chesters J. 1999. A possible role for cyclins in the zinc requirements during G1 and G2 phases of the cell cycle. *J. Nutr. Biochem.* 10(5):279–90
48. Chesters JK, Petrie L, Travis AJ. 1990. A requirement for $Zn^{2+}$ for the induction of thymidine kinase but not ornithine decarboxylase in 3T3 cells stimulated from quiescence. *Biochem. J.* 272(2):525–27
49. Chesters JK, Petrie L, Vint H. 1989. Specificity and timing of the $Zn^{2+}$ requirement for DNA synthesis by 3T3 cells. *Exp. Cell Res.* 184(2):499–508
50. Cho HM, Ryu JR, Jo Y, Seo TW, Choi YN, et al. 2019. Drp1-Zip1 interaction regulates mitochondrial quality surveillance system. *Mol. Cell* 73(2):364–76.e8
51. Colomar-Carando N, Meseguer A, Company-Garrido I, Jutz S, Herrera-Fernández V, et al. 2019. Zip6 transporter is an essential component of the lymphocyte activation machinery. *J. Immunol.* 202(2):441–50
52. Conti M, Beavo J. 2007. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu. Rev. Biochem.* 76:481–511
53. Cooper GS, Bynum MLK, Somers EC. 2009. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. *J. Autoimmun.* 33(3–4):197–207
54. Curcio JS, Silva MG, Silva Bailão MG, Báo SN, Casaletti L, et al. 2017. Identification of membrane proteome of *Paracoccidioides lutzii* and its regulation by zinc. *Future Sci. OA* 3(4):FSO232
55. Dai F-Z, Yang J, Chen X-B, Xu M-Q. 2013. Zinc finger protein A20 inhibits maturation of dendritic cells resident in rat liver allograft. *J. Surg. Res.* 183(2):885–93
56. D'Amico E, Zanghì A, Romano A, Sciandra M, Palumbo GAM, Patti F. 2019. The neutrophil-to-lymphocyte ratio is related to disease activity in relapsing remitting multiple sclerosis. *Cells* 8(10):1114
57. Daniel AG, Farrell NP. 2014. The dynamics of zinc sites in proteins: electronic basis for coordination sphere expansion at structural sites. *Metallomics* 6(12):2230–41
58. Dardenne M, Prasad A, Bach J-F. 2013. Zinc and thymulin. In *Trace Elements in Clinical Medicine* [*Proceedings of the Second Meeting of the International Society for Trace Element Research in Humans (ISTERH)*], ed. H Tomita, pp. 177–82. Tokyo: Springer
59. DeRuisseau KC, Cheuvront SN, Haymes EM, Sharp RG. 2002. Sweat iron and zinc losses during prolonged exercise. *Int. J. Sport Nutr. Exerc. Metab.* 12(4):428–37
60. Dierichs L, Kloubert V, Rink L. 2018. Cellular zinc homeostasis modulates polarization of THP-1-derived macrophages. *Eur. J. Nutr.* 57(6):2161–69
61. Dowd PS, Kelleher J, Guillou PJ. 1986. T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. *Br. J. Nutr.* 55(1):59–69
62. Dubben S, Hönscheid A, Winkler K, Rink L, Haase H. 2010. Cellular zinc homeostasis is a regulator in monocyte differentiation of HL-60 cells by 1α,25-dihydroxyvitamin $D_3$. *J. Leukoc. Biol.* 87(5):833–44
63. Duvall E, Wyllie AH. 1986. Death and the cell. *Immunol. Today* 7(4):115–19
64. Elmes ME. 1977. Apoptosis in the small intestine of zinc-deficient and fasted rats. *J. Pathol.* 123(4):219–23
65. Eron SJ, MacPherson DJ, Dagbay KB, Hardy JA. 2018. Multiple mechanisms of zinc-mediated inhibition for the apoptotic caspases-3, -6, -7, and -8. *ACS Chem. Biol.* 13(5):1279–90
66. Fanzo JC, Reaves SK, Cui L, Zhu L, Lei KY. 2002. p53 protein and p21 mRNA levels and caspase-3 activity are altered by zinc status in aortic endothelial cells. *Am. J. Physiol. Cell Physiol.* 283(2):C631–38

67. Faure H, Peyrin JC, Richard MJ, Favier A. 1991. Parenteral supplementation with zinc in surgical patients corrects postoperative serum-zinc drop. *Biol. Trace Elem. Res.* 30(1):37–45
68. Fenstermacher KJ, DeStefano JJ. 2011. Mechanism of HIV reverse transcriptase inhibition by zinc: formation of a highly stable enzyme-(primer-template) complex with profoundly diminished catalytic activity. *J. Biol. Chem.* 286(47):40433–42
69. Fenwick PK, Aggett PJ, Macdonald D, Huber C, Wakelin D. 1990. Zinc deficiency and zinc repletion: effect on the response of rats to infection with *Trichinella spiralis*. *Am. J. Clin. Nutr.* 52(1):166–72
70. Fischer HJ, Sie C, Schumann E, Witte A-K, Dressel R, et al. 2017. The insulin receptor plays a critical role in T cell function and adaptive immunity. *J. Immunol.* 198(5):1910–20
71. Fong LYY, Mancini R, Nakagawa H, Rustgi AK, Huebner K. 2003. Combined cyclin D1 overexpression and zinc deficiency disrupts cell cycle and accelerates mouse forestomach carcinogenesis. *Cancer Res.* 63(14):4244–52
72. Fosmire GJ. 1990. Zinc toxicity. *Am. J. Clin. Nutr.* 51(2):225–27
73. Fossati G, Mazzucchelli I, Gritti D, Ricevuti G, Edwards SW, et al. 1998. In vitro effects of GM-CSF on mature peripheral blood neutrophils. *Int. J. Mol. Med.* 1(6):943–51
74. Fraker PJ, King LE. 2004. Reprogramming of the immune system during zinc deficiency. *Annu. Rev. Nutr.* 24:277–98
75. Fraker PJ, King LE, Laakko T, Vollmer TL. 2000. The dynamic link between the integrity of the immune system and zinc status. *J. Nutr.* 130(Suppl. 5):1399–406
76. Fraker PJ, Lill-Elghanian DA. 2004. The many roles of apoptosis in immunity as modified by aging and nutritional status. *J. Nutr. Health Aging* 8(1):56–63
77. Franklin RB, Costello LC. 2009. The important role of the apoptotic effects of zinc in the development of cancers. *J. Cell. Biochem.* 106(5):750–57
78. Friedman RL, Manly SP, McMahon M, Kerr IM, Stark GR. 1984. Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. *Cell* 38(3):745–55
79. Fukada T, Kambe T, ed. 2019. *Zinc Signaling*. Singapore: Springer. 2nd ed.
80. Fukushima T, Horike H, Fujiki S, Kitada S, Sasaki T, Kashihara N. 2009. Zinc deficiency anemia and effects of zinc therapy in maintenance hemodialysis patients. *Ther. Apher. Dial.* 13(3):213–19
81. Gammoh NZ, Rink L. 2017. Zinc in infection and inflammation. *Nutrients* 9(6):624
82. Ghaffari J, Khalilian A, Salehifar E, Khorasani E, Rezaii MS. 2014. Effect of zinc supplementation in children with asthma: a randomized, placebo-controlled trial in northern Islamic Republic of Iran. *East. Mediterr. Health J.* 20(6):391–96
83. Gore A, Moran A, Hershfinkel M, Sekler I. 2004. Inhibitory mechanism of store-operated $Ca^{2+}$ channels by zinc. *J. Biol. Chem.* 279(12):11106–11
84. Grazioso CF, Isalgué M, de Ramírez I, Ruz M, Solomons NW. 1993. The effect of zinc supplementation on parasitic reinfestation of Guatemalan schoolchildren. *Am. J. Clin. Nutr.* 57(5):673–78
85. Green DR, Kroemer G. 2009. Cytoplasmic functions of the tumour suppressor p53. *Nature* 458(7242):1127–30
86. Gruber K, Maywald M, Rosenkranz E, Haase H, Plumakers B, Rink L. 2013. Zinc deficiency adversely influences interleukin-4 and interleukin-6 signaling. *J. Biol. Regul. Homeost. Agents* 27(3):661–71
87. Günther V, Lindert U, Schaffner W. 2012. The taste of heavy metals: gene regulation by MTF-1. *Biochim. Biophys. Acta* 1823(9):1416–25
88. Gupta P, Rapp F. 1976. Effect of zinc ions on synthesis of herpes simplex virus type 2–induced polypeptides. *Proc. Soc. Exp. Biol. Med.* 152(3):455–58
89. Haase H, Ellinger S, Linseisen J, Neuhäuser-Berthold M, Richter M. 2020. Revised D-A-CH-reference values for the intake of zinc. *J. Trace Elem. Med. Biol.* 61:126536
90. Haase H, Hebel S, Engelhardt G, Rink L. 2015. The biochemical effects of extracellular $Zn^{2+}$ and other metal ions are severely affected by their speciation in cell culture media. *Metallomics* 7(1):102–11
91. Haase H, Rink L. 2009. Functional significance of zinc-related signaling pathways in immune cells. *Annu. Rev. Nutr.* 29:133–52
92. Haase H, Rink L. 2014. Zinc signals and immune function. *Biofactors* 40(1):27–40
93. Hasan R, Rink L, Haase H. 2013. Zinc signals in neutrophil granulocytes are required for the formation of neutrophil extracellular traps. *Innate Immun.* 19(3):253–64

94. Hasan R, Rink L, Haase H. 2016. Chelation of free $Zn^{2+}$ impairs chemotaxis, phagocytosis, oxidative burst, degranulation, and cytokine production by neutrophil granulocytes. *Biol. Trace Elem. Res.* 171(1):79–88
95. He L, He T, Farrar S, Ji L, Liu T, Ma X. 2017. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell. Physiol. Biochem.* 44(2):532–53
96. Heller RA, Sun Q, Hackler J, Seelig J, Seibert L, et al. 2020. Prediction of survival odds in COVID-19 by zinc, age and selenoprotein P as composite biomarker. *Redox Biol.* 38:101764
97. Henderson LM, Chappell JB, Jones OT. 1988. Superoxide generation by the electrogenic NADPH oxidase of human neutrophils is limited by the movement of a compensating charge. *Biochem. J.* 255(1):285–90
98. Hennigar SR, Kelley AM, McClung JP. 2016. Metallothionein and zinc transporter expression in circulating human blood cells as biomarkers of zinc status: a systematic review. *Adv. Nutr.* 7(4):735–46
99. Hershfinkel M. 2018. The zinc sensing receptor, ZnR/GPR39, in health and disease. *Int. J. Mol. Sci.* 19(2):439
100. Higashimura Y, Takagi T, Naito Y, Uchiyama K, Mizushima K, et al. 2020. Zinc deficiency activates the IL-23/Th17 axis to aggravate experimental colitis in mice. *J. Crohns Colitis* 14(6):856–66
101. Hoeger J, Simon T-P, Beeker T, Marx G, Haase H, Schuerholz T. 2017. Persistent low serum zinc is associated with recurrent sepsis in critically ill patients: a pilot study. *PLOS ONE* 12(5):e0176069
102. Hojyo S, Miyai T, Fujishiro H, Kawamura M, Yasuda T, et al. 2014. Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength. *PNAS* 111(32):11786–91
103. Hönscheid A, Rink L, Haase H. 2009. T-lymphocytes: a target for stimulatory and inhibitory effects of zinc ions. *Endocr. Metab. Immune Disord. Drug Targets* 9(2):132–44
104. Hou S, Vigeland LE, Zhang G, Xu R, Li M, et al. 2010. $Zn^{2+}$ activates large conductance $Ca^{2+}$-activated $K^+$ channel via an intracellular domain. *J. Biol. Chem.* 285(9):6434–42
105. Hu H, Bandell M, Petrus MJ, Zhu MX, Patapoutian A. 2009. Zinc activates damage-sensing TRPA1 ion channels. *Nat. Chem. Biol.* 5(3):183–90
106. Hu J, Yang Z, Wang J, Yu J, Guo J, et al. 2016. Zinc chloride transiently maintains mouse embryonic stem cell pluripotency by activating Stat3 signaling. *PLOS ONE* 11(2):e0148994
107. Huang T, Yan G, Guan M. 2020. Zinc homeostasis in bone: zinc transporters and bone diseases. *Int. J. Mol. Sci.* 21(4):1236
108. Huber KL, Hardy JA. 2012. Mechanism of zinc-mediated inhibition of caspase-9. *Protein Sci.* 21(7):1056–65
109. Hwang JJ, Kim HN, Kim J, Cho D-H, Kim MJ, et al. 2010. Zinc(II) ion mediates tamoxifen-induced autophagy and cell death in MCF-7 breast cancer cell line. *Biometals* 23(6):997–1013
110. Ishida T. 2019. Review on the role of $Zn^{2+}$ ions in viral pathogenesis and the effect of $Zn^{2+}$ ions for host cell–virus growth inhibition. *Am. J. Biomed. Sci. Res.* 2(1):28–37
111. Jansen J, Karges W, Rink L. 2009. Zinc and diabetes—clinical links and molecular mechanisms. *J. Nutr. Biochem.* 20(6):399–417
112. Jayawardena R, Ranasinghe P, Galappatthy P, Malkanthi R, Constantine G, Katulanda P. 2012. Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. *Diabetol. Metab. Syndr.* 4(1):13
113. Joisten N, Proschinger S, Rademacher A, Schenk A, Bloch W, et al. 2021. High-intensity interval training reduces neutrophil-to-lymphocyte ratio in persons with multiple sclerosis during inpatient rehabilitation. *Mult. Scler.* 27(7):1136–39
114. Jörnvall H, Bergman T. 2013. Zinc alcohol dehydrogenases. In *Encyclopedia of Metalloproteins*, ed. RH Kretsinger, VN Uversky, EA Permyakov, pp. 2349–54. New York: Springer
115. Kabu K, Yamasaki S, Kamimura D, Ito Y, Hasegawa A, et al. 2006. Zinc is required for FcεRI-mediated mast cell activation. *J. Immunol.* 177(2):1296–305
116. Kahmann L, Uciechowski P, Warmuth S, Plümäkers B, Gressner AM, et al. 2008. Zinc supplementation in the elderly reduces spontaneous inflammatory cytokine release and restores T cell functions. *Rejuvenation Res.* 11(1):227–37
117. Kaltenberg J, Plum LM, Ober-Blöbaum JL, Hönscheid A, Rink L, Haase H. 2010. Zinc signals promote IL-2-dependent proliferation of T cells. *Eur. J. Immunol.* 40(5):1496–503

118. Kambe T, Matsunaga M, Takeda T-A. 2017. Understanding the contribution of zinc transporters in the function of the early secretory pathway. *Int. J. Mol. Sci.* 18(10):2179
119. Karlsen TH, Sommerfelt H, Klomstad S, Andersen PK, Strand TA, et al. 2003. Intestinal and systemic immune responses to an oral cholera toxoid B subunit whole-cell vaccine administered during zinc supplementation. *Infect. Immun.* 71(7):3909–13
120. Keller SR, Scott HM, Mastick CC, Aebersold R, Lienhard GE. 1995. Cloning and characterization of a novel insulin-regulated membrane aminopeptidase from Glut4 vesicles. *J. Biol. Chem.* 270(40):23612–18
121. Kim JE, Yoo SR, Jeong MG, Ko JY, Ro YS. 2014. Hair zinc levels and the efficacy of oral zinc supplementation in patients with atopic dermatitis. *Acta Derm. Venerol.* 94(5):558–62
122. King JC, Brown KH, Gibson RS, Krebs NF, Lowe NM, et al. 2015. Biomarkers of Nutrition for Development (BOND): zinc review. *J. Nutr.* 146(4):S858–85
123. King LE, Fraker PJ. 2000. Variations in the cell cycle status of lymphopoietic and myelopoietic cells created by zinc deficiency. *J. Infect. Dis.* 182(Suppl. 1):16–22
124. King LE, Fraker PJ. 2002. Zinc deficiency in mice alters myelopoiesis and hematopoiesis. *J. Nutr.* 132(11):3301–7
125. Kitamura H, Morikawa H, Kamon H, Iguchi M, Hojyo S, et al. 2006. Toll-like receptor–mediated regulation of zinc homeostasis influences dendritic cell function. *Nat. Immunol.* 7(9):971–77
126. Kloubert V, Blaabjerg K, Dalgaard TS, Poulsen HD, Rink L, Wessels I. 2018. Influence of zinc supplementation on immune parameters in weaned pigs. *J. Trace Elem. Med. Biol.* 49:231–40
127. Kloubert V, Rink L. 2015. Zinc as a micronutrient and its preventive role of oxidative damage in cells. *Food Funct.* 6(10):3195–204
128. Kloubert V, Wessels I, Wolf J, Blaabjerg K, Janssens V, et al. 2021. Zinc deficiency leads to reduced interleukin-2 production by active gene silencing due to enhanced CREMα expression in T cells. *Clin. Nutr.* 40(5):3263–78
129. Knoell DL, Julian MW, Bao S, Besecker B, Macre JE, et al. 2009. Zinc deficiency increases organ damage and mortality in a murine model of polymicrobial sepsis. *Crit. Care Med.* 37(4):1380–88
130. Kochańczyk T, Drozd A, Krężel A. 2015. Relationship between the architecture of zinc coordination and zinc binding affinity in proteins: insights into zinc regulation. *Metallomics* 7(2):244–57
131. Kocyła A, Adamczyk J, Krężel A. 2018. Interdependence of free zinc changes and protein complex assembly: insights into zinc signal regulation. *Metallomics* 10(1):120–31
132. Kocyła A, Tran JB, Krężel A. 2021. Galvanization of protein-protein interactions in a dynamic zinc interactome. *Trends Biochem. Sci.* 46(1):64–79
133. Kogan S, Sood A, Garnick MS. 2017. Zinc and wound healing: a review of zinc physiology and clinical applications. *Wounds* 29(4):102–6
134. Kolenko V, Uzzo RG, Bukowski R, Bander NH, Novick AC, et al. 1999. Dead or dying: necrosis versus apoptosis in caspase-deficient human renal cell carcinoma. *Cancer Res.* 59(12):2838–42
135. Kondaiah P, Yaduvanshi PS, Sharp PA, Pullakhandam R. 2019. Iron and zinc homeostasis and interactions: Does enteric zinc excretion cross-talk with intestinal iron absorption? *Nutrients* 11(8):1885
136. Korant BD, Kauer JC, Butterworth BE. 1974. Zinc ions inhibit replication of rhinoviruses. *Nature* 248(449):588–90
137. Krenn BM, Gaudernak E, Holzer B, Lanke K, van Kuppeveld FJM, Seipelt J. 2009. Antiviral activity of the zinc ionophores pyrithione and hinokitiol against picornavirus infections. *J. Virol.* 83(1):58–64
138. Kretsinger RH, Uversky VN, Permyakov EA, ed. 2013. *Encyclopedia of Metalloproteins*. New York: Springer
139. Krones C, Klosterhalfen B, Fackeldey V, Junge K, Rosch R, et al. 2004. Deleterious effect of zinc in a pig model of acute endotoxemia. *J. Investig. Surg.* 17(5):249–56
140. Kulik L, Maywald M, Kloubert V, Wessels I, Rink L. 2019. Zinc deficiency drives Th17 polarization and promotes loss of Treg cell function. *J. Nutr. Biochem.* 63:11–18
141. Lanke K, Krenn BM, Melchers WJG, Seipelt J, van Kuppeveld FJM. 2007. PDTC inhibits picornavirus polyprotein processing and RNA replication by transporting zinc ions into cells. *J. Gen. Virol.* 88(Part 4):1206–17
142. Lazarczyk M, Dalard C, Hayder M, Dupre L, Pignolet B, et al. 2012. EVER proteins, key elements of the natural anti–human papillomavirus barrier, are regulated upon T-cell activation. *PLOS ONE* 7(6):e39995

143. Lazarczyk M, Pons C, Mendoza J-A, Cassonnet P, Jacob Y, Favre M. 2008. Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses. *J. Exp. Med.* 205(1):35–42
144. Lee M-G, Choi M-A, Chae S, Kang M-A, Jo H, et al. 2019. Loss of the dermis zinc transporter ZIP13 promotes the mildness of fibrosarcoma by inhibiting autophagy. *Sci. Rep.* 9(1):15042
145. Lee S-J, Cho KS, Koh J-Y. 2009. Oxidative injury triggers autophagy in astrocytes: the role of endogenous zinc. *Glia* 57(12):1351–61
146. Lee S-J, Koh J-Y. 2010. Roles of zinc and metallothionein-3 in oxidative stress–induced lysosomal dysfunction, cell death, and autophagy in neurons and astrocytes. *Mol. Brain* 3(1):30
147. Lee Y-M, Lim C. 2008. Physical basis of structural and catalytic Zn-binding sites in proteins. *J. Mol. Biol.* 379(3):545–53
148. Levaot N, Hershfinkel M. 2018. How cellular $Zn^{2+}$ signaling drives physiological functions. *Cell Calcium* 75:53–63
149. Li X, Chen S, Mao L, Li D, Xu C, et al. 2019. Zinc improves functional recovery by regulating the secretion of granulocyte colony stimulating factor from microglia/macrophages after spinal cord injury. *Front. Mol. Neurosci.* 12:18
150. Lienau S, Rink L, Wessels I. 2018. The role of zinc in calprotectin expression in human myeloid cells. *J. Trace Elem. Med. Biol.* 49:106–12
151. Likoswe BH, Phiri FP, Broadley MR, Joy EJM, Patson N, et al. 2020. Inflammation adjustment by two methods decreases the estimated prevalence of zinc deficiency in Malawi. *Nutrients* 12(6):1563
152. Liu C, DeRoo EP, Stecyk C, Wolsey M, Szuchnicki M, Hagos EG. 2015. Impaired autophagy in mouse embryonic fibroblasts null for Krüppel-like factor 4 promotes DNA damage and increases apoptosis upon serum starvation. *Mol. Cancer* 14:101
153. Liu M-J, Bao S, Gálvez-Peralta M, Pyle CJ, Rudawsky AC, et al. 2013. ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB. *Cell Rep.* 3(2):386–400
154. Liuzzi JP, Guo L, Chang S-M, Cousins RJ. 2009. Krüppel-like factor 4 regulates adaptive expression of the zinc transporter Zip4 in mouse small intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 296(3):G517–23
155. Liuzzi JP, Guo L, Yoo C, Stewart TS. 2014. Zinc and autophagy. *Biometals* 27(6):1087–96
156. Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, et al. 2005. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. *PNAS* 102(19):6843–48
157. Liuzzi JP, Narayanan V, Doan H, Yoo C. 2018. Effect of zinc intake on hepatic autophagy during acute alcohol intoxication. *Biometals* 31(2):217–32
158. Liuzzi JP, Pazos R. 2020. Interplay between autophagy and zinc. *J. Trace Elem. Med. Biol.* 62:126636
159. Liuzzi JP, Yoo C. 2013. Role of zinc in the regulation of autophagy during ethanol exposure in human hepatoma cells. *Biol. Trace Elem. Res.* 156(1–3):350–56
160. Lo MN, Damon LJ, Wei Tay J, Jia S, Palmer AE. 2020. Single cell analysis reveals multiple requirements for zinc in the mammalian cell cycle. *eLife* 9:e51107
161. Loh SN. 2010. The missing zinc: p53 misfolding and cancer. *Metallomics* 2(7):442–49
162. Lowe NM, Fekete K, Decsi T. 2009. Methods of assessment of zinc status in humans: a systematic review. *Am. J. Clin. Nutr.* 89(6):S2040–51
163. Ma H, Su Le, Yue H, Yin X, Zhao J, et al. 2015. HMBOX1 interacts with MT2A to regulate autophagy and apoptosis in vascular endothelial cells. *Sci. Rep.* 5:15121
164. Maares M, Haase H. 2016. Zinc and immunity: an essential interrelation. *Arch. Biochem. Biophys.* 611:58–65
165. Maares M, Haase H. 2020. A guide to human zinc absorption: general overview and recent advances of in vitro intestinal models. *Nutrients* 12(3):762
166. Maares M, Keil C, Straubing S, Robbe-Masselot C, Haase H. 2020. Zinc deficiency disturbs mucin expression, *O*-glycosylation and secretion by intestinal goblet cells. *Int. J. Mol. Sci.* 21(17):6149
167. Mahdaviroshan M, Golzarand M, Taramsari MR, Mahdaviroshan M. 2013. Effect of zinc supplementation on serum zinc and calcium levels in postmenopausal osteoporotic women in Tabriz, Islamic Republic of Iran. *East. Mediterr. Health J.* 19(3):271–75

168. Maret W. 2017. Zinc in cellular regulation: the nature and significance of "zinc signals." *Int. J. Mol. Sci.* 18(11):2285
169. Maret W, Jacob C, Vallee BL, Fischer EH. 1999. Inhibitory sites in enzymes: zinc removal and reactivation by thionein. *PNAS* 96(5):1936–40
170. Martin SJ, Mazdai G, Strain JJ, Cotter TG, Hannigan BM. 1991. Programmed cell death (apoptosis) in lymphoid and myeloid cell lines during zinc deficiency. *Clin. Exp. Immunol.* 83(2):338–43
171. Mattingly PC, Mowat AG. 1982. Zinc sulphate in rheumatoid arthritis. *Ann. Rheum. Dis.* 41(5):456–57
172. Maywald M, Rink L. 2017. Zinc supplementation induces $CD4^+CD25^+Foxp3^+$ antigen-specific regulatory T cells and suppresses IFN-γ production by upregulation of Foxp3 and KLF-10 and downregulation of IRF-1. *Eur. J. Nutr.* 56(5):1859–69
173. Maywald M, Wang F, Rink L. 2018. The intracellular free zinc level is vital for Treg function and a feasible tool to discriminate between Treg and activated Th cells. *Int. J. Mol. Sci.* 19(11):3575
174. Maywald M, Wessels I, Rink L. 2017. Zinc signals and immunity. *Int. J. Mol. Sci.* 18(10):2222
175. McDonald CM, Suchdev PS, Krebs NF, Hess SY, Wessells KR, et al. 2020. Adjusting plasma or serum zinc concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *Am. J. Clin. Nutr.* 111(4):927–37
176. Méplan C, Richard MJ, Hainaut P. 2000. Metalloregulation of the tumor suppressor protein p53: Zinc mediates the renaturation of p53 after exposure to metal chelators in vitro and in intact cells. *Oncogene* 19(46):5227–36
177. Metz CHD, Schröder AK, Overbeck S, Kahmann L, Plümäkers B, Rink L. 2007. T-helper type 1 cytokine release is enhanced by in vitro zinc supplementation due to increased natural killer cells. *Nutrition* 23(2):157–63
178. Mir E, Hossein-Nezhad A, Bahrami A, Bekheirnia MR, Javadi E, et al. 2007. Serum zinc concentration could predict bone mineral density and protect osteoporosis in healthy men. *Iran. J. Public Health* 36(Suppl. 1):30–36
179. Miura Y, Endo T. 2016. Glycomics and glycoproteomics focused on aging and age-related diseases: glycans as a potential biomarker for physiological alterations. *Biochim. Biophys. Acta* 1860(8):1608–14
180. Miyai T, Hojyo S, Ikawa T, Kawamura M, Irié T, et al. 2014. Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development. *PNAS* 111(32):11780–85
181. Mnatsakanyan H, Sabater i Serra R, Salmeron-Sanchez M, Rico P. 2019. Zinc maintains embryonic stem cell pluripotency and multilineage differentiation potential via AKT activation. *Front. Cell Dev. Biol.* 7:180
182. Mocchegiani E, Fabris N. 1995. Age-related thymus involution: Zinc reverses in vitro the thymulin secretion defect. *Int. J. Immunopharmacol.* 17(9):745–49
183. Mocchegiani E, Muzzioli M, Giacconi R, Cipriano C, Gasparini N, et al. 2003. Metallothioneins/PARP-1/IL-6 interplay on natural killer cell activity in elderly: parallelism with nonagenarians and old infected humans. Effect of zinc supply. *Mech. Ageing Dev.* 124(4):459–68
184. Muneoka S, Goto M, Nishimura T, Enomoto K, Kadoshima-Yamaoka K, Tomimori Y. 2019. G protein–coupled receptor 39 agonist improves concanavalin A–induced hepatitis in mice. *Biol. Pharm. Bull.* 42(8):1415–18
185. Muzzioli M, Stecconi R, Moresi R, Provinciali M. 2009. Zinc improves the development of human $CD34^+$ cell progenitors towards NK cells and increases the expression of GATA-3 transcription factor in young and old ages. *Biogerontology* 10(5):593–604
186. Nakashima-Kaneda K, Matsuda A, Mizuguchi H, Sasaki-Sakamoto T, Saito H, et al. 2013. Regulation of IgE-dependent zinc release from human mast cells. *Int. Arch. Allergy Immunol.* 161(Suppl.):44–51
187. Noh S, Lee SR, Jeong YJ, Ko KS, Rhee BD, et al. 2015. The direct modulatory activity of zinc toward ion channels. *Integr. Med. Res.* 4(3):142–46
188. Noulas C, Tziouvalekas M, Karyotis T. 2018. Zinc in soils, water and food crops. *J. Trace Elem. Med. Biol.* 49:252–60
189. Ollig J, Kloubert V, Taylor KM, Rink L. 2019. B cell activation and proliferation increase intracellular zinc levels. *J. Nutr. Biochem.* 64:72–79
190. Ollig J, Kloubert V, Weßels I, Haase H, Rink L. 2016. Parameters influencing zinc in experimental systems in vivo and in vitro. *Metals* 6(3):71

191. Orlov AP, Orlova MA, Trofimova TP, Kalmykov SN, Kuznetsov DA. 2018. The role of zinc and its compounds in leukemia. *J. Biol. Inorg. Chem.* 23(3):347–62
192. Pawlitzki M, Uebelhör J, Sweeney-Reed CM, Stephanik H, Hoffmann J, et al. 2018. Lower serum zinc levels in patients with multiple sclerosis compared to healthy controls. *Nutrients* 10(8):967
193. Pitt SJ, Stewart AJ. 2015. Examining a new role for zinc in regulating calcium release in cardiac muscle. *Biochem. Soc. Trans.* 43(3):359–63
194. Plum LM, Brieger A, Engelhardt G, Hebel S, Nessel A, et al. 2014. PTEN-inhibition by zinc ions augments interleukin-2-mediated Akt phosphorylation. *Metallomics* 6(7):1277–87
195. Plum LM, Rink L, Haase H. 2010. The essential toxin: impact of zinc on human health. *Int. J. Environ. Res. Public Health* 7(4):1342–65
196. Porter NJ, Christianson DW. 2019. Structure, mechanism, and inhibition of the zinc-dependent histone deacetylases. *Curr. Opin. Struct. Biol.* 59:9–18
197. Prasad AS. 1985. Clinical and biochemical manifestation zinc deficiency in human subjects. *J. Pharmacol.* 16(4):344–52
198. Prasad AS. 2000. Effects of zinc deficiency on Th1 and Th2 cytokine shifts. *J. Infect. Dis.* 182(Suppl. 1):62–68
199. Prasad AS. 2014. Zinc: an antioxidant and anti-inflammatory agent: role of zinc in degenerative disorders of aging. *J. Trace Elem. Med. Biol.* 28(4):364–71
200. Prasad AS, Bao B, Beck FWJ, Kucuk O, Sarkar FH. 2004. Antioxidant effect of zinc in humans. *Free Radic. Biol. Med.* 37(8):1182–90
201. Prasad AS, Beck FWJ, Bao B, Fitzgerald JT, Snell DC, et al. 2007. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am. J. Clin. Nutr.* 85(3):837–44
202. Prasad AS, Miale A, Farid Z, Sandstead HH, Schulert AR. 1963. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. *J. Lab. Clin. Med.* 61:537–49
203. Qadri F, Ahmed T, Wahed MA, Ahmed F, Bhuiyan NA, et al. 2004. Suppressive effect of zinc on antibody response to cholera toxin in children given the killed, B subunit–whole cell, oral cholera vaccine. *Vaccine* 22(3–4):416–21
204. Qi S, He J, Zheng H, Chen C, Jiang H, Lan S. 2020. Zinc supplementation increased bone mineral density, improves bone histomorphology, and prevents bone loss in diabetic rat. *Biol. Trace Elem. Res.* 194(2):493–501
205. Qiu M, Chen Y, Chu Y, Song S, Yang N, et al. 2013. Zinc ionophores pyrithione inhibits herpes simplex virus replication through interfering with proteasome function and NF-κB activation. *Antiv. Res.* 100(1):44–53
206. Quintal SM, dePaula QA, Farrell NP. 2011. Zinc finger proteins as templates for metal ion exchange and ligand reactivity. Chemical and biological consequences. *Metallomics* 3(2):121–39
207. Rajagopalan S, Winter CC, Wagtmann N, Long EO. 1995. The Ig-related killer cell inhibitory receptor binds zinc and requires zinc for recognition of HLA-C on target cells. *J. Immunol.* 155(9):4143–46
208. Ranasinghe P, Wathurapatha WS, Ishara MH, Jayawardana R, Galappatthy P, et al. 2015. Effects of zinc supplementation on serum lipids: a systematic review and meta-analysis. *Nutr. Metab.* 12:26
209. Rasker JJ, Kardaun SH. 1982. Lack of beneficial effect of zinc sulphate in rheumatoid arthritis. *Scand. J. Rheumatol.* 11(3):168–70
210. Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. 2019. The role of zinc in antiviral immunity. *Adv. Nutr.* 10(4):696–710
211. Reiterer G, MacDonald R, Browning JD, Morrow J, Matveev SV, et al. 2005. Zinc deficiency increases plasma lipids and atherosclerotic markers in LDL-receptor-deficient mice. *J. Nutr.* 135(9):2114–18
212. Rice JM, Zweifach A, Lynes MA. 2016. Metallothionein regulates intracellular zinc signaling during $CD4^+$ T cell activation. *BMC Immunol.* 17(1):13
213. Rink L, ed. 2011. *Zinc in Human Health*. Amsterdam: IOS Press
214. Rolles B, Maywald M, Rink L. 2018. Influence of zinc deficiency and supplementation on NK cell cytotoxicity. *J. Funct. Foods* 48:322–28

215. Roscioli E, Jersmann HP, Lester S, Badiei A, Fon A, et al. 2017. Zinc deficiency as a codeterminant for airway epithelial barrier dysfunction in an ex vivo model of COPD. *Int. J. Chron. Obstruct. Pulmon. Dis.* 12:3503–10
216. Rosenkranz E, Maywald M, Hilgers R-D, Brieger A, Clarner T, et al. 2016. Induction of regulatory T cells in Th1-/Th17-driven experimental autoimmune encephalomyelitis by zinc administration. *J. Nutr. Biochem.* 29:116–23
217. Rosenkranz E, Metz CHD, Maywald M, Hilgers R-D, Weßels I, et al. 2016. Zinc supplementation induces regulatory T cells by inhibition of Sirt-1 deacetylase in mixed lymphocyte cultures. *Mol. Nutr. Food Res.* 60(3):661–71
218. Rossi L, Migliaccio S, Corsi A, Marzia M, Bianco P, et al. 2001. Reduced growth and skeletal changes in zinc-deficient growing rats are due to impaired growth plate activity and inanition. *J. Nutr.* 131(4):1142–46
219. Sacan O, Turkyilmaz IB, Bayrak BB, Mutlu O, Akev N, Yanardag R. 2016. Zinc supplementation ameliorates glycoprotein components and oxidative stress changes in the lung of streptozotocin diabetic rats. *Biometals* 29(2):239–48
220. Sakakibara Y, Sato S, Kawashima Y, Someya Y, Shirato K, et al. 2011. Different recovery responses from dietary zinc-deficiency in the distribution of rat granulocytes. *J. Nutr. Sci. Vitaminol.* 57(2):197–201
221. Sakurai H, Adachi Y. 2005. The pharmacology of the insulinomimetic effect of zinc complexes. *Biometals* 18(4):319–23
222. Salari S, Khomand P, Arasteh M, Yousefzamani B, Hassanzadeh K. 2015. Zinc sulphate: a reasonable choice for depression management in patients with multiple sclerosis: a randomized, double-blind, placebo-controlled clinical trial. *Pharmacol. Rep.* 67(3):606–9
223. Sandstead HH, Freeland-Graves JH. 2014. Dietary phytate, zinc and hidden zinc deficiency. *J. Trace Elem. Med. Biol.* 28(4):414–17
224. Sanna A, Firinu D, Zavattari P, Valera P. 2018. Zinc status and autoimmunity: a systematic review and meta-analysis. *Nutrients* 10(1):68
225. Santos HO, Teixeira FJ, Schoenfeld BJ. 2020. Dietary versus pharmacological doses of zinc: a clinical review. *Clin. Nutr.* 39(5):1345–53
226. Schubert C, Guttek K, Grüngreiff K, Thielitz A, Bühling F, et al. 2014. Oral zinc aspartate treats experimental autoimmune encephalomyelitis. *Biometals* 27(6):1249–62
227. Scott DA. 1934. Crystalline insulin. *Biochem. J.* 28(4):1592–1602.1
228. Scott ME, Koski KG. 2000. Zinc deficiency impairs immune responses against parasitic nematode infections at intestinal and systemic sites. *J. Nutr.* 130(5):S1412–20
229. Seo H-J, Cho Y-E, Kim T, Shin H-I, Kwun I-S. 2010. Zinc may increase bone formation through stimulating cell proliferation, alkaline phosphatase activity and collagen synthesis in osteoblastic MC3T3-E1 cells. *Nutr. Res. Pract.* 4(5):356–61
230. Seo H-M, Kim YH, Lee JH, Kim JS, Park YM, Lee JY. 2017. Serum zinc status and its association with allergic sensitization: the fifth Korea National Health and Nutrition Examination Survey. *Sci. Rep.* 7(1):12637
231. Shalini S, Dorstyn L, Dawar S, Kumar S. 2015. Old, new and emerging functions of caspases. *Cell Death Differ.* 22(4):526–39
232. Shen H, MacDonald R, Bruemmer D, Stromberg A, Daugherty A, et al. 2007. Zinc deficiency alters lipid metabolism in LDL receptor deficient mice treated with rosiglitazone. *J. Nutr.* 137(11):2339–45
233. Solomons NW. 2001. Dietary sources of zinc and factors affecting its bioavailability. *Food Nutr. Bull.* 22(2):138–54
234. Stennicke HR, Salvesen GS. 1997. Biochemical characteristics of caspases-3, -6, -7, and -8. *J. Biol. Chem.* 272(41):25719–23
235. Stoncius LV, Ashrafi SH, Meyer J. 1985. Ultrastructure of mast cells in the hyperplastic buccal mucosa of the zinc-deficient rat. *J. Oral Pathol.* 14(5):375–82
236. Strand TA, Hollingshead SK, Julshamn K, Briles DE, Blomberg B, Sommerfelt H. 2003. Effects of zinc deficiency and pneumococcal surface protein A immunization on zinc status and the risk of severe infection in mice. *Infect. Immun.* 71(4):2009–13

237. Strieder-Barboza C, Baker NA, Flesher CG, Karmakar M, Neeley CK, et al. 2019. Advanced glycation end-products regulate extracellular matrix–adipocyte metabolic crosstalk in diabetes. *Sci. Rep.* 9(1):19748
238. Subramanian Vignesh K, Landero Figueroa JA, Porollo A, Caruso JA, Deepe GS Jr. 2013. Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. *Immunity* 39(4):697–710
239. Suzuki T, Kajita Y, Katsumata S-I, Matsuzaki H, Suzuki K. 2015. Zinc deficiency increases serum concentrations of parathyroid hormone through a decrease in serum calcium and induces bone fragility in rats. *J. Nutr. Sci. Vitaminol.* 61(5):382–90
240. Taylor KM, Hiscox S, Nicholson RI, Hogstrand C, Kille P. 2012. Protein kinase CK2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel ZIP7. *Sci. Signal.* 5(210):ra11
241. te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. 2010. $Zn^{2+}$ inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLOS Pathog.* 6(11):e1001176
242. Trame S, Wessels I, Haase H, Rink L. 2018. A short 18 items food frequency questionnaire biochemically validated to estimate zinc status in humans. *J. Trace Elem. Med. Biol.* 49:285–95
243. Truong-Tran AQ, Carter J, Ruffin RE, Zalewski PD. 2001. The role of zinc in caspase activation and apoptotic cell death. *Biometals* 14(3–4):315–30
244. Tupe R, Kulkarni A, Adeshara K, Sankhe N, Shaikh S, et al. 2015. Zinc inhibits glycation induced structural, functional modifications in albumin and protects erythrocytes from glycated albumin toxicity. *Int. J. Biol. Macromol.* 79:601–10
245. Uchida R, Xiang H, Arai H, Kitamura H, Nishida K. 2019. L-type calcium channel–mediated zinc wave is involved in the regulation of IL-6 by stimulating non-IgE with LPS and IL-33 in mast cells and dendritic cells. *Biol. Pharm. Bull.* 42(1):87–93
246. Valés-Gómez M, Erskine RA, Deacon MP, Strominger JL, Reyburn HT. 2001. The role of zinc in the binding of killer cell Ig-like receptors to class I MHC proteins. *PNAS* 98(4):1734–39
247. Valko M, Morris H, Cronin MTD. 2005. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* 12(10):1161–208
248. Vallee BL, Auld DS. 1993. Cocatalytic zinc motifs in enzyme catalysis. *PNAS* 90(7):2715–18
249. Vallee BL, Galdes A. 1984. The metallobiochemistry of zinc enzymes. *Adv. Enzymol. Relat. Areas Mol. Biol.* 56:283–430
250. Vogel-González M, Talló-Parra M, Herrera-Fernández V, Pérez-Vilaró G, Chillón M, et al. 2021. Low zinc levels at clinical admission associates with poor outcomes in COVID-19. *Nutrients* 13(2):562
251. von Bülow V, Rink L, Haase H. 2005. Zinc-mediated inhibition of cyclic nucleotide phosphodiesterase activity and expression suppresses TNF-α and IL-1β production in monocytes by elevation of guanosine 3′,5′-cyclic monophosphate. *J. Immunol.* 175(7):4697–705
252. Wan Y, Petris MJ, Peck SC. 2014. Separation of zinc-dependent and zinc-independent events during early LPS-stimulated TLR4 signaling in macrophage cells. *FEBS Lett.* 588(17):2928–35
253. Wang C, Zhang R, Wei X, Lv M, Jiang Z. 2020. Metalloimmunology: the metal ion–controlled immunity. In *Advances in Immunology in China B*, ed. C Dong, Z Jiang, pp. 187–241. Amsterdam: Elsevier
254. Wang J, Zhao H, Xu Z, Cheng X. 2020. Zinc dysregulation in cancers and its potential as a therapeutic target. *Cancer Biol. Med.* 17(3):612–25
255. Wang L, Wang F-S, Gershwin ME. 2015. Human autoimmune diseases: a comprehensive update. *J. Intern. Med.* 278(4):369–95
256. Wang Z, Wang J, Cao D, Han L. 2020. Correlation of neutrophil-to-lymphocyte ratio with the prognosis of non-ST-segment elevation in patients with acute coronary syndrome undergoing selective percutaneous coronary intervention. *J. Int. Med. Res.* 48(10):0300060520959510
257. Wei C-C, Luo Z, Hogstrand C, Xu Y-H, Wu L-X, et al. 2018. Zinc reduces hepatic lipid deposition and activates lipophagy via $Zn^{2+}$/MTF-1/PPARα and $Ca^{2+}$/CaMKKβ/AMPK pathways. *FASEB J.* 32(12):6666–80
258. Wessels I. 2017. Epigenetics and minerals: an overview. In *Handbook of Nutrition, Diet, and Epigenetics*, ed. V Patel, V Preedy, pp. 1–19. Basel, Switz.: Springer

259. Wessels I, Cousins RJ. 2015. Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 309(9):G768–78
260. Wessels I, Haase H, Engelhardt G, Rink L, Uciechowski P. 2013. Zinc deficiency induces production of the proinflammatory cytokines IL-1β and TNFα in promyeloid cells via epigenetic and redox-dependent mechanisms. *J. Nutr. Biochem.* 24(1):289–97
261. Wessels I, Maywald M, Rink L. 2017. Zinc as a gatekeeper of immune function. *Nutrients* 9(12):1286
262. Wessels I, Pupke JT, von Trotha K-T, Gombert A, Himmelsbach A, et al. 2020. Zinc supplementation ameliorates lung injury by reducing neutrophil recruitment and activity. *Thorax* 75(3):253–61
263. Wessels I, Rink L. 2020. Micronutrients in autoimmune diseases: possible therapeutic benefits of zinc and vitamin D. *J. Nutr. Biochem.* 77:108240
264. Wessels I, Rolles B, Rink L. 2020. The potential impact of zinc supplementation on COVID-19 pathogenesis. *Front. Immunol.* 11:1712
265. Williams RB, Chesters JK. 1970. The effects of early zinc deficiency on DNA and protein synthesis in the rat. *Br. J. Nutr.* 24(4):1053–59
266. Wilson M, Hogstrand C, Maret W. 2012. Picomolar concentrations of free zinc(II) ions regulate receptor protein–tyrosine phosphatase β activity. *J. Biol. Chem.* 287(12):9322–26
267. Wong CP, Dashner-Titus EJ, Alvarez SC, Chase TT, Hudson LG, Ho E. 2019. Zinc deficiency and arsenic exposure can act both independently or cooperatively to affect zinc status, oxidative stress, and inflammatory response. *Biol. Trace Elem. Res.* 191(2):370–81
268. Wong CP, Rinaldi NA, Ho E. 2015. Zinc deficiency enhanced inflammatory response by increasing immune cell activation and inducing IL6 promoter demethylation. *Mol. Nutr. Food Res.* 59(5):991–99
269. Wong SHK, Shih RSM, Schoene NW, Lei KY. 2008. Zinc-induced G2/M blockage is p53 and p21 dependent in normal human bronchial epithelial cells. *Am. J. Physiol. Cell Physiol.* 294(6):C1342–49
270. World Health Organization (WHO). 2003. The World Health report 2002. *Midwifery* 19(1):72–73
271. WHO. 2020. *Cancer*. Fact sheet, WHO. **https://www.who.int/news-room/fact-sheets/detail/cancer**
272. Xie W, Xue Q, Niu L, Wong K-W. 2020. Zinc transporter SLC39A7 relieves zinc deficiency to suppress alternative macrophage activation and impairment of phagocytosis. *PLOS ONE* 15(7):e0235776
273. Xie Z, Wu H, Zhao J. 2020. Multifunctional roles of zinc in Alzheimer's disease. *NeuroToxicology* 80:112–23
274. Yakoob MY, Theodoratou E, Jabeen A, Imdad A, Eisele TP, et al. 2011. Preventive zinc supplementation in developing countries: impact on mortality and morbidity due to diarrhea, pneumonia and malaria. *BMC Public Health* 11(Suppl. 3):23
275. Yu M, Lee W-W, Tomar D, Pryshchep S, Czesnikiewicz-Guzik M, et al. 2011. Regulation of T cell receptor signaling by activation-induced zinc influx. *J. Exp. Med.* 208(4):775–85
276. Zha Q-B, Wei H-X, Li C-G, Liang Y-D, Xu L-H, et al. 2016. ATP-induced inflammasome activation and pyroptosis is regulated by AMP-activated protein kinase in macrophages. *Front. Immunol.* 7:597
277. Zhao N, Wang X, Zhang Y, Gu Q, Huang F, et al. 2013. Gestational zinc deficiency impairs humoral and cellular immune responses to hepatitis B vaccination in offspring mice. *PLOS ONE* 8(9):e73461
278. Zhu B, Wang J-Y, Zhou J-J, Zhou F, Cheng W, et al. 2017. PML-RARα stabilized by zinc in human acute promyelocytic leukemia NB4 cells. *J. Inorg. Biochem.* 175:92–100
279. Zhuang X, Pang X, Zhang W, Wu W, Zhao J, et al. 2012. Effects of zinc and manganese on advanced glycation end products (AGEs) formation and AGEs-mediated endothelial cell dysfunction. *Life Sci.* 90(3–4):131–39