Nutrition Review

Stephen R. Hennigar, PhD, and James P. McClung, PhD

Nutritional Immunity: Starving Pathogens of Trace Minerals

Abstract: *Nutritional immunity is a process by which a host organism sequesters trace minerals in an effort to limit pathogenicity during infection. Circulating concentrations of minerals, such as iron and zinc, decline rapidly and dramatically with the inflammation associated with infection. The decline in iron and zinc is thought to starve invading pathogens of these essential elements, limiting disease progression and severity. The mechanisms contributing to the hypoferremia and hypozincemia of inflammation and potential interventions that exploit this process for the management of infection will be discussed.*

Keywords: zinc; iron; hepcidin; inflammation; infection

Trace minerals including iron and zinc are essential for the survival of all living organisms. In fact, approximately half of all proteins and enzymes require trace minerals to confer function. These minerals are incorporated into metalloproteins and metalloenzymes used for critical biological processes, such as oxygen transport, cell growth and differentiation, and protection against oxidative stress. Pathogens (eg, gram-negative and gram-positive bacteria, viruses, and fungi) must acquire trace minerals in order to replicate and cause disease. As both host and pathogen require trace minerals for optimal health and survival, effective mechanisms to compete for and acquire these elements during infection become paramount.

Nutritional Immunity

Vertebrate immune systems have evolved sophisticated defense mechanisms to protect against invading pathogens. A primary line of host defense is to sequester and starve invading pathogens of trace minerals.

Vertebrate immune systems have evolved sophisticated defense mechanisms to protect against invading pathogens. A primary line of host defense is to sequester and starve invading pathogens of trace minerals.

The relationship between trace minerals and immunity was first elucidated in the 1940s when Schade and Caroline (1944 and 1946) discovered transferrin, an iron-binding protein in egg whites and human plasma.[1,2] They found that transferrin bound iron, thereby sequestering iron from pathogens and preventing microbial growth. In 1975, Weinberg termed this process *nutritional immunity*.[3] Although nutritional immunity originally referred to host-mediated sequestering of iron, it is now known that other trace minerals, including zinc and manganese, are also sequestered to protect against invading pathogens.[4] In this process, intestinal assimilation of minerals is reduced, and

DOI: 10.1177/1559827616629117. From the US Army Research Institute of Environmental Medicine, Military Nutrition Division, Natick, Massachusetts. Address correspondence to James P. McClung, PhD, US Army Research Institute of Environmental Medicine, Military Nutrition Division, 10 General Greene Ave, Natick, MA 01760; e-mail: james.p.mcclung8.civ@mail.mil.

free minerals are sequestered in storage tissues, primarily the liver, thereby rapidly limiting the circulating minerals available for uptake and use by pathogens. The rapid decline in circulating mineral concentrations is underscored by observations that infusion of humans with the endotoxin lipopolysaccharide (LPS), the outer membrane component responsible for the deleterious effects of gram-negative bacterial infections, reduces circulating levels of iron and zinc by >50% within hours.[5,6] Consequently, pathogens have evolved mechanisms to circumvent nutritional immunity. The objective of this article is to review mechanisms by which hosts and pathogens withhold and acquire 2 trace minerals, iron and zinc.

Host Mechanisms for Starving Pathogens of Iron

Hepcidin, a 25-amino acid peptide hormone, is secreted by the liver in response to inflammatory stimuli. Interestingly, hepcidin was first identified for its antimicrobial properties (hep-, liver; cidin, antibacterial).[7,8] In the initial studies, Ganz and colleagues[8] noted a >100-fold increase in urinary hepcidin concentrations in a patient who developed a systemic infection. Substantial research now indicates that hepcidin exerts these effects by regulating iron homeostasis (reviewed in Ganz[9]). Infection and the resulting inflammatory stimuli, particularly interleukin-6 (IL-6), stimulate hepcidin transcription in the liver through Jak2/STAT3 signaling. In turn, hepcidin signals for the internalization and degradation of the iron exporter, ferroportin. Hepcidin-mediated declines in ferroportin inhibit iron transfer into circulation from the enterocytes, macrophages, and iron-storing hepatocytes, thereby withholding iron from invading pathogens.

Existing iron in circulation is predominantly complexed with heme, which is bound by hemoglobin within red blood cells, and myoglobin in muscle. Transferrin binds and delivers iron to cells for receptor-mediated uptake and use, or storage in ferritin, a large intracellular protein capable of storing ~4500 iron atoms. During infection, neutrophils secrete lactoferrin, a structurally related iron-binding glycoprotein, in order to scavenge extracellular iron. These proteins limit the availability of free iron, thus affecting the ability of invading pathogens to replicate and cause disease. Hosts also use the regulation of transporters, such as natural resistance-associated macrophage protein 1 (NRAMP1), to withhold iron from pathogens (reviewed in Wessling-Resnick[10]). NRAMP1 is localized to lysosomes and phagosomes in monocytes, macrophages, and T lymphocytes where it functions to export iron (and manganese) into the cytosol, thereby limiting the availability of iron to pathogens that enter the cell and survive within phagosomes. This is apparent in NRAMP1 knockout mice, which have a reduced inflammatory response to infection and inhibited iron recycling.[11,12]

Host Mechanisms for Starving Pathogens of Zinc

A number of transporters and binding proteins have been implicated in the sequestration of zinc from pathogens. Animal studies have assessed the liver transcript abundance of all 24 mammalian zinc transporters and the zinc-binding protein metallothionein in response to LPS or turpentine (a well-characterized inflammatory stimulus) injection.[13,14] Metallothionein and the zinc transporter Zrt-Irt-like Protein 14 (ZIP14; *SLC39A14*) demonstrated the greatest magnitude of change, increasing ~35- and ~3.5-fold in LPS- and turpentine-injected mice, respectively. Similar to hepcidin, the increase in ZIP14 is mediated by IL-6.[13] This suggests that ZIP14 sequesters circulating zinc in the liver during infection/inflammation and metallothionein serves to bind the resulting intracellular zinc. It should be noted that ZIP14 is also capable of transporting transferrin- and non-transferrin-bound iron; however, ZIP14 knockout mice injected with LPS have normal circulating zinc but remain hypoferremic,[14] suggesting that zinc is the preferred substrate for ZIP14 during infection/inflammation.

The zinc-binding protein calprotectin confers another mechanism by which hosts limit the availability of zinc to pathogens.[15] Calprotectin is the most abundant antimicrobial protein in neutrophils, making up ~40% of the cytoplasmic neutrophil content, and is secreted at the site of inflammation. In fact, calprotectin is used clinically to monitor the severity of intestinal inflammation in patients with inflammatory bowel diseases. Calprotectin is upregulated in zinc-limiting conditions[16] and functions by binding 2 zinc (or manganese) atoms, thereby chelating and starving microbes of these essential minerals.[4,15] Importantly, calprotectin mutants that are unable to bind zinc or manganese are ineffective in reducing bacterial growth.[17]

Pathogenic Mechanisms for Acquiring Iron and Zinc

Recent research evidence indicates that pathogens have developed multiple mechanisms to overcome iron and zinc sequestration in response to infection and inflammation. For example, siderophores, low-molecular-weight (<1 kDa) iron-chelators, are secreted by pathogens and bind and chelate iron with a higher affinity than transferrin.[18] Siderophore-iron complexes are then scavenged by pathogens via cell surface receptors and the bound iron is released through ferric reductases, reducing ferric (Fe^{3+}) to the soluble ferrous (Fe^{2+}) form for use as a nutrient source. In fact, pathogenic strains that produce more siderophores are hypervirulent, whereas strains that synthesize defective siderophores are less virulent.[18] To combat siderophores, hosts produce siderocalin, also known as lipocalin 2. Siderocalin binds and sequesters siderophores, preventing their uptake by pathogens. Pathogens also employ iron uptake systems and receptors that directly bind iron-binding proteins such as transferrin, lactoferrin, and hemoglobin. For example, pathogens can lyse red blood cells, liberating hemoglobin and removing heme for uptake via receptors. Perhaps most interesting is *Borrelia burgdorferi*, the

tick-borne parasite responsible for Lyme disease. *Borrelia burgdorferi* is the only known organism to circumvent any requirement for iron and has evolved to use manganese instead.[19]

Although zinc was identified as an essential nutrient for the growth of *Aspergillus niger* in 1869 (~1 century prior to its essentiality for humans),[20] the mechanisms employed by pathogens to acquire zinc are not well described. Delivery of zinc for uptake by pathogens occurs via zinc-binding siderophores, known as "zincophores."[21] For example, *Yersinia pestis*, the gram-negative bacteria that causes bubonic plague, utilizes Ybt as a zincophore and ZnuABC to acquire zinc and cause lethal infection in a septicemic plague mouse model.[22] Zinc uptake occurs via high-affinity zinc transporters, including ZnuABC in bacteria and ZRT1 in fungi. Interestingly, *Salmonella typhimurium* has evolved to overcome calprotectin-mediated zinc chelation and thrive in the inflamed gut by expressing ZnuABC,[23] thereby offering a competitive advantage over rival commensal bacteria.

Status and Susceptibility to Infection

In practice, nutritional immunity provides a mechanism by which low circulating levels of iron and zinc may be protective, whereas higher levels may be associated with increased rates of infection and faster disease progression/death. For example, individuals with iron overload due to hemochromatosis or who require frequent transfusions due to thalassemia or other chronic anemias are highly susceptible to infection.[24-26] Conversely, iron supplementation may increase virulence and susceptibility to infection in iron deficient and iron-normal individuals (reviewed in Weinberg[26]). It should be emphasized that these mechanisms are especially important during bouts of acute inflammation (minutes to days) and for those who reside in areas with endemic infectious diseases, such as malaria and tuberculosis. These geographic regions also have a higher rate of iron deficiency. Thus, the decision to supplement to correct poor iron status and associated effects on human health, to include neuropsychological and physical performance and pregnancy outcomes, must be considered in light of the possible impairment in the ability of their immune systems to fight infection.

Potential consequences of the repeated sequestration of trace minerals includes the reduction in mineral status observed with repeated bouts of acute inflammation, such as unaccustomed exercise, or the inflammation associated with chronic disease (days to years), such as obesity. Intense or unaccustomed activity is associated with declines in iron status (reviewed in McClung and Murray-Kolb[27]) and increased inflammation and serum hepcidin concentrations.[28] Moreover, several studies dating back >50 years have demonstrated the association between obesity and hypoferremia and hypozincemia in human and rodent models.[29-33] Similar to exercise, increased adiposity is associated with an elevation in pro-inflammatory stimuli, including IL-6 and hepcidin (reviewed in Tussing-Humphreys et al[34]). Interestingly, obese individuals are often more susceptible to infection despite reduced trace mineral status, perhaps due to the increased requirement of immune cells for iron and zinc.

Interventions and Perspectives

The Red Queen Hypothesis of coevolution states that constant adaptation is required to maintain comparative evolutionary fitness.[35] Nutritional immunity is one example of this hypothesis, as the host and pathogen must constantly evolve mechanisms to obtain trace minerals in order to gain a competitive advantage. A better understanding of the mechanisms employed by pathogens and hosts in the battle for trace minerals will contribute to the design of novel therapeutics that exploit the pathogen's requirement for nutrients in order to combat infection. For example, siderophore-mediated drug delivery uses antibiotics covalently linked to siderophores, collectively referred to as sideromycins. In this "Trojan horse" approach, the siderophore component of the siderophore-antibiotic conjugate is recognized and both are taken up by the pathogen. Moreover, recent work has demonstrated that *Escherichia coli* Nissle 1917, a probiotic strain isolated from a soldier during World War I who appeared resistant to an outbreak of diarrhea, overcomes gastroenteritis induced by *Salmonella typhimurium* by competing for iron.[36] *Escherichia coli* Nissle 1917 is used to treat or prevent a variety of intestinal disorders, such as ulcerative colitis and Crohn's disease, suggesting that this mechanism can be used in the development of future probiotics for the treatment of various conditions. Studies investigating the competition between host and pathogen for iron, zinc, and other nutrients involved in nutritional immunity may identify additional therapeutic targets to aid in host defense.

Acknowledgments

This article was supported in part by the US Army Military Research and Material Command and appointment to the US Army Research Institute of Environmental Medicine administered by the Oak Ridge Institute for Science and Education (to SRH) through an interagency agreement between the US Department of Energy and the US Army Medical Research and Material Command.

Authors' Note

Approved for public release; distribution is unlimited. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations or commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations. AJLM

References

1. Schade AL, Caroline L. Raw hen egg white and the role of iron in growth inhibition of *Shigella dysenteriae, Staphylococcus aureus, Escherichia coli* and *Saccharomyces cerevisiae*. *Science*. 1944;100:14-15.
2. Schade AL, Caroline L. An iron-binding component in human blood plasma. *Science*. 1946;104:340.
3. Weinberg ED. Nutritional immunity. Host's attempt to withhold iron from microbial invaders. *JAMA*. 1975;231:39-41.

4. Kehl-Fie TE, Skaar EP. Nutritional immunity beyond iron: a role for manganese and zinc. *Curr Opin Chem Biol.* 2010;14:218-224.
5. Kemna E, Pickkers P, Nemeth E, van der Hoeven H, Swinkels D. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. *Blood.* 2005;106:1864-1866.
6. Gaetke LM, McClain CJ, Talwalkar RT, Shedlofsky SI. Effects of endotoxin on zinc metabolism in human volunteers. *Am J Physiol.* 1997;272:E952-E956.
7. Krause A, Neitz S, Magert HJ, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett.* 2000;480:147-150.
8. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem.* 2001;276:7806-7810.
9. Ganz T. Hepcidin and iron regulation, 10 years later. *Blood.* 2011;117:4425-4433.
10. Wessling-Resnick M. Nramp1 and other transporters involved in metal withholding during infection. *J Biol Chem.* 2015;290:18984-18990.
11. Valdez Y, Grassl GA, Guttman JA, et al. Nramp1 drives an accelerated inflammatory response during Salmonella-induced colitis in mice. *Cell Microbiol.* 2009;11:351-362.
12. Soe-Lin S, Apte SS, Andriopoulos B Jr , et al. Nramp1 promotes efficient macrophage recycling of iron following erythrophagocytosis in vivo. *Proc Natl Acad Sci U S A.* 2009;106:5960-5965.
13. Liuzzi JP, Lichten LA, Rivera S, et al. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. *Proc Natl Acad Sci U S A.* 2005;102:6843-6848.
14. Beker Aydemir T, Chang SM, Guthrie GJ, et al. Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia). *PLoS One.* 2012;7:e48679.
15. Corbin BD, Seeley EH, Raab A, et al. Metal chelation and inhibition of bacterial growth in tissue abscesses. *Science.* 2008; 319:962-965.
16. Mazzatti DJ, Uciechowski P, Hebel S, et al. Effects of long-term zinc supplementation and deprivation on gene expression in human THP-1 mononuclear cells. *J Trace Elem Med Biol.* 2008;22:325-336.
17. Kehl-Fie TE, Chitayat S, Hood MI, et al. Nutrient metal sequestration by calprotectin inhibits bacterial superoxide defense, enhancing neutrophil killing of Staphylococcus aureus. *Cell Host Microbe.* 2011;10:158-164.
18. Holden VI, Bachman MA. Diverging roles of bacterial siderophores during infection. *Metallomics.* 2015;7:986-995.
19. Posey JE, Gherardini FC. Lack of a role for iron in the Lyme disease pathogen. *Science.* 2000;288:1651-1653.
20. Prasad AS. Discovery of human zinc deficiency: its impact on human health and disease. *Adv Nutr.* 2013;4:176-190.
21. Hood MI, Skaar EP. Nutritional immunity: transition metals at the pathogen-host interface. *Nat Rev Microbiol.* 2012;10:525-537.
22. Bobrov AG, Kirillina O, Fetherston JD, Miller MC, Burlison JA, Perry RD. The Yersinia pestis siderophore, yersiniabactin, and the ZnuABC system both contribute to zinc acquisition and the development of lethal septicaemic plague in mice. *Mol Microbiol.* 2014;93:759-775.
23. Liu JZ, Jellbauer S, Poe AJ, et al. Zinc sequestration by the neutrophil protein calprotectin enhances *Salmonella* growth in the inflamed gut. *Cell Host Microbe.* 2012;11:227-239.
24. Khan FA, Fisher MA, Khakoo RA. Association of hemochromatosis with infectious diseases: expanding spectrum. *Int J Infect Dis.* 2007;11:482-487.
25. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. *J Nutr.* 2001;131:616S-633S.
26. Weinberg ED. Iron availability and infection. *Biochim Biophys Acta.* 2009;1790:600-605.
27. McClung JP, Murray-Kolb LE. Iron nutrition and premenopausal women: effects of poor iron status on physical and neuropsychological performance. *Annu Rev Nutr.* 2013;33:271-288.
28. McClung JP, Martini S, Murphy NE, et al. Effects of a 7-day military training exercise on inflammatory biomarkers, serum hepcidin, and iron status. *Nutr J.* 2013;12:141.
29. Wenzel BJ, Stults HB, Mayer J. Hypoferraemia in obese adolescents. *Lancet.* 1962;2:327-328.
30. Seltzer CC, Mayer J. Serum iron and iron-binding capacity in adolescents. II. Comparison of obese and nonobese subjects. *Am J Clin Nutr.*1963;13:354-361.
31. Atkinson RL, Dahms WT, Bray GA, Jacob R, Sandstead HH. Plasma zinc and copper in obesity and after intestinal bypass. *Ann Intern Med.* 1978;89:491-493.
32. Chandra RK, Kutty KM. Immunocompetence in obesity. *Acta Paediatr Scand.* 1980;69:25-30.
33. Levine AS, McClain CJ, Handwerger BS, Brown DM, Morley JE. Tissue zinc status of genetically diabetic and streptozotocin-induced diabetic mice. *Am J Clin Nutr.* 1983;37:382-386.
34. Tussing-Humphreys L, Pusatcioglu C, Nemeth E, Braunschweig C. Rethinking iron regulation and assessment in iron deficiency, anemia of chronic disease, and obesity: introducing hepcidin. *J Acad Nutr Diet.* 2012;112:391-400.
35. Barber MF, Elde NC. Buried treasure: evolutionary perspectives on microbial iron piracy. *Trends Genet.* 2015;31:627-636.
36. Deriu E, Liu JZ, Pezeshki M, et al. Probiotic bacteria reduce salmonella typhimurium intestinal colonization by competing for iron. *Cell Host Microbe.* 2013;14:26-37.