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Bioinorganic Chemistry of Zinc in relation to the Immune system

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Abstract

Zinc is well-known to have a central role in human inflammation and immunity and is itself an anti-inflammatory and antiviral agent. Despite its massively documented role in such processes, the underlying chemistry of zinc in relation to specific proteins and pathways of the immune system has not received much focus. This short review provides an overview of this topic, with emphasis on the structures of key proteins, zinc coordination chemistry, and probable mechanisms involved in zinc-based immunity, with some focus points for future chemical and biological research.

Keywords: Zinc; immune system; inflammation; zinc proteins; virus infections

Short biography: The author received his Ph.D. in theoretical biochemistry from Lund University in 2004 (supervisor: Ulf Ryde) and worked as a postdoc at Yale with Prof. William Jorgensen and at Stanford with Prof. Ed Solomon. Since 2007 working at DTU in Denmark with a wide range of research interests and about 130 published papers in computational (bio)chemistry, with a focus on bioinorganic and physical chemistry of proteins related to evolution and disease.
List of abbreviations

ACE2: Angiotensin-converting enzyme 2; membrane-bound zinc peptidase that catalyzes hydrolysis of angiotensin II and is the entry point of some coronaviruses, incl. SARS-CoV-2.

ADAM: A disintegrin and metalloproteinase; important group of zinc peptidases.

CD: Circular Dichroism spectroscopy

DFT: Density Functional Theory

EPR: Electron Pair Magnetic Resonance

IFNγ: Interferon gamma; a cytokine important for macrophage activation and MHC production.

HCV: Hepatitis C virus

HIV: Human Immunodeficiency virus

HLA: Human leukocyte antigen; cell surface proteins divided into MHCI and MHCII groups

LPS: Lipopolysaccharide, a major constituent of gram-negative bacterial membranes.

MHCI: Major histocompatibility complex class I; presents antigens from inside the cell.

MHCII: Major histocompatibility complex class II; presents antigens from outside the cell.

MMP: Matrix metalloproteases. Important group of zinc proteases.

MT: Metallothionein (MT1, MT2, MT3, MT4)

NF-κB: Nuclear factor κ-light chain enhancer of activated B-cells, important for cytokine production.

NK cell: Natural Killer cell

NMR: Nuclear Magnetic Resonance

NSP: Non-Structural Protein. Viral proteins involved in virus replication.

SAG: Superantigen, antigen that has major impact on immune system activation.

SOD-1: Cu,Zn-Superoxide-dismutase 1

TCR: T-cell receptor

TLR: Toll-like receptor

TNF-α: Tumor necrosis factor alpha; important cytokine (small immune signaling protein).

UV-VIS: Ultraviolet-Visible absorption spectroscopy

WBC: white blood cell (see Table 1 for a list).

ZAP: Zinc-finger Antiviral Protein. Important human antiviral protein.

ZIP: Zrt- and Irt-like Protein; Zinc transport protein

ZnT: Zinc transporter
1. Introduction

Zinc is an essential transition metal for living organisms and plays an important role in the human immune system. Previous reviews have described the immunology of zinc from a mainly biological and clinical perspective, and the zinc biochemistry more generally, whereas this paper attempts to provide a focused overview of the biochemistry of zinc relating to the immune system, with emphasis on the structural chemistry of zinc proteins.

Zinc (Z = 30) is the last metal of the 3d-transition series, is thiophilic relative to several other 3d-transition metal ions, and always occurs in its particularly stable, redox-inactive divalent form, Zn(II), which involves a complete d-shell 3d^{10} configuration that prevents any d-orbital directionality in a ligand field. Accordingly, the ideal geometries of Zn(II) in coordination chemistry is tetrahedral for coordination number 4, trigonal bipyramid for coordination number 5, and octahedral for coordination number 6, with tetrahedral most often preferred. The special properties of zinc (singular accessible spin and redox state, high effective nuclear charge, borderline Lewis-acidity, moderate lability of its ligands, high electronegativity and thiophilicity) makes it unique among the s-block and d-block metals applied in humans and highly complementary to the redox-inactive oxophilic Ca^{2+}, Mg^{2+}, Na^+, and K^+.

Experimental zinc biochemistry is challenged by the fact that Zn(II) is spectroscopically silent both to electron-pair magnetic resonance spectroscopy (EPR) (as the ion does not contain unpaired electrons) and UV-VIS spectroscopy of d-d transitions (which do not occur due to the full d-shell). However, the structural biology of zinc proteins explored by X-ray diffraction and data on zinc-binding affinities (dissociation constants, K_d) are very rich. The proteins can be studied spectroscopically with other metal ions such as Co(II), and they can be studied computationally by classical polarizable force fields and quantum chemical calculations, which are probably more accurate for zinc than e.g. iron, cobalt and manganese, where spin produces errors in relative chemical energies.

Zinc is a required cofactor of several hundred human enzymes covering all the six main enzyme classes, and potentially plays additional structural or regulatory roles in up to 10% of all human proteins, including e.g. zinc finger motifs in transcription factors. Many of these proteins are involved in homeostasis, anti-oxidant stress, metabolism, inflammation and cell life-death processes, and several of them have central roles in the human immune system, as reviewed below.
2. Brief overview of human zinc homeostasis

2.1. Zinc distribution and requirements

Zinc homeostasis has been excellently reviewed elsewhere\textsuperscript{[36-41]} so this section is mainly for context. The human body contains approximately 1–4 grams of zinc\textsuperscript{[9,42]} By far the most (~90%) in muscles and bones.\textsuperscript{[43]} Of the small amount of the total body's zinc that is circulating in blood (~1%), by far the most (~60–80%) is bound at moderate strength to serum albumin, whereas a minority (20–30%) is strongly bound to α2-macroglobulin.\textsuperscript{[44]} This makes zinc homeostasis very distinct from e.g. that of iron, which features the dedicated transport and storage proteins transferrin\textsuperscript{[45]} and ferritin.\textsuperscript{[46]}

Inside cells, approximately half of the zinc is located in cytoplasm bound to proteins or in vesicles, and roughly a third of the zinc is located in the nucleus, with the remaining zinc ions residing in the plasma membrane proteins.\textsuperscript{[38,41,47]} This intracellular zinc concentration $[\text{Zn}^{2+}]$ has a negligible "free" part (nano- to picomolar), below 1 zinc ion at any given time in a typical cell,\textsuperscript{[48]} due to the presence of high-affinity zinc proteins.\textsuperscript{[49]} The labile zinc is bound to amino acids and some water molecules on surfaces of proteins, whereas the non-labile pool is bound to high-affinity sites in proteins.\textsuperscript{[50]} Zn(II) affinities of protein sites vary by 12 orders of magnitude, with Zn(II) dissociation constants ($K_d$) ranging from $10^{-7}$ M (low-affinity) to $10^{-19}$ M (high-affinity).\textsuperscript{[23]}

Regular intake of zinc (typically of the order of 10 mg) is needed because the storage of zinc is not substantial compared to some other metal ions.\textsuperscript{[9,43]} Blood zinc levels are easily measured and thus used to diagnose zinc deficiency: Zinc concentrations below 10 micromolar typically imply zinc deficiency, with normal levels being ~10-20 micromolar.\textsuperscript{[47,51]} Zn deficiency is quite common, typically in elderly, but also in some cases in children.\textsuperscript{[4]} Typical manifestations of zinc deficiency include sense abnormalities and hair loss, and more importantly various types of lesions (e.g. skin lesions) and impairment of both innate and acquired immune responses.\textsuperscript{[43,52]}

2.2. Proteins controlling zinc homeostasis: ZnT, ZIP, and MT

The three main proteins of zinc homeostasis are zinc transporters (ZnT), zinc importer Zrt-/Irt-like proteins (ZIP), and metallothioneins (MT).\textsuperscript{[36]} Figure 1 shows representative structures of human ZnT8 (PDB: 6XPE\textsuperscript{[53]}), ZIP (bacterial ortholog PDB: 5TSA\textsuperscript{[54]}), and the α-domain of human MT2 (PDB: 1MHU\textsuperscript{[55]}). The architectures of the three proteins illustrate that ZnT (Figure 1A) and ZIP (Figure 1B) are transmembrane helical transport proteins, whereas MT proteins are small soluble disordered proteins (Figure 1C). Some features of the proteins are discussed below.
ZnT proteins (ZnT1 to ZnT10, coded by corresponding genes called *SLC30A1-10*) transport zinc from the cytoplasm of the cell into compartments within the cell or to the outside of the cell, whereas ZIP (Zrt- and Irt-like proteins) control the opposite process of importing zinc to the cytoplasm from compartments within the cell or from outside. The cryo-electron microscopy structure of human ZnT8 in both the outward- and inward-facing conformations was published in 2020 (Figure 1A). It displays a dimer topology with each subunit having six transmembrane helices and a highly conserved zinc site bound within this transmembrane domain, probably (although not perfectly clear due to resolution) in a tetrahedral geometry coordinated by His106, Asp110, His220, and Asp224. Three additional zinc ions coordinated by histidine, glutamate, and cysteine in the cytosolic and C-terminal domains outside the membrane are plausibly involved in modulation of zinc transport.

The genetically inherited disorder of zinc deficiency Acrodermatitis enteropathica, which causes skin lesions and is fatal without life-long zinc supplementation, is caused by mutation of the gene encoding the ZIP4 zinc transporter, and provides the hallmark example of ZIP’s role in zinc homeostasis. ZIP can transport zinc ions across both outer an inner membranes, but they differ in their localization and probably their detailed transport mechanisms. Thus, human ZIP2 is expressed in...
the outer plasma membrane and works in a pH- and concentration-dependent way.\textsuperscript{[57]} ZIP is also implied in co-transport of HCO\textsubscript{3}\textsuperscript{−}.\textsuperscript{[57,58]}

The crystal structure of a ZIP ortholog (PDB: 5TSA; \textbf{Figure 1B})\textsuperscript{[54]} revealed the full topology the eight transmembrane helices, with the four inner helices forming a central pore containing multiple zinc/cadmium ions each bound to two or three inwards-pointing aspartate, glutamate, glutamine, and histidine side-chains. The low coordination number probably reflects additional binding of solvent molecules in low-affinity sites suitable for transmembrane transport.\textsuperscript{[23]}

Four MT isoforms exist in humans: Broadly expressed MT1 and MT2, and tissue-specific MT3 and MT4. They bind up to 20\% of intracellular zinc as a buffer and act as chaperones for some zinc proteins.\textsuperscript{[59–62]} Their buffering function is due to an ability to bind and release zinc reversibly via two distinct \(\alpha\)- and \(\beta\)-domains incorporating up to four and three divalent metal ions, respectively, a feature that has been studied in detail by spectroscopy and DFT.\textsuperscript{[28,62–66]} The \(\alpha\)-domain of human MT2\textsuperscript{[55]} is shown in \textbf{Figure 1C}. Both domains use cysteines as ligand (nine for the \(\beta\)-domain and 11 for the \(\alpha\)-domain). While one Zn(II) has \(K_d \sim 10^{-8}\) M, the affinity of four of the Zn(II) ions is \(K_d \sim 10^{-12}\) M,\textsuperscript{[67]} remarkably close to average in the wide range of Zn(II),\textsuperscript{[23]} supporting a moderate-affinity Zn(II) buffer function. The non-cooperative binding of metal ions to the MT clusters\textsuperscript{[66]} and protein-enforced asymmetry provide the sites of the \(\alpha\)- and \(\beta\)-domains with a gradual hierarchy of affinities appropriate for buffering function.\textsuperscript{[65]}

Changes in Zn(II) availability is monitored by metal-response element-binding transcription factor-1 (MTF-1), which binds Zn(II) in its zinc-finger domain and controls the expression of genes involved in zinc homeostasis.\textsuperscript{[68]} Changes in MT expression and zinc dyshomeostasis have been associated with several diseases such as cancer and Alzheimer's disease,\textsuperscript{[69–71]} and to gum diseases relating to bacterial infections and inflammation.\textsuperscript{[72]}

In relation to immune defense, MT proteins play an important role in regulating zinc levels that e.g. inhibit the important protein complex NF-\(\kappa\)B (nuclear factor \(\kappa\)-light-chain-enhancer of activated B cells)\textsuperscript{[73]} that controls transcription and cytokine production in relation to immune processes.\textsuperscript{[74]} More generally, MT seems to be crucial for redistributing zinc resources in favor of the host organism's many zinc-dependent defense mechanisms and away from invading pathogens needing them for transcription.\textsuperscript{[75]} Consistent with such a role, MT proteins are expressed more upon viral infections including hepatitis C virus,\textsuperscript{[76,77]} influenza,\textsuperscript{[78]} measles,\textsuperscript{[79]} and HIV.\textsuperscript{[80]}
3. Zinc's role in immunity

3.1. Overview of the immune system and white blood cells
An overview of white blood cells (WBC) and their main functions is given in Table 1. The human immune system consists of two parts – the adaptive and innate systems. The innate immune response is the first line of defense against microorganisms. This defense is achieved by e.g. natural killer cells and phagocytes (neutrophils, macrophages) that identify molecular fingerprints of the microorganisms via single-pass membrane proteins, so-called toll-like receptors (TLRs).[81] The adaptive immune system includes learning from infections, notably via antigens presented on cell surfaces that lead to production of antibodies (proteins encapsulating pathogens for neutralization and targeting for destruction) and long-term immunological memory. Inflammation is mediated by cytokines including TNF-α and interleukins produced by cells such as macrophages, T-cells and B-cells.[82]

Of particular notice for the discussion below are: i) the neutrophils, the most abundant WBC central to the innate immune system, circulating the blood stream and providing early response by eating injured and infected cells;[83,84] ii) the mast cells, which control inflammation signals to alert and modulate other WBCs;[85,86] iii) dendritic cells, which present antigens to T-cells and thus help convert innate immune responses into acquired memory upon T-cell and memory B-cell activation;[87] iv) macrophages that eat cells and present antigens of ingested cells to T-cells;[88] v) T-helper (CD4+) cells that recognize antigens on antigen-presenting cells;[89] vi) cytotoxic (CD8+) T-cells that kill infected cells after recognizing antigens on their surface;[90] vii) B-cells that produce antibodies and keep information on historically encountered antigens;[91,92] and viii) natural killer cells that kill other cells without "approval" from antibodies or antigens present on the cell's surface.[93] Less abundant WBCs like eosinophils[94–96] and basophils[97] are not discussed below.

As part of the innate defense, phagocytes (e.g. neutrophils and macrophages) engulf the pathogens and neutralize them via enzymes and radicals, and neutrophils at early stages of infection release antimicrobial compounds (a process called degranulation)[84] and neutrophil extracellular traps consisting of DNA chromatin web with high affinity for pathogen proteins.[98] However, the development of long-term immunity that reduce pathogen outbreaks to population-wise more tolerable seasonal epidemics is facilitated by the adaptive immune memory of T- and B-cells, which are substantially modulated by zinc,[9,99–102] as described below. The B-cells are mainly responsible for the expressing antibodies and antimicrobial peptides,[91,92] whereas T-cells control the communication and memory formation via reaction towards antigen-presenting cells.[90]
The two classes of Major Histocompatibility Complexes (MHC) are central to the immune system's molecular recognition.[103] They are structurally and functionally related but have specialized towards handling separate types of antigens: Antigen-presenting cells such as dendritic cells and B-cells display molecular fragments derived from extracellular pathogen proteins in MHCII receptors such as HLA-DP and HLA-DR. These antigen-receptor complexes are then recognized by T-helper CD4+ cells that then activate other WBCs for a general defense. In contrast, fragments presented by MHC receptors arise from inside cells and are thus indicators of infection of the specific cell and accordingly recognized by cytotoxic CD8+ T-cells that can kill the infected cell.[103] Natural killer cells also interrogate cells via MHC and require them to prove authenticity by presenting human molecular "self-antigens", and thus the system ensures both positive and negative quality control of cells.[103]

3.2. Zinc's role in immune function as seen from [Zn2+] changes

Zinc is well-known to play a major role in the human immune system.[1,6,7,11–14,104–106] Zinc's role in regulating inflammation signals, notably via NF-κB related cytokine production, has been reviewed elsewhere.[107] Since the seventies it has been known that zinc deficiency leads to a variety of immune weaknesses in mice.[108] Since the eighties it has been known that zinc modulates human immune cells.[109] Zinc is a known contributing cause of lymphopenia, a state with low levels of white blood cells.[110]

While the role of Zn and MT in viral infections is well-established,[75,76,111–113] a decrease of cytoplasmic [Zn2+] by expression of ZnT also occurs upon bacterial infections,[40] and zinc nanoparticles are being increasingly studied for the use against bacterial infections.[114–117] [Zn2+] increases when WBCs are subject to infection or inflammatory stimulation, induced by lipopolysaccharide (LPS),[118] a major constituent of gram-negative bacterial membranes.[119]

Clinical examples of zinc's beneficial role in controlling various diseases and infections are beyond the scope of this review, but many examples have been compiled e.g. by Read et al.[75], Rink and Gabriel,[11] and Wessels et al.[120]

The major redistribution of [Zn2+] during infection partly reflects the increased need for proteins that require zinc either directly or via their synthesis (zinc finger transcription factors and the large zinc proteome itself[32]). Redistribution of [Zn2+] is a consistent feature of major non-infection diseases such as Alzheimer's disease,[69,121] cancer,[40,122] and allergy.[123] Some of this redistribution may be a protective feature of increased requirement for zinc-dependent proteins, and some may reflect
pathogenic processes. During active infection, MT plays a crucial part in this redistribution of available zinc resources to the relative benefit of the host organism.\textsuperscript{[75,124]}

However, in addition to optimal allocation of [Zn\textsuperscript{2+}] for the human host, redistribution may also partly reflect a scorched-earth tactics to deprive pathogens of critical zinc. Given its systemic importance, adequate supply of [Zn\textsuperscript{2+}] is vital not just to the human host but also to the pathogens, both for general housekeeping, with typical bacteria needing zinc for \(\sim\)5\% of their proteomes,\textsuperscript{[125]} and directly for proliferation, with e.g. virus gene replication highly dependent on [Zn\textsuperscript{2+}].\textsuperscript{[126]}

Plausibly due to the host defense relocating available zinc resources, zinc availability seems more important to the human host defense than the effects it has on pathogen survival and proliferation, at least when the host defense works normally and applies this focused [Zn\textsuperscript{2+}] redistribution.\textsuperscript{[13]} During conditions of functional zinc deficiency, the immune defense may shift from a more communicative coordinated response to a basic innate defensive, as seen in some studies.\textsuperscript{[127]}

3.3. Effects of zinc on specific WBC functions
Evidence of zinc's importance to specific immune cells, monitored from either zinc supplementation, zinc deficiency, or changes in zinc transport protein expression, is summarized in Table 2. Some key examples are highlighted below.

The importance of zinc supply to mast cells is evident from changes in ZnT4 and ZnT5 expression.\textsuperscript{[128,129]} Mast cells contain comparatively large amounts of zinc, and modulation of mast cells, notably caspase activation (Zn is a well-known caspase inhibitor) and degranulation, is zinc-dependent via the high-affinity IgE receptor.\textsuperscript{[129,130]} Correspondingly, Zn also inhibits phosphatase activity leading to enhanced interleukin expression in human epithelial cells.\textsuperscript{[131]}

Innate immunity via neutrophil and NK cell function is well-known to be partly zinc-dependent. Zn(II) facilitates neutrophil activity via protein kinase C and metabolism of reactive oxygen species.\textsuperscript{[132]} It has been long known that zinc deficiency impairs NK cell production.\textsuperscript{[133]} Zinc is important both for maturation of NK cells and for their cytotoxic function,\textsuperscript{[134]} a process readily reversed by zinc supplementation.\textsuperscript{[135]} It has also been shown that NK cells require zinc for recognition of HLA-C MHCI receptors, a central part of their function that enables them to identify target cells.\textsuperscript{[136]}

Dendritic cells that present antigens of pathogens to alert the immune system also depend on zinc for optimal TLR signaling, as shown by studies using the TLR4 agonist lipopolysaccharide (LPS).\textsuperscript{[152]} In other studies, mutations in the zinc finger transcription factor Ikaros were found to reduce human dendritic cell numbers, pointing to a role in dendritic cell development.\textsuperscript{[137]}
Figure 2. Zinc sites regulating MHCII and T-cell receptor interactions. (A) Superantigen (streptococcal pyrogenic exotoxin C) bound to the MHCII receptor HLA-DR, with zoom-in on the zinc site (PDB: 1HQR).\textsuperscript{[138]} Green = MHCII HLA-DR alpha chain (chain A). Blue = MHCII HLA-DR beta chain (chain B). Purple = myelin basic protein (chain C). Yellow = superantigen (chain D). (B) Src-family tyrosine kinase Lck zinc-mediated complex with T-cell CD4 co-receptor (PDB: 1Q68). (C) T-cell receptor CD8α-Lck-Zn(II) complex (PDB: 1Q69).

Upon some bacterial infections, superantigens (SAG) play a major role in the inflammatory process via interaction with human MHCII.\textsuperscript{[138]} SAGs made by some pathogens lead to massive non-specific activation of T-cells, in contrast to most antigens that only activate a very small fraction of specific T-cells.\textsuperscript{[139,140]} SAGs are plausibly produced by the pathogen as a strategy to over-activate non-specific (and thus potentially less efficient) T-cells.\textsuperscript{[140]} MHCII molecules, which present pathogen molecules on the cell surface to the immune system, have two binding sites for bacterial SAGs; one of these is a zinc-dependent site located on the β chain of MHC (PDB: 1HQR) (Figure 2A).\textsuperscript{[138]}

Whereas mature B- and T-cells tend to be less directly affected by zinc levels, zinc deficiency is generally associated with impaired cell development.\textsuperscript{[9,141,142]} Mature T-cells experience high ZIP8 expression, especially upon activation, which helps regulating T-cell IFN-γ expression.\textsuperscript{[143]} Zinc also regulates T-cell receptor interactions directly\textsuperscript{[102]} and enhances T-cell cytokine production.\textsuperscript{[144]} Last but not least, Zn(II) directly mediates the recognition of CD4 and CD8 T-cell co-receptors: The Src-family tyrosine kinase Lck is required for both T-cell development and infection-based activation of T-cells, via zinc-dependent interaction with the CD4 and CD8 co-receptors that define the CD4+ "helper" CD8+ "killer" T-cells.\textsuperscript{[145]}

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The role of zinc and MT in differentiating and maturing T-cells is well-established.\cite{101,102,146-148} Cytoplasmic zinc levels increase upon T cell receptor (TCR) triggering, dependent on ZIP6,\cite{102} and CD4\(^+\) T-cell activation is associated with increased Zn and MT.\cite{149,150} Structures of the CD4-Lck-Zn(II) (PDB: 1Q68; Figure 2B) and CD8\(\alpha\)-Lck-Zn(II) (PDB: 1Q69; (Figure 2C) complexes\cite{151} indicate a probable mode of the zinc-TCR interaction: These structures contain a cross-linking tetrahedral zinc site coordinated by four cysteines, two from the CD4/CD8 co-receptor, and two from Lck. A recent study indicates that zinc deficiency in aging mice is a partial reason for CD4\(^+\) T-cell-related immunosenescence, the phenomenon that aging tends to impair immune defense.\cite{152}

Whereas T-cell levels are impaired in mouse models of zinc deficiency, an impact on mature B-cells is not evident.\cite{153} However, Zn homeostasis is essential for B-cell development, and these processes are ZIP10-mediated.\cite{110} ZIP10-deficiency in mature B-cells attenuate both T-cell–dependent and –independent immune responses.\cite{99} Recently, an autosomal recessive immunodeficiency disease featuring absent B cells was identified in five unrelated families resulting from loss-of-function mutations in the gene SLC39A7, which encodes ZIP7,\cite{154} providing a direct genotype-phenotype relationship for zinc in B-cell development and in signaling via the B-cell antigen receptor.\cite{11}

Finally, macrophages that eat infected cells are also highly sensitive to zinc homeostasis. Zn has been shown to enhance phagocyte function during Salmonella infection.\cite{155} ZIP10 regulates the survival of macrophages via a Zn- and p53-dependent mechanism.\cite{156} Removal of MT impairs macrophage function in mice.\cite{157} Zinc deficiency in rats leads reduces zinc finger transcription factor GATA-3, which in turn is required for production of T-helper cells, and via these, production of cytokines required by immature macrophages to convert into active M2 macrophages.\cite{158} Recent studies of M2-macrophages suggest that they help to promote wound healing but weaken antimicrobial defenses in a process that depends on MT3 and interleukin (IL)-4.\cite{72}

4. Human zinc proteins important to immune function

4.1. Zinc fingers

Since zinc is systemically required for both invading pathogens and the host cell's housekeeping as well as immune defense, the three main homeostatic proteins ZnT, ZIP, and MT, have been consistently implied in a variety of immune defense mechanisms, as summarized above. However, in addition to the general control over [Zn\(^{2+}\)] for general housekeeping and WBC functioning, zinc also play specific roles in proteins related to immune processes, one important example being zinc fingers.\cite{159,160}
Due to their special affinity for nucleic acids, zinc fingers play a central role in binding to RNA or DNA to modify replication; they can target genetic material for degradation,[159,160] and this can even be achieved with simple artificial zinc finger proteins.[161] Zinc-finger motifs with full peptide fold can bind Zn(II) with very high affinity, up to \( K_d \) of \( 10^{-15} \) M,[162] but lower values are probably more common.[23]

Since pathogens require expansion of their genomes in the host, zinc-finger transcription factors are central to combatting infection.[113,163] Zinc finger nucleases, i.e. restriction enzymes that combine a zinc finger DNA-binding and a DNA-cleavage domain, can make helper T-cells tolerant to HIV-1.[164] Zinc finger proteins are important in differentiating helper T-cells and cytotoxic T-cells.[165] The promyelocytic leukemia zinc finger regulates development of NK cells,[166] and cKrox has been identified as a central regulator of CD4 differentiation.[167] The Ikaros zinc finger transcription factor, which is important to dendritic cell development,[137] is also important to T-cell development[168,169] and regulation of mature B-cells.[170] The zinc finger protein Bcl-6 (B-cell lymphoma 6 protein) helps to control helper-T cell early development[171,172] and is involved in stress-dependent control over the fate of germinal B-cells.[173]

CCCH zinc finger proteins (containing tetrahedral Zn(II) sites with three cysteines and one histidine as ligands) such as tristetraprolin (Zfp36), roquin 1 and regnase 1 play important roles in cytokine mRNA degradation.[163] For example, tristetraprolin specifically binds and targets for degradation adenine- and uridine-rich (AU-rich) elements within the 3′-untranslated region of some cytokine mRNAs such as TNF-α.[174] It should be noted that MT is important for constituting zinc finger transcription factors with zinc,[175,176] which arguably provides the main link between zinc deficiency, MT overexpression, and zinc finger function in relation to infections.

4.2. Cu-Zn superoxide dismutase 1

Cu-Zn superoxide dismutase 1 (SOD1) is one of the three human superoxide dismutases that convert superoxide into oxygen and water in two reaction steps inside cells. SOD1 plays a major role in various inflammation, stress, immune and disease processes and is critical for conversion of oxygen-based free radicals during normal oxidative metabolism.[177–179] As a primary anti-oxidant stress protein, its expression has been related to life span in *S. cerevisae* and *Drosophila*,[180] and its misfolding is implied in the motor-neuron degenerative disease ALS.[179,181,182]

During Influenza A virus infections, SOD1 levels have been found to correlate inversely with expression of viral genes, indicating a protective effect of the enzyme.[183] This may simply be because
SOD1 expression generally correlates with zinc availability, or because SOD1 is recruited as protective measure against radical warfare waged on pathogens. Antigen-dependent human T-cell activation leads to intracellular redistribution of SOD-1 in T-cells upon constitition of TCRs.\[184\] Immunomodulatory effects have also been observed with the extracellular non-zinc SOD-3 isoform.\[185,186\]

DFT\[187–189\], QM-MM\[190\], and bioinformatics\[191,192\] studies of SOD1 have been performed, as have extensive NMR and CD spectroscopy studies helping to understand the very high dimer stability of SOD1,\[193\] and many site-directed mutagenesis studies on protein stability and folding.\[194–196\] In the binuclear site both Zn and Cu are bound to three histidines and a bridging histidine, and the importance of Zn(II) for moderating the catalytic properties of Cu(I)/Cu(II) is thus important: The \(K_d\) of Zn(II) has been estimated at \(10^{-13}\) M, and is impaired by disease-causing amino acid mutations.\[197\]

4.3. Zinc peptidases
Zinc peptidases are essential for turning over the proteome, working directly on peptide bonds in hard-to-digest protein and peptide substrates.\[198\] As such, they play a major role in all processes where proteins and peptides are subject to turnover. The main superfamilies are gluzincin, inuzincin, metzincin, carboxypeptidase, and DD carboxypeptidase.\[198\] Many zinc peptidases have been studied mechanistically both by spectroscopy\[15,22,199\] and quantum chemical computations,\[200–203\] and the mechanism of their function is relatively well-understood.

Among the zinc peptidases, the matrix metalloproteases (MMP) and ADAM (a disintegrin and metalloproteinase) are particularly relevant to the overall homeostasis of cells and immune function: They are transmembrane endopeptidases (i.e. they hydrolyse non-terminal peptide bonds).\[204\] As an important example, ADAM17 is also known as tumor necrosis factor-\(\alpha\)-converting enzyme (TACE), because its substrate, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), is a major immunomodulatory cytokine released by macrophages during infection as a central part of inflammatory signaling.\[205,206\] ADAMs and MMPs, via their conversion of cytokines, play an important role in modulating inflammatory responses and the level of immune system alertness.\[204–208\]

The main roles in relation to the immune system are in degrading proteins and peptides of invading pathogens together with other non-zinc proteases such as lysosomal cysteine proteases (e.g. cathepsin b). However even the non-zinc proteases may require zinc for transcription in many cases, as e.g. seen for cathepsin b in breast cancer cells,\[209\] and free [Zn\(^{2+}\)] conversely often works as an inhibitor of them (IC\(_{50}\) ~ 160 nM for cathepsin at physiological pH\[210\]), which indicates how strongly zinc affects the proteasome. The intracellular zinc peptidases degrade pathogen proteins into fragments.
that are then complexed with MHCII and presented on surface of the cell for recognition by T-cells leading to activation of the immune system. The extracellular zinc proteases, e.g., MMPs, degrade pathogen substrates produced from macrophage activity and human proteins of inactivated infected cells, as required for reconstituting tissue. Some pathogen peptidases (which are themselves zinc peptidases) directly target human MMPs during infection.[211]

5. Zinc proteins and virus infections

5.1. Importance of zinc to viral infections
The acknowledgement of the role of zinc in infections is quite old.[212,213] Zinc supplements are well-accepted to decrease morbidity of respiratory infections in children in the developing world.[214,215] Zinc's ability to inhibit virus infection has been recognized for more than five decades.[216] In vitro, zinc inhibits replication of viruses such as herpes simplex virus,[217–219] hepatitis C virus,[220] Picorna virus,[221,222] syncytial virus,[214] Semliki Forest virus,[223] influenza,[224] and highly pathogenic coronaviruses such as SARS-CoV-1[225] and SARS-CoV-2,[226] with a list of examples in Table 3. Since this review's focus is the structural chemistry and bioinorganic chemistry of zinc in relation to immune function, the reader is referred to other reviews for a more complete overview.[75]

As discussed above, current understanding suggests that, although the virus needs zinc for several proteins and replication, zinc also serves as an inhibitor of several enzymes[210], and the host can use MT to redistribute zinc in favor of the human immune system's zinc requirements, such that zinc supply overall tends to be beneficial, except if patients studied were not already zinc deficient.[75] The structural chemistry of zinc covers both examples where zinc is to the advantage of the human host and to the advantage of the invading virus, with prominent examples of both given below.

5.2. ACE2
Several coronaviruses such as SARS-CoV-1 and SARS-CoV-2 have their main entry point into human cells via their spike protein binding to the single-pass membrane-bound dipeptidyl zinc carboxydipeptidase angiotensin-converting enzyme 2 (ACE2).[227–229] ACE2 is important for regulating blood pressure and works to lower it by hydrolysing of the potent vasoconstrictor angiotensin II into the vasodilator angiotensin and is thus of clinical importance to e.g. cardiovascular disease.[230–232]
Figure 3. Zinc proteins related to virus infections. (A) Human ACE2; membrane zinc peptidase and surface receptor for SARS-CoV-1 and SARS-CoV-2 cell entry (PDB: 1R42).[^233] (B) Hepatitis C virus non-structural protein NS5A (PDB: 1ZH1).[^234] (C) NSP10 from Sars-CoV-1 (PDB: 2FYG).[^235]

Figure 4. Structural Zinc sites with presumed antiviral effects. (A) Zn(II)-bound influenza virus hemagglutinin (PDB: 6LKS).[^236] (B) Zn(II)-bound SARS-CoV-2 main protease (PDB: 7DK1).[^226] (C) Human zinc finger antiviral protein RNA binding domain in complex with RNA (PDB: 6UEI).[^237]
The structure of ACE2 is shown in Figure 3A (PDB: 1R42). In the active site, Zn(II) is coordinated by His374, His378, Glu402, and one water molecule as in classical carboxypeptidases, where the water ligand serves as nucleophile attacking the carbonyl carbon of the peptide bond that is hydrolyzed, and the Zn(II) ion enhances its nucleophilicity typically by lowering the pKa to produce a hydroxide nucleophile and binds the carbonyl oxygen to enhance the electrophilicity of the carbonyl center.

The zinc site is situated in the active site cleft flanked by transmembrane helices, not directly accessible to the virus spike protein, which binds to the extracellular part. ACE2 binding occurs via the open state of the spike protein where the spike-protein receptor-binding domain is in an upwards conformation. Whereas zinc carboxypeptidases have been studied in detail by mechanistic and computational bioinorganic chemistry, ACE2 has not, to the author's knowledge.

5.3. Viral zinc proteins
Considering how small viral genomes tend to be, the number of viral zinc proteins is notable, with examples ranging from measles and HIV to Epstein-Barr virus, Ebola virus, and coronaviruses. These zinc proteins commonly play a central role in virus replication, and it is thus of interest to the human host organism to prevent the binding of zinc to them either by depleting available local Zn(II) or by therapeutically ejecting Zn(II) from the proteins, as discussed below.

In terms of mechanistic bioinorganic chemistry, substantial work was done on the Zn(II)-binding HIV-1 nucleocapsid Protein NCp7, which is important for replicating and packaging the HIV genome and contains two CCHC zinc finger motifs. The Zn(II) affinities of the two sites were determined by fluorescence spectroscopy, with $K_d \sim 10^{-14}$ M and $10^{-9}$ M. $K_d \sim 10^{-14}$ M suggests possible extraction of zinc from the human host MT to the viral protein, which would make biologically sense and has indeed been observed in vitro. The structure of this protein has been described in detail (e.g. PDB: 2JZW) and it was studied early on by DFT by Maynard et al., who provided a physical-chemistry basis for electrophilic reagents attacking cysteine 29 of the protein as an anti-HIV therapy. X-ray Absorption Spectroscopy and time-dependent DFT has also been used to study gold compounds as zinc-ejection anti-HIV therapies targeting NCp7.

Another illustrative example of structural and mechanistic studies of such proteins is nonstructural protein 5A (NS5A) of hepatitis C virus (HCV). Its structure (PDB: 1ZH1) is shown in Figure 3B. NS5A is part of the multi-subunit membrane-bound replicase complex of the virus. The dimer structure has the zinc site of each monomer located close to the dimer interface.
The zinc sites, which are involved in controlling the replication event by binding to RNA, are required for the replication process, are tetrahedral and coordinated by four cysteines. This protein has been recently studied by molecular dynamics and DFT computations, in order to explore the binding of zinc chelator disulfiram, which was previously suggested as antiviral zinc-ejecting therapy in HCV infections.

Coronaviruses contain zinc sites in some of their non-structural proteins involving in replication of the virus. For example, SARS-CoV-1 NSP10 is a zinc protein that plays a main role in virus replication. The structure of this protein has revealed two zinc sites (PDB: 2FYG), shown in Figure 3C. One of these sites is a CCCH zinc finger in a unique conformation, whereas the other is a 4C-type zinc finger related to the gag-knuckle group. The NSP12, NSP7, and NSP8 containing viral RNA-dependent RNA polymerase complex of SARS-CoV-2 also contains zinc sites (7BV1, not shown), although the mechanistic importance of these remains to be clarified, but analogy suggests that they have similar essential roles on SARS-CoV-2 replication.

5.4. Antiviral zinc sites

While zinc is required in zinc fingers of many viral proteins, the mechanism of free zinc's inhibition of virus replication is also of major interest but remains poorly explored both mechanistically and structurally. However, a recent study of Zn(II)-bound influenza virus hemagglutinin (PDB: 6LKS), shown in Figure 4A, gives important clues to such a mechanism, with Zn(II) binding to Glu68 and His137 at the head regions of the trimers inducing conformational changes in hemagglutinin similar to those induced by low pH, an observation that may underly the observation of zinc's inhibitory effect on influenza virus.

Correspondingly relevant to the ongoing SARS-CoV-2 pandemic, a recently published structure of Zn(II)-bound SARS-CoV-2 main protease (Mpro) features Zn(II) bound in a tetrahedral coordination geometry in a solvent-exposed catalytic site to cysteine and histidine as well as two water molecules (PDB: 7DK1), as shown in Figure 4B. The main protease (Mpro) of SARS-CoV-2 has been shown to be inhibited by Zn(II), with an dissociation constant $K_D = 7.6 \cdot 10^{-7}$ M. This affinity is low but typical of Zn(II) binding to relatively solvent-exposed sites in proteins, and much lower than in protein-buried high-affinity zinc sites.
5.5. Human Zinc finger antiviral protein

Whereas many viral proteins directly require zinc for their function, the human host generally seems to have larger advantage of adequate zinc supply than the virus. While many mechanisms can contribute to this tendency, including a human-centric allocation of available zinc resources by ZnT, MT, and ZIP proteins,[75] direct anti-viral defense by zinc proteins is evident from the studies of the human zinc-finger antiviral protein (ZAP), which is well-known to help fight multiple virus infections, as documented e.g. for hepadnaviruses (e.g. hepatitis B)[260], filoviruses (e.g. Ebola),[261], toga/alphaviruses (e.g. Semliki Forest virus and Ross River virus),[262] retroviruses (e.g. HIV),[263] and orthomyxoviruses (e.g. influenza).[264]

ZAP is a cytoplasmic zinc-finger protein expressed in mammals that recognizes non-self nucleic acids that may signify an invading virus, and works as a restriction factor on the RNA replication.[265] ZAP specifically binds to cytosine-guanine (CpG) dinucleotides that are specifically rare in mammals but common in many viruses, possibly as an evolutionary adaptation.[266] The new RNA-ZAP complex conformation enables ribonucleases to degrade the RNA molecule, thus preventing virus replication.[265] During its function, recent studies suggest that ZAP also activate and facilitate cytokine production by T-cells.[267] ZAP can also bind longer CpG oligonucleotides that tend to be associated with dendritic cell maturation,[268,269] but does so at lower affinity, possibly due to electrostatic mismatch in the binding pocket.[237]

The structure of human ZAP N-terminal RNA binding domain (Figure 4C) (PDB: 6UEI[237]) is mainly helical and contains four different 3C1H zinc finger sites in the typical tetrahedral coordination geometry. The complex with an RNA substrate shows how the binding to CpG dinucleotides is conformationally favored, which provides a rationale for the selective processing of non-human RNA which for many viruses is more CG rich.[237] As a sidenote of relevance to the ongoing pandemic, although SARS-CoV-2 seems adapted to have a lower CG-content of its mRNA, ZAP has also been found to restrict this virus.[270] These studies provide a structural and biological basis for the importance of ZAP that can hopefully be studied more in the future.
6. Antibacterial function of zinc

In addition to the general role of zinc in maintaining an adequate immune response against pathogens, zinc also has a direct inhibitory effect on various bacteria. In terms of direct antibacterial toxicity, Cu is probably more antibacterial than Zn, which has a more complex response. However this changes with specific forms of Zn: One of the most cited and well-documented of these effects is for zinc oxide nanoparticles. While relatively safe for humans, these particles tend to inhibit growth of bacteria inversely to the size of the nano-particles. The effect of zinc oxides has been known for decades and is larger for zinc vs. other metals such as Cu, Al, and Ti, as shown for Staphylococcus aureus.

Several mechanisms of this effect have been proposed. E. coli cells were shown to have their membranes damaged by the ZnO nanoparticles, which were also diffusing into the bacterial cells. The permeation of the bacterial membranes may lead to accumulation of reactive oxygen species or Zn\(^{2+}\) ions solvated from the nanoparticles, which work to limit bacterial growth. Another study found that methicillin-resistant S. aureus was inhibited by a complex set of pathways involving up-regulation of pyrimidine biosynthesis and carbohydrate degradation, and reduced amino acid synthesis that might relate to metabolic pathway disruptions. Finally, studies of S. pneumoniae suggest that Zn outcompetes other metal ions such as Mn which leads to functional metal ion deficiency.
of the bacteria. These various mechanisms may be context- and species-dependent and suggest more work into determining the contribution of each type of mechanism in specific applications.

7. Summary and conclusions

This review paper has attempted to give an overview of the bioinorganic chemistry of zinc in the immune system, with some inclusion of the essential biology, which is however already discussed in much detail by other excellent reviews, but with particular emphasis on the structural and mechanistic bioinorganic chemistry that has been less discussed before. It is evident that Zn(II) plays a very central role in immune function, and the involved proteins and biochemical mechanisms relating to these functions are increasingly emerging, although far from fully understood.

The main processes and proteins discussed in the paper are summarized in Figure 5 for quick overview. Given the ongoing importance of the immune system both to an aging human population and in the light of threats from emerging pathogens, the bioinorganic chemistry of zinc proteins relating to immunity is probably going to be a large topic in future research. It is the author’s opinion that there is an enormous need and at the same time sufficient structural basis for more elaborate mechanistic and computational studies of many of the proteins mentioned, as a basis for rationalizing and predicting the biological and medicinal impacts of zinc in relation to disease. It is the hope that the above focused overview will help to motivate and provide a starting point for such future research.

Conflicts of interest statement. The author has no conflicts of interests associated with this work.

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1728.


19552.


48576–48587.


# Table 1. Overview of white blood cells (WBC).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells</th>
<th>Primary functions</th>
</tr>
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<tbody>
<tr>
<td><strong>Myeloid cells</strong></td>
<td></td>
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<tr>
<td></td>
<td>neutrophils (granulocytes)</td>
<td>Most abundant WBC (~50%). Early-response phagocytic (eating) cells; part of the innate immune system.[83,84]</td>
</tr>
<tr>
<td></td>
<td>eosinophils (granulocytes)</td>
<td>1-3% of WBC. Diverse roles in modulating inflammation and host protection.[94-96]</td>
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<tr>
<td></td>
<td>mast cells (granulocytes)</td>
<td>Control inflammation signals to alert and modulate other white blood cells; important in allergies.[85,86]</td>
</tr>
<tr>
<td></td>
<td>basophils (granulocytes)</td>
<td>Typically &lt;1% of WBC. Like mast cells they control inflammation signals and coordinate immune response.[97]</td>
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<tr>
<td></td>
<td>dendritic cells (monocytes)</td>
<td>Presenting antigens on their cell surface to the T-cells.[87]</td>
</tr>
<tr>
<td></td>
<td>macrophages (monocytes)</td>
<td>Phagocytic (eats cells), important both during development and normal tissue repair and as a defense against pathogens.[88]</td>
</tr>
<tr>
<td><strong>Lymphoid cells</strong></td>
<td>T-helper (CD4(^+)) cells</td>
<td>Recognize antigens from MHCII, and then recruit B-cells, memory T cells, macrophages, and other cells as part of the adaptive immune response.[89]</td>
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<tr>
<td></td>
<td>Memory T-cells</td>
<td>Play a role in homeostasis and immune memory like the memory B-cells.[282]</td>
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<td></td>
<td>Cytotoxic (CD8(^+)) T-cells</td>
<td>Kills infected cells that present antigens via MHCI on their cell surface.[90]</td>
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<td></td>
<td>Plasma B-cells</td>
<td>Produce antibodies in the blood that are then transported to infection sites and neutralize pathogens.[92]</td>
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<td></td>
<td>Memory B-cells</td>
<td>Essential to the adaptive immune system by remembering molecular recognition of historic antigens to enable efficient secondary infection responses.[91]</td>
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<td></td>
<td>Natural killer cells</td>
<td>As opposed to cytotoxic T-cells they kill stressed cells even without antibodies bound or MHCI markers (as required by T-cells), making them fast and non-selective during early infection protection and against cancer.[93]</td>
</tr>
</tbody>
</table>
Table 2. Examples of zinc's role in specific immune cells and processes.

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Mechanism</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Neutrophils</td>
<td>Neutrophil function</td>
<td>Zn(II) facilitates neutrophil activity via protein kinase C and</td>
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<td></td>
<td></td>
<td>metabolism of reactive oxygen species. [132]</td>
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<tr>
<td>Granulocytes, monocytes, T-cells</td>
<td>Cytokine expression</td>
<td>Zn supplementation leads to higher cytokine expression. [13] MT</td>
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<td></td>
<td></td>
<td>facilitates allocation of WBCs to inflammation sites. [124]</td>
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<tr>
<td>Dendritic cells</td>
<td>Antigen detection</td>
<td>Toll-like receptor signaling is Zn-dependent. [52]</td>
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<tr>
<td>Immature T-cells</td>
<td>T-cell maturation</td>
<td>Zn deficiency impairs thymic function [13] MT regulates T-cell</td>
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<td></td>
<td></td>
<td>differentiation. [148]</td>
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<tr>
<td>Mature CD4⁺ T-helper cells</td>
<td>T-cell activation</td>
<td>CD4⁺ T-cell activation is associated with increased Zn and MT. [149]</td>
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<td></td>
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<td>Zn regulates T-cell receptors. [102] CD4⁺ T-cells increased by Zn after</td>
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<td>stem cell transplantation [150]</td>
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<td></td>
<td>Cytokine production</td>
<td>ZIP8 regulates T-cell IFN-γ expression. [143] Zn enhances T-cell</td>
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<td></td>
<td></td>
<td>cytokine production. [144]</td>
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<td></td>
<td>SAG T-helper cell recognition</td>
<td>MHCII molecules have two binding sites for bacterial SAGs; one is a</td>
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<td></td>
<td></td>
<td>zinc-dependent. [138]</td>
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<tr>
<td>Mature T- and B-cells</td>
<td>T/B-cell communication</td>
<td>Zip10-deficiency in mature B-cells attenuate both T-cell–dependent and</td>
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<td></td>
<td></td>
<td>–independent immune responses. [99]</td>
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<tr>
<td>Developing B-cells</td>
<td>B-cell function</td>
<td>Zn uptake via ZIP10 is essential for B-cell survival in early B-cell</td>
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<td></td>
<td></td>
<td>development. [110] Immunodeficiency featuring absent B-cells results</td>
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<td></td>
<td></td>
<td>from mutations in ZIP7. [154]</td>
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<tr>
<td>Phagocytes</td>
<td>Phagocyte enhancement</td>
<td>Zn enhances phagocyte function during Salmonella infection. [155] ZIP10</td>
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<td></td>
<td></td>
<td>regulates macrophage survival via Zn and p53. [156] MT knockout</td>
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<td></td>
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<td>impairs macrophage function in mice. [157] Rat macrophage-T-helper</td>
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<td>cell communication is impaired by Zn deficiency. [158]</td>
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<tr>
<td>Human epithelial cells</td>
<td>Epithelial cell Stress response</td>
<td>Zn inhibits phosphatase activity leading to enhanced interleukin</td>
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<td></td>
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<td>expression. [131]</td>
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<tr>
<td>NK cells</td>
<td>Recognition by NK cells</td>
<td>NK cells require zinc for recognition of HLA-C MHCI receptors. [136]</td>
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<td></td>
<td></td>
<td>Zinc is important for NK cell maturation. [134]</td>
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<tr>
<td>Mast cells</td>
<td>Mast cell function</td>
<td>Mast cell function relates to changes in ZnT4 and ZnT5. [128, 129]</td>
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<td></td>
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<td>Caspase inactivation and degranulation is Zn-dependent. [129, 130]</td>
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<tr>
<td>Disease-related virus</td>
<td>Observations/mechanism</td>
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<tr>
<td>Influenza</td>
<td>Zinc oxide nanoparticles inhibit virus in vitro. [224]</td>
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<tr>
<td>Herpes simplex virus (HSV)</td>
<td>Zn inhibits HSV DNA polymerase. [218] Zinc inactivates virus in vitro. [219]</td>
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<td></td>
<td>Zinc supply via ionophores inhibit virus replication. [217]</td>
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<tr>
<td>Picorna virus</td>
<td>Zinc supply via ionophores inhibit virus replication. [221]</td>
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<td></td>
<td>Zinc inhibited Foot-and-Mouth Disease Virus replication in primary calf kidney cells. [222]</td>
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<tr>
<td>Semliki Forest virus</td>
<td>Zinc treatment enhanced host resistance in infected mice [223]</td>
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<tr>
<td>Salmonella</td>
<td>Zinc treatment enhanced host resistance in turkeys [155]</td>
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<tr>
<td>Respiratory syncytial virus (RSV)</td>
<td>Zinc treatment (but not other divalent cations) reduced virus replication [214]</td>
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<tr>
<td>HIV-1</td>
<td>MT and intracellular zinc modulate immune-activated monocytes and increases resistance to cell death. [80]</td>
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<tr>
<td>Hepatitis C</td>
<td>MT accumulates in the nucleus of HCV-infected cells, knockout of MT1+MT2 increases viral loads [76]</td>
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<tr>
<td>Rhinovirus</td>
<td>Zn inhibits virus replication. [216]</td>
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<tr>
<td>SARS-CoV-1</td>
<td>Zn inhibits polymerase activity in vitro; Zn inhibits viral replication in cell culture. [225]</td>
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<tr>
<td>SARS-CoV-2</td>
<td>Zn inhibits the main protease (MPro) in vitro virus replication. [226]</td>
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</table>

\(^{a}\) The data reported in the table are mainly supportive of in vitro biochemical and cellular functions. Evidence of therapeutic effect requires large randomized human trials.
Zinc's role in human immunity has been extensively covered from a biological perspective. The structural, thermodynamic, and inorganic chemistry of zinc in relation to proteins and pathways of the immune system has received less attention and is the topic of the present review.

**Keywords:** zinc; immune system; zinc-finger anti-viral protein; coordination chemistry, protein structure