Zinc Sequestration: Arming Phagocyte Defense against Fungal Attack

Kavitha Subramanian Vignesh1,2, Julio A. Landero Figueroa3, Aleksey Porollo4, Joseph A. Caruso3, George S. Deepe, Jr.2,5*

1 Department of Molecular Genetics, Biochemistry, Microbiology and Immunology, University of Cincinnati, Cincinnati, Ohio, United States of America, 2 Division of Infectious Diseases, College of Medicine, University of Cincinnati, Cincinnati, Ohio, United States of America, 3 University of Cincinnati/Agilent Technologies Metallomics Center of the Americas, Department of Chemistry, University of Cincinnati, Cincinnati, Ohio, United States of America, 4 Divisions of Rheumatology and Biomedical Informatics, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, United States of America, 5 Veterans Affairs Hospital, Cincinnati, Ohio, United States of America

Introduction

The innate immune system employs various defense mechanisms to combat invading microbes. From a pathogen perspective, access to adequate nutrition is one of the fundamental requirements for survival within the host. The ability to counter microbial survival by restricting basic elements of growth, extending from amino acids to sugars and metals, is referred to as nutritional immunity [1]. The mechanisms of Zn acquisition, transport, and storage have been investigated in both prokaryotic and eukaryotic systems. In this review, the total amount of zinc regardless of its chemical form will be referred to as Zn, and the labile fraction as Zn2+. From an immunological perspective, the primary focus has been on the impact of Zn regulation on the numbers and function of lymphocytes and phagocytes and their correlation with susceptibility to infections, but a dissection of the molecular details in these processes has been lacking. More recently, understanding the Zn modulatory mechanisms and how they drive host-pathogen interactions at the molecular level has been a subject of intense scrutiny. This review will accentuate existing and novel insights into the roles of Zn in nutritional immunity and in phagocyte defenses against fungi.

Zinc Takes Center Stage: A Common Requisite in Host-Pathogen Interactions

Regulation of Zn homeostasis is essential for several host functions at multiple levels: i) for cellular processes including, but not limited to, transcription, translation, catalysis, and cell division; ii) for counteracting Zn2+ deficiency or excess; and iii) for immunomodulatory responses in host-pathogen interactions. An estimated 10% or 2,800 proteins in the human genome are Zn-dependent, implying a critical role for this metal in biological functions [2]. In the immune system, Zn regulation is of paramount importance as the development and function of innate and adaptive arms of immunity are influenced by this metal [3]. Zn homeostasis established by a balance in Zn2+ flux, intracellular distribution, and storage impacts phagocytosis, leukocyte recruitment, cytokine production, glycolysis, and oxidation triggered in response to immune signals. Aberrant Zn regulation in the circulation or in cells mitigates robust immune activation and leads to suboptimal host defenses. For example, Zn deficiency in humans with the genetic disorder acrodermatitis enteropathica is caused by Zn malabsorption and characterized by increased susceptibility to infections. An excess of Zn2+ diminishes T cell mitogenic responses [4]. Thus, an intact immune response requires strict Zn2+ regulation.

The fundamental requirement of Zn2+ for the function of several enzymes, transcription factors, and structural proteins [3] is evident not only in mammals but also in bacteria and fungi [6], in principal, due to the redox-inert property of this metal [7]. Zn2+ enhances the synthesis of toxic secondary metabolites such as Aspergillus flavus mycotoxins that inhibit phagocytosis and cytotoxicity of T cells [8–10]. Zn2+-dependent superoxide dismutases (SODs) produced by Cryptococcus neoformans, Histoplasma capsulatum, and Candida albicans are critical for scavenging superoxide radicals produced by phagocytes [11–13]. These factors underscore the significance of Zn acquisition and distribution for fungal pathogenesis and survival within the host. Thus, the struggle for Zn2+ between host and pathogen impacts survival of the invader and defense by the immune system.

Zinc Acquisition Strategies: Host versus Fungi

The immune system maintains Zn equilibrium via transporters, storage, and binding mechanisms (Figure 1). While lower eukaryotes such as fungi possess fewer Zn2+ transporters [14], mammals have 24 transporters, called ZIPs (Slc39a, importers) and ZNTs (Slc30a, exporters). Some transporters manifest a ubiquitous expression pattern in several host cells, and others exhibit tissue specificity and function irreplaceably in Zn2+ transport. For example, Slc30a1 is widely expressed in >12 organs, while Slc39a4 expression is restricted to the small intestine and kidney and is absolutely essential for dietary Zn absorption [15]. Spatial organization of the transporters regulates Zn2+ in the cytosol and intracellular compartments including Golgi, mitochondria, and zincosomes that are a source of exchangeable metal during deficiency [16]. The remarkable complexity in Zn2+ transporters reflects the need for strict homeostasis and a regulatory system that responds to different biological stimuli in an organelle-, cell-, and tissue-specific manner. For example, interleukin-6 induces Zn2+ import via ZIP14 in hepatocytes [17].

Citation: Vignesh KS, Figueroa JAL, Porollo A, Caruso JA, Deepe GS, Jr (2013) Zinc Sequestration: Arming Phagocyte Defense against Fungal Attack. PLoS Pathog 9(12): e1003815. doi:10.1371/journal.ppat.1003815

Editor: Joseph Heitman, Duke University Medical Center, United States of America

Published December 26, 2013

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This work was supported by grants from the NIH AI-094971, AI-106269 and a Merit Review Award from the Veterans Affairs. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: george.deepe@uc.edu

PLOS Pathogens | www.plospathogens.org

December 2013 | Volume 9 | Issue 12 | e1003815
while granulocyte macrophage-colony stimulating factor (GM-CSF) triggers Zn$^{2+}$ uptake via ZIP2 in macrophages [18]. The dependence of mammals on dietary sources for the metal implies the need for mechanisms that efficiently acquire Zn$^{2+}$ and maintain regulated distribution in organ systems. Metallothioneins (MTs) comprise a class of metal binding proteins that regulate Zn$^{2+}$ and prevent intoxication. MTs bind Zn$^{2+}$ with picomolar affinity through seven binding sites, one of which is more readily exchangeable, and interactions with glutathione, ATP, or GTP mediate Zn$^{2+}$ release [19]. These properties facilitate a controlled exchange mechanism in infected phagocytes, where Zn$^{2+}$ access to the microorganism needs to be restricted. Thus, phagocytes possess manifold mechanisms to manipulate Zn resources during infection.

To establish infection, fungi must adapt to limited nutrient availability upon encounter with the host. Upon phagocytosis, *C. albicans* triggers a transcriptional response signature reflecting a state of nutrient deprivation within macrophages [20]. For
pathogenic fungi, gaining entry into the host is associated with a transition from a possibly Zn\(^{2+}\)-sufficient external environment to a lower Zn\(^{2+}\)-containing milieu. Similarly, for opportunistic fungi such as *C. albicans*, the shift from a commensal to a pathogenic state may be accompanied by a dramatic paucity in Zn\(^{2+}\), primarily due to Zn\(^{2+}\) restriction in the extracellular environment [22]. *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Blastomyces dermatitidis* thrive in soil containing 30–350 \(\mu\text{M}\) bioavailable Zn\(^{2+}\) [23]. Within macrophages, they are confronted with an environment containing only picomolar quantities of freely exchangeable Zn\(^{2+}\) [7]. To thwart host defenses and establish infection, fungi must exert strategies to sense and respond to metal scarcity caused by sequestration into intracellular niches or binding to host proteins via high affinity interactions. Mechanisms responding to Zn\(^{2+}\) availability in fungi include proteins that are directly affected by the presence or absence of Zn\(^{2+}\) availability in fungi include proteins that are directly affected by the presence or absence of Zn\(^{2+}\).
Zn\(^{2+}\). For instance, Zn\(^{2+}\) inhibits DNA-binding activity of the Zn-responsive activator protein, Zap1p; however, a limiting milieu leads to transcription of Zap1p-dependent Zn\(^{2+}\) acquisition machinery. The upregulation of ZRT1 and ZRT2 transporters by Zap1p under a Zn\(^{2+}\)-deficient state is critical, as the absence of these importers diminishes fungal pathogenesis [14,24]. In Cryptococcus gattii, an ortholog of Zap1 is upregulated by Zn\(^{2+}\) deficiency. Genetic deletion of ZAP1 impairs growth in a Zn\(^{2+}\)-limiting environment, and mice infected with ZAP1 mutant yeasts exhibit increased survival [25]. These findings emphasize a role for Zn\(^{2+}\) regulation in fungal virulence.

Although extracellular fungi do not directly compete for the pool of Zn\(^{2+}\) within cells, they must secure the metal from a restricted environment in infected tissue. A. fumigatus possesses Zrt1A and Zrt1B analogous to Zrt1p and Zrt2p that facilitate Zn\(^{2+}\) uptake in a low-pH environment during deficiency [26]. In a specialized mechanism described as the “zincophore system,” C. albicans hyphae sequester host Zn\(^{2+}\) by secretion of pH-regulated antigen-1, which reassociates with Zrt1p for subsequent import [24]. Thus, multiple Zn\(^{2+}\) acquisition strategies in fungi collectively diminish vulnerability to host immunity. To establish virulence in vivo, these factors must contribute persistently to cope with metal scarcity induced by the immune system.

**Host Zinc Pool: Restricted Access**

Microbes are extremely sensitive to metal availability, and phagocytes have mastered mechanisms to curtail pathogen access to Zn\(^{2+}\). Despite our knowledge of Zn homeostasis, the manner in which the innate system modulates Zn\(^{2+}\) regulatory proteins in the context of fungal interactions and its influence on survival has been sparingly investigated.

In macrophages, a dual stimulus involving GM-CSF and H. capsulatum infection potently induces Zn\(^{2+}\) influx by ZIP2. The enhanced Zn\(^{2+}\) uptake may reflect at least two possibilities: i) a stress response during infection to support macrophage functions such as increased transcription, and ii) a mechanism to deprive extracellular yeasts of Zn\(^{2+}\) analogous to the induction of hypoxia in bacterial sepsis. This phenomenon can be viewed as an opportunity for H. capsulatum to exploit Zn\(^{2+}\) elevation to capture more labile Zn\(^{2+}\) within the host. However, despite increased influx, GM-CSF creates a state of “deprived” intracellular Zn\(^{2+}\) by two mechanisms. First, GM-CSF causes Zn\(^{2+}\) localization in the Golgi, a shift associated with expression of exporters ZNT4 and ZNT7 that export cytosolic Zn\(^{2+}\) into the Golgi. Second, signaling via signal transducer and activator of transcription STAT3 and STAT5 triggers the production of MTs that constrict the labile Zn\(^{2+}\) pool by binding the metal [18]. These studies highlight a fundamental Zn\(^{2+}\) sequestration property of transporters and MTs, which starve the pathogen and orches-

**Zinc Regulation: An Impact beyond Nutritional Immunity**

Regulation of Zn\(^{2+}\) shapes the functional attributes of innate defense, impacting phagocyte function beyond nutritional immunity. GM-CSF-activated macrophages counter pathogen attack by eliciting a dual defense strategy comprising Zn\(^{2+}\) restriction to H. capsulatum, while concurrently enhancing phagocyte effector function. Zn\(^{2+}\) abates superoxide production by NADPH oxidase (Nox) by inhibiting hydrogen voltage-gated channel HV1. Fungi scavenge superoxide radicals via Zn and Cu or Mn dependent SODs [11,12]. In activated macrophages, MTs bind Zn\(^{2+}\) and create an environment deficient in Zn\(^{2+}\) ions, in effect, sustaining HV1 and Nox function (Figure 2). In this milieu, H. capsulatum is susceptible to ROS [18], presumably due to an infec
tual Zn and Cu dependent SOD response. The extent of Zn\(^{2+}\) deprivation by MTs results in effective superoxide production, and may simultaneously compromise fungal SOD-mediated defenses.

Collectively, Zn\(^{2+}\) restriction drives antifungal defense through a concurrent twofold effect: first, it induces Zn\(^{2+}\) starvation in the pathogen, and second, it strengthens oxidative burst-mediated defenses of the innate system. Thus, innate immunity is equipped with a variety of Zn\(^{2+}\) restriction strategies that function cooperatively to eliminate pathogens. As novel roles for metals are being described in dictating the outcome of immune regulation, we may be only beginning to appreciate the prominence of trace metals in immune defenses against infection.
10. Yoshida LS, Abe S, Tsunawaki S (2000) Fungal gliotoxin targets the onset of superoxide-generating NADPH oxidase of human neutrophils. Biochem Biophys Res Commun 268: 716–723.
11. Youseff BH, Holbrook ED, Smolnicky KA, Rappleye CA (2012) Extracellular superoxide dismutase protects \textit{H serpentica} yeast cells from host-derived oxidative stress. PLoS Pathog 8: e1002713. doi:10.1371/journal.ppat.1002713.
12. Hwang CS, Rhie GE, Oh JH, Huh WK, Yim HS, et al. (2002) Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of \textit{Candida albicans} against oxidative stresses and the expression of its full virulence. Microbiology 148: 3705–3713.
13. Cox GM, Harrison TS, McDade HC, Taborda CP, Heinrich G, et al. (2003) Superoxide dismutase influences the virulence of \textit{Candida albicans} by affecting growth within macrophages. Infect Immun 71: 173–180.
14. Wilson D, Citiulo F, Hube B (2012) Zinc exploitation by pathogenic fungi. PLoS Pathog 8: e1003034. doi:10.1371/journal.ppat.1003034.
15. Liuzzi JP, Cousins RJ (2004) Mammalian zinc transporters. Annu Rev Nutr 24: 131–172.
16. Eide DJ (2006) Zinc transporters and the cellular trafficking of zinc. Biochim Biophys Acta 1763: 711–722.
17. Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, et al. (2005) Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozinemia of the acute-phase response. Proc Natl Acad Sci U S A 102: 6843–6848.
18. Subramanian Vignesh K, Landero Figueroa JA, Porollo A, Caruso JA, Deepe GS Jr (2009) Zinc transporters and zinc influence IFN-gamma expression in activated human T cells. J Leukoc Biol 86: 337–348.
19. Maret W (2000) The function of zinc metallothionein: a link between cellular zinc and redox state. J Nutr 130: 1455S–1458S.
20. Lorenz MC, Benda JA, Fink GR (2004) The iron acquisition system of \textit{Candida albicans} mediates the toxicity of human macrophages. Eukaryot Cell 3: 1076–1087.
21. Rajapaksha RM, Tobose-Kaplon MA, Baath E (2004) Metal toxicity affects fungal and bacterial activities in soil differently. Appl Environ Microbiol 70: 2966–2973.
22. Corbin BD, Sceley EH, Raab A, Feldmann J, Miller MR, et al. (2008) Metal chelation and inhibition of bacterial growth in tissue abscesses. Science 319: 962–965.
23. Schulte EE (2004) Soil and applied zinc. In: Understanding plant nutrients. Wisconsin Cooperative Extension Publications. Available: http://corn.agronomy.wisc.edu/Management/pdfs/a250a.pdf. Accessed 30 November 2013.
24. Citiulo F, Jacobsen DI, Miramon P, Schild L, Brunke S, et al. (2012) \textit{Candida albicans} scavenges host zinc via Pral during endothelial invasion. PLoS Pathog 8: e1002777. doi:10.1371/journal.ppat.1002777.
25. Schneider RD, Foçaforma GdSS, Kmetzsch L, Schrank A, Vainstein MH, et al. (2012) Zap1 regulates zinc homeostasis and modulates virulence in \textit{Cryptococcus gattii}. PLoS ONE 7: e43773. doi:10.1371/journal.pone.0043773.
26. Vicente-Ilancontrera R, Moreno MA, Leal F, Caderiz JA (2005) The zrfA and zrfB genes of \textit{Aspergillus fumigatus} encode the zinc transporter proteins of a zinc uptake system induced in an acid, zinc-depleted environment. Eukaryot Cell 4: 837–848.
27. Murgia G, Vespignani I, Cerese J, Nobili F, Persozzi G (1999) Cloning, expression, and vesicular localization of zinc transporter ZnT4 in intestinal tissue and cells. Am J Physiol 277: G1251–G1239.
28. Aydemir TB, Liuzzi JP, McClellan S, Cousins RJ (2009) Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-gamma expression in activated human T cells. J Leukoc Biol 86: 337–348.
29. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, et al. (2009) Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against \textit{Candida albicans}. PLoS Pathog 5: e1000639. doi:10.1371/journal.ppat.1000639.
30. Ramirez-Ortiz ZG, Lee CK, Wang JP, Boon L, Specht CA, et al. (2011) A nonredundant role for plasmacytoid dendritic cells in host defense against the human fungal pathogen \textit{Aspergillus fumigatus}. Cell Host Microbe 9: 415–424.
31. Botella H, Peyron P, Levillain F, Poincloux R, Poquet Y, et al. (2011) Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. Cell Host Microbe 10: 248–259.