The importance of dietary and environmental zinc for human health can be ignored only at significant peril to child well-being throughout the world

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Update on Zinc Biology by Noel W. Solomons

Key insights

Zinc is a cardinal element with a profound impact on public health, and zinc status is emerging as a key parameter that dictates human physiology and disease. Deficiency of zinc is widespread and affects growth and development, organ function and immunity.

Current knowledge

Zinc is an essential trace mineral with critical roles as a structural component of proteins, an enzymatic co-factor, and transcriptional regulator in a wide array of cellular and biochemical processes. Indeed, 3% of the human genome consists of genes that encode for zinc finger proteins, underscoring the importance of zinc across a range of functions. Research on the roles of zinc-containing proteins is beginning to paint a clearer picture of zinc homeostasis, which in turn is leading to a greater understanding of various pathologies such as immune dysfunction and linear growth retardation.

Practical implications

The expanding body of research has led to new insights on the role of zinc, helping to clarify the mechanisms of bone growth and host defence against pathogens. In addition to its key roles in the development and regulation of innate and adaptive immunity, zinc is also a central element in the concept of 'nutritional immunity'. The importance of dietary zinc, therefore, underpins public health and is of particular importance in children.

Zinc is an essential element in a variety of processes during human growth and development, and in normal tissue function.

Recommended reading

Klug A: The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annu Rev Biochem* 2010;79:213–231.

Update on Zinc Biology

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Key Messages

- There is a growing understanding of the cellular and molecular function of zinc that explains the manifestations and limitations that occur with nutritional zinc insufficiency.
- Zinc participates in the regulation of genetic expression through its role in nuclear transcription factors called zinc finger proteins.
- Zinc has a role in elongation and maintenance of bone at the levels of the regulation of the hormonal axis and of signaling within the cellular elements of cartilage and bone.

Key Words

Zinc · Zinc fingers · Zinc transporters · Metallothioneins · Metalloenzymes

Abstract

Zinc has become a prominent nutrient of clinical and public health interest in the new millennium. Functions and actions for zinc emerge as increasingly ubiquitous in mammalian anatomy, physiology and metabolism. There is undoubtedly an underpinning in fundamental biology for all of the aspects of zinc in human health (clinical and epidemiological) in pediatric and public health practice. Unfortunately, basic science research may not have achieved a full understanding

as yet. As a complement to the applied themes in the companion articles, a selection of recent advances in the domains homeostatic regulation and transport of zinc is presented; they are integrated, in turn, with findings on genetic expression, intracellular signaling, immunity and host defense, and bone growth. The elements include ionic zinc, zinc transporters, metallothioneins, zinc metalloenzymes and zinc finger proteins. In emerging basic research, we find some plausible mechanistic explanations for delayed linear growth with zinc deficiency and increased infectious disease resistance with zinc supplementation.

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The Emergence of Zinc Nutrition

This supplement issue of *Annals of Nutrition and Metabolism* is dedicated to the topic of zinc nutrition in pediatrics and juvenile public health. This is an increasingly relevant theme in pediatric nutrition in the new millennium. To set the stage for contemporary reviews of clinical and epidemiological aspects of zinc, it is worthwhile to develop a wider and deeper perspective on recent advances in zinc biology. This is the aim of this first contribution.

The first indication of the essentiality of zinc as a nutrient came from the observation by the French physiologist, Raulin, in the mid-19th century that this element was necessary for the growth of *Aspergillus niger* (bread mold).

Early in the 20th century, studies at the University of Wisconsin established the requirement of zinc for adequate growth of laboratory rodents. It was not, however, until the middle of the same century that the chemical analysis of zinc had advanced to the point of feasible routine measurement in clinical contexts. A series of patients in Boston, suffering from alcoholic cirrhosis, were found to have exceptionally low circulating levels of zinc. At about the same time, in the Middle Eastern countries Iran and Egypt, international teams of investigators described a unique variety of dwarfism, associated with hypogonadism and delayed sexual maturation, in adolescent boys from nomadic groups moving among the oases of remote desert regions. Supplementation with zinc in the context of a balanced diet produced rapid linear growth recovery and advancement of puberty. In the 1970s, a relationship of zinc deficiency and the lethal infantile dermatological condition known as acrodermatitis enteropathica, with primary disorders in the skin and hair, on the one hand, and in gastrointestinal function, on the other hand, was recognized. A similar complex of findings was reported simultaneously in patients being maintained for prolonged periods of time with the fledgling technique of total (exclusive) parenteral alimentation providing an infusion of all energy and protein sources, without paying attention to micronutrients. Oral supplementation with zinc in acrodermatitis enteropathica and infusion of zinc in total parenteral alimentation resulted in resolution of the integumentary and malabsorptive lesions, with prolonged survival in both settings. It was in 1973 that the World Health Organization first ventured a detailed estimated dietary requirement for the nutrient [1]. The historical aspects of the discovery and emergence of zinc in biology and human nutrition have been recently reviewed [2, 3].

Historically, however, it has been hard for zinc to get traction in the public health domain. An anecdote from the personal experience of the author attests to this fact. This author, working in Guatemala on issues of zinc status and bioavailability beginning in the 1970s, was told by several officials of the Pan American Health Organizations in the mid-1980s that zinc was irrelevant to the public health concerns of the Latin American region. The decade of the 1980s was, however, becoming the age of 'hidden hunger' [4], that is, a renaissance of concern for micronutrient

malnutrition. Among the principle nutrients of interest were iron, iodine and vitamin A for pediatric nutrition, with folate emerging as a concern for women at a fertile age. It was only with the founding of the International

Zinc Nutrition Consultative Group (IZiNCG) in 2000, the first year of the new millennium, that the diverse threads of evidence of zinc as a pervasive and serious public health problem across the world began to be woven into a tapestry that captured the attention of global policy makers [5].

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Inorganic Chemistry of Zinc

Zinc is the 24th most abundant element in the earth's crust, at 0.004%. Along with iron, copper, manganese and nickel among others, zinc is a member of the transition metal series of the periodic table of elements. It has atomic number 30 and an atomic weight of 65.38, a combination of five stable isotopes of fixed natural abundance. Zinc has a totally filled *d* electronic orbital shell and two electrons in its outer *s* shell. As such, zinc is unique among transition metals for its robust stability in the divalent oxidation state as a cation (Zn⁺⁺). In this respect, it is neither an oxidizing nor reducing agent and does not participate in redox functions in physiology.

Zinc forms a number of salts and compounds. Zinc oxide is a whitish powder, poorly soluble in water at a neutral pH. Zinc sulfate forms bluish granules; it is highly soluble in water and has a strong metallic taste. Compounds of zinc with small molecules form the acetate or the gluconate. It will complex with chelators such as ethylenediaminetetraacetic acid (EDTA). Chelates with amino acids, as in the bisglycinate, are popular presentations for oral supplements.

Organic Chemistry of Zinc

The organic chemistry of zinc revolves primarily around its involvement in the configuration and function of an extensive set of enzymes and a growing variety of nuclear transcription factors.

Zinc Metalloproteins and Metalloenzymes

Zinc is an essential component of numerous proteins (zinc metalloproteins, zinc metalloenzymes). A pioneer

Table 1. Enzymes recently identified and characterized as zinc metalloenzymes

<i>JAB1/MPN/MOV34 metalloenzyme</i> [7] Catalyzes: Catalyzes the cleavages of ubiquitin-like small proteins from protein conjugates. Comment: Related proteins are found throughout living species.
<i>Cobalamin-dependent methionine synthase (MetH)</i> [8] Catalyzes: Catalyzes methyl transfer from N(5)-methyl-tetrahydrofolate to form homocysteine. Comment: A zinc ion is instrumental at the active site and has a role in dissociative homocysteine binding. Relevant to mammalian biology.
<i>UDP-3-O-((R)-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC)</i> [9] Catalyzes: Catalyzes the deacetylation of uridine diphosphate(UDP)-3-O-((R)-3-hydroxymyristoyl)-N-acetylglucosamine to form UDP-3-O-myristoylglucosamine and acetate. Comment: Alternative binding of divalent iron and divalent zinc. Relevant to Gram-negative bacteria.

in the field, Bert Vallee, provided a definition in 1969: 'Metalloenzymes are catalytically active metalloproteins that contain stoichiometric amounts of firmly bound, biologically active metal atoms' [6]. Their advent in the 1950s was met with the same skepticism with which a role for zinc in public health would later be received. Classical enzymologists of the day insisted that organic moieties could not have a stoichiometric relationship with metal ions and accused the discoverers of exogenous contamination of their chemical preparations during purification. Emerging techniques such as X-ray crystallography soon firmly confirmed the validity of metalloenzymes. Zinc is able to complex within the molecule with negatively charged moieties such as cysteine and histidine to provide structural bridges, maintaining the three-dimensional configuration of peptides.

Estimates concerning the amount of zinc metalloproteins in existence vary from 100 to 1,000, depending on how widely across living species one cares to include them for calculation. They are represented in all six classical classes of enzymes. Biochemists continue to isolate zinc metalloenzymes relevant to mammalian biology which conform with the Parisi-Vallee postulates [6]. Table 1 illustrates several of the enzymes most recently identified as zinc metalloenzymes in the continuing elucidation of their extent and functions in nature [7–9]. Meanwhile, studies on the active site of carbonic anhydrase, the first of the zinc metalloenzymes identified, and its mode of operation continue to advance six decades later [10].

Zinc Finger Proteins

The other important class of zinc proteins is that of 'zinc fingers' or zinc finger proteins (ZFPs), which are small but complex and convoluted peptides usually located in the cell nucleus. They were first recognized in the

mid-1980s as a DNA-binding motive in the nuclei of cells from the African clawed frog, *Xenopus laevis* [11, 12], with zinc ions complexing with cysteine and histidine residues in the peptide sequence. This gave a finger-like, three-dimensional configuration to the protein. It was progressively recognized, thereafter, that many combinations of the negatively charged amino acids (e.g. Cys₂His₂, Cys₄, Cys₆, etc.) could form complexes in ZFPs. Moreover, not all zinc-containing nuclear transcription factors necessarily had a 'finger-like' shape and form. Some were more like 'knuckles', whereas others were like twisted ribbon, and there was another series in the form of the treble clef sign on a music sheet. The propensity to bind to the genetic backbone of the cell nucleus was soon recognized to have functional implications for the regulation (expression or suppression) of the transcription of the genomic code to messenger RNA to form peptides [13].

Zinc Homeostasis: Regulation of Extra- and Intracellular Transport

Homeostasis is the physiological principle of an organism regulating systems to maintain them in optimal balance within the constant fluxes provoked by new exposures and ongoing losses. When the supply is scarce, the appropriate regulatory response is to increase the efficiency of acquisition and blunt losses; when the supply is excessive, the opposite responses are in order. Depending on the inherent critical essentiality or toxic potential of a nutrient, the homeostatic mechanisms can have more or less evolutionary fine tuning. Zinc homeostasis has some homologies with and some differences from the regulatory mechanisms of its fellow transition metal, iron. Considering the well-characterized system of the latter element [14], iron's strong oxidant nature and nutritive role

for dangerous pathogens requires that it be tightly controlled in absorption and cellular localization while at the same time being made safely available to the body and to its cells for the critical roles in oxidative metabolism. It is a common wisdom that zinc is much less dangerous, but every bit as essential, leading to a homeostatic system geared toward upregulating for availability and distribution. Indeed, the most important and relevant among many developments in the basic science of zinc biology has been the isolation, characterization and elucidation of the roles of the mechanistic elements of zinc homeostasis. This begins with the actual zinc transporters and binding proteins themselves [15, 16].

Zinc Transporters and Binders Transport Proteins

Two distinct but interacting classes of zinc transport proteins have been identified and studied extensively during the past decade in terms of the location of their genes, structure and sequence, putative functions and regulation by host zinc status, hormonal factors and interactions with other transporters or binders. As described in a comprehensive, classic review by Lichten and Cousins [15] in 2009, biologists have identified 24 distinct transporters across two classes. The first class is the zinc transporter (ZnT) class, whose family members are enumerated from ZnT1 to ZnT10. These tend to be involved in exporting zinc ions from a cytosolic location either into cellular organelles or out of cells themselves; they contain histidine residues as the complexing entities. The second class is that of the Zrt-, Irt-like protein (ZIP). This class has the opposite function: it transports the metal either from the extracellular space or the organellar lumen into the cytoplasm [15]. The explicit functions of many of the zinc transporters currently elude scientific understanding. For about half of the ZIP proteins and a handful of the ZnT transporters, physiological or pathophysiological roles and implications have been documented [15]. These are summarized in table 2.

Binding Proteins

The major intracellular binding protein for zinc is metallothionein (MT), a 61-amino acid peptide rich in sulfur-containing cysteine residues. The sulfur is the focal point of the complexing of divalent metal ions. Its physiological role is to regulate the levels of a number of divalent metals in mammals. The sulfhydryl-rich environment derives from up to 20 cysteine residues in the molecule; these complex a range of divalent metals, including copper, cadmium and mercury as well as zinc [17].

Table 2. Established functions of members of the zinc transport protein families

<i>Zinc transporter (ZnT)</i>	
ZIP4	intestinal zinc transport
ZIP5	intestinal zinc transport pancreatic release of endogenous zinc
ZIP6	metastatic breast cancer
ZIP7	metastatic breast cancer
ZIP10	metastatic breast cancer renal zinc reabsorption
ZIP14	hypo zincemia of inflammation
<i>Zrt-, Irt-like protein (ZIP)</i>	
ZnT1	intestinal zinc transport pancreatic release of endogenous zinc renal zinc reabsorption
ZnT2	pancreatic release of endogenous zinc breast milk zinc in lactation
ZnT8	insulin processing

As compiled from Lichten and Cousins [15].

There are four isotypes of MT proteins, with MT-1 and MT-2 present in all cells of the body. They regulate zinc and copper's intracellular levels and flux and detoxify heavy metals; MTs are involved in nuclear transcription and play a role in immune function through their sequestration of metals [18]. In addition, they have specific roles in the transport of metals through the intestinal mucosa. Not only can higher zinc levels within the cell induce MT synthesis, but so can other factors, such as p53 protein, metal-responsive transcription factor 1, and intracellular glutathione concentrations [19]. Finally, as pointed out by Lichten and Cousins [15], there is a co-regulation interaction involving members of the MT-1 family of binders and individual elements of the zinc transporter classes.

Intracellular Zinc Transport

Once in the body, cells have the ability to accumulate and concentrate extracellular metal ions. A zinc pool in individual cells and the transport related to maintaining and moving this pool are emerging concepts in zinc biology. This has been reviewed by Eide [20], who points out that, with the exception of specialized cells (cells of the prostate and neurons), 'there is remarkable similarity in the concentration of zinc; each corresponds to a total cellular zinc concentration of 0.1–0.5 mM' [20]. Once in the cells, zinc localizes in various organelles; it is present in metalloenzymes in the mitochondria. Many zinc metalloproteins are secreted or are resident within the secretory pathway, which naturally includes the organelles of

the endoplasmic reticulum, the Golgi body and the secretory vesicles.

It is generally assumed that ionic zinc must be involved in intracellular signaling. The exact concentration of 'free' zinc within cells is not precisely known but is believed to be in extraordinarily low concentrations (10^{-5} to 10^{-12} M), that is 2–8 times below the total concentration of primarily protein-bound zinc [20]. Eide [20] suggests two possible scenarios for an impact of ionic zinc: (1) either it has a kind of metal chaperone mechanism or (2) there is a 'toggle-switch' function; Eide favors the latter theory, in which there is a dysphasic nature to the uptake of zinc into the cell followed later by its export. This would allow for a dynamic and pulsatile cycle of overfilling and excessive emptying of the zinc pool. With initial uptake, 'free' zinc ions could theoretically accumulate to less extreme concentrations and plausibly act in signal transmission.

Intracellular Signaling

The fluxes of zinc, both across the cell and within the cell, set up signaling mechanisms, making it part of a role as a first or second messenger. This has been shown to be important in relation to insulin secretion in diabetes mellitus and its control primarily by ZnT8 in association with MT-1 and the zinc transporter ZnT8, which supplies pancreatic β cells with zinc. Zinc supplementation stimulates the expression of the transporter. Recent investigations in various laboratories [21, 22] provide evidence suggesting that zinc flux in the islet of Langerhans cells of the pancreas exercises an important influence on the secretion of insulin. This is opening up the possibility of oral zinc as an adjunct to glucose control in diabetes mellitus.

Finally, although a single cell line may be immortal, the exponential nature of cell proliferation requires a programmed and gene-directed cell death (apoptosis) in order to keep a stable number of cells in a compartment or tissue. Cellular apoptosis needs to be distinguished from injury-related cell death, known as necrosis. In an early recognition of intracellular signaling, Truong-Tran et al. [23] presented evidence that a specific pool (or pools) of intracellular labile zinc regulates apoptosis and that systemic changes in zinc levels in the body, as related to dietary intake, altered physiological states or disease, can influence cell susceptibility to apoptosis in a zinc-dependent manner.

Intestinal Absorption of Zinc

Unlike in iron, the intestinal absorption of zinc is determined by the current dietary intake of the nutrient, not by the host's status. The principle that whole-body zinc homeostasis is governed by the gastrointestinal tract's adjustment of the endogenous zinc losses to the amount absorbed is the basis of the contemporary estimates of human dietary zinc recommendations [24]. This relationship is not linear, and the net efficiency in zinc uptake decreases with increasing daily intake. This unique difference

in homeostatic regulation of zinc absorption can now increasingly be understood by recent revelations related to the cellular mechanisms in the enterocyte.

The zinc transporters in enterocytes, ZIP4 and ZnT1 [15], respond appropriately to

dietary zinc availability and are responsible for a saturable, energy-dependent and regulated uptake of zinc [25]. The former transporter is expressed on the luminal surface of the enterocyte, facilitating uptake from the lumen; the latter is located on the basolateral surface of the cell, involved in export of the nutrient into the body. When intraluminal concentrations of zinc rise to about 2 mM, corresponding to about 10 mg per day of zinc intake, a non-saturable, passive diffusion mechanism governs uptake [26]. Within the cell, excess zinc is sequestered by MT and lost into the feces with the desquamation of the enterocytes in normal turnover. Finally, the fact that competitive interaction in intestinal absorption exists for iron and zinc and copper and zinc implicates a role for another apical transport protein, divalent metal transporter 1 (DMT1) [26].

Zinc and Genetics: Locking and Unlocking the Genome

The transcendental biological event at the close of the last century was the deciphering of the human genome and of the genomes of other animal and plant species. The discovery of the double-helical structure of DNA in the 1950s had provided the clue as to how genetic information could be coded and translated into the structural representation in the sequence of the amino acids in peptides and proteins. Cracking the genome revealed the detailed extent of the hereditary information. A central role for zinc in the millisecond-to-millisecond interpretation and expression of genomic information is being elucidated.

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ZFPs in Transcriptional Regulation

The leader of the research group that uncovered ZFPs, Aaron Klug [27], has recently summarized almost two decades of discovery with a focus on their transcriptional regulation of information in the genome. Genes encoding the ZFP constitute 3% of the entire human genome. Klug [27] points out the evolutionary genius of the novel principle of DNA recognition embodied in the structural conformation of the ZFP: 'Whereas other DNA binding proteins generally make use of the two-fold symmetry of the double helix, zinc fingers can be linked linearly in tandem to recognize nucleic acid sequences of varying lengths. This modular design offers a large number of combinatorial possibilities for the specific recognition of DNA (or RNA)' [27]. As such, in association with the chromatin of the nucleus, the ZFP can interact with the genetic material, repressing or activating the transcription expression of mRNA to interpret the genome's coded protein-sequencing potential.

Swamynathan [28] reviews the roles of a specific, ubiquitous variety of ZFP to illustrate its extensive participation in the control of translation of genetic information into protein expression. The author remarks: 'Krüppel-like factors (KLFs), members of the zinc-finger family of transcription factors capable of binding GC-rich sequences, have emerged as critical regulators of important functions all over the body. They are characterized by a

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highly conserved C-terminal DNA-binding motif containing three C2H2 zinc-finger domains, with variable N-terminal regulatory domains' [28]. In the human genome alone, there are 17 known KLFs. He further illustrates their pervasiveness by listing almost a dozen developmental events or cellular processes regulated by the KLF class of ZFPs. These are outlined in table 3 [28].

Focusing on a single moiety, John and Garrett-Sinha [29] present an illustrative example of a ZFP with far-reaching transcriptional functions to interpret genomic information in many tissues and species, namely B lymphocyte-induced maturation protein 1 (Blimp-1). As discussed below, Blimp-1 is intimately related to human

Table 3. Examples of the developmental events or cellular processes regulated by the Krüppel-like zinc finger transcription factors

- Erythropoiesis
- Cardiac remodeling
- Adipogenesis
- Stem cell maintenance
- Epithelial barrier formation
- Control of cell proliferation and neoplasia
- Flow-mediated endothelial gene expression
- Skeletal and smooth muscle development
- Gluconeogenesis
- Monocyte activation
- Goblet cell development in intestine and conjunctiva
- Regeneration of retinal neurons
- Neonatal lung development

As compiled from Swamynathan [28].

Table 4. Comprehensive range of regulatory roles for the ZFP Blimp-1 beyond its primary function in plasma cell maturation

- Homeostasis of effector T cells (humans)
- Tumor suppression in germinal center-derived B cells (humans)
- Specification of primordial germ cells (mouse)
- Specification of muscle fiber type (zebrafish)

As compiled from John and Garrett-Sinha [29].

immunity, regulating terminal differentiation of B lymphocytes to antibody-secreting plasma cells. Its transcriptional function is generally that of a repressor. Table 4 outlines additional functions across biology [29].

ZFPs in Posttranscriptional Regulation

The gene-encoded transcription factor of Wilms' tumor 1 (WT1) is a protein with four classical Cys₂His₂ zinc fingers at the C-terminus end of the protein chain. As a conventional transcription factor, it has a number of recognized functions related to interacting, through its zinc finger, with nuclear DNA as a conventional placer in transcriptional regulation. There is recent evidence, however, that this same zinc finger configuration can bind and interact with selected mRNA targets. This represents an example of a (non-genomic) posttranscriptional regulatory role as well [30]. The authors conclude that 'WT1's complex roles in development and disease now need to be understood in terms of both DNA and mRNA targets' [30].

Zinc, Immunity and Host Defenses against Pathogens

Both epidemiological and clinical experiences indicate an important role of zinc in immunologically mediated host defense. Mechanistic insights at the cellular and nuclear level are helping to link the observations at the level of host disease resistance.

Zinc and B Lymphocytes

For decades, manipulation of zinc status in laboratory animals has altered the function of the B lymphocyte (bursa-derived) lines. Modern technology, including specific knock-out mice, is revealing more molecular detail, including the involvement of zinc in ZFPs. One recent example, among many, relates to early lymphoid development. Regulation mechanisms operate in the germinal centers to differentiate B and T cells and later to promote their maturation. A ZFP transcription factor, recently renamed as leukemia/lymphoma-related factor (LRF), forms an obligate dimer in the nucleus of B lymphocytes [31]. It is part of a larger zinc finger transcriptional repressor family and regulates mature B cell lineage fate and humoral immune responses via distinctive mechanisms [32].

In another example, the zinc finger goes to ‘zinc knuckles’ in the same domain of B cells for the DNA binding to target genes; this relates to ‘early B cell factor 1’ (EBF1), another transcription factor essential for both B lymphopoiesis and mature B cell function [33]. EBF1 is a requisite component of the B lymphocyte transcriptional network and is essential for specification of the B cell lineage. It is important for the differentiation of the line from stem cells through the immature progenitor stages to the mature lymphocyte [34].

There is also evidence that EBF1 is a regulating transcriptional factor, acting as a repressor for a major primary effector of immune function to the extreme maturation of the lymphocyte lineage. This refers to Blimp-1, the master gene for plasma cell differentiation. Blimp-1 is itself a ZFP and a repressor-type transcription factor. Plasma cells, of course, are the terminal cells that produce specific protective antibodies. Kikuchi et al. [34] summarize their conclusions: ‘These results suggest that EBF1 takes part in transcriptional regulations of the Blimp-1 gene in immature B cells, and may play a key role in B cell differentiation’ [34].

To close the circle from ZFPs interacting in lymphopoiesis to development, there is yet another example: zinc finger protein 521 (ZNF521), which is a 30-zinc finger moiety. It has been shown by Mega et al. [35] to control B cell development by inhibiting the activity of EBF1. This would act in the opposite direction of EBF1 by influencing plasma cell maturation; on the one hand, one has unopposed EBF1 acting towards suppression, and on the other hand, a counter-balance of ZNF521-opposed EBF1 influencing expression of maturation.

Zinc and T Lymphocytes

A critical involvement of a ZFP was discovered for T lymphocytes in a serendipitous manner. A transgenic mouse in a laboratory in San Francisco was found with a T cell system that did not develop beyond an early thymocyte stage [36]. The thymus gland itself was vestigial and hypocellular. The defect was localized on the mouse chromosomes and, using that guidance, the authors deduced that zinc finger BTB-POZ domain protein 1 (Zbtb1) was the actor in suppression. The sequence was confirmed by recapitulating the original phenotype in a knock-out mouse. Zbtb1 also appeared to have a minor role in the development of other lymphoid cells, including the B cells and the natural killer T (NKT) cell line.

Cytotoxic T cells are an important vigilante in adaptive immunity. This line has a role in host defense by attacking cells that are not recognized as ‘self’, that is as deriving from the host organism itself. This recognition is provided by the major histocompatibility complex (MHC) loci on the surface of cells, which present their antigenic evidence for compatibility (the epitope) for sampling by the cytotoxic T cells. Another class of zinc finger-like proteins, somewhat unique for their non-nuclear (cytosolic) location of action, affects the adaptive immune response. This family of endoplasmic reticulum aminopeptidase enzymes (ER aminopeptidases) derives its unique configurations from a complex with an ionic zinc [37]. Their essential role is to prepare the peptide fragments within the cell that will eventually become part of the epitope presentation on the cell’s outer-surface MHC system as the ‘calling card’ for self-recognition by the T cell surveillance.

Finally, the development of all of the functions of NKT cells is under the control of the transcriptional regulator

Both epidemiological and clinical experiences indicate an important role of zinc in immunologically mediated host defense.

promyelocytic leukemia zinc finger (PLZF) [38]. This includes their activation and their secretion of the various cytokine mediators. One of these cytokines, interleukin-4 (IL4), produced by PLZF-expressing cells, causes some CD8 T cells to take on the innate-like functional features of the NKT cells themselves.

Zinc and Nutritional Immunity

In addition to the innate immune system and the adaptive (acquired) immune system, to which both of the aforementioned lymphocyte classes belong, a mechanism called ‘nutritional immunity’ [39, 40] comes into play in the context of nutrients, their deficiency and regulatory responses. This phenomenon arises with nutrients that are essential both to potential pathogens and to the hosts. Nutritional immunity was originally conceived and described in terms of iron, and the microbes and parasites with high requirements for this element [39].

LeGrand and Alcock [41] have performed a sophisticated, novel analysis and update of the nutrient-dependent aspects of the acute-phase response (APR), mediated by cytokines. They focus on two of the components of APR: (1) fever and (2) the mediated sequestration of iron as well as that of zinc. They argue that there is a differential susceptibility of the host and the pathogen to the stressors, capable of causing harm to both the pathogen and the host. In what the authors term ‘immune brinksmanship’, they see an evolutionary basis for the mutually stressful components of the APR. They regard this as a coordinated system of mobilizing endogenous stressors through the APR. Stress increases expression of MT, diminishing circulating and cytosolic zinc concentrations and limiting its availability. In this analysis, the authors suggest that – as with iron – withholding zinc from pathogens is an additional component of the overall nutritional immunity domain of defense.

Expression profiling studies, using microarray analysis across the genome, have been conducted in cellular models related to pediatric septic shock, most commonly related to meningococemia [42]. This modeling predicts therapeutic promise in the opposite direction with respect to circulating zinc. The upregulation of the enzyme (metalloproteinase-8) which breaks down intracellular MT is a consequence of sepsis. Conserving cellular MT is seen as the protective response for survival. In the context

of the conjectures surrounding ‘nutritional immunity’ mentioned above, this would reinterpret the stereotypical decline in zinc as an incidental consequence of the accumulation of MT within the APR. This model identified zinc supplementation for the inhibition of the activity of this enzyme as a potential and novel therapeutic approach in sepsis [42].

Zinc and Bone Growth

All of the companion articles in this issue allude to zinc deficiency and linear growth retardation. The biology of bone growth is intimately linked to the potential for zinc deprivation to have a negative impact. Extreme short stature in humans (stunting) is characterized by the relative conservation of the dimensions of the head, neck and trunk, with a major contraction of the long bones of the lower extremities. Although bones require constituent nutrients, e.g. amino acids, calcium, phosphorus and magnesium, dysfunction in the well-described hormonal cascade for regulating bone formation at the growth plates [43] can limit bone growth. Growth hormone and insulin-like growth factor (IGF) are important messengers of the trophic signals leading to bone elongation. As recently shown in zinc-deficient children [44], IGF is sensitive to the zinc status of the host. Zinc supplementation corrected the hormonal axis and led to significant linear growth.

At the cellular level in the functional cells of bone, MT is important in regulating bone growth [45]. Zinc deficiency leads to lower chondrocyte proliferation, reduced metaphysis heights, along with increased osteoclast density. MT plays a role in the regulation of the zinc pool for bone growth. Thus, credible mechanisms underlie the limitation of dietary zinc as a factor for maximal bone growth during the crucial interval of early life.

Zinc has a potent stimulatory effect on osteoblastic bone formation and an inhibitory effect on osteoclastic bone resorption. The former has been demonstrated experimentally in cultured osteoblasts exposed to different levels of zinc, which was reflected by calcium deposition in association with increased alkaline phosphatase activity [46]. Yamaguchi and Weitzmann [47] designed a more complex experiment aimed at the bone resorption component, in which they examined the role of nuclear factor κ -light-chain enhancer of activated B cells (NF- κ B)

Withholding zinc from pathogens is an additional component of the overall nutritional immunity domain of defense.

as activated by the cytokine tumor necrosis factor α (TNF α). They summarize their findings as follows: 'Our data show that zinc suppressed osteoclast differentiation and promoted osteoblast mineralization and did indeed act as a potent NF- κ B activation antagonist in both osteoclast and osteoblast precursors. Importantly, zinc antagonized NF- κ B activation driven by TNF α , a potent inflammatory mediator of bone resorption and suppressor of bone formation in vitro and in vivo' [47].

Finally, the genetic regulation of the cellular elements of bone is the domain of transcription factors. Jensen et al. [48] have summarized the situation as follows: 'A large and growing number of transcription factors make important contributions to the precise control of osteoblast formation and function. It has become increasingly clear that these diverse transcription factors and the signals that regulate their activity cannot be viewed as discrete, separate signaling pathways. Rather, they form a highly interconnected, cooperative network that permits gene expression to be closely regulated' [48]. Researchers at the Harvard Medical School have filled in part of this gap in regulatory contributors within the realm of zinc biology; it involves the aforementioned 30-zinc ZFP, ZFP521. They demonstrate its expression in periosteal cells, chondroblasts, prehypertrophic chondrocytes, osteoblast pre-

cursors, and osteocytes, exerting a positive growth and maturation effect in bone [49].

Conclusion

The importance of dietary and environmental zinc for human health can be ignored only at significant peril to child well-being throughout the world. All of the applied aspects of zinc in clinical pediatrics and public health are based – or should be based – on the fundamentals of the basic biology of the trace element. Research findings related to the role of zinc throughout the gamut of human physiology and metabolism are expanding in an exponential manner. It is a challenge to keep pace with developments from such diverse fields while relating them to the practical problems in nutrition and health. A selected array of advances related to child growth (bone elongation) and host defense (immunology) merits increased recognition and elucidation due to their relevance for pediatric health.

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