Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 24 (2013) 353–359

Journal of Nutritional Biochemistry

Increased inflammatory response in aged mice is associated with age-related zinc deficiency and zinc transporter dysregulation☆

Carmen P. Wong[a], Kathy R. Magnusson[b,c], Emily Ho[a,c,*]

[a]School of Biological and Population Health Sciences, Oregon State University, OR 97331, USA
[b]Department of Biomedical Sciences, Oregon State University, Corvallis, OR 97331, USA
[c]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA

Received 31 March 2012; received in revised form 25 June 2012; accepted 3 July 2012

Abstract

Aging is a complex process associated with physiological changes in numerous organ systems. In particular, aging of the immune system is characterized by progressive dysregulation of immune responses, resulting in increased susceptibility to infectious diseases, impaired vaccination efficacy and systemic low-grade chronic inflammation. Increasing evidence suggest that intracellular zinc homeostasis, regulated by zinc transporter expression, is critically involved in the signaling and activation of immune cells. We hypothesize that epigenetic alterations and nutritional deficits associated with aging may lead to zinc transporter dysregulation, resulting in decreases in cellular zinc levels and enhanced inflammation with age. The goal of this study was to examine the contribution of age-related zinc deficiency and zinc transporter dysregulation on the inflammatory response in immune cells. The effects of zinc deficiency and age on the induction of inflammatory responses were determined using an *in vitro* cell culture system and an aged mouse model. We showed that zinc deficiency, particularly the reduction in intracellular zinc in immune cells, was associated with increased inflammation with age. Furthermore, reduced Zip 6 expression enhanced proinflammatory response, and age-specific Zip 6 dysregulation correlated with an increase in Zip 6 promoter methylation. Furthermore, restoring zinc status via dietary supplementation reduced aged-associated inflammation. Our data suggested that age-related epigenetic dysregulation in zinc transporter expression may influence cellular zinc levels and contribute to increased susceptibility to inflammation with age.
Published by Elsevier Inc.

Keywords: Aging; Epigenetics; Immunity; Inflammation; Zinc

1. Introduction

Aging of the immune system results in a progressive dysregulation of immune responses including immunosenescence, where there is a gradual decline in both cellular and humoral immune responses, and increased susceptibility to infectious diseases and compromised vaccination efficacy in the elderly [1]. At the same time, "inflamm-aging," a low-grade systemic chronic inflammation characterized by constitutively elevated levels of proinflammatory cytokines in blood, is commonly observed in the elderly population [2,3]. Chronic inflammation has been implicated in the promotion of many age-related diseases including cancer, cardiovascular disease and auto-immune diseases. In addition, increases in inflammatory mediators in the blood are significant predictors of morbidity and mortality in aged individuals [4–6].

Zinc is an essential micronutrient required for many cellular processes, especially for the normal development and function of the immune system [7–9]. National surveys indicate that a significant portion of the aged population has inadequate zinc intake [10–13], and a decline in zinc status, as shown by plasma zinc concentrations, is observed with increasing age [14–17]. There are remarkable similarities between the hallmarks of zinc deficiency and age-related immunological dysfunction, both characterized by impaired immune responses and systemic chronic inflammation. Thus age-related zinc deficiency may play a significant role in age-associated dysregulation of immune function and may be a contributing factor in age-related inflammation and associated morbidities [18,19]. Importantly, zinc has anti-inflammatory properties and low zinc status is associated with increased susceptibility to infections and exaggerated inflammatory responses [20–23]. Recent studies indicate that intracellular zinc homeostasis is critically involved in the signaling events in immune cells, and the regulation of cellular zinc in these immune cells is mediated by changes in the expression of specific zinc transporters [24–26]. Zinc transporters comprises a family of multiple transmembrane spanning domain proteins that are encoded by two solute-linked carrier (SLC) gene families: SLC30 (ZnT) and SLC39 (Zip)

☆ Funding support: Oregon Agricultural Experiment Station (OR00735), Environmental Health Science Center at Oregon State University (NIEHS P30 ES00210), Oregon State University General Research Fund, Linus Pauling Institute, and National Institute on Aging, NIH (R01 AG016322).

* Corresponding author. School of Biological and Population Health Sciences, Oregon State University, Corvallis, OR 97331, USA. Tel.: +1 541 737 9559.
E-mail address: Emily.Ho@oregonstate.edu (E. Ho).

0955-2863/$ - see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.jnutbio.2012.07.005

[27,28]. ZnT and Zip family zinc transporters have opposing roles in regulating cellular zinc homeostasis; ZnT transporters reduce cytosolic zinc bioavailability by promoting zinc efflux and Zip transporters function by increasing cytosolic zinc. In the context of inflammation, stimulation of immune cells with inflammatory stimuli such as lipopolysaccharide (LPS) results in changes in cellular zinc that is mediated by alterations in zinc transporter expression [29]. Thus alterations and/or dysregulation of zinc transporter expression with age could potentially affect zinc homeostasis in immune cells and contribute to immune dysfunction and chronic inflammation [18,30].

The mechanisms contributing to age-related zinc loss and age-related inflammation are unclear. Accumulating evidence indicates that epigenetic dysregulation is a common feature of aging, characterized by global DNA hypomethylation and gene-specific promoter hypermethylation or hypomethylation, as well as alteration in histone modifications [31–34]. In the immune system, age-associated epigenetic modifications such as DNA methylation have been shown to affect immune cell activation and may also contribute to the decline of cellular zinc with age, as several zinc transporters have been shown to be susceptible to epigenetic regulation [35–38]. At the same time, nutrient deficits such as zinc deficiency may further modulate epigenetic regulation [39–41]. The goal of the current study was to examine the contribution of age-related zinc deficiency and zinc transporter dysregulation on the inflammatory response in immune cells using an *in vitro* cell culture system and an aged mouse model. We hypothesized that age-related decreases in cellular zinc levels, in part, are mediated by epigenetic alterations that result in zinc transporter dysregulation and contribute to enhanced inflammation with age. Moreover, enhancing zinc status in aged mice should mitigate age-related inflammation.

2. Materials and methods

2.1. Cell culture, in vitro zinc depletion and LPS treatments

Human monocytic cell line THP-1 was obtained from American Type Tissue Collection (Manassas, VA, USA). Cells were grown in RPMI 1640 culture medium with 10% fetal bovine serum (FBS) and maintained in humidified incubators with 5% CO_2 at 37°C. Zinc-deficient (ZD) media were prepared, as previous published, using a chelation strategy in which zinc was removed from FBS by incubating with 10% Chelex 100 (wt/vol) (Sigma, St. Louis, MO, USA) overnight at 4°C with constant stirring [42]. THP-1 cells were cultured in zinc-adequate (ZA) medium (RPMI with 10% Chelex-treated FBS and 4 μM $ZnCl_2$) or ZD medium (RPMI with 10% Chelex-treated FBS) for up to 14 days, and media were changed every 3 to 4 days. Mineral levels in tissue culture media and THP-1 cells were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and/or FluoZin-3 flow cytometry where appropriate. ZA or ZD THP-1 cells were treated with phorbol 12-myristate 13-acetate at 5 ng/ml for 48 h to induce the cells to differentiate into macrophages. Differentiated THP-1 macrophages were treated with 0, 10 or 100 ng/ml LPS (Sigma) for 6 h and harvested for zinc and gene expression analyses. Previous time-course studies indicated that LPS induced a rapid proinflammatory response (data not shown), and the 6-h time point was chosen to reflect optimal induction time for various genes of interest.

2.2. Animals, diets and study design

Female C57Bl/6 mice at various ages (2–26 months) were purchased from the aged rodent colonies at the National Institute on Aging (Bethesda, MD, USA). Mice were housed in a temperature- and humidity-controlled environment and were fed standard rodent diet where appropriate, or randomly assigned to a purified ZA diet containing 30 mg/kg zinc, or a zinc-supplemented (ZS) diet containing 300 mg/kg zinc that was previously shown to be well tolerated and were able to normalize plasma zinc levels in old mice to levels similar to that of young mice [43,44]. Purified ZA and ZS diets were purchased from Research Diets (New Brunswick, NJ, USA). Mice were fed ZA or ZS diets for 3 weeks. Food and water were provided ad libitum. Dietary intakes and body weights of all mice were monitored throughout the entire study. Mice were euthanized by CO_2 asphyxiation at the termination of the experiments, and sera and tissues were collected. The animal protocol was approved by the Oregon State University Institutional Laboratory Animal Care and Use Committee. Tissues were processed immediately for *ex vivo* stimulation and/or differentiation or were preserved in RNALater (Life Technologies, Grand Island, NY, USA) for DNA and RNA isolation.

2.3. Ex vivo differentiation of bone marrow-derived dendritic cells and macrophages

Bone marrow (BM)-derived dendritic cells (BMDCs) and macrophages (BMM) were differentiated *ex vivo* according to published protocols [45,46]. BM cells were flushed and collected from the femurs and tibias of young and old mice. Red blood cells (RBCs) were removed using RBC lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM EDTA). BMs were adjusted to 1.5×10^6 cells/ml in RPMI media containing 10% FBS. BMDC and BMM were differentiated in media containing 20 ng/ml granulocyte/macrophage colony stimulating factor (GM-CSF) and 20 ng/ml macrophage colony stimulating factor (M-CSF), respectively (Peprotech, Rocky Hill, NJ, USA). Nonadherent cells were removed on days 2 and 4, and BMDC and BMM cells were harvested on day 7 for intracellular zinc analysis and siRNA transfections, respectively.

2.4. Inflammatory response assays

For *ex vivo* splenocyte stimulation, spleens collected from young and old mice were made into single-cell suspension, and RBCs were removed using RBC lysis buffer. To determine how age and zinc status affect inflammatory response on a per cell basis, an equal number of splenocytes from young or old mice were seeded at 5×10^6 cells per well in 24-well culture plates and treated with 0, 0.1 and 1 μg/ml LPS for 6 h and harvested for zinc and gene expression analyses. Serum interleukin (IL)-6 levels were detected using mouse IL6 Ready-SET-Go ELISA kit from eBioscience (San Diego, CA, USA).

2.5. Total and intracellular zinc measurements

Total zinc concentrations were determined using ICP-OES, as previously described, with minor modification [47]. Briefly, samples (plasma or cell pellets) were digested in 1 ml 70% ultrapure nitric acid and incubated overnight. Incubated samples were diluted with chelex-treated nanopure water to a final concentration of 7% nitric acid, centrifuged and analyzed using the Prodigy High Dispersion ICP-OES instrument (Teledyne Leeman Labs, Hudson, NH, USA) against known standards. Intracellular zinc levels were determined using FluoZin-3 acetoxymethyl ester (FluoZin-3), a cell permeable, zinc-specific fluorescent indicator that measures intracellular free zinc (Molecular Probes, Eugene, OR, USA), according to published methods [48]. Cells were labeled with 1 μM FluoZin-3 for 30 minutes at 37°C and washed once in phosphate-buffered saline. Intracellular zinc levels, as determined by FluoZin-3 mean fluorescence intensity, were analyzed by flow cytometry. Data were acquired using FACSCalibur (BD Biosciences, San Jose, CA, USA). Data analyses were performed using Summit software (DakoCytomation, Fort Collins, CO, USA).

2.6. Proinflammatory and zinc transporter gene expression

Total RNA from tissues and treated cells were isolated using TRIzol (Invitrogen). Total RNA was reverse transcribed into cDNA using SuperScript III First-Strand Synthesis SuperMix for quantitative real-time polymerase chain reaction (qRT-PCR) (Invitrogen). Real-time PCR was performed using the following PCR primers: human tumor necrosis factor α (TNF) (forward: 5′-CCCCAGGGACCTCTCTCTAATC-3′, reverse: 5′-GGTTTGCTACAACATGGGCTACA-3′), human IL1β (forward: 5′-CCTGTCCTGCGTGTTGAAAGA-3′, reverse: 5′-GGGAACTGGGCAGACTCAAA-3′), human GAPDH (forward: 5′-CGAGATCCCTCCAAAATCAA-3′, reverse: 5′-TTCACACCCATGACGAACAT-3′), mouse TNFα (forward: 5′-CTGTAGCCCACGTCGTAGCA-3′, reverse: 5′-GTGTGGGTGAGGAGCACGTA-3′), mouse IL1β (forward: 5′-AAGATGAAGGGCTGCTTCCAA-3′, reverse: 5′-TGAAGGAAAAGAAGGTGCTCATG-3′), mouse Zip 6 (forward: 5′-AAGTGAGAAGAAGGCAGAAATCC-3′, reverse: 5′-GGAGAAGATGTAACAGAGCATCG-3′), mouse ZnT 1 (forward: 5′-TGGATGTACAAGTAAATGGGAATCT-3′, reverse: 5′-GTCTTCAGTACAACCCTTCCAGTTA-3′), or mouse 18S ribosomal RNA (18S) (forward: 5′-CCGCAGCTAGGAATAATGGAAT-3′, reverse: 5′-CGAACCTCCGACTTTCGTTCT-3′). Real-time PCR reactions were performed using Fast SYBR Green Mastermix (Invitrogen) on 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Gene copies were determined using the standard curve method. A standard curve was generated from serial dilutions of purified plasmid DNA that encoded for each gene of interest. Data represent the copy number of the gene of interest normalized to the copy number of GAPDH (for human) or 18S (for mouse) housekeeping genes.

2.7. Zip 6 gene silencing

BM macrophages were harvested and transfected with Zip 6-specific siRNA (Ambion Silencer siRNA S98750) using siPORT Amine and Ambion Silencer siRNA Transfection kit (Applied Biosystems). Control cells were transfected with scrambled siRNA (Ambion Silencer Select Negative Control No. 1). Transfected cells were seeded in 24-well tissue culture plates at 3×10^5 cells per well for 24 h and treated with 0, 10 and 100 ng/ml LPS for 6 h and harvested for gene expression analyses.

2.8. Genomic DNA isolation and DNA methylation analyses

Genomic DNA was isolated using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA). Global DNA methylation was measured using SuperSense Methylated DNA Quantification Kit (Epigentek, Brooklyn, NY, USA) and was reported as relative fluorescence units (RFUs) per 100 ng genomic DNA. Zip 6 promoter methylation was determined using Zip 6-specific EpiTect Methyl Profiler qPCR assay (Qiagen), which

Download English Version:

https://daneshyari.com/en/article/8337634

Download Persian Version:

https://daneshyari.com/article/8337634

Daneshyari.com