Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation

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Wessels I, Cousins RJ. Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation. Am J Physiol Gastrointest Liver Physiol 309: G768–G778, 2015. First published August 13, 2015; doi:10.1152/ajpgi.00179.2015.—Integrity of the immune system is particularly dependent on the availability of zinc. Recent data suggest that zinc is involved in the development of sepsis, a life-threatening systemic inflammation with high death rates, but with limited therapeutic options. Altered cell zinc transport mechanisms could contribute to the inflammatory effects of sepsis. Zip14, a zinc importer induced by proinflammatory stimuli, could influence zinc metabolism during sepsis and serve as a target for therapy. Using cecal ligation-and-puncture (CLP) to model polymicrobial sepsis, we narrowed the function of ZIP14 to regulation of zinc homeostasis in hepatocytes, while hepatic leukocytes were mostly responsible for driving inflammation, as shown by higher expression of IL-1β, TNFα, S100A8, and matrix metalloproteinase-8. Using Zip14 knockout (KO) mice as a novel approach, we found that ablation of Zip14 produced a delay in development of leukocytosis, prevented zinc accumulation in the liver, altered the kinetics of hypozincemia, and drastically increased serum IL-6, TNFα, and IL-10 concentrations following CLP. Hence, this model revealed that the zinc transporter ZIP14 is a component of the pathway for zinc redistribution that contributes to zinc dyshomeostasis during polymicrobial sepsis. In contrast, using the identical CLP model, we found that supplemental dietary zinc reduced the severity of sepsis, as shown by amelioration of cytokines, calprotectins, and blood bacterial loads. We conclude that the zinc transporter ZIP14 influences aspects of the pathophysiology of nonlethal polymicrobial murine sepsis induced by CLP through zinc delivery. The results are promising for the use of zinc and its transporters as targets for future sepsis therapy.

zinc transport; sepsis; zinc metabolism; cytokines

SEPSIS IS ONE OF THE LEADING inflammatory diseases, causing millions of deaths annually and producing immense costs for health systems worldwide (22, 28). Despite intense research, the mechanisms underlying sepsis are not completely understood, and treatment is limited to symptomatic approaches, with limited success (44). An association between altered zinc homeostasis and the severity of the inflammatory reactions has been suggested by several studies using various disease models and systems. These include studies of LPS-induced endotoxemia, acute stress, and sepsis in pigs (8, 27), fowl (26, 38), rodents (14, 15, 40), and human subjects (17).

The acute response to endotoxin in vivo includes hypozincemia and altered kinetics of zinc distribution to specific tissues (8). Such changes in body zinc redistribution are believed to be controlled through selective regulation of zinc transport pathways. Two zinc transporter (ZnT) families comprising 24 members (the ZnT family with 10 members and the Zip family with 14 members), exhibiting differential modes of regulation and cell-type expression, alter zinc metabolism to meet dietary and physiological needs (29). On the basis of our previous experiments on the responsiveness of zinc homeostasis to cytokine/hormonal stimuli, we hypothesized that microbial attack would drastically alter expression of specific zinc/Zip transporters in mice. Begum et al. (7) showed that LPS induced a novel gene in monocytes that was subsequently identified as Zip8. Experiments focused on the liver, and use of a quantitative PCR (qPCR) screen of individual transporter gene transcripts showed that Zip14 was the most highly induced transporter in the liver of mice treated with LPS to induce endotoxemia (31). Subsequently, Zip14 was documented to be expressed in multiple tissues of mice under a variety of physiological conditions, including endotoxemia (2, 3, 30). The liver has been a prime target of these investigations. Liver dysfunction is a major factor that contributes to the severity of sepsis (6, 35). This often fatal condition is accompanied by endotoxemia and drastic changes in cytokine production and secretion (1, 13, 23, 47).

On the basis of the responsiveness of Zip14 expression to endotoxin, we hypothesized that this zinc transporter would be induced in the liver during sepsis and, hence, would contribute to the altered zinc homeostasis and functional outcomes observed under such conditions. Using Zip14 knockout (KO) mice as a novel approach, we report that the zinc transporter ZIP14 is a component of the pathway for zinc that contributes to zinc dyshomeostasis during polymicrobial sepsis. Our experiments demonstrate the involvement of ZIP14 in the response to sepsis and the beneficial anti-inflammatory effects of supplemental dietary zinc during sepsis.

MATERIALS AND METHODS

Animals and diets. Male and female mice of the C57BL/6 strain were used at 8–20 wk of age. Genotypes were Zip14+/+ [wild-type (WT)] and Zip14−/− (KO). Derivation and characterization of mice of the KO genotype are described elsewhere (2, 3). Animals of only one sex were used to generate a specific data set. The mice were fed a commercial rodent diet (Harlan Teklad 7912), except for one series of experiments in which a normal-zinc [zinc-adequate (ZnA), 30 mg Zn/kg] or a zinc-supplemented [high-zinc (ZnH), 180 mg Zn/kg] diet was fed (3). Mice were euthanized by exsanguination via cardiac puncture up to 72 h after cecal ligation-and-puncture (CLP) or sham operation. Isoflurane anesthetic was used for all procedures. Buprenorphine (0.1 mg/kg sc) was used for analgesia as needed. Protocols were approved by the University of Florida Institutional Animal Care and Use Committee.

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CLP. Polymicrobial sepsis was induced by CLP according to established methodology (11). Briefly, a laparotomy was performed under anesthesia, and the cecum was exteriorized and ligated 1 cm from the distal end. The cecum was punctured once with a 27-gauge syringe needle and then returned to the abdominal cavity, and the surgical site was closed with staples. For sham operation, a laparotomy was performed and the cecum was exteriorized, but without ligation or puncture. No mortality resulted from puncture with the 27-gauge needle.

Biochemical analyses. TNFα and IL-10 levels were measured by enzyme-linked immunosorbent assay (eBioscience, San Diego, CA). IL-6, matrix metalloproteinase (MMP)-9, S100A8, and S100A9 levels were quantified using a customized magnetic multiplexing assay (R & D Systems, Minneapolis, MN). Plasma alanine aminotransferase (ALT) was measured colorimetrically (37).

Isolation of leukocytes and parenchymal cells from liver. The mice were anesthetized, and the abdomen was wiped with 70% ethanol. The outer skin of the peritoneum was cut to expose the peritoneal cavity. After excision by cardiac puncture, the gallbladder was removed, and the inferior vena cava was severed. The liver was perfused by injection of 10 ml of ice-cold PBS into the hepatic portal vein using a 27-gauge needle. Thereafter, the liver and spleen were removed and placed into ice-cold FACS buffer (PBS and 5% BSA). Single-cell suspensions from the liver were generated by forcing the tissue through a 70-μm cell strainer using the plunger of a 5-ml syringe. An aliquot of this cell suspension was saved for analysis of bacterial load, and the remaining cells were washed twice with ice-cold FACS buffer. The cell pellet was resuspended in 20 ml of isotonic Percoll (33.75%) at room temperature and centrifuged at 700 g for 12 min. Hepatocytes (HP) were collected as a disk-like sheet floating on top of the Percoll gradient, washed in wash buffer (Williams’ 43 Medium E and 10 mM HEPES, pH 7.3), and used to generate RNA for expression analysis. The leukocyte-containing pellet was suspended in 4 ml of Tris-buffered NH₄Cl (TAC) buffer (17 mM Tris and 140 mM NH₄Cl) for 10 min, underlayered with 1 ml of FCS-10 mM EDTA, and centrifuged again. The cells were washed again and then suspended in FACS-EDTA buffer (FACS buffer and 5 mM EDTA) for further analysis; these cells were designated the hepatic leukocyte (HL) fraction. The spleen was passed through a cell strainer using a plunger as described above. An aliquot of the homogenate was used to analyze bacterial load. These methods were adapted from procedures designed by Wang et al. (43). To measure viability, cells were stained with propidium iodide (Sigma-Aldrich) and analyzed using flow cytometry as described elsewhere (19).

Immunoblotting. Polyclonal rabbit antibody against ZIP14 was raised in-house as described previously (31). The rabbit IgG fractions were affinity-purified. Liver tissue samples were flash-frozen in liquid nitrogen upon collection. Frozen liver tissue was homogenized in lysis buffer (20 mM Tris-HCl, 1% Triton X-100, 10% glycerol, 137 mM NaCl, and 2 mM EDTA) containing protease inhibitor cocktail (Santa Cruz Biotechnology, Santa Cruz, Santa Cruz, CA) and sodium vanadate as a phosphatase inhibitor. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Transfer to a nitrocellulose membrane was confirmed by Pierce red staining. Immunoreactivity was visualized by enhanced chemiluminescence.
microbial peptides by the liver (Fig. 1B) within the first 24 h following CLP. Liver *Il-6* and *Tnfα* mRNA and transcripts for both components of the calprotectin heterodimer (*S100A8* and *S100A9*) in the liver of these mice that underwent CLP surgery were markedly increased (Fig. 1B). In contrast, WBC showed modest changes in *Il-6*, *Tnfα*, *S100A8*, and *S100A9* mRNA expression (data not shown). The liver showed significant increases in *Il-10* mRNA and *Mmp-9* transcripts. These responses are considered anti-inflammatory and antimicrobial, respectively.

Results obtained for mRNA expression were comparable to significant increases in serum *Il-6*, *MMP-9*, and *S100A9* proteins following CLP (Fig. 1C). Levels of *S100A8*, *Tnfα*, and *IL-10* were also elevated in the serum of CLP mice but did
not reach statistical significance (data not shown). Protein levels of all mediators peaked between 3 and 24 h after CLP and returned to basal levels after 48 h. These results underline the differential contribution of the liver and WBC to the inflammatory response and levels of serum mediators during sepsis.

The response of parameters (tissue and serum zinc and Mt-1 mRNA expression) examined following CLP with a single puncture using a 25-gauge needle was greater (data not shown). Some mortality was observed when the 25-gauge needle was used. Hence, a single puncture with the 27-gauge needle, where no mortality was observed, was used in all the experiments reported here.

Zip14 expression in the liver is strongly elevated throughout sepsis. CLP induced a transient wave of changes in ZnT mRNA in the liver. Of all 14 Zips and 10 zinc transporters tested, maximal increases in Zip4, Zip6, Zip10, and Zip14 mRNAs were detected at 1, 9, 24, and 72 h post-CLP, respectively (Fig. 2A). Changes in liver Zip8 mRNA were minimal over the entire 72-h period post-CLP. The increases in Zip4, Zip6, and Zip10 expression were transient and returned to near-normal levels by 72 h. In contrast, Zip14 mRNA increased by 9 h after CLP and was the only transporter mRNA that was constantly elevated in CLP animals compared with sham-operated animals throughout the 72-h period post-CLP (Fig. 2A). Western blots confirmed upregulation for ZIP14 protein (Fig. 2B). Expression of the other transporters only slightly changed at the protein level (data not shown). Therefore, these data suggest that ZIP14 is upregulated to sustain hepatic zinc transport during sepsis.

Differences in zinc homeostasis, cytokines, and host defensive mediators during sepsis involve HP and HL. The observation of a sequential induction of zinc transporter and cytokine transcripts in total liver RNA extracts raises the question about the contributions of individual cell types. To answer this question, HP (parenchymal cells) and HL were separated and analyzed for relative expression of transcripts for markers of zinc homeostasis, cytokines, and host defensive factors. Purity of the isolated cell populations was established through expression of the lineage markers hepcidin (HP) and F4/80 (HL) as assessed using qPCR. Viability of ~90% was confirmed using propidium iodine staining (data not shown). Higher expression of Mt-1 and Zip14 mRNA and a higher intracellular labile zinc content were found in HP than HL (Fig. 3A). This finding, along with the increase in total liver zinc concentrations after CLP (Fig. 1A), demonstrates the significance of HP in zinc redistribution to the liver during sepsis. While Zip4 mRNA appeared to be primarily expressed by HP, Zip6 mRNA expression was restricted to HL. Both cell types expressed Zip10 following CLP (Fig. 3B). Transcripts for the inflammatory markers Tnfa, Il-1β, and S100A8 were elevated in HL by 9 h post-CLP. Il-10 mRNA expression in HP was initially high, but by 9 h, levels were comparable to those in HL. Il-6 was expressed by both cell types (Fig. 3B). Mmp-8 mRNA expression was examined and found to be higher in HL than HP after CLP. These data suggest that, in this in vivo model of poly-microbial sepsis, HP functions to regulate zinc homeostasis and also contributes to inflammation via IL-6 and IL-10 production.

Zinc homeostasis in response to sepsis is significantly changed in Zip14 KO mice. The availability of the Zip14 KO mouse strain allowed us to examine the role of ZIP14-mediated zinc redistribution in the response to CLP-induced sepsis. The response of serum zinc concentration in the KO mice following CLP was different from that in the WT mice. Specifically, serum zinc concentration was significantly depressed (P < 0.01) only at 24 h after CLP in the KO mice (Fig. 4A). The delayed response in mice when ZIP14 is not produced suggests that the initial hypozincemia of polymicrobial sepsis is the result of a compensatory mechanism involving other zinc transporters (Fig. 4A). In support of this hypothesis, levels of
Zip4, Zip6, and Zip10 mRNA expression were significantly elevated in the KO mice compared with the WT mice at 24 h post-CLP (Fig. 4A). The increase in WBC counts in the WT mice was inversely related to the extent of hypozincemia. The WBC counts in blood from the KO mice did not closely follow the serum zinc levels, however (Fig. 4A). This suggests that hypozincemia influences the level of circulating WBC. In contrast, ablation of Zip14 prevented the accumulation of total liver zinc in the WT mice after CLP (Fig. 4B). The Zip14 ablation caused an induction of Mt-1 mRNA following CLP, suggesting that either the KO mice retained a pool of zinc that activated metal-responsive transcription factor 1 (MTF-1) or the induction is mediated by cytokines, e.g., the increase in circulating IL-6. The necessity of ZIP14 for hepatic zinc accumulation (Fig. 4A) suggests that ZIP14 may function in intracellular processing/utilization of zinc, as well as uptake at the cell surface. Surprisingly, hepcidin mRNA expression was significantly stronger in KO animals after CLP than in WT.
animals (Fig. 4C). The high hepcidin mRNA levels in the KO mice, perhaps a reflection of elevated IL-6, are not consistent with the similar levels of NHI in liver of both genotypes (Fig. 4D). Apoptosis of liver cells, based on the marker programmed death-ligand 1 (Pd-l1) mRNA, was significantly increased in the septic KO animals and was decreased in WBC (Fig. 4C). On the other hand, liver cell proliferation, based on Pcna mRNA, was significantly decreased in the liver of KO animals. It was lower, but not significantly, in WBC as well.

Knockout of Zip14 results in altered cytokine production, but not sepsis progression. Western analysis proved that ZIP14 is not produced in the liver of the KO mice in response to CLP. In contrast, cell activation, indicated by STAT3 phosphorylation, was comparably enhanced in both genotypes (Fig. 5A). Analysis of cytokine mRNA expression in the liver revealed a decrease in Tnfα and significant decreases in Il-6, Il-1β, and of Il-10 in the KO mice (Fig. 5B). In contrast, the proinflammatory response by WBC from the KO mice remained unchanged, except Il-10 mRNA expression was significantly decreased. Most surprisingly, plasma levels of IL-6, IL-10, and TNFα were significantly higher in septic KO mice than in the WT animals, despite lower mRNA expression (Fig. 5C). Increased inflammatory markers in serum suggest a disadvantage during sepsis in the KO mice. No changes were detected for serum ALT or bacterial load in liver, spleen, or blood, however (Fig. 5D). In addition, none of the KO animals died or showed signs of more severe disease than the WT mice. Hence, the Zip14
null mutation did not appear to enhance the progression of mild sepsis over the time course of our study.

Zinc supplementation reduces sepsis progression. The Zip14 KO model provided new and valuable information on the role of zinc transport to and within the liver during sepsis. To more directly characterize the effects of zinc in mild sepsis, we fed WT mice the ZnA or ZnH diet for 1 wk prior to CLP. Figure 6A illustrates an increase in liver zinc in sham-operated mice fed the ZnH diet and a greater increase by 24 h after CLP. The significant drop in serum zinc following CLP was prevented in the ZnH diet-fed mice compared with ZnA diet-fed mice. However, no significant changes in Zip14 and Mt-1 mRNA expression were observed in HP, HL, and WBC from mice fed the ZnA diet compared with those fed the ZnH diet (Fig. 6B). Mmp-9 mRNA expression was significantly decreased in HP and HL and negligible in WBC. The ZnH diet significantly decreased Il-6 mRNA in HP, Tnfα mRNA in HL, and S100A9 mRNA in WBC, demonstrating the anti-inflammatory effect of zinc (Fig. 6B). We found significantly decreased levels of Tnfα, S100A8, and S100A9 in serum from ZnH diet-fed mice compared with ZnA diet-fed mice (Fig. 6C). Moreover, IL-6, MMP-9, and IL-10 were decreased, but not significantly. In concordance with the ameliorated inflammatory response, ALT levels in the serum

![Image](http://ajpgi.physiology.org/)

**Zip14 TRANSPORTER INFLUENCES MURINE POLYMICROBIAL SEPSIS**

Fig. 5. Zip14 ablation alters the hepatic inflammatory response to sepsis. Zip14 KO and WT mice were killed 24 h after CLP or sham operation. A: Western analysis of ZIP14 and STAT3 phosphorylation (pSTAT3) in liver of CLP and sham-operated mice. Blots are representative of results from 3 independent experiments; tubulin and STAT3 were used as loading controls. B: Il-6, Tnfα, Il-1β, and Il-10 mRNA expression in liver and WBC. Data were normalized to TBp mRNA. Values are means ± SE (n = 3 mice per group). C: IL-6, MMP-9, S100A8, and S100A9 concentrations in serum analyzed using a customized Luminex assay and TNFα and IL-10 plasma concentrations analyzed by ELISA. Values are means ± SE (n = 3 mice per group). D: serum alanine aminotransferase (ALT) activity (n = 3 mice per group) and bacterial load (colony-forming units (cfu)/ml) of liver homogenate, spleen homogenate, and whole blood (n = 3 mice per group). Values are means ± SE. *P < 0.05 vs. KO [by ANOVA/Bonferroni’s test (B and C) and ANOVA/Tukey’s test (D)]. Significantly different values do not share the same letter (a, b).

![Graph](http://ajpgi.physiology.org/)

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were significantly decreased in the ZnH diet-fed mice. Interestingly, we also found a lower bacterial load in the spleen and blood from mice fed the ZnH diet. These results clearly point to a benefit of prior zinc supplementation for the outcomes in this mouse model of sepsis.

**DISCUSSION**

The inflammatory acute-phase response is a complex physiological process involving various cell types and proinflammatory, as well as anti-inflammatory, phases. The ultimate goal is clearance of the insult, usually a pathogen or injury, and reestablishment of homeostasis. If homeostasis is not reestablished, as during sepsis, tissue damage, organ failure, and death are the final consequences (1, 13, 23). In this report we connect inflammation of sepsis to changes in zinc homeostasis. The murine model used here focuses primarily on sepsis up to 24 h post-CLP, when leukocytosis occurs and inflammatory stimuli, including IL-6, IL-1β, and TNFα, increase. Zinc is needed for liver metabolism and protection; however, the exact role of this micronutrient in the immune response to sepsis is unknown.

Unique to this study is the identification of a cascade of Zip4, Zip6, and Zip10 mRNA expression in the liver of septic mice. These changed on a temporal basis and were cell type-specific, but they played only a minor role in generating hypozincemia. Zip14 was the major transporter responsible for zinc redistribution during sepsis. Therefore, results from this polymicrobial sepsis model are in agreement with results from LPS-induced endotoxemia (2, 30, 31) and HP in vitro (30, 31). In addition, we narrowed the main function in regulation of zinc homeostasis to HP, while HL were mostly responsible for driving inflammation, as shown by higher expression of IL-1β, Tnfα, S100A8, and Mmp-8. While these factors have been shown to be elevated during sepsis, their origin has not been assigned to specific cell types of the liver, nor were they previously related to zinc redistribution.

Of significance was the inverse correlation of WBC in the blood to the decrease in serum zinc. This finding supports the hypothesis that hypozincemia is an activating signal for inflammation, as shown by higher expression of IL-1β, Tnfα, Il-6, and Mmp-8. While these factors have been shown to be elevated during sepsis, their origin has not been assigned to specific cell types of the liver, nor were they previously related to zinc redistribution.

Clinical evidence from human studies suggests that lower plasma TNFα, IL-6, and IL-10 levels correlate with survival in severely septic patients (47). Similarly, plasma TNFα correlates with severity of sepsis in human patients (12). Our murine sepsis data show that both HP and HL contribute to systemic IL-6 levels, while HL contribute to systemic TNFα and IL-10. Survival was not an issue in our experiments, since relatively mild conditions were used. Comparison of Tnfα, Il-6, and Il-10 transcripts in liver cells of KO vs. WT mice after CLP with serum protein levels shows opposite effects. This could relate to the times of RNA sampling vs. serum sampling. Expression by splenocytes would be an explanation as well. Alternatively, mechanisms mediating resolution of the inflammatory response, including expression of antagonists to proinflammatory mediators, such as IL-1 receptor antagonist, IL-6 receptor, and TNF receptor, might be disturbed. These possibilities remain to be tested. Nevertheless, the marked increases in serum TNFα, IL-6, and IL-10 suggest that progression of sepsis is influenced by Zip14 expression. More extensive studies are needed to evaluate the physiological role of ZIP14 in a severe sepsis
model. In addition, in future studies the influence of sex needs to be investigated using this mutant model. Nevertheless, our data support the concept that zinc, as transported by ZIP14, influences cytokine levels through an as-yet-identified mechanism during sepsis. Our finding that supplemental dietary zinc generally reduces cytokine levels in CLP mice supports this hypothesis.

Evidence from genome expression assays indicates that metallothionein and Zip8 are among highly upregulated genes in nonsurvivors of pediatric septic shock (46). Those patients exhibited hypozincemia, while surviving patients had normal serum zinc levels. Those findings support the idea that a zinc-related component is involved in the severity of sepsis. At first glance, accumulation of high amounts of zinc might be surprising, as it could be the perfect environment also for bacterial growth. However, our data reveal that most of the zinc is directly taken up by HP, possibly causing a transient zinc deficiency in the intracellular space and blood vessels supplying the liver. On the basis of serum ALT levels, the highly compromised transfer of zinc to the liver in the KO mice did not lead to cell damage. However, greater Pd-H expression supports the notion that HP are in a protective metabolic state (49).

Dietary zinc deficiency has been shown to accentuate organ damage in a murine model of severe sepsis, resulting in high mortality (25). Conversely, short-term (3 days) dietary zinc supplementation in another model of severe murine sepsis decreased mortality and lowered indexes of sepsis, including bacterial load (36). The mechanism of the influence of zinc on sepsis is not fully known. The MMPs play a role in tissue injury of sepsis (33, 42). Calprotectin (S100A8 and S100A9) inhibits microbial growth through zinc chelation (9). Calprotectin may also inhibit MMPs by binding zinc, which is essential for enzymatic activity (24). Zinc may also inhibit cytokine production through a variety of mechanisms (16, 21).

Our very positive effects of zinc supplementation suggest that timing of zinc as a therapy is very important. In a clinical setting with human septic patients, zinc supplementation could be monitored by measurement of peripheral blood mononuclear cell (PBMC) metallothionein mRNA levels (2).

There has been some controversy regarding the value of murine models of human sepsis (39, 41) for which PBMCs were used as a source of the RNA for transcriptome profiling by microarrays. Nevertheless, significant similarities exist, as mRNAs for MMPs, calprotectins, specific cytokines, and nutrient transport proteins in mice and human PBMCs were detected by these profiling experiments (41). Consequently, significant advances continue to be made with the murine CLP-induced sepsis model, for example, the role of IL-3 (44) and TNF receptor shedding from hepatocytes (12). This is particularly the situation in experiments at the organ/tissue level, e.g., the liver, as shown in our present experiments, rather than transcriptome analysis using circulating blood cells.

The focus of this report is on the liver, which is considered a key organ subject to dysfunction in sepsis (6, 35). The experiments presented here, the first with a zinc transporter KO model, suggest that zinc and the zinc transporter ZIP14 influence aspects of the pathophysiology of nonlethal polymicrobial murine sepsis induced by CLP. Ablation of Zip14 produced a delay in development of leukocytosis, prevented zinc accumulation in the liver, altered the kinetics of hypozincemia, and drastically increased serum IL-6, TNFα, and IL-10 concentration following CLP. To identify zinc-responsive factors modulated by acute sepsis, mice were fed the ZnH diet for 1 wk. The ZnH diet reduced cytokines, calprotectins (S100A8 and S100A9), serum ALT, and blood bacterial loads. Supplemental zinc at this level did not appear to produce toxicity. These data indicate that 1) zinc has an anti-inflammatory role during sepsis, in that it attenuates the proinflammatory response, and 2) ZIP14, when located at the cell surface, has an important transport function, in that it transports zinc into cells for sites of action.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.J.C. and I.W. developed the concept and designed the research; R.J.C. and I.W. drafted the manuscript; R.J.C. and I.W. edited and revised the manuscript; R.J.C. and I.W. approved the final version of the manuscript; I.W. performed the experiments; I.W. analyzed the data; I.W. prepared the figures.

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