REVIEW

Zinc and its role in age-related inflammation and immune dysfunction

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Zinc is an essential micronutrient required for many cellular processes, especially for the normal development and function of the immune system. Zinc homeostasis and signaling are critical in immune activation, and an imbalance in zinc homeostasis is associated with the development of chronic diseases. Zinc deficiency causes significant impairment in both adaptive and innate immune responses, and promotes systemic inflammation. The elderly are a population particularly susceptible to zinc deficiency. National surveys indicate that a significant portion of the aged population has inadequate zinc intake, and a decline in zinc status is observed with age. There are remarkable similarities between the hallmarks of zinc deficiency and immunological dysfunction in aged individuals. Both zinc deficiency and the aging process are characterized by impaired immune responses and systemic low grade chronic inflammation. It has been hypothesized that age-related zinc deficiency may be an important factor contributing to immune dysfunction and chronic inflammation during the aging process. In this review, we discuss the effects of zinc status on aging, potential molecular and epigenetic mechanisms contributing to age-related decline in zinc status, and the role of zinc in age-related immune dysfunction and chronic inflammation.

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1 Introduction

Aging is a complex process associated with physiological changes in numerous organ systems. In particular, aging of the immune system, or immunosenescence, results in a progressive dysregulation of immune responses, affecting multiple immune cell types involved in both adaptive and innate immunity. Immunosenescence is characterized by involution of the thymus, defective lymphocyte maturation and function, and a gradual decline in both cellular and humoral immune responses, resulting in increased susceptibility to infectious diseases and compromised vaccination efficacy in the elderly [1]. At the same time, aging is associated with ''inflamm-aging'', a low-grade systemic chronic inflammation characterized by constitutively elevated levels of proinflammatory cytokines [2, 3]. Inflammation is a physiological process mediated by the innate immune system to protect against pathogen infections, environmental insults, and to promote wound healing. However, aberrant or excess inflammation is detrimental, as overproduction and prolonged exposure to inflammatory mediators (including inflammatory cytokines, prostaglandins, and reactive oxygen and nitrogen species) can result in changes in cell proliferation, apoptosis, angiogenesis, and genetic instability including alterations to DNA repair, increased DNA damage, and aberrant DNA methylation [3, 4]. Accumulating evidence indicates that chronic inflammation is commonly observed in the elderly, and increases in inflammatory mediators in the blood are significant predictors of morbidity and mortality in aged individuals [5]. Chronic inflammation has been correlated to

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Abbreviations: **AE,** acrodermatitis enteropathica; **DC,** dendritic cells; **DNMT,** DNA methyltransferase; **EAR,** estimated average requirement; **LPS,** lipopolysaccharide; **MT,** metallothionein; **MTF-1,** metal-regulatory transcription factor 1; **NFκB,** nuclear factor-kappaB; **SLC,** solute-linked carrier; **TCR,** T cell receptor

the promotion of many age-related diseases. For example, elevated levels of proinflammatory cytokines such as IL-6 and TNFα have been associated with age-related diseases such as cardiovascular disease, type-2 diabetes, osteoporosis and autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus [6]. Further, epidemiological studies indicate that up to 25% of all cancers are associated with inflammation [7]. Thus there is a critical need to develop strategies to reduce age-associated chronic inflammation to improve the quality of life for the elderly. While the etiology of immunosenescence and inflamm-aging is multi-faceted, we postulate that nutritional deficits such as zinc deficiency that are prevalent in the elderly may play an important role in contributing to the perturbations in the immune system with age. Further, improving zinc status in the elderly via dietary zinc supplementation may reverse and correct age-related immune dysfunction and chronic inflammation resulting in improved health outcomes.

Zinc is an essential micronutrient required for many biological processes including growth and development, neurological function, reproduction, and immunity. In particular, the importance of zinc for the immune system is underscored by the effects of zinc deficiency, which results in immune dysfunction including thymic atrophy, lymphopenia, impaired adaptive immunity, and chronic inflammation [8–10]. Low zinc status is associated with increased susceptibility to infections and exaggerated inflammatory responses, and zinc imbalance may contribute to the onset and/or progression of diseases including asthma, diabetes, and Alzheimer's diseases [11]. Individuals particularly at risk for zinc deficiency include the elderly, vegetarians, and patients with chronic illnesses. In particular, low zinc status in the elderly has been shown to contribute to age-associated dysregulation of immune function, and may be a contributing factor in age-related inflammation and associated morbidities [12]. However, the precise mechanisms linking zinc, age, and inflammation are unclear. In this review, we will highlight the effects of zinc status on aging, potential mechanisms that contribute to age-related decline in zinc status, and the role of zinc in immune responses with emphasis on age-related immune dysfunction and chronic inflammation. We will also discuss the potential role of epigenetic control on zinc homeostasis, and how epigenetic alteration with age may contribute to age-related zinc deficiency and inflammation.

2 Zinc status and aging

Zinc is a key component for the function of numerous proteins, including zinc-containing metalloenzymes and zinc-associated transcription factors. It is the most abundant trace intracellular element, and is an essential micronutrient required for numerous biological functions, including cell proliferation, differentiation, and reproduction. In addition, zinc is essential for the normal development and function of the immune system [9]. There is little storage for zinc in the human body, and bioavailable zinc from food or supplements must replenish the zinc pool on a regular basis. Alterations in zinc uptake, retention, or secretion can quickly lead to zinc deficiency and affect zinc-dependent functions. Zinc deficiency can significantly depress adaptive immune response, and susceptible individuals have impaired host defense and are at increased risk to opportunistic infections [8]. At the same time, zinc deficiency has been shown to increase systemic inflammation [13, 14].

Severe zinc deficiency mostly occurs in developing countries and is rare in the United States. On the other hand, marginal zinc deficiency is a potentially widespread problem, as a significant proportion of the US population does not consume adequate zinc. Approximately 12% of the US population does not consume the estimated average requirement (EAR) for zinc, but national surveys show that the prevalence of inadequate zinc intake is even higher among individuals above 50 years of age than any other population [15–18]; 40% of men and 45% of women consume less than the EAR [19]. Studies also show that zinc status, as shown by plasma zinc concentrations, declines with age [20–23]. However, the decline in status may not be solely attributed to decreases in dietary zinc intake. Utilization, distribution, and absorption of zinc may also be altered with age. For example, elderly women consuming zinc supplements did not exhibit increases in plasma zinc levels. In contrast, younger cohorts receiving the same zinc supplements had significant increases in plasma zinc [24]. Zinc deficiency in the elderly has a profound effect on the immune system, rendering them susceptible to infections and chronic inflammation [25, 26]. It is not known why plasma zinc levels fall with aging, but, some propose that it is related to a decline in zinc absorption. However, comparisons of zinc absorption between young and older individuals fail to show a consistent affect of age. Of the five studies done [27–31], three found lower zinc absorption in older than younger adults, two did not. Different study designs, methods, and diets likely account for the divergent results. In a study of the response to zinc loading (100 mg/d for 9 months), Wastney and co-workers found that total zinc absorption increased more in older than younger men with these high doses [32]. Studies of age and zinc absorption in experimental animals are also inconsistent [33, 34]. Thus, although plasma zinc concentrations are consistently lower in older individuals, there is no consistent evidence that this decline is due to a decrease in the ability to regulate zinc absorption. Regardless, there is mounting evidence that subclinical zinc deficiency may be more widespread in an elderly population and there may be an enhanced need for zinc with age. An alternative explanation for the decline in plasma zinc with age may be due to an enhanced uptake of zinc by cells due to altered expression of the zinc binding protein metallothionein (MT) and zinc transporters, resulting in tissue-specific zinc redistribution and/or changes in intracellular zinc pools.

Zinc transporters comprise a family of multiple transmembrane spanning domain proteins that are encoded by two solute-linked carrier (SLC) gene families: *SLC30* (a.k.a. ZnT) and *SLC39* (a.k.a. Zip). Evaluation of the human genome identified 24 human zinc transporters, consisting of 10 *SLC30* and 14 *SLC39* family members [35, 36]. In general, ZnT and Zip family members have opposing roles in regulating cellular zinc homeostasis. ZnT transporters reduce cytosolic zinc bioavailability by promoting zinc efflux in conditions of excess, while Zip transporters function by increasing cytosolic zinc during deficient states. Members of both families exhibit tissue and cell specific expression and possess differential responsiveness to dietary zinc as well as to physiologic stimuli including cytokines [35, 37]. The expression of zinc transporters has been profiled in immune cell types and their regulation plays an important role in specific immune cell activation. Cousins and colleagues evaluated the gene expression of zinc transporters in peripheral blood monocytes, T-lymphocytes, and granulocytes following modest dietary zinc supplementation in healthy young males [38]. They observed that zinc supplementation induced significant alterations in transcript abundance of individual transporter within different immune cell types and further, that considerable differences exist between immune cell populations (up to 270-fold). In particular, ZnT1 mRNA and metallothionein-1 (MT-I) expression increased with zinc supplementation, while expression of the importers Zip-1, -3 and -8 decreased. Most strikingly, zinc-mediated alteration in zinc transporter gene expression enhanced the response of different leukocyte subsets to relevant immune challenges. Specifically, activated monocytes, granulocytes, and T lymphocytes in zinc supplemented individuals had significantly increased TNFα, IL1β, and IFNγ transcript levels, respectively. Overbeck et al similarly observed that intracellular zinc homeostasis in different leukocyte subsets is regulated by distinct patterns of zinc exporter expression, and postulated that this leads to different immune subsets having varying susceptibilities to zinc deficiency [39]. Further, recent data indicate that intracellular zinc signaling is involved in immune cells activation, and is regulated via changes in the expression of zinc transporters (to be discussed in further detail in next section) [40–42]. Thus alterations in zinc transporter expression are intimately linked to zinc homeostasis and immune function, and dysregulation of zinc transporter expression may contribute to low zinc status and immune dysfunction observed with aging. Zinc imbalance and perturbed expression of zinc transporters have also been associated with various age-related chronic diseases. Alterations of zinc transporters ZnT1, ZnT4, and ZnT6 have been reported in the brains of preclinical Alzheimer's disease subjects [43]. In diabetes, disease susceptibility is associated with reduced zinc status and zinc transporter dysregulation, and zinc supplementation is protective against both Type 1 and Type 2 diabetes [44]. In pancreatic islets, insulin production by β-cells is tightly regulated ZnT8. It is postulated that changes in ZnT8 abundance or zinc transport activity will affect zinc accumulation as well as insulin storage and release, resulting in impaired glucose-induced insulin response and promote progression from glucose intolerance to diabetes [45]. Indeed, susceptibility to Type 2 diabetes is associated with ZnT8, and interestingly, ZnT8 is also a major autoantigen in autoimmune Type 1 diabetes [46, 47]. Thus alteration in the expression and regulation of zinc transporters is a potential contributing factor leading to age-associated decline in zinc status, immune dysfunction, and the development of age-related chronic diseases.

To date, little is known regarding the effects of age on the expression and function of zinc transporters. Age- related epigenetic changes has been proposed as one potential mechanism that may result in age-related dysregulation of zinc transporter expression with age (to be discussed in further detail in later section). Epigenetic alterations associated with aging includes a progressive decline in DNA methylation in cells and tissues (global DNA hypomethylation), coupled with site-specific promoter hypermethylation at select genes, resulting in gene silencing [48, 49]. It has been postulated that aberrant methylation of zinc transporter promoter regions may modulate the expression and function of select zinc transporters [50]. The effects of age on zinc transporter expression, particularly in immune tissues, remains to be investigated. This will help determine the underlying molecular mechanisms contributing to altered zinc metabolism during aging, and will clarify whether low zinc status observed with age results from diminished zinc absorption, and/or a redistribution of zinc to different tissues/cellular compartments due to aberrant zinc transporter expression and function.

3 Zinc in immune responses

It has been well established that zinc is necessary for the normal function of the immune system. The clinical manifestation of severe zinc deficiency is highlighted in patients suffering from acrodermatitis enteropathica (AE), a rare genetic disorder resulting in defective zinc uptake and severe zinc deficiency [51]. AE patients suffer from dermatitis, diarrhea, and growth retardation. In addition, AE patients have characteristic immune defects including thymic atrophy and lymphopenia. Both conditions lead to impaired host defense, resulting in recurrent viral, bacterial, and fungal infections [52]. Nutritional zinc deficiency results in similar immune dysfunction. For example, plasma zinc level in children is a predictor of diarrheal and respiratory morbidity [53]. Children with low plasma zinc concentration have increased diarrhea prevalence and severity, and have a higher mean prevalence rate of acute lower respiratory tract infections compared to children with normal plasma zinc. In hemodialysis patients, impaired immune response to Diphtheria vaccination is associated

with decreased serum zinc concentration [54]. In the elderly, patients with reduced zinc status have higher frequencies of respiratory infections [55–57] and gastrointestinal disease [58]. A multitude of defects in both adaptive and innate immunity contributes to impaired immune response resulting from zinc deficiency.

Changes in zinc status affect multiple immune cell types involved in both innate and adaptive immunity and has been extensively reviewed [9, 59]. Innate immunity is the immune mechanism that provides immediate but non-specific defense against pathogens and infections, and involves natural killer cells, mast cells, eosinophils, basophils, and phagocytic cells such as macrophages and neutrophils. In vivo, zinc deficiency has been shown to affect the functions of cells involved in innate immune response. For example, mouse peritoneal macrophages from zinc deficient mice have reduced phagocytosis and parasite killing capacities [60]. Zinc deficiency also reduces the activity of natural killer cells, and decreases chemotaxis and oxidative burst by neutrophils [12, 61]. In addition, zinc is involved in mast cell degranulation and cytokine production, and zinc depletion inhibits mast cell activation [62]. Zinc deficiency also affects immune cells involved in adaptive immunity. Induction of an adaptive immune response involves the recognition of antigen-specific lymphocytes to antigen, such as during viral infection or immunization, and the subsequent development of immunological memory [63, 64]. T and B cells are the primary mediators of adaptive immunity, and are involved in cell-mediated and antibody immune responses, respectively. Significant amount of work have demonstrated the importance of zinc in adaptive immunity [9]. Zinc deficiency causes a reduction in B cell lymphopoiesis resulting in reduced number of pre-B and immature B cells due to accelerated apoptosis, but the absolute numbers of mature B cells are not significantly affected. However, antibody response, particularly against T cell-dependent antigens is affected by zinc deficiency [65, 66]. Numerous defects are observed in the T cell compartment with zinc deficiency. T cell lymphopoiesis takes place in the thymus and is significantly affected by zinc deficiency. Thymic atrophy is a hallmark of zinc deficiency, caused by substantial loss of pre-T cells due to enhanced apoptosis [67]. The activity of thymulin, a hormone that regulates the differentiation of immature T cells in the thymus and the function of mature T cells in the periphery, is also decreased with zinc deficiency [68]. In the periphery, zinc deficient T cells have reduced cell proliferation upon mitogen stimulation, reduced precursor number and activity of $CD8^+$ cytotoxic T cells, and impaired $CD4^+$ T cells functions, resulting in altered T helper (Th) cells function, and an imbalance of Th1/Th2 cytokines secretion [69, 70]. While zinc deficiency results in the impairment of antibody-mediated and cell-mediated immune responses, increased autoreactivity has been observed in zinc deficient animals due to impaired immune tolerance and inefficient removal of autoreactive T cells [71].

Recent studies indicate that intracellular zinc homeostasis is critically involved in the signaling events in immune cells, and is regulated by changes in zinc transporter expression [42, 72]. Two classes of intracellular zinc signaling have been observed in immune cells, involving transcription-independent early zinc signaling in which intracellular zinc levels change rapidly (within minutes) upon stimulation, and transcription-dependent late zinc signaling where intracellular zinc levels are altered several hours after stimulation [10]. In T cells, the regulation of T cell receptor (TCR) signaling is controlled by activation-induced zinc influx, where cytoplasmic zinc concentration increases within 1 min after TCR engagement. Zinc influx is mediated by the zinc transporter Zip6, and Zip6 gene silencing inhibits T cell activation [42]. In monocytes, immediate increase in intracellular zinc was observed upon lipopolysaccharide (LPS) treatment, and preventing zinc influx with the zinc-specific chelator TPEN blocks LPS-induced monocyte activation [40]. The function of dendritic cells (DC), a potent antigen presenting cell that is involved in driving the inflammatory process, is also regulated by zinc homeostasis. In DC, a reduction in intracellular zinc mediated via downregulation of Zip6 and upregulation of ZnT1 expression, is required for upregulation of major histocompatibility complex class II and costimulatory molecules [41]. Alteration of zinc transporter expression and/or zinc homeostasis in immune cells may represent one mechanism by which age-associated zinc deficiency contributes to immune dysfunction. Mocchegiani and collaborators have postulated that intracellular zinc levels are compromised during aging due to increased expression of MT [73]. MT is a major storage protein for zinc, thus age-related increases in MT expression could results in increased sequestration of zinc. Taken together, zinc is intimately involved at multiple levels in the development and function of the immune system, including lymphopoiesis, the development of adaptive and innate immune response, and controlling intracellular signaling events and immune cell activation. It is noteworthy that immune dysfunction observed with age (thymic atrophy, reduced antibody and cellular immune responses, increased inflammation and autoimmunity) bear remarkable similarities to the hallmarks of zinc deficiency, and low zinc status associated with age is likely a contributing factor in age-related immune dysfunction.

4 Zinc as an anti-inflammatory agent

Similar to aging, the decline of adaptive and innate immunity with zinc deficiency is accompanied by a heightened proinflammatory response. In both in vitro and in vivo models, zinc deficiency has been associated with increases in inflammatory cytokines and other markers of inflammation. In an in vitro prostate cancer model, depletion of intracellular zinc increases the expression of

proinflammatory cytokines IL-6 and IL-8, and may contribute to tumor progression [74]. In a mouse model of polymicrobial sepsis, zinc deficiency increases systemic inflammation, resulting in increased organ damage and mortality [14]. In allergic airway inflammation, a murine model of asthma, zinc deficient mice have increased allergic inflammation, as determined by higher airway hyperresponsiveness and eosinophilia [75]. In addition, zinc deficiency also increases oxidative stress and DNA damage [76–82], which also play a role in promoting the inflammatory process. Zinc may modulate the proinflammatory response by targeting Nuclear Factor KappaB (NFκB), a transcription factor that is the master regulator of proinflammatory responses [83]. Activation of NFκB results in the phosphorylation and degradation of the inhibitory protein IκB mediated by IκB kinase, allowing nuclear translocation and subsequent nuclear binding of the NFκB complex to consensus sequences and the induction of gene transcription, including the production of proinflammatory cytokines. Both dietary zinc deficiency and the depletion of intracellular zinc with a zinc-specific chelator result in increased activation of NFκB, and the upregulation of NFκB-controlled proinflammatory cytokine expression [13, 74]. In aging, dysregulated NFκB signaling is proposed to be a culprit that result in age-related chronic inflammation [84, 85], and may potentially be exacerbated by low zinc status in the elderly.

While zinc deficiency results in systemic inflammation, zinc supplementation has been shown to have potent anti-inflammatory effects. For example, zinc supplementation significantly decreases proinflammatory immune cell infiltration into the lung during allergic inflammation, and normalizes Zip1 and Zip14 expression that are altered with acute inflammation [75]. In another study, short term zinc repletion reverses sepsis-induced proinflammatory response and reduces associated tissue damage in zinc deficient mice [14]. Moreover, zinc supplementation suppresses sepsis-induced NFκB activation [13]. The suppressive effects of zinc on NFκB activation is mediated by blocking IκB kinase activity and inhibiting the phosphorylation and degradation of IκB [86]. In addition, the ability of zinc supplementation to inhibit activation of NFκB may be related to the activation of A20, an endogenous negative regulator of NFκB [87, 88]. In humans, several zinc supplementation studies have shown to be associated with improved immune functions and decreased inflammation. For example, zinc supplementation in the elderly reduced the incidence of infections [23], increased delayed type hypersensitivity reaction [89, 90], and increased T cell function [91]. Moreover, zinc supplementation in humans exerts anti-inflammatory effects. Zinc supplementation has been associated with decreased inflammatory responses in populations susceptible to zinc deficiency, including sickle cell patients and in children [92, 93]. In the elderly, age-related increase in proinflammatory markers including C-reactive proteins and inflammatory cytokines, are corrected by zinc supplementation [94–96]. However, the beneficial effects of zinc supplementation on immune function in the elderly have been inconsistent. Some of the discrepancies may be attributed to differences in zinc dose and timing (for review see [12, 97]). It has also been postulated that polymorphisms in inflammatory genes such as IL-6, a pro-inflammatory cytokine and regulator of MT expression, may alter responses to zinc supplementation in the elderly [97]. This research suggests that genetic variation in pro-inflammatory genes such as IL-6 renders individuals more susceptible to intracellular zinc loss and these are the subjects that may benefit the most from zinc supplementation [98]. Regardless, improving zinc status in the elderly population may be of significant benefits in improving overall immune function, and reducing age-related inflammation and associated morbidities.

5 Zinc, aging and epigenetics

It is clear that nutrient-gene interactions may exist that alter an individual's susceptibility to zinc deficiency and associated immune defects with age. In addition, there are possible epigenome alterations that may play a role in age-related zinc decline. The role of epigenetic alteration during the aging process has gained increasing attention in recent years. Epigenetics is the study of the regulation of gene activity that is not dependent on nucleotide sequence; this may include heritable changes in gene activity and expression, but also long-term alterations in the transcriptional potential of a cell that are not heritable [99]. Epigenetic phenomena include, but are not limited to, covalent modifications of histones, methylation of cytosines in DNA, and gene regulation by non-coding RNA. These features are potentially reversible and affect the expression of genes. For example, methylation of CpG islands in promoter elements is a major epigenetic controlling event for gene silencing [99–101]. In recent years, it has become increasingly clear that in addition to genetic changes, the epigenome is equally as critical as the DNA to controlling gene expression and healthy human development. In the context of the immune system, epigenetic modifications, including DNA methylation and histone modifications, have been shown to control immune function [102]. In particular, epigenetic modifications appear to regulate the differentiation of various immune cell populations critical in the development, regulation, and maintenance of immune response, including memory T cells [103], T-helper cells [104], regulatory T cells [105], and DC [106]. Aberrant DNA methylation can lead to hyperactivation or silencing of genes while histone modifications can result in either transcriptional activation or repression, depending on the histone mark. Accumulating evidence indicates 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 [49, 107, 108]. Furthermore, alteration to the

epigenome has been proposed to be involved in the pathogenesis of various human diseases, including various age-related diseases such as chronic inflammation, autoimmunity, and cancer [3, 109–111]. For example, Agrawal et al recently demonstrated that epigenetic modifications in aged human DNA increase its immunogenicity and increases DC activation, and may be a potential mechanism leading to age-associated increase in autoimmune and proinflammatory responses [109]. In another study, promoter methylation status of IL-6 in rheumatoid arthritis patients has been shown to be significantly hypomethylated compared to control subjects, indicating that differential methylation may be involved in IL-6 regulation and disease pathogenesis [112]. In addition to age-related epigenetic modifications, specific nutrients can also modulate epigenetic regulation and alter disease susceptibility. Several studies revealed that maternal exposures to nutritional deficiencies lead to significant and persistent effects in their offspring. For example, studies using data from the WWII Dutch winter famine found that under- nutrition during the mid to late gestational periods was associated with increased risk of glucose intolerance and diabetes in middle age [113, 114]. Importantly, maternal zinc deficiency contributes to an increased risk for several chronic diseases, such as metabolic syndrome and diabetes later in life in the offspring [115, 116]. Marginal zinc deficiency in pregnant rats resulted in sustained defects in immune function in the offspring, that do not readily reverse with zinc supplementation and the defect can last through several generations [117]. Maternal zinc deficiency also caused persistent defects in insulin secretion and glucose intolerance in the offspring of rats. This work has prompted interest in the possibility that nutritional deficiencies, such as zinc, may cause epigenetic modifications resulting in ''fetal programming'' and increased susceptibility to disease.

The link of zinc deficiency to alterations in the epigenome is apparent with several epigenetic mechanisms. In particular, the strongest evidence is for the role of zinc in DNA methylation. Methylation of cytosines in CpG rich DNA is catalyzed by DNA methyltransferase (DNMT) enzymes. Emerging data suggests a requirement for zinc in pathways that both create and regulate DNA methylation and histone modification events. DNMTs, as well as chromatin modifying enzymes such as histone methyltransferase and histone deacetylases are zinc-containing enzymes [118, 119]. Zinc also acts as key cofactor in several enzymes involved in the methionine/transsulfuration pathway; a key pathway for generating methyl donation equivalents such as S-adenosyl-methionine and betaine. Betaine-homocysteine methyltransferase and methionine synthase are also zinc enzymes [120]. Serine hydroxymethyltransferase, a key enzyme in folate metabolism and helps transfer methyl units from serine into the methionine cycle is regulated by zinc-dependent transcription factors including metal-regulatory transcription factor 1 (MTF-1). Together this strongly suggests that zinc likely has an important function in maintaining methylation status in the cell. Thus, zinc deficiency may cause a methyl deficiency, similar to other methyl donors like folate, resulting abnormal gene expression and developmental defects. In rats, zinc deficiency decreased turnover of S-adenosyl-methionine and depressed DNA and histone methylation in the liver [121]. In agouti mice, supplementation with methyl donors (choline, betaine, folate, B12, methionine) and zinc epigenetically regulated the expression of agouti in the offspring [122]. In addition, oxidative stress also can lead to alterations in DNA methylation [123]. Replacement of guanine with the oxygen radical adduct 8-hydroxyguanine markedly alters methylation of adjacent cytosines [124]. Interestingly, several zinc transporters such as ZnT5 and Zip8 contain CpG islands in their promoter elements and are susceptible to epigenetic regulation. For example, hypermethylation of the CpG island of Zip8 gene causes epigenetic silencing and down-regulation of Zip8 expression, resulting in a decrease in cadmium accumulation [125], and promoter methylation of ZnT5 is associated with reduced expression of an associated reporter gene [50]. Coneyworth et. al. have proposed that age-related methylation of ZnT5 contributes to the decline of zinc status with age and downstream health consequences [50]. It is possible that age-related epigenetic modifications, either with age itself or in combination with age-related zinc deficiency could impact key regulatory mechanisms that exacerbate intracellular zinc loss and immune dysregulation. In particular, epigenetic dysregulation of zinc transporters during the aging process may be one contributing factor resulting in age-related zinc deficiency. It is a likely possibility that age-related alterations in the epigenome contribute to age-related zinc decline, and together age and zinc deficiency cause additional epigenetic changes that result in immune dysfunction and other related age-related pathologies. Further delineation of age-related gene changes at the epigenome level, and their link to nutritional deficiencies is an emerging area of research.

6 Concluding remarks

Elderly individuals over 65 years of age represent 13% of the U.S. population; they are the most rapidly growing population segment. National surveys show that their dietary zinc intakes are inadequate. Coupled with impaired utilization of zinc with age, the elderly are a population highly susceptible to zinc deficiency and that many of their health problems are conditions associated with poor zinc nutriture. The importance of zinc in the normal development and function of the immune system and the negative impact of zinc deficiency on immune response is well established. Immune defects associated with aging, including immunosenescence and chronic inflammation, are remarkably similar to the hallmarks of zinc deficiency. Furthermore, imbalance in zinc homeostasis and zinc transporter dysregulation may be one of the underlying etiologies in

age-related diseases. Thus the decline in zinc status with age may play a significant role in immune dysfunction and the progression of age-related chronic diseases. However to date, the precise factors contributing to age-related zinc deficiency remain poorly defined. We postulate that alteration in the expression and regulation of zinc transporters, in particular epigenetic dysregulation, during the aging process is an important contributing factor towards age-associated decline in zinc status. As the elderly population increases, demands on the health care system and related costs will increase exponentially. The identification of the underlying molecular mechanisms by which zinc status alters immune function in the elderly population will aid in defining optimal zinc nutrition for this susceptible population and may help establish age-specific requirements for zinc, reduce disease burden, and improve the quality of life for the elderly.

7 References

[1] Gruver, A. L., Hudson, L. L., Sempowski, G. D., Immunosenescence of ageing. *J. Pathol.* 2007, *211*, 144–156.

[2] Franceschi, C., Capri, M., Monti, D., Giunta, S. et al., Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech. Ageing Dev.* 2007, *128*, 92–105.

[3] Provinciali, M., Barucca, A., Cardelli, M., Marchegiani, F., Pierpaoli, E., Inflammation, aging, and cancer vaccines. *Biogerontology* 2010, *11*, 615–626.

[4] Schetter, A. J., Heegaard, N. H., Harris, C. C., Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. *Carcinogenesis* 2010, *31*, 37–49.

[5] Kundu, J. K., Surh, Y. J., Inflammation: gearing the journey to cancer. *Mutation Res.* 2008, *659*, 15–30.

[6] Ahmad, A., Banerjee, S., Wang, Z., Kong, D. et al., Aging and inflammation: etiological culprits of cancer. *Curr. Aging Sci.* 2009, *2*, 174–186.

[7] Hussain, S. P., Harris, C. C., Inflammation and cancer: An ancient link with novel potentials. *Int. J. Cancer* 2007, *121*, 2373–2380.

[8] Fischer Walker, C., Black, R. E., Zinc and the risk for infectious disease. *Annu. Rev. Nutr.* 2004, *24*, 255–275.

[9] Fraker, P. J., King, L. E., Reprogramming of the immune system during zinc deficiency. *Annu. Rev. Nutr.* 2004, *24*, 277–298.

[10] Fukada, T., Yamasaki, S., Nishida, K., Murakami, M., Hirano, T., Zinc homeostasis and signaling in health and diseases : Zinc signaling. *J. Biol. Inorg. Chem.* 2011.

[11] Devirgiliis, C., Zalewski, P. D., Perozzi, G., Murgia, C., Zinc fluxes and zinc transporter genes in chronic diseases. *Mutation Res.* 2007, *622*, 84–93.

[12] Haase, H., Rink, L., The immune system and the impact of zinc during aging. *Immun. Ageing* 2009, *6*, 9.

[13] Bao, S., Liu, M. J., Lee, B., Besecker, B. et al., Zinc modulates the innate immune response in vivo to polymicrobial sepsis through regulation of NF-kappaB. *Am. J. Physiol.* 2010, *298*, L744–L754.

[14] Knoell, D. L., Julian, M. W., Bao, S., Besecker, B. et al., Zinc deficiency increases organ damage and mortality in a murine model of polymicrobial sepsis. *Crit. Care Med.* 2009, *37*, 1380–1388.

[15] Mares-Perlman, J. A., Subar, A. F., Block, G., Greger, J. L., Luby, M. H., Zinc intake and sources in the US adult population: 1976-1980. *J. Am. Coll. Nutr.* 1995, *14*, 349-357.

[16] Ma, J., Betts, N. M., Zinc and copper intakes and their major food sources for older adults in the 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII). *J. Nutr.* 2000, *130*, 2838–2843.

[17] Ervin, R. B., Kennedy-Stephenson, J., Mineral intakes of elderly adult supplement and non–supplement users in the third national health and nutrition examination survey. *J. Nutr.* 2002, *132*, 3422-3427.

[18] Prasad, A. S., Fitzgerald, J. T., Hess, J. W., Kaplan, J. et al., Zinc deficiency in elderly patients. *Nutrition* 1993, *9*, 218–224.

[19] Institute of Medicine, P. o. M., *Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, National Academy Press, Washington, DC 2002.

[20] Hotz, C., Peerson, J. M., Brown, K. H., Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976-1980). *Am. J. Clin. Nutr.* 2003, *78*, 756–764.

[21] Ravaglia, G., Forti, P., Maioli, F., Nesi, B. et al., Blood micronutrient and thyroid hormone concentrations in the oldest-old. *J. Clin. Endocrinol. Metab.* 2000, *85*, 2260–2265.

[22] Cakman, I., Rohwer, J., Schutz, R. M., Kirchner, H., Rink, L., Dysregulation between TH1 and TH2 T cell subpopulations in the elderly. *Mech Ageing Dev.* 1996, *87*, 197–209.

[23] Prasad, A. S., Beck, F. W., Bao, B., Fitzgerald, J. T. et al., Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am. J. Clin. Nutr.* 2007, *85*, 837–844.

[24] Kim, J., Paik, H. Y., Joung, H., Woodhouse, L. R. et al., Effect of dietary phytate on zinc homeostasis in young and elderly Korean women. *J. Am. Coll. Nutr.* 2007, *26*, 1–9.

[25] Prasad, A. S., Zinc: Role in immunity, oxidative stress and chronic inflammation. *Curr. Opin. Clin. Nutr. Metabol Care* 2009, *12*, 646–652.

[26] Barnett, J. B., Hamer, D. H., Meydani, S. N., Low zinc status: a new risk factor for pneumonia in the elderly? *Nutr. Rev.* 2010, *68*, 30–37.

[27] Turnlund, J. R., Durkin, N., Costa, F., Margen, S., Stable isotope studies of zinc absorption and retention in young and elderly men. *J. Nutr.* 1986, *116*, 1239–1247.

[28] August, D., Janghorbani, M., Young, V. R., Determination of zinc and copper absorption at three dietary Zn-Cu ratios by using stable isotope methods in young adults and elderly subjects. *Am. J. Clin. Nutr.* 1989, *50*, 1457–1463.

[29] Couzy, F., Kastenmayer, P., Mansourian, R., Guinchard, S. et al., Zinc absorption in healthy elderly humans and the effect of diet. *Am. J. Clin. Nutr.* 1993, *58*, 690–694.

[30] Kim, J., Paik, H. Y., Joung, H., Woodhouse, L. R. et al., Zinc supplementation reduces fractional zinc absorption in young and elderly korean women. *J. Am. Coll. Nutr.* 2004, *23*, 309–315.

[31] Ali, S. A., Lowe, N. M., Jack, C. I. A., Reid, M. D. et al., Zinc absorption in the healthy elderly. *Unpublished data*.

[32] Wastney, M. E., Ahmed, S., Henkin, R. I., Changes in regulation of human zinc metabolism with age. *Am. J. Physiol.* 1992, *263*, R1162–R1168.

[33] Coudray, C., Feillet-Coudray, C., Rambeau, M., Tressol, J. C. et al., The effect of aging on intestinal absorption and status of calcium, magnesium, zinc, and copper in rats: a stable isotope study. *J. Trace Elem. Med. Biol.* 2006, *20*, 73–81.

[34] Mooradian, A. D., Song, M. K., The intestinal zinc transport in aged rats. *Mechanisms Ageing Dev.* 1987, *41*, 189–197.

[35] Liuzzi, J. P., Cousins, R. J., Mammalian zinc transporters. *Annu. Rev. Nutr.* 2004, *24*, 151–172.

[36] Eide, D. J., The SLC39 family of metal ion transporters. *Pflugers Arch.* 2004, *447*, 796–800.

[37] Kambe, T., Yamaguchi-Iwai, Y., Sasaki, R., Nagao, M., Overview of mammalian zinc transporters. *Cell. Mol. Life. Sci.* 2004, *61*, 49–68.

[38] Aydemir, T. B., Blanchard, R. K., Cousins, R. J., Zinc supplementation of young men alters metallothionein, zinc transporter, and cytokine gene expression in leukocyte populations. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 1699–1704.

[39] Overbeck, S., Uciechowski, P., Ackland, M. L., Ford, D., Rink, L., Intracellular zinc homeostasis in leukocyte subsets is regulated by different expression of zinc exporters ZnT-1 to ZnT-9. *J. Leuko. Biol.* 2008, *83*, 368–380.

[40] Haase, H., Ober-Blobaum, J. L., Engelhardt, G., Hebel, S. et al., Zinc signals are essential for lipopolysaccharide-induced signal transduction in monocytes. *J. Immunol.* 2008, *181*, 6491–6502.

[41] Kitamura, H., Morikawa, H., Kamon, H., Iguchi, M. et al., Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. *Nat. Immunol.* 2006, *7*, 971–977.

[42] Yu, M., Lee, W. W., Tomar, D., Pryshchep, S. et al., Regulation of T cell receptor signaling by activation-induced zinc influx. *J. Exp. Med.* 2011, *208*, 775–785.

[43] Lyubartseva, G., Smith, J. L., Markesbery, W. R., Lovell, M. A., Alterations of zinc transporter proteins ZnT-1, ZnT-4 and ZnT-6 in preclinical Alzheimer's disease brain. *Brain Pathol.* 2009, *20*, 343–350.

[44] Taylor, C. G., Zinc, the pancreas, and diabetes: Insights from rodent studies and future directions. *Biometals* 2005, *18*, 305–312.

[45] Chistiakov, D. A., Voronova, N. V., Zn(2+)-transporter-8: A dual role in diabetes. *BioFactors (Oxford, England)* 2009, *35*, 356–363.

[46] Sladek, R., Rocheleau, G., Rung, J., Dina, C. et al., A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 2007, *445*, 881–885.

[47] Wenzlau, J. M., Juhl, K., Yu, L., Moua, O. et al., The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc. Natl. Acad. Sci. USA* 2007, *104*, 17040–17045.

[48] Issa, J. P., Age-related epigenetic changes and the immune system. *Clinical Immunol.* 2003, *109*, 103–108.

[49] Maegawa, S., Hinkal, G., Kim, H. S., Shen, L. et al., Widespread and tissue specific age-related DNA methylation changes in mice. *Genome Res.* 2010, *20*, 332–340.

[50] Coneyworth, L. J., Mathers, J. C., Ford, D., Does promoter methylation of the SLC30A5 (ZnT5) zinc transporter gene contribute to the ageing-related decline in zinc status? *Proc. Nutr. Soc.* 2009, *68*, 142–147.

[51] Van Wouwe, J. P., Clinical and laboratory diagnosis of acrodermatitis enteropathica. *Eur. J. Pediatr.* 1989, *149*, 2–8.

[52] Fraker, P. J., King, L. E., Laakko, T., Vollmer, T. L., The dynamic link between the integrity of the immune system and zinc status. *J. Nutr.* 2000, *130*, 1399S–1406S.

[53] Bahl, R., Bhandari, N., Hambidge, K. M., Bhan, M. K., Plasma zinc as a predictor of diarrheal and respiratory morbidity in children in an urban slum setting. *Am. J. Clin. Nutr.* 1998, *68*, 414S–417S.

[54] Kreft, B., Fischer, A., Kruger, S., Sack, K. et al., The impaired immune response to diphtheria vaccination in elderly chronic hemodialysis patients is related to zinc deficiency. *Biogerontology* 2000, *1*, 61–66.

[55] Hamer, D. H., Sempertegui, F., Estrella, B., Tucker, K. L. et al., Micronutrient deficiencies are associated with impaired immune response and higher burden of respiratory infections in elderly Ecuadorians. *J. Nut.* 2009, *139*, 113–119.

[56] Meydani, S. N., Barnett, J. B., Dallal, G. E., Fine, B. C. et al., Serum zinc and pneumonia in nursing home elderly. *Am. J. Clin. Nutr.* 2007, *86*, 1167–1173.

[57] Meydani, S. N., Hamer, D. H., Zinc supplementation in elderly nursing home residents. *Clin. Infect. Dis.* 2009, *49*, 479.

[58] Pepersack, T., Rotsaert, P., Benoit, F., Willems, D. et al., Prevalence of zinc deficiency and its clinical relevance among hospitalised elderly. *Arch. Gerontol. Geriatr.* 2001, *33*, 243–253.

[59] Ibs, K. H., Rink, L., Zinc-altered immune function. *J. Nutr.* 2003, *133*, 1452S–1456S.

[60] Wirth, J. J., Fraker, P. J., Kierszenbaum, F., Zinc requirement for macrophage function: effect of zinc deficiency on

uptake and killing of a protozoan parasite. *Immunology* 1989, *68*, 114–119.

[61] Allen, J. I., Perri, R. T., McClain, C. J., Kay, N. E., Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. *J. Lab. Clin. Med.* 1983, *102*, 577–589.

[62] Kabu, K., Yamasaki, S., Kamimura, D., Ito, Y. et al., Zinc is required for Fc epsilon RI-mediated mast cell activation. *J. Immunol.* 2006, *177*, 1296–1305.

[63] McHeyzer-Williams, L. J., McHeyzer-Williams, M. G., Antigen-specific memory B cell development. *Annu. Rev. Immunol.* 2005, *23*, 487–513.

[64] Castellino, F., Germain, R. N., Cooperation between CD4+ and CD8+ T cells: when, where, and how. *Annu. Rev. Immunol.* 2006, *24*, 519–540.

[65] DePasquale-Jardieu, P., Fraker, P. J., Interference in the development of a secondary immune response in mice by zinc deprivation: persistence of effects. *J. Nutr.* 1984, *114*, 1762–1769.

[66] Moulder, K., Steward, M. W., Experimental zinc deficiency: Effects on cellular responses and the affinity of humoral antibody. *Clin. Exp. Immunol.* 1989, *77*, 269–274.

[67] Fraker, P. J., King, L. E., A distinct role for apoptosis in the changes in lymphopoiesis and myelopoiesis created by deficiencies in zinc. *Faseb J.* 2001, *15*, 2572–2578.

[68] Prasad, A. S., Meftah, S., Abdallah, J., Kaplan, J. et al., Serum thymulin in human zinc deficiency. *J. Clin. Invest.* 1988, *82*, 1202–1210.

[69] Beck, F. W., Prasad, A. S., Kaplan, J., Fitzgerald, J. T., Brewer, G. J., Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. *Am. J. Physiol.* 1997, *272*, E1002–E1007.

[70] Dowd, P. S., Kelleher, J., Guillou, P. J., T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. *Brit. J. Nutr.* 1986, *55*, 59–69.

[71] Finamore, A., Roselli, M., Merendino, N., Nobili, F. et al., Zinc deficiency suppresses the development of oral tolerance in rats. *J. Nutr.* 2003, *133*, 191–198.

[72] Murakami, M., Hirano, T., Intracellular zinc homeostasis and zinc signaling. *Cancer Sci.* 2008, *99*, 1515–1522.

[73] Mocchegiani, E., Giacconi, R., Cipriano, C., Costarelli, L. et al., Zinc, metallothioneins, and longevity--effect of zinc supplementation: zincage study. *Ann. N. Y. Acad. Sci.* 2007, *1119*, 129–146.

[74] Golovine, K., Uzzo, R. G., Makhov, P., Crispen, P. L. et al., Depletion of intracellular zinc increases expression of tumorigenic cytokines VEGF, IL-6 and IL-8 in prostate cancer cells via NF-kappaB-dependent pathway. *The Prostate* 2008, *68*, 1443–1449.

[75] Lang, C., Murgia, C., Leong, M., Tan, L. W. et al., Anti-inflammatory effects of zinc and alterations in zinc transporter mRNA in mouse models of allergic inflammation. *Am. J. Physiol.* 2007, *292*, L577–L584.

[76] Song, Y., Chung, C. S., Bruno, R. S., Traber, M. G. et al., Dietary zinc restriction and repletion affects DNA integrity in healthy men. *Am. J. Clin. Nutr.* 2009, *90*, 321–328.

[77] Sakaguchi, S., Iizuka,Y., Furusawa, S., Ishikawa, M. et al., Role of Zn(2+) in oxidative stress caused by endotoxin challenge. *Eur. J. Pharmacol.* 2002, *451*, 309–316.

[78] Taylor, C. G., Bray, T. M., Effect of hyperoxia on oxygen free radical defense enzymes in the lung of zinc-deficient rats. *J. Nutr.* 1991, *121*, 460–466.

[79] Yousef, M. I., El-Hendy, H. A., El-Demerdash, F. M., Elagamy, E. I., Dietary zinc deficiency induced-changes in the activity of enzymes and the levels of free radicals, lipids and protein electrophoretic behavior in growing rats. *Toxicology* 2002, *175*, 223–234.

[80] Shaheen, A. A., el-Fattah, A. A., Effect of dietary zinc on lipid peroxidation, glutathione, protein thiols levels and superoxide dismutase activity in rat tissues. *Int. J. Biochem. Cell Biol.* 1995, *27*, 89–95.

[81] Canali, R., Vignolini, F., Nobili, F., Mengheri, E., Reduction of oxidative stress and cytokine-induced neutrophil chemoattractant (CINC) expression by red wine polyphenols in zinc deficiency induced intestinal damage of rat. *Free Radic. Biol. Med.* 2000, *28*, 1661–1670.

[82] Song, Y., Leonard, S. W., Traber, M. G., Ho, E., Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. *J. Nutr.* 2009, *139*, 1626–1631.

[83] Hayden, M. S., Ghosh, S., Shared principles in NF-kappaB signaling. *Cell* 2008, *132*, 344–362.

[84] Chung, H. Y., Cesari, M., Anton, S., Marzetti, E. et al., Molecular inflammation: underpinnings of aging and age-related diseases. *Ageing Res. Rev.* 2009, *8*, 18–30.

[85] Salminen, A., Huuskonen, J., Ojala, J., Kauppinen, A. et al., Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. *Ageing Res. Rev.* 2008, *7*, 83–105.

[86] Jeon, K. I., Jeong, J. Y., Jue, D. M., Thiol-reactive metal compounds inhibit NF-kappa B activation by blocking I kappa B kinase. *J. Immunol.* 2000, *164*, 5981–5989.

[87] Prasad, A. S., Bao, B., Beck, F. W., Sarkar, F. H., Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-kappaB. *Nutrition* 2011, *27*, 816–823.

[88] Ho, E., Quan, N., Tsai, Y. H., Lai, W., Bray, T. M., Dietary zinc supplementation inhibits NFkappaB activation and protects against chemically induced diabetes in CD1 mice. *Exp. Biol. Med. (Maywood)* 2001, *226*, 103–111.

[89] Cossack, Z. T., T-lymphocyte dysfunction in the elderly associated with zinc deficiency and subnormal nucleoside phosphorylase activity: effect of zinc supplementation. *Eur. J. Cancer Clin. Oncol.* 1989, *25*, 973–976.

[90] Duchateau, J., Delepesse, G., Vrijens, R., Collet, H., Beneficial effects of oral zinc supplementation on the immune response of old people. *Am. J. Med.* 1981, *70*, 1001–1004.

[91] Fortes, C., Forastiere, F., Agabiti, N., Fano, V. et al., The effect of zinc and vitamin A supplementation on immune

response in an older population. *J. Am. Ger. Soc.* 1998, *46*, 19–26.

[92] Bao, B., Prasad, A. S., Beck, F. W., Snell, D. et al., Zinc supplementation decreases oxidative stress, incidence of infection, and generation of inflammatory cytokines in sickle cell disease patients. *Transl. Res.* 2008, *152*, 67–80.

[93] Sandstead, H. H., Prasad, A. S., Penland, J. G., Beck, F. W. et al., Zinc deficiency in Mexican American children: influence of zinc and other micronutrients on T cells, cytokines, and antiinflammatory plasma proteins. *Am. J. Clin. Nutr.* 2008, *88*, 1067–1073.

[94] Bao, B., Prasad, A. S., Beck, F. W., Fitzgerald, J. T. et al., Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. *Am. J. Clin. Nutr.* 2010, *91*, 1634–1641.

[95] Kahmann, L., Uciechowski, P., Warmuth, S., Plumakers, B. et al., Zinc supplementation in the elderly reduces spontaneous inflammatory cytokine release and restores T cell functions. *Rejuv. Res.* 2008, *11*, 227–237.

[96] Mariani, E., Cattini, L., Neri, S., Malavolta, M. et al., Simultaneous evaluation of circulating chemokine and cytokine profiles in elderly subjects by multiplex technology: relationship with zinc status. *Biogerontology* 2006, *7*, 449–459.

[97] Mocchegiani, E., Malavolta, M., Costarelli, L., Giacconi, R. et al., Zinc, metallothioneins and immunosenescence. *Proc. Nutr. Soc.* 2010, *69*, 290–299.

[98] Mocchegiani, E., Giacconi, R., Costarelli, L., Muti, E. et al., Zinc deficiency and IL-6 -174G/C polymorphism in old people from different European countries: effect of zinc supplementation. *ZINCAGE study. Exp. Gerontol.* 2008, *43*, 433–444.

[99] Wolffe, A. P., Matzke, M. A., Epigenetics: Regulation through repression. *Science* 1999, *286*, 481–486.

[100] Turker, M. S., Bestor, T. H., Formation of methylation patterns in the mammalian genome. *Mutation Res.* 1997, *386*, 119–130.

[101] Jones, P. A., Baylin, S. B., The fundamental role of epigenetic events in cancer. *Nature Rev.* 2002, *3*, 415–428.

[102] van den Elsen, P. J., van Eggermond, M. C., Wierda, R. J., Epigenetic control in immune function. *Adv. Exp. Med. Biol. 711*, 36–49.

[103] Northrop, J. K., Thomas, R. M., Wells, A. D., Shen, H., Epigenetic remodeling of the IL-2 and IFN-gamma loci in memory CD8 T cells is influenced by CD4 T cells. *J. Immunol.* 2006, *177*, 1062–1069.

[104] Wilson, C. B., Rowell, E., Sekimata, M., Epigenetic control of T-helper-cell differentiation. *Nat. Rev. Immunol.* 2009, *9*, 91–105.

[105] Lal, G., Bromberg, J. S., Epigenetic mechanisms of regulation of Foxp3 expression. *Blood* 2009, *114*, 3727–3735.

[106] Bluml, S., Zupkovitz, G., Kirchberger, S., Seyerl, M. et al., Epigenetic regulation of dendritic cell differentiation and function by oxidized phospholipids. *Blood* 2009, *114*, 5481–5489.

[107] aKawakami, K., Nakamura, A., Ishigami, A., Goto, S., Takahashi, R., Age-related difference of site-specific histone modifications in rat liver. *Biogerontology* 2009, *10*, 415–421.

[108] Grolleau-Julius, A., Ray, D., Yung, R. L., The role of epigenetics in aging and autoimmunity. *Clin. Rev. Allergy Immunol.* 2009, *39*, 42–50.

[109] Agrawal, A., Tay, J., Yang, G. E., Agrawal, S., Gupta, S., Age-associated epigenetic modifications in human DNA increase its immunogenicity. *Aging* 2010, *2*, 93–100.

[110] Valinluck, V., Sowers, L. C., Inflammation-mediated cytosine damage: a mechanistic link between inflammation and the epigenetic alterations in human cancers. *Cancer Res.* 2007, *67*, 5583–5586.

[111] Issa, J. P., CpG-island methylation in aging and cancer. *Curr. Top. Microbiol. Immunol.* 2000, *249*, 101–118.

[112] Nile, C. J., Read, R. C., Akil, M., Duff, G. W., Wilson, A. G., Methylation status of a single CpG site in the IL6 promoter is related to IL6 messenger RNA levels and rheumatoid arthritis. *Arthritis Rheumatism* 2008, *58*, 2686–2693.

[113] Ravelli, A. C., van der Meulen, J. H., Michels, R. P., Osmond, C. et al., Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998, *351*, 173–177.

[114] Roseboom, T. J., van der Meulen, J. H., Ravelli, A. C., Osmond, C. et al., Effects of prenatal exposure to the Dutch famine on adult disease in later life: An overview. *Mol. Cell. Endocrin.* 2001, *185*, 93–98.

[115] Maret, W., Sandstead, H. H., Possiblea roles of zinc nutriture in the fetal origins of disease. *Exp. Gerontol.* 2008, *43*, 378–381.

[116] Keen, C. L., Hanna, L. A., Lanoue, L., Uriu-Adams, J. Y. et al., Developmental consequences of trace mineral deficiencies in rodents: acute and long-term effects. *J. Nutr.* 2003, *133*, 1477S–1480S.

[117] Beach, R. S., Gershwin, M. E., Hurley, L. S., Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. *Science* 1982, *218*, 469–471.

[118] Fatemi, M., Hermann, A., Pradhan, S., Jeltsch, A., The activity of the murine DNA methyltransferase Dnmt1 is controlled by interaction of the catalytic domain with the N-terminal part of the enzyme leading to an allosteric activation of the enzyme after binding to methylated DNA. *J. Mol. Biol.* 2001, *309*, 1189–1199.

[119] Somoza, J. R., Skene, R. J., Katz, B. A., Mol, C. et al., Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. *Structure* 2004, *12*, 1325–1334.

[120] Evans, J. C., Huddler, D. P., Jiracek, J., Castro, C. et al., Betaine-homocysteine methyltransferase: zinc in a distorted barrel. *Structure* 2002, *10*, 1159–1171.

[121] Wallwork, J. C., Duerre, J. A., Effect of zinc deficiency on methionine metabolism, methylation reactions and

protein synthesis in isolated perfused rat liver. *J. Nutr.* 1985, *115*, 252–262.

[122] Wolff, G. L., Kodell, R. L., Moore, S. R., Cooney, C. A., Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *Faseb J.* 1998, *12*, 949–957.

[123] Hitchler, M. J., Domann, F. E., An epigenetic perspective on the free radical theory of development. *Free Radic. Biol. Med.* 2007, *43*, 1023–1036.

[124] Franco, R., Schoneveld, O., Georgakilas, A. G., Panayiotidis, M. I., Oxidative stress, DNA methylation and carcinogenesis. *Cancer Lett.* 2008, *266*, 6–11.

[125] Fujishiro, H., Okugaki, S., Yasumitsu, S., Enomoto, S., Himeno, S., Involvement of DNA hypermethylation in down-regulation of the zinc transporter ZIP8 in cadmium-resistant metallothionein-null cells. *Toxicol. Appl. Pharmacol.* 2009, *241*, 195–201.

