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Metallothionein: a Potential Link in the Regulation of Zinc in Nutritional Immunity

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Abstract Nutritional immunity describes mechanisms for withholding essential transition metals as well as directing the toxicity of these metals against infectious agents. Zinc is one of these transition elements that are essential for both humans and microbial pathogens. At the same time, Zn can be toxic both for man and microbes if its concentration is higher than the tolerance limit. Therefore a "delicate" balance of Zn must be maintained to keep the immune cells surveilling while making the level of Zn either to starve or to intoxicate the pathogens. On the other hand, the invading pathogens will exploit the host Zn pool for its survival and replication. Apparently, different sets of protein in human and bacteria are involved to maintain their Zn need. Metallothionein (MT)—a group of low molecular weight proteins, is well known for its Zn-binding ability and is expected to play an important role in that Zn balance at the time of active infection. However, the differences in structural, functional, and molecular control of biosynthesis between human and bacterial MT might play an important role to determine the proper use of Zn and the winning side. The current review explains the possible involvement of human and bacterial MT at the time of infection to control and exploit Zn for their need.

Keywords Inflammation · Glucocorticoid hormone · Metallothionein · Metalloproteases · Nutritional immunity · Zinc toxicity

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Introduction

To prevent pathogenesis of infectious microorganisms, humans restrict access to essential metals in a process known as nutritional immunity. Broadly, nutritional immunity describes mechanisms for withholding essential transition metals as well as directing the toxicity of these metals against infectious agents. Scope of nutritional immunity has broaden from its original concept of referring to iron (Fe) to include other transition metals such as zinc (Zn), copper (Cu), and manganese (Mn) [1]. While Fe and Cu are known to have redox potential and are involved in large number of oxidoreductases or other electron transfer proteins, Zn plays critical role in structural as well as catalytic proteins both in eukaryotes and prokaryotes [2]. Zn is frequently incorporated into metalloenzymes, storage proteins, and transcription factors and become the second most abundant transition metal in most living systems after Fe. For example, ~80% enzymes in archaea and bacteria are Zn-containing proteins while those in eukaryotes are ~50%. However, Zn-binding proteins, including Zn-dependent transcription factors, make up a larger proportion of the total proteome in eukaryotes as compared to bacteria and archaea [3]. Thus, Zn is essential for both humans and microbial pathogens to survive. At the same time, Zn can be toxic if its concentration is higher than the tolerance limit both for man and microbes.

Cells of the human body use a number of sophisticated mechanisms to maintain intracellular and extracellular Zn homeostasis. Role of Zn in life processes has been thoroughly reviewed [4–6]. Dietary Zn deficiency results in loss of immune function and resistance to infection suppressing thymic function, T lymphocyte development, lymphocyte proliferation, and T cell-dependent B cell functions [7]. At the same time, to acquire the required amount of Zn in Zn-deficient conditions and to prevent lethal effects of Zn in Zn excess

conditions, pathogenic bacteria also use a number of mechanisms to maintain key cellular processes including growth and replication [8–10].

Therefore, it is expected that a "delicate" balance must be maintained at the time of infection that in one hand limit Zn availability to the pathogens; at the same time, the Zn level should be good enough to cause toxicity to the pathogens as well activation to the immune cells (Fig. 1). The site of infection might govern the strategy to be adopted by the invading pathogens since Zn availability may vary in different tissues. For example, group A Streptococcus is suggested to face Zn toxicity during colonization of the nasopharynx, but Zn deprivation on the skin [9]. Apparently, different sets of proteins in human and bacteria are involved in maintaining the balance. A family of low molecular weight proteins, namely, metallothionein (MT), a bonafide Zn-binding protein that is ubiquitously present in both prokaryotic and eukaryotic organisms, is also expected to play an important role in that balance, i.e., in nutritional immunity.

MTs in human are primarily involved in homeostasis of essential metals such as Zn and Cu, and detoxifying of toxic metals, such as Cd and mercury (Hg) [11–14]. In the last few decades, MT expression in humans was linked with a number of inducers (or initiators) such as heavy metals, endotoxins, cytokines, glucocorticoids (GCs), reactive oxygen species (ROS), and toxic organic compounds [15–20]. Expression of MT in human tissues is also induced during different pathological condition [15, 21]. In bacteria, MTs are mainly involved in metal resistance, for example, Cd resistance by *Escherichia coli* [22] and *Salmonella enterica* [23], lead resistance by *Providencia vermicola* [24], and Cu resistance by *Mycobacterium tuberculosis* [25].

However, the role of MT in the cross talk between human MT and bacterial MT in nutritional immunity more particularly at the time of active infection is largely unknown. The role of bacterial MT in Zn speciation and homeostasis is also largely unknown [10]. The current review will attempt to propose possible involvement of human and bacterial MT at the time of infection to control and exploit Zn with special reference to nutritional immunity. The focus of the current review will be on the synthesis or degradation of MT in response to infectious diseases, the human-MT mediated Zn homeostasis in response to infectious insult, role of MT in directing Zn to activate immune response against the infection, mechanism of exploitation of as well as resistance against host Zn pool by the infectious agents, any possible competition between host (human) MT and bacterial MT for host Zn pool, and host-MT-mediated changes at the site of infection (Fig. 2).

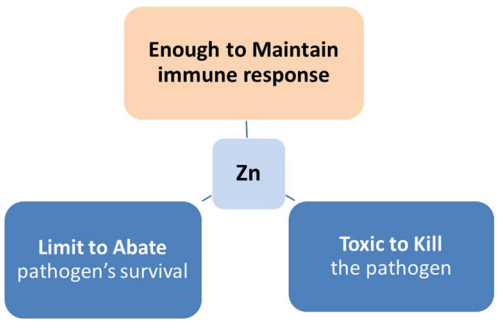

Fig. 1 Using zinc at the time of infection. Immune cells of the host require enough Zn supply to maintain immune response against the pathogenic insult. While the Zn availability must be limited to abate survival and proliferation of the pathogens, and at the same time, free Zn must incur toxic insult to the pathogens to kill them

Zn Distribution and Homeostasis in Human

Zinc is widely distributed in various tissues in human with a total amount of 1.4–2.3 g in adults, 85% of which are localized in the muscles and bones, 11% in the skin and liver, and the remaining 4% in other tissues [26]. Highest concentration of Zn is present in the retina and choroid of the eye, followed by the prostate, bones, liver, and kidneys [27–29]. Virtually, all Zn is intracellular: 30–40% in the nucleus; 50% in the cytosol, organelles, and specialized vesicles; and the remainder is associated with cell membranes [30]. In human, plasma Zn maintains a homeostatic level of approximately 10–18 mol/L that represents only 0.1% of total body Zn [31]. In human, the global Zn storage is mediated by hormones such as glucagon and epinephrine that in turn can increase MT expression and Zn storage in liver [32].

The number of in vivo Zn-binding proteins in humans was estimated to be 2800, corresponding to 10% of the human proteome. Among these, the most abundant class of Zn-binding proteins is that of Zn fingers, with Cys4 and Cys2-His2 binding coordination [3, 33].

The intracellular homeostasis and distribution of Zn is controlled by specialized sets of proteins: Zn^{2+} importer family (14 ZIPs, solute-linked transporter (SLC) 39A) and Zn^{2+} transporter family (10 ZnTs, SLC 30A) transporters [34]. ZnTs generally transport Zn^{2+} out of the cytosol, whereas ZIPs import them from cellular compartments or the extracellular space into the cytosol [35]. Most ZnTs are present in intracellular compartments, such as endosomes, Golgi, or endoplasmic reticulum, while only ZnT1 appears to be located at the plasma membrane as it is the primary regulator of cellular Zn efflux [36]. Most ZIPs are observed at the plasma membrane; however, Zip7 is located at the Golgi apparatus [37]. The localization of some ZIPs changes according to Zn availability or physiologic conditions. Zip5 has a basolateral plasma membrane orientation in polarized cells during dietary zinc sufficiency [38, 39]. Similarly, ZIP14 is mobilized to the sinusoidal membrane of the mouse hepatocyte during acute inflammation and, therefore, increases zinc uptake as a component of the acute phase response [35, 40].

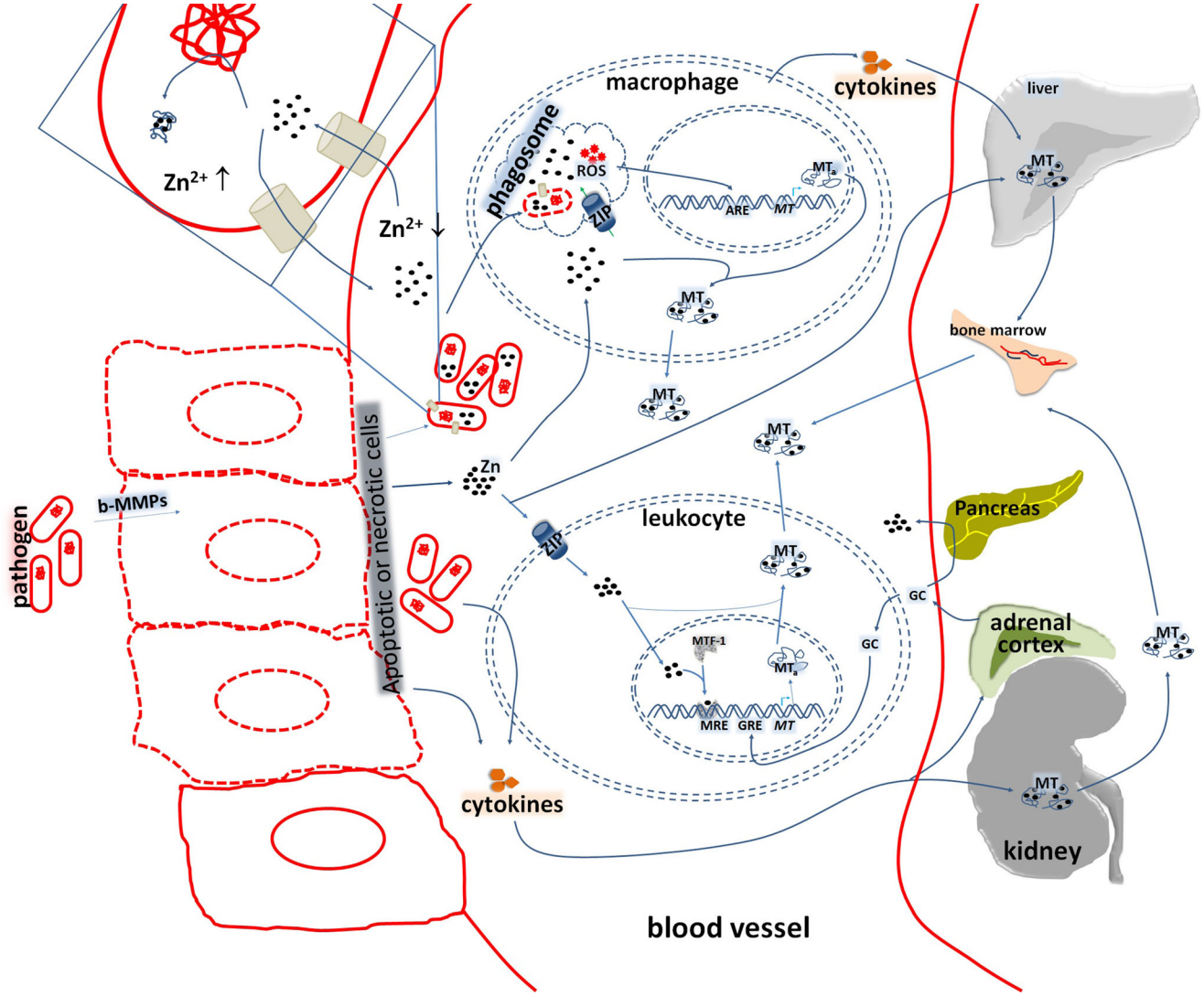

Fig. 2 Potential roles of MT in nutritional immunity in controlling Zn availability for invading pathogens. Most bacterial pathogens use metalloproteinases to invade host tissue. The invaded tissues are degraded due to apoptosis or necrosis and release Zn. Increased Zn at the site of infection results in upregulated expression of ZIP by the circulating leukocytes or infiltrated inflammatory cells, resulting in upregulated expression of MT through the activation of metal-responsive elements (MREs). These intracellular MTs protect leukocytes from the increased influx of Zn at the site of infections. Inflammatory cytokines released at the site of infection may eventually induce adrenal cortex to release glucocorticoid (GC) hormones. Once GC reached the circulation, MT synthesis in leukocytes can be induced through GRE. Kidney also responds to the inflammatory cytokines and secretes more MT in the circulation. Pathogenic invasion also redistributes Zn from serum to liver. With the increased amount of Zn in the liver, MT biosynthesis in hepatocytes is upregulated. Both hepatic and renal MT may induce bone marrow hematopoietic stem cells to produce more circulatory leukocytes. At the site of infection, macrophages also face increased Zn and reactive oxygen species (ROS) during phagocytosis. Both the Zn and ROS can induce MT biosynthesis. The resulting upregulated MT protects the phagocytes from Zn toxicity as well as minimize the Zn level for the invading pathogens. Depending on the Zn starvation (*downward arrow*) or Zn excess (*upward arrow*) condition, invading bacteria may upregulate either Zn-influx or Zn-efflux mechanism, respectively (*inset*). Some of eh cytoplasmic Zn induce bacterial MT biosynthesis that in turn help the bacteria to maintain required Zn content

Zn in Host Immunity Against Infectious Diseases

Zinc regulates an array of developmental and functional aspects of cell-mediated immunity involving neutrophils, NK cells, and macrophages; cytokine production by immune cells; and the growth and function of T and B cells. Zn also mediates protection from the adverse effect of ROS that are produced not only during metabolism but also during inflammatory processes. Free intracellular Zn^{2+} is essential in extravasation to the site of the infection and uptake and killing of microorganisms by neutrophils [41]. Role of Zn in immunity has been thoroughly reviewed [1, 42–44]. A number of important roles of Zn in immunity that are relevant to the focus of the current review are highlighted below.

Zinc modulates the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) signaling pathway.

NF-κB influences the expression of pro-inflammatory cytokines (e.g., interleukin (IL)-1b, IL-6, IL-8, tumor necrosis factor alpha (TNF-α), and MCP-1), chemokines, acute phase proteins (CRP and fibrinogen), matrix metalloproteinases (MMPs), adhesion molecules, growth factors, and other factors involved in inflammatory response, such as COX-2 and iNOS [45, 46]. Zinc importer ZIP8 (SLC39A8) is the most significantly upregulated transporter in response to cytokines, bacteria, and sepsis. ZIP8 increases cytosolic Zn content by promoting extracellular uptake or release from subcellular organelles. The thiol-reactive cytosolic Zn induces NF-κB inhibition downstream from MAPKs; hence, ZIP8-mediated Zn influx works as a negative feedback regulator of NF-κB in response to infection [47, 48].

In human, a number of proteins that exert their antimicrobial effects are Zn dependent. Such as, the cathelicidin LL-37, secretion of which by intestinal epithelial cells is Zn dependent [49], shows antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcal* species and *E. coli*, and *Candida albicans* [50]. Zinc-dependent secretory proteins, namely, human peptidoglycan recognition proteins (PGLYRPs), inhibit many Gram-positive and Gram-negative bacteria [51]. Biological functions of thymulin, a serum Zn-dependent thymus-specific hormone, binds receptors on T cells, induces T cell markers, and promotes allogenic cytotoxicity, suppressor T cell functions, and IL-2 production [52]. Furthermore, Zinc is also crucial for the balance between the different T cell subsets [31, 43, 44]. Paradoxically, the activity of NADPH oxidase, involved in the destruction of pathogens after phagocytosis, may be inhibited by both Zn-deficient and Zn excess conditions.

Inflammatory processes during active infectious stage are associated with remarkable changes in Zn homeostasis. During the active infectious stage, a rapid decrease of the serum Zn level takes place due to its redistribution from plasma into organs, predominantly the liver. An upregulated expression of ZIP14 in liver in response to the pro-inflammatory cytokine IL-6 has been shown to mediate the redistribution [40], thus limit Zn availability for the invading pathogens. Furthermore, the Zn chelation by calprotectin is mostly released by the leukocytes and has been shown to suppress the reproduction of bacteria and *C. albicans* [53]. At the same time, an increased Zn concentration in macrophages can intoxicate phagocytosed microorganisms [7, 44]. Again, increased intracellular hepatocyte Zn promotes energy metabolism, neutralizes ROS, and guarantees the synthesis of acute phase proteins in the liver [54, 55] that are needed to fight the pathogens (Fig. 2).

Zn reduces the incidence and severity of diarrhea and acute lower respiratory tract infections in infants and children [56, 57] as well as the incidence of *Staphylococcus aureus* pneumonia, *Streptococcus* pneumonia tonsillitis, and *E. coli* urinary tract infections in sickle cell anemia patients [58]. Zn supplementation significantly decreases the incidence of infections in elderly subjects [59]. Moreover, Zn augments monocyte adhesion to endothelial cells in vitro and affects production of pro-inflammatory cytokines, such as IL-1b, IL-6, and TNF-α. Zn aids NK cells to recognize major histocompatibility complex (MHC) class I, and the lytic activity. In vitro, moderate Zn supplementation increases the differentiation of $CD34^+$ cells toward NK cells and their cytotoxic activity.

Immune suppression in Zn-deficient conditions is well documented with an increased susceptibility to various infectious agents, including *F. tularensis* [60], *Listeria monocytogenes*, *Salmonella enteritidis*, *M. tuberculosis*, and many viruses, protozoan parasites, and eukaryotes [7, 61–63]. A delayed production of protective antibodies in Zn-deficient condition has been reiterated.

Zn in Pathogenesis and Virulence

Zinc is essential to the survival of a pathogen in the host. Bacteria are predicted to incorporate Zn into 5–6% of all proteins [3]. A number of Zn-dependent virulence factors contribute to the survival and pathogenesis of the invading bacteria (Table 1).

Zinc-dependent microbial metalloproteases are a group of well-documented virulence factors and one of the four major groups of extracellular proteases. The other three groups of microbial proteases include serine proteases (EC 3.4.21), cysteine (or thiol) proteases (EC 3.4.22), and aspartate proteases (EC 3.4.23). Metalloprotease typically exhibits broad proteolytic specificity that facilitates the pathogen to disrupt physiological barriers to invade host, degrade key signaling intermediates, and release metals from host metalloproteins. Cytokines or interleukins that are important for the neutrophils and macrophage recruitment at the site of infection can be disrupted by bacterial metalloproteases to avoid immune clearance. A list of well-known virulence factors that belong to the Zn-dependent bacterial metalloproteases are presented in Table 1. These virulence factors augment pathogenesis of the respective pathogens in various ways.

Zn as a Regulator of MT Biosynthesis and Induction

In human cells, MT biosynthesis is regulated by metal (MRE), antioxidant (ARE), and glucocorticoid (GRE) response elements. Thus, the divalent trace elements such as Zn, ROS, and stress hormones such as GC are potent MT inducers in human cells [88–91]. Zn has a direct impact on the MT biosynthesis and induction. Zn binds MRE-binding transcription factors (MTFs) and activates MRE. After Zn occupancy, MTF-1 binds specifically to the MRE sequence to initiate transcription of MT genes. The requirement of additional Zn for the binding of the MTF-1 with its promoter in cell-free system attests the definitive role of Zn in MT biosynthesis [90, 92–94]. Induction of MT by other

Table 1 Zn-dependent bacterial metalloproteases that act as virulence factor

Virulence factor	Source pathogens	Mechanism of actions	Reference
vEP	*Vibrio fulnificus*	Activate prothrombin and act as fibrinolytic enzyme to facilitate the development of systemic infection	[64]
ZmpB	*Burkholderia cenocepacia*	Proteolysis of alpha-1 proteinase inhibitor, alpha(2)-macrogobulin, type IV collagen, fibronectin, lactoferrin, transferrin, and immunoglobulins	[65]
Flavastacin	*Flavobacterium meningosepticum*	Exerts endopeptidase activity against variety of cytokines and cytoskeletal proteins	[66, 67]
VV protease VV hemolysin	*Vibrio vulnificus*	Edematous and hemorrhagic skin damage hemolysis and cytolysis to facilitate bacterial invasion from the intestine to the blood stream	[68, 69]
λ-toxin	*Clostridium perfringens*	Degrades collagen, fibronectin, fibrinogen, IgA, and C3 component. Increases vascular permeability and hemorrhagic edema	[70]
Pseudolysin	*Pseudomonas aeruginosa*	Degrade IgA	[71, 72]
Fragilysin	*Bacteroides fragilis*	The enterotoxin causes tissue destruction and facilitate bacterial invasion	[73–75]
IgA protease	*Streptococcus sanguis Seratia marcescens*	Degrade Ig	[76, 77]
Mirabilysin	*Proteus mirabilis*	Degrade IgG, IgA	[78, 79]
Pseudolysin	*P. aeruginosa*	Degrade IgG	[80]
InhA1	*Bacillus anthracis*	Cleave prothrombin and factor X to induce clotting disrupt endothelial cell by cleaving MAP kinases	[81–85]
Serratia 56 K protease	*Serratia marcescens*	Cleaves human lysozyme and human serum transferrin, rat tropocollagen. Capable of degrading defense-oriented humoral proteins and tissue constituents. Toxic to fibroblasts	[77]
Protease	*Legionella pneumophila*	Degrade IL-2 and cleave CD4 on human T cells, thus impedes T cell activation	[86]
Npr599 and InhA	*Bacillus anthracis*	Degrade host tissues, increase barrier permeability, and/or modulate host defenses	[87]

elements such as Cd and Cu [90, 95–97] is also Zn dependent as these metals displace Zn from the Zn-containing protein which in turn allows the free Zn to induce MT expression [98, 99].

A number of steps might be involved in Cu- and Cd-induced expressions of MT genes. Firstly, Cd and Cu may displace Zn from the binding sites of Zn-containing metalloproteins including MT. Subsequently, free Zn may bind to the Zn finger of MTF-1 and regulate the expression of MT gene [98, 99]. GRE within the promoter region of the MT gene can act independently to induce MT transcription in the presence of GC, a stress hormone [100–102]. ARE also plays an important role in the induced expression of MT in response to ROS, such as hydrogen peroxide [100, 103]. Notably, both GC and ROS are increased in an active infectious state. Furthermore, maintaining the physiological concentrations of Zn is necessary to avoid oxidative stress, since both Zn deficiency and Zn overload are pro-oxidant conditions [104].

Differences Between Human and Bacterial MT

To date, four major isoforms, namely, MT-1, MT-2, MT-3, and MT-4, have been identified in human. In human, MT-1 and MT-2 were detected in all organs [105, 106]; MT-3 in the brain, lung, kidney, and reproductive organs [107–111]; and MT-4 in differentiating stratified squamous epithelial cells [112]. In human, eight functional MT-1 isogenes have been identified, namely, MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-1H, MT-1M, and MT-1X [113, 114].

The very first MT-like proteins in bacteria were identified in the marine cyanobacterium *Synechococcus* sp. (strain RRIMP N1) and later in the freshwater strain *Synechococcus* TX-20 [91, 115]. These bacterial MTs were considerably different from human MTs, as they contain aromatic amino acid His [116]. Bacterial MTs do not have any significant sequence homology with human MTs, except for a high Cys content. In 1990s, the gene for MT from *Synechococcus* PCC7942) was sequenced

(*SmtA*), along with the gene for the metal-responsive transcription factor SmtB, and the operator-promoter region between the two genes [117, 118]. A glutathione S-transferase (GST)-fusion protein of SmtA expressed in *E. coli* revealed that SmtA is capable of binding Zn^{2+}, Cd^{2+}, Cu^{2+}, and Hg^{2+} [118].

The determination of the pH values for half-displacement of bound metal suggested for SmtA in comparison with mammalian MTs a relatively higher affinity for Zn^{2+} and a relatively lower affinity for Cd^{2+}. Later, it was confirmed that the purified SmtA binds four Zn^{2+} or Cd^{2+}, where nine Cys residues participate in the metal binding [119]. In 2008, Gold et al. (2008) reported a copper-binding MT (MymT), of ~5 kDa, in *M. tuberculosis* expression of which can be induced by other metals such as Zn, Cd, Co, and Ni [120]. The MymT can also be induced by nitrosative and oxidative stress, as well as mildly acidic conditions and cell wall perturbation.

The inorganic core of SmtA strongly resembles the Zn4Cys11 cluster of mammalian MT, despite different amino acid sequences. In SmtA, four Zn^{2+} binds in a Zn4Cys9His2 cluster. InSmtA, the two ZnCys3His sites and one of the ZnCys4 sites readily exchange Zn^{2+} for exogenous Cd^{2+}, while the remaining ZnCys4 remains inert [121]. This metal binding behavior of SmtA is different from most of the MTs where metal binding is generally kinetically labile [122, 123]. Moreover, "all-cysteine" MTs bind Cd^{2+} 10 times more strongly than Zn^{2+}, and stoichiometric amounts of Cd^{2+} are usually sufficient to displace all of the Zn^{2+} from MT under similar conditions to those used here [122].

Fighting for Zn: Man vs. Microbes

The human body is a rich reservoir of Zn and needs to maintain Zn homeostasis within the physiological range (as discussed earlier). A number of microbial pathogens have evolved to exploit the Zn reserve in different organs of the human body. As a countermeasure, human body uses a number of defense mechanism to limit the availability of free Zn but also to maintain Zn homeostasis. Zn-binding proteins in eukaryotes, namely, psoriasin [124], calgranulin C [125, 126] and calprotectin [127–129], exert their antimicrobial potential through Zn^{2+} chelation. The same families of proteins also have pro-inflammatory properties causing inflammation-mediated pathologies [130]. Ironically, a number of pathogens such as *S. enterica* [131, 132], *Campylobacter jejuni* [133], *Haemophilus influenzae* [134], *L. monocytogenes* [8], and *Streptococcus pneumoniae* [114] can counteract the antimicrobial potential of those proteins in different ways.

In an active infectious state, neutrophils that are recruited at the site of infection secrete calprotectin, which mainly binds Zn and Mn [135, 136], thus make the Zn unavailable for the microbial invaders. Interestingly, *Neisseria meningitidis* was shown to scavenge calprotectin-chelated Zn, thus evade neutrophil-mediated killing [137]. Again, detection of the bacterial invaders via lipopolysaccharide can induce IL-6 expression, which in turn increases MT expression and reduces free Zn^{2+} [138, 139]. Paradoxically, GC signaling can either induce MT biosynthesis, hence reducing the free Zn^{2+} or induce Zn^{2+} secretion from pancreatic cells aiding microbial Zn^{2+} feast [94]. MT controls human matrix metalloproteinases (MMPs) by regulating Zn [140]. Host-derived MMP controls influx of effector cells, killing of pathogens, resolution of inflammation, and remodeling of extracellular matrix [141].

Bacterial pathogen uses three strategies to combat host-imposed Zn starvation or poisoning: (i) transcriptional regulation by metal-sensing metalloregulatory proteins; (ii) Zn efflux and acquisition across cell membranes; and (iii) Zn sparing (increase the expression of non-Zn-requiring proteins to replace essential Zn-dependent enzymes and proteins), and allocation of Zn to Zn-requiring enzymes, processes that are governed by Zn speciation in the cytoplasm [10]. An invading pathogen acquires host Zn mostly (90%) from skeletal muscle and bone and for the rest from the liver and kidneys [142, 143]. Intracellular Zn in these tissues is present at 100–500 μM, a large portion of which is bound to MTs [144, 145]. Only a small part of the total body Zn, i.e., 0.1%, is present in blood serum (1.25 μg/mL serum) that are bound to albumin (73–91%), macroglobin (9–27%), or various serum proteins and amino acids (2–8%) [146–148].

In bacterial cells, the total cell-associated Zn is in the millimolar range; however, the bioavailable Zn in the bacterial cytoplasm is predicted to be in the picomolar to nanomolar range [10, 149]. In one hand, Zn buffering between this 10^6-fold concentration difference tells the overcapacity of bacterial cell to chelate Zn, while the mechanism of which remains unknown. Many bacterial pathogens such as *L. monocytogenes*, *S. enterica*, *Brucella abortus*, and *Yersinia pestis* depend on the ATP-binding cassette (ABC) transporters to acquire Zn from human host [8, 131, 150]. These ABC transporters, common across Gram-positive and Gram-negative bacteria [151], contains three components: the periplasmic binding protein ZnuA binds a single Zn^{2+} with high affinity, the ZnuB permease that actively transports Zn through the inner membrane, and the ZnuC ATPase provides energy by ATP hydrolysis [152, 153]. In contrast to Zn starvation condition, bacteria such as *M. tuberculosis* uses Zn-efflux pumps to survive in macrophages [154]. Using liver homogenate, Choudhuri et al. (1992) showed that at a lysosomal pH of around 4.7, about 60% of Zn can be displaced from MT, thereby making it susceptible to degradation [155]. Hence, an increasing Zn excess condition due to its release from MT and the subsequent degradation of the apo-MT might overthrow the Zn-efflux pumps of the invading pathogen. In a severely Zn starvation condition, the Zn-free (apo) form of Zn uptake repressor (Zur) of most bacteria shows low affinity for the operator and overlapping promoter regions of

high-affinity Zn uptake system(s) [10]. In addition, the Zn efflux systems are repressed by the apo form of the Zn efflux repressor, ZntR in Zn-limited conditions. With an increased bioavailable Zn, the Zn-bound form of Zur binds to the operator site, thus preventing transcription of the Zn uptake systems [156]. Likewise, the efflux regulator, ZntR, binds Zn (in Zn-excess condition) and allosterically activates transcription of Zn-specific P-type ATPase efflux transporter (zntA) [157].

Metal-specific outer membrane also functions in Zn uptake in Gram-negative bacteria [158, 159]. For example, Neisseria ZnuD might be capable of transporting free, hydrated Zn^{2+}, as suggested by the structural and computational studies [159]. In an escalation in the Zn acquisition "arms race" between microbe and host, an outer-membrane porin-designated CbpA, a candidate bacterial receptor for CP-Zn complexes, is thought to capture this CP-bound Zn, consistent with a direct role in Zn piracy [137].

Competition Between Host MT and Bacterial MT for Host-Zn Pool

While Zn in human MTs is bound to Cys residues, the same in bacterial MTs can be bound to Cys and aromatic amino acid, His [119]. MT binds Zn exceptionally strongly owing to the exclusive coordination of the metal with cysteine sulfur ligands (stability constant of Zn_7MT-2 = 3.2×10^{13} M^{-1} at pH 7.4). Again, the Zn-binding constants of most of the enzymes studied are at least 1000 times lower than that of MT [160]. Commonly, Zn^{2+} forms tetrahedral complex involving His, Glu or Asp, and Cys, in metalloproteins. The side chains of residues are capable of binding one or two Zn^{2+} [161, 162]. Notably, up to 20% of intracellular Zn are complexed by MTs [163, 164]. In mammalian MT, Zn^{2+} are bound tetrahedrally to Cys in both domains. Zn-S cluster with in MT is very sensitive to changes of cellular redox state. Therefore, a shift to more oxidizing environment releases Zn from MT, whereas a shift to more reducing environment leads Zn binding to apo-MT [165, 166]. Thus, Zn^{2+}, only rapidly released by MTs, is able to play its relevant function against oxidative stress and participate in immune responses.

In healthy human serum, MT-1 plus MT-2 (MT-1/2) concentration (n = 200) could be as low as 10 ng/mL and as high as >90 ng/mL [167]. Earlier, it was reported that the MT-1/2 concentration in human serum could be in the range of 10–30 ng/mL [168] with an average of 23 ± 4.6 ng/mL [169]. However, an increased level of MT-1/2 was detected in various liver diseases such as chronic hepatitis [167].

Changes of Host-MT Expression in Response to Infectious Diseases

Bacterial Infection and MT Expression

A number of evidence has shown the link between the MT expression in different human organs in relation to bacterial infectious diseases. Given the fact that there is instant increase of hepatic MT expression in response to bacterial infection, an effect that is generally mediated by endotoxin (lipopolysaccharide (LPS)), leads to classify MTs as acute phase proteins [170, 171]. Bacterial lipopolysaccharide-induced MT overexpression in liver is often mediated by pro-inflammatory cytokines, including IL-1, IL-6, TNF-α, interferon (IFN)-γ [172], nitric oxide [173], and the stress hormone glucocorticoids [174].

MT expression in inflammatory bowel diseases (IBD) is somewhat inconclusive. In organ biopsies of the IBD patients, MT expression was generally lower, such as in ulcerative colitis and Crohn's disease, compared to the control specimens [175–178]. However, MT overexpression was observed in fibroblasts and intestinal epithelial cells of ulcerative and fissural lesions in ulcerative colitis and Crohn's disease [179]. Since MT expression depends on the time and degree of inflammation as well as on the tissue of origin, hence the inconsistencies could be explained by different sampling [170].

Viral Infection and MT Expression

O'Connor et al. (2014) reported a significant upregulation of MT genes when compared to the IFN-stimulated genes in hepatitis-C virus-infected liver biopsies of IFNL-3 rs8099917 responders [180]. Fibrosis scores were also inversely correlated with MT levels in the liver biopsies. The higher MT expression in the responders was seen as reason for the improved HCV clearance, hence was linked with clinical relevance. In a murine experimental model of coxsackievirus infection, MT expression was increased by fivefold ($P < 0.01$) in liver and kidneys, and in spleen by 34% ($P < 0.05$) [181].

Role of Host MT in Directing Zn to Activate Immune Response Against the Infection

Proliferation of lymphocytes in the presence of concanavalin A or lipopolysaccharides [182–184], and proliferation of cytotoxic T lymphocytes (CTLs) in mixed lymphocyte reactions, can be augmented by MT [185]. The exo-MT was suggested to facilitate the proliferation of immature T cells, but suppress their terminal differentiation [185].

Macrophages treated with the in vitro exo-MT produce superoxide through respiratory burst to destroy antigen [183]. In PBL, ROS is produced during respiratory burst as a self-defense mechanism [186–188]. In PBL, pre-synthesized

MTs from their precursors or freshly synthesized MT induced by the dietary Zn [95, 189] provide protection against apoptosis, necrosis, or DNA breakdown caused by ROS. Zn supplementation may also help to prevent oxidative damage of DNA due to arsenic exposure by induction of MT expression [190, 191]. Furthermore, transportation of MT to the cell membrane is necessary for their immunoregulatory properties, where Zn is involved in transporting MT to the cell membrane and regulating T cell [192].

Summary: Host Winning Factor in Zn Regulation Using MT

Fighting for the Zn in nutritional immunity using MT offers a number of advantages for the human host. For example, (i) the number of Zn atoms bound per MT is higher in human MT (seven Zn) compared to that of bacterial MT (four Zn); (ii) exchange of Zn between free Zn^{2+} and MT-bound Zn is thermodynamically favorable for human MT, as at least one MT-bound Zn in bacterial MT is unlikely to be released; (iii) at the time of infection, human MT synthesis can be upregulated by a number of infection related responses such as ROS, and GC; (iv) bioavailable Zn^{2+} in bacterial cells remains in picomolar to nanomolar range, while in immune cells such as lymphocytes and macrophages, that amount may range in micromolar level; and (v) in response to infection resulting in the redistribution of Zn, upregulated MT biosynthesis is not limited to the site of infection but can be observed by number of organs such as kidney and liver. Thus, it is expected that at the time of active infection, upregulated biosynthesis of human MT might play a major role in nutritional immunity.

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