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REPROGRAMMING OF THE IMMUNE SYSTEM DURING ZINC DEFICIENCY

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■ **Abstract** Thymic atrophy, lymphopenia, and compromised cell- and antibody-mediated responses that cause increased rates of infections of longer duration are the immunological hallmarks of zinc deficiency (ZD) in humans and higher animals. As the deficiency advances, a reprogramming of the immune system occurs, beginning with the activation of the stress axis and chronic production of glucocorticoids that accelerate apoptosis among pre-B and -T cells. This reduces lymphopoiesis and causes atrophy of the thymus. In contrast, myelopoiesis is preserved, thereby providing protection for the first line of immune defense or innate immunity. Changes in gene expression for cytokines, DNA repair enzymes, zinc transporters, signaling molecules, etc., suggest that cells of the immune system are attempting to adapt to the stress of suboptimal zinc. Better understanding of the molecular and cellular changes made in response to inadequate zinc should lead to the development of immunotherapeutic interventions.

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INTRODUCTION

As of two decades ago, it was evident that suboptimal nutriture had an adverse impact on host defense. However, the mechanisms whereby specific nutrients might alter the function of cells of the immune system were unknown. Because zinc deficiency (ZD) is a frequent dietary problem and accompanies many chronic diseases, it seemed to be an especially relevant model for determining the impact of a single element nutritional deficiency on immune function at the cellular and molecular level (30). The original discovery of dietary zinc deficiency in humans was made in the Middle East (58, 59), and was shown to exist in many underdeveloped nations including Brazil, Bangladesh, Guatemala, Indonesia, and Peru (1, 9, 11, 29, 30, 57, 58). In the United States, investigators have demonstrated subsets of Americans such as pregnant teenagers, children from low-income homes, low-birth-weight infants, some of the elderly, etc., are also at risk of dietary zinc deficiency (30, 37). However, it is important to also remember that chronic diseases such as gastrointestinal disorders, chronic diarrhea, renal disease, sickle cell anemia, cirrhosis, some cancers, cystic fibrosis, and pancreatic insufficiency have been shown to lead to suboptimal zinc status (9, 17, 24, 42, 43, 58, 64, 67). These disease states are associated with increased infections of prolonged duration, a clear indication of compromised immunity (24, 58). The genetic disease *Acrodermatitis enteropathica* is more rare and impairs absorption of zinc from the diet, leading to skin lesions or *Acrodermatitis* that is characteristic of zinc deficiency (58). It is important to note that this disease provides the hallmarks for zinc deficiency because thymic atrophy, lower numbers of circulating lymphocytes, poor delayed-type hypersensitivity responses, and high numbers of infections accompany this disease (18, 54). For this reason, a number of labs have used models of zinc deficiency for their immunological studies, and it now represents the best-characterized nutritional-immunological paradigm.

IMPAIRMENT OF ANTIBODY-MEDIATED AND CELL-MEDIATED RESPONSES BY ZINC DEFICIENCY

Antibody-Mediated Responses

Many investigators began their studies by utilizing the advantages offered by inbred mice in order to define in detail the effect of ZD on various facets of the immune system. Indeed, the mouse has proven to be a highly reliable model for the human immune system. The resulting literature now consists of hundreds of papers and reviews (24, 36).

Initial studies noted that as acute zinc deficiency advanced in young adult mice, one could expect to observe highly atrophied thymuses within 30 days. Whereas a normal thymus might be 30 mg in a well-fed mouse, it would range from 12 to 2 mg in marginally zinc-deficient (MZD) and severely zinc-deficient mice (SZD) that weighed 75% to 78% and 68% to 72%, respectively, of mice fed a zinc-adequate (ZA) diet (38, 41, 42). Because inanition accompanies ZD as it progresses one is obliged to include restricted-fed mice (48). Regrettably, the latter mice rarely provide any useful insight because they are closer to ZA mice for all parameters evaluated. Data also indicated that the numbers of lymphocytes in the peripheral blood and spleen were substantially reduced among ZD mice (13). Thus, the thymic atrophy and lymphopenia that were hallmarks of ZD in humans were also readily observed in ZD mice by many labs (13, 19, 24, 36). To assess actual immune defense capacity, mice were also inoculated with sublethal doses of the parasite *Trypanosoma cruzi*, which causes Chagas disease in humans (22). The ZD mice exhibited blood parasitemias that were nearly 50-fold greater than that of ZA controls. Large numbers of the ZD mice died, whereas none of the ZA or restricted-fed mice died. It was a remarkable demonstration of the degree to which a single-element nutritional deficiency could compromise host defense against an infection.

Defenses against other pathogens and infectious agents exhibit similar impairment for ZD rodents, providing quantitative evidence that suboptimal zinc could lead to intensification of infections with prolonged duration (24, 62). In subsequent experiments mice were immunized with sheep red blood cells. These cells required both functional T-helper cells and B cells and a hapten attached to either lipopolysaccharide or Ficoll, which required predominantly B cells for responses. Substantial reductions of 40% to 70% were noted in the ability of ZD to produce specific antibody responding cells or plaques to these various antigens (13, 23, 24). Taken together, the data confirmed that substantial reductions in antibody-mediated defense capacity was created by the deficiency.

However, it was also noted that the number of anti-sheep red blood cell plaques generated was normal when considered on a per-million lymphocyte basis in the spleen. In other words, although the absolute number of responding lymphocytes was down, the remaining population of responding cells when normalized appeared to be responding appropriately (13). This finding suggested that the residual splenic lymphocytes in ZD mice were reasonably functional. Moreover, flow cytometry

indicated no major change in the phenotypic distribution of the major classes and subclasses of T and B cells (40).

Tolerance

A recent experiment provides insight into the impact of ZD on the regulatory aspects of the immune response. Rats made zinc deficient for 28 days were repeatedly administered ovalbumin orally to induce tolerance (20). However, the ZD mice did not become tolerized and exhibited a dysregulation of cytokine expression and lack of clonal deletion when compared to controls. This suggests that periods of ZD might enhance the possibility of autoimmune disease by contributing to the inefficient removal of anti-self or nonsense clones that are routinely generated in the marrow.

Cell-Mediated Responses

In the case of cell-mediated reactions, the response of natural killer (NK) cells was less intense in ZD rodents and humans (1, 19, 64). Tumors in some cases appeared to grow faster in ZD mice presumably because of reduced defense (21). Several labs demonstrated that suboptimal zinc status also altered delayed-type hypersensitivity reactions in rodents and humans (54). However, these results are controversial because there is concern that human subjects may have been previously exposed to the antigens used in the tests. Thus, the data are confounded by unknown degrees of rebound in memory-type responses. Whether one uses rodents or humans, many of the assays used to evaluate cell-mediated responses are, unfortunately, still problematic. Therefore, the impact of a deficiency in zinc cell-mediated responses key to cancer and viral defense remain less well defined.

Our lack of information on the impact of ZD on viral defense mechanisms is especially troublesome because the work of Dr. M. Beck demonstrates that both Coxsackie and influenza viruses have a significantly accelerated rate of mutation in mice that are selenium deficient (6, 7). The accelerated mutations could be due to greater production of oxygen radicals as well as reduced defense allowing for greater proliferation of mutagenized viral subsets. Though the increase might be slight, it is conceivable that periods of zinc deficiency might also increase the incidence of mutations leading to cancer and/or chronic viral diseases. Indeed, a number of labs have shown that suboptimal zinc causes DNA strand breaks (2, 33–35). Though immune repair was rapid in repleted ZD adult mice, the long-term impact of ZD on the regulatory aspects of immunity is not really known (23).

Underinvestigated Areas of Immune Defense

Of additional concern is how little we know of the impact of zinc deficiency on mucosal immunity. ZD is well known for creating diarrhea, which suggests a disruption of gut immunity (9, 58). It undoubtedly affects pulmonary immunology, but the degree to which a ZD host may be more susceptible to pneumonia, tuberculosis,

SARS, etc., is not known in quantitative terms. Memory responses are, of course, vital to providing long-term protection against previously encountered infections and to providing protection via vaccinations. There is insufficient information on the impact of marginal or severe zinc deficiency on the generation and maintenance of memory responses. This is of special concern in the case of young children or the elderly, where suboptimal immune defense capacity along with suboptimal nutriture might combine synergistically to yield inadequate protective responses to vaccines. Whether or not periods of zinc deficiency alter the number or status of memory cells present in adults is also of significant concern. Indeed, with the exception of a few preliminary studies, we know little of the effect of zinc on the complement system, acute phase response, inflammatory responses, or the hypersensitivity responses. It is sobering to realize that after 30 years of effort by many labs, we have only scratched the surface on how changes in zinc status impact the various branches and cells of the immune system.

ROLE OF ELEVATED GLUCOCORTICOIDS GENERATED DURING ZINC DEFICIENCY

Neuroendocrine Changes Initiated by Zinc Deficiency

Several years ago it was clearly demonstrated that glucocorticoids, in particular corticosterone, were chronically elevated in zinc-deficient mice and that adrenalectomies or removal of these steroids prevented the thymus from atrophying during zinc deficiency (15, 16). Since these studies were done, glucocorticoid-induced apoptosis of thymocytes became a classical system for the study of cell death (12). Thus, it became evident that the chronically produced glucocorticoids found in ZD might be accelerating apoptosis in early T and B cells. Prior to this, adrenalectomies were used to also ascertain whether the chronically elevated glucocorticoids in zinc-deficient mice played any role in depletion of the marrow of developing B cells or thymic atrophy (25). Mice were adrenalectomized or sham operated. Zinc-deficient sham mice had a sixfold elevation of corticosterone levels and a thymus 65% the size of the adrenalectomized zinc-deficient mice. Moreover, the sham-operated zinc-deficient mice exhibited a reduction of 50% in the proportion of pre-B cells in the marrow compared to adrenalectomized zinc-deficient mice (25). However, the adrenalectomized zinc-deficient mice had nearly normal levels of pre-B, immature, and mature B cells and were analogous to sham-operated zinc-adequate mice for all these variables. Thus, removal of corticosterone via adrenalectomy provided substantial protection for B-lineage cells developing in the marrow and T cells in the thymus of zinc-deficient mice. Clearly, chronically elevated corticosterone, generated as zinc deficiency advances, plays a key role in the observed lymphopenia and thymic atrophy of zinc deficiency.

The above experiments demonstrated that an acute form of zinc deficiency made significant neuroendocrine changes to include activating the hypothalamus-pituitary-adrenocorticoid axis or the stress axis. Subsequently, protein-energy

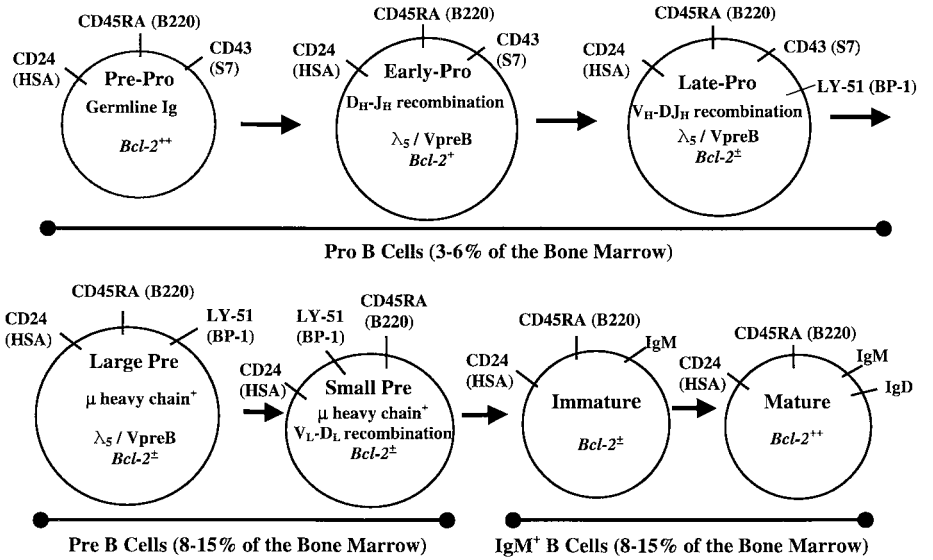
malnourished (PEM) rodents and humans were also shown to have elevated glucocorticoids (44, 55, 63, 66). In this regard it is interesting to point out that there are many parallels between PEM and ZD. Besides elevated glucocorticoids, both deficiencies cause thymic atrophy and lymphopenia, and reduce cell- and antibody-mediated responses (18, 24, 28, 44, 55, 63, 66). Moreover, children suffering from PEM have low serum zinc (28). Conversely, as discussed above, ZD is accompanied by inanition or reduced intake of calories and protein as it advances (48). This began the recognition that some acute forms of malnutrition constituted a stress that made significant neuroendocrine changes. These findings were analogous to the observations of clinicians who found that patients under the stress of trauma, surgery, burns, etc., also produced elevated quantities of corticosterone and cortisol (52, 53). Thus, to a degree ZD constitutes a natural stress.

IMPACT OF ZINC DEFICIENCY ON LYMPHOPOIESIS

B Cell Lymphopoiesis

The atrophy of the thymus and decline in the number of peripheral and splenic lymphocytes in the ZD mouse paralleled observations made in zinc-deficient humans (1, 54, 64). It seemed very probable that the deficiency had altered lymphopoiesis or the production of lymphocytes. In mature adults, the bone marrow is the site of production of all the cells of the B lineage as well as the earliest of T cells that finish maturation in the thymus (31). The marrow and the thymus are thus the so-called primary immune tissues. We were particularly interested in these tissues because there had never been an examination of the effect of a nutritional deficiency on lymphopoiesis or hematopoiesis using modern tools of immunology. When considering nutritional relationships, it is important to remember that the bone marrow is the largest tissue of the body; it is required on a daily basis to produce all the cells in the blood, including lymphocytes, neutrophils, monocytes, platelets, red blood cells, eosinophils, and basophils (61). Whether in mouse or man, the marrow is a large tissue and must, therefore, be a major user of nutrients. Thus, it was important to determine how the marrow might change or reprogram hematopoietic processes in response to an inadequate supply of zinc and the presence of chronically elevated glucocorticoids.

The lineage subsets for cells of the B lineage and their respective markers are outlined in Figure 1 (31). The earliest protein to appear on early B cells is CD45RA or the pan B cell marker $B220^{+}$. It appears on the so-called pre-pro B cells that express germ line Ig. Note, however, this very early B cell expresses reasonable amounts of the antiapoptotic protein Bcl-2 (50). This may be because they are not yet of any danger to the body, which could be the case for pre- and immature B cells that upon completing Ig gene rearrangement might express anti-self or nonsense molecules. The latter cells are normally eliminated apoptotically. Thus, these lineages express little Bcl-2 and could be exquisitely sensitive to suboptimal zinc (Zn) and steroids if they accelerated apoptosis (Figure 2) (12). B cells that



Bone marrow B lymphocyte development scheme

Figure 1 There are many subsets of cells within the B lineage developing in the marrow. The array of phenotypic markers, rearrangement of the Ig gene, and expression of the antiapoptotic protein Bcl-2 is depicted for the various members of this group.

successfully acquire acceptable forms of immunoglobulin (IgM and IgD) move out to the periphery to become naive B-cells ready to engage in antibody-mediated responses. Analogous lineages and diversity of Bcl-2 expression exists for early T cells that are in the process of developing and rearranging the genes for the T-cell receptor.

Using multicolor flow cytometry along with a DNA stain, it was possible to not only assess changes in the quantitative distribution or proportion of these B-cell subsets using markers shown in Figure 1, but to ascertain whether they maintained their cell cycle status during ZD. Subsequent to 30 days of suboptimal zinc (0.5–0.8 μg Zn/g diet), the B-cell compartments of young adult mice were greatly reduced due to collective losses of 50% to 70% among the pre-B and immature B cells (Figure 3) (42, 56). Mature B cells survived reasonably well. Surprisingly, an increase of 20% to 50% in the pro-B cells was noted in the depleted B-cell compartment (Figure 3) (56). The greater loss of pre- and immature B cells correlated with the lower expression of the antiapoptotic protein Bcl-2 (Figure 1) (50). This was an indication that heightened apoptosis among precursor B and T cells caused by low Zn and/or chronic exposure to Gc could be occurring. Recall that adrenalectomies or removal of Gc provided substantial protection to the primary immune tissues (16, 25). However, the ability of natural or endogenously

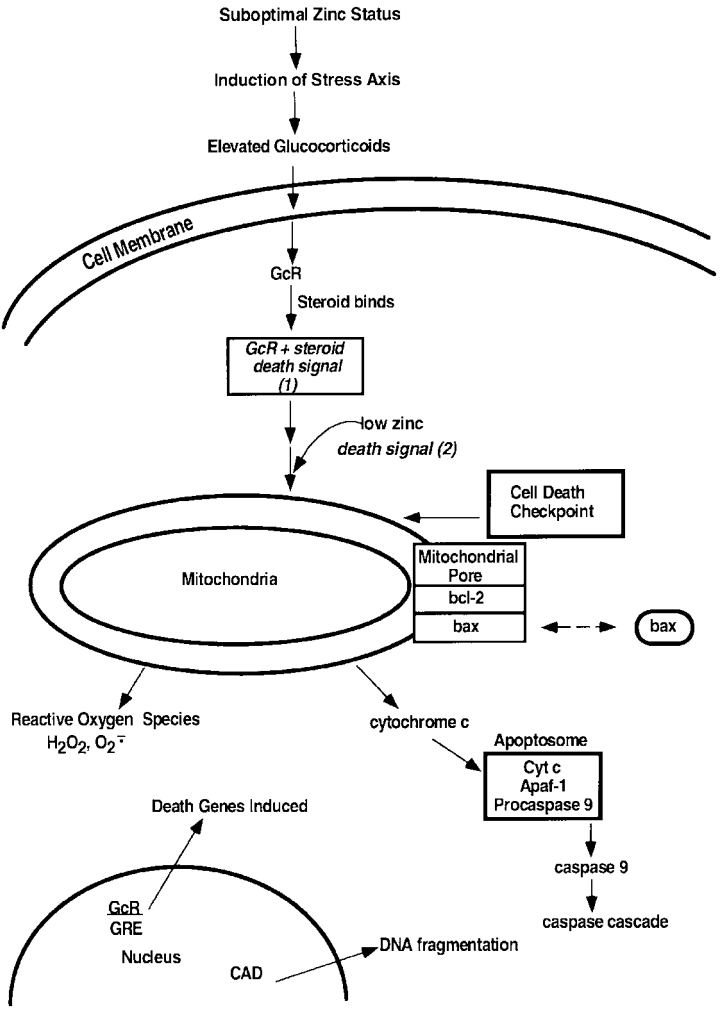


Figure 2 A classical apoptotic pathway that is activated by glucocorticoids (Gc) and suboptimal nutriture is outlined. Gc generated by the stress of suboptimal zinc binds to their cytosolic receptor (GcR), whose translocation and induction of death genes is regulated by the ratio of expression of the antiapoptotic Bcl-2 family members versus the proapoptotic members such as bax. The latter molecules affect whether or not cytochrome c is released from the mitochondria, a regulatory point in the death pathway. If cytochrome c is released it forms a complex in the cytosol called an apoptosome, which activates caspases, a family of proteolytic enzymes. These enzymes digest and alter the cytoskeleton, causing the cell to condense in size. They also activate a caspase-activated DNase (CAD), which cleaves the genomic material into 200 base pair fragments. The exact role of suboptimal zinc in the death pathway has not yet been defined.

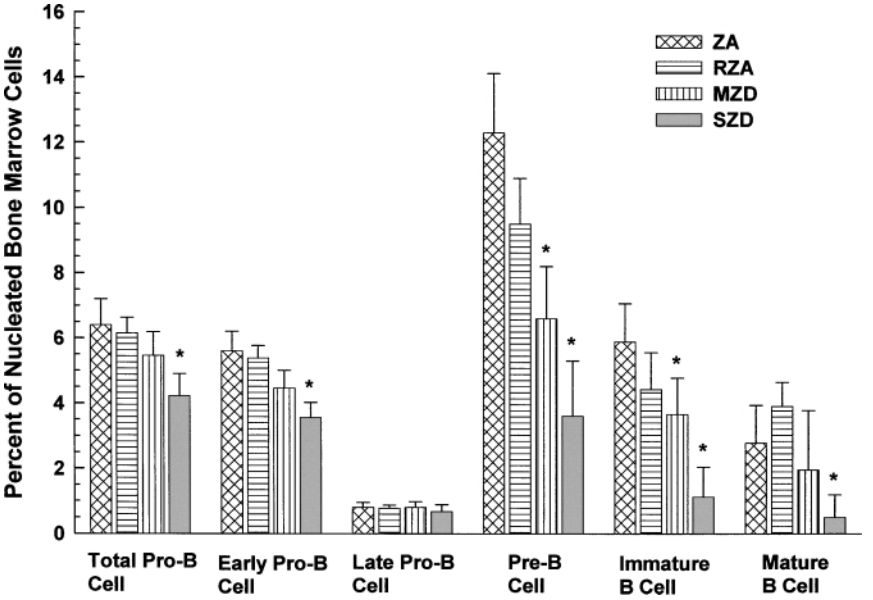


Figure 3 Typical changes in the distribution of subsets of cells within the B lineage in the marrow created by suboptimal zinc in young adult mice. Young adult mice were fed a zinc-adequate diet (ZA), restricted-fed adequate diet (RZA), or a zinc-deficient diet for 30 days after which zinc-deficient mice were subdivided into MZD and SZD subsets, as previously identified. Losses were greatest among pre-B cells that do not express Bcl-2 (Figure 1) and may be more susceptible to apoptosis.

produced Gc to induce apoptosis during stresses such as ZD was not yet known. Quantities of Gc analogous to that generated in ZD mice did, however, cause substantial apoptosis among precursor B and T cells both in vitro and in vivo (26, 27). Thus, the hypothesis emerged that heightened apoptosis played a role in lymphopenia and thymic atrophy.

Thymopoietic Processes

The other primary tissue that is essential to lymphopoiesis is the thymus, where T-cells are produced presumably via seeding of organ with very early pre-pro T cells from the marrow. Indeed, though the markers are different, the scheme of development, rearrangement of the T-cell receptor, and changes in expression of Bcl-2 are analogous to the B-cell scheme shown in Figure 1. Young adult A/J mice were placed on ZA or ZD diet, but sacrificed at a point when their thymuses were partially atrophied (e.g., 12.6 mg $\pm$ 2.0 mg for ZD (38%) versus 33.5 mg $\pm$ 2.7 mg for ZA). Here again, ZD caused substantial losses in the double positive $CD4^+CD8^+$ or pre-T cells that normally account for 80% of the cells of the thymus. These data also affirmed the hypothesis that ZD might be enhancing apoptosis

among these vulnerable cell lineages. As with B cells, the pro-T cells survived to a greater degree to become a 50% greater proportion of the residual cells (41). Mature $CD4^+$ helper and $CD8^+$ cytolytic T cells also were quite resistant to ZD and survived well in the otherwise atrophying thymus (41). Thus, the survival-death pattern among developing T cells in the thymus paralleled the pattern observed for developing B cells in the marrow (Figure 3).

Cell Death Pathways

The scheme provided in Figure 2 is a depiction of a common and well-defined apoptotic pathway that is probably applicable to ZD (32). Glucocorticoids definitely utilize this Bcl-2 modulated pathway, binding to a cytosolic receptor that can then move to the nucleus, inducing death genes in vulnerable cell types. This pathway is also used when cells are deprived of sera or essential nutrients (12). It is regulated by the Bcl-2 family of antiapoptotic proteins and its homologs. Cells expressing high levels of Bcl-2 family members, especially in the mitochondria, resist apoptosis, keeping mitochondrial pores closed. In the case of cells with low Bcl-2 expression or substantial expression of proapoptotic family members such as bax, the pore is thought to open, releasing cytochrome c (32). This causes formation of a complex called the apoptosome and activation of a family of caspases that digest the cytoskeleton and nuclear matrix of the cell. It also causes cleavage of a caspase-dependent DNase (CAD) that digests the genome into 200 base pair fragments (32). Thus, this is a multilayered, programmed form of death designed to rapidly destroy the cell. As a result of these events, the cell condenses and rapidly inverts its membrane. The latter causes digestion of the apoptotic cells by phagocyte cells in a well-functioning immune system (12). Knowledge of this pathway led to the supposition that the endogenously produced glucocorticoids perhaps acting in synergy with low zinc would initiate this death pathway during ZD, causing accelerated death among the pre-T and pre-B cells that express little Bcl-2 (Figure 2).

ACCELERATED APOPTOSIS AMONG PRE-T AND -B CELLS

Directly demonstrating heightened apoptosis among precursor cells in ZD mice was problematic because phagocytic cells rapidly remove apoptotic cells. To circumvent these problems, the thymuses from the experiment described previously were used because of the large percentage of pre-T cells (83%) that would be undergoing apoptosis during ZD if the theory were correct. They were processed into single-cell suspensions, and macrophages were removed. It was assumed that at the time of harvest more death signals would have been sent to pre-T cells in ZD mice than in ZA mice. Multiparameter FACS was used to simultaneously assay for phenotype and apoptosis. The data revealed that the degree of apoptosis in pre-T cells ($CD4^+CD8^+$) was 30% or threefold higher than that of pre-T cells from ZA mice (9%) (see Figure 4). In the two subsequent experiments performed

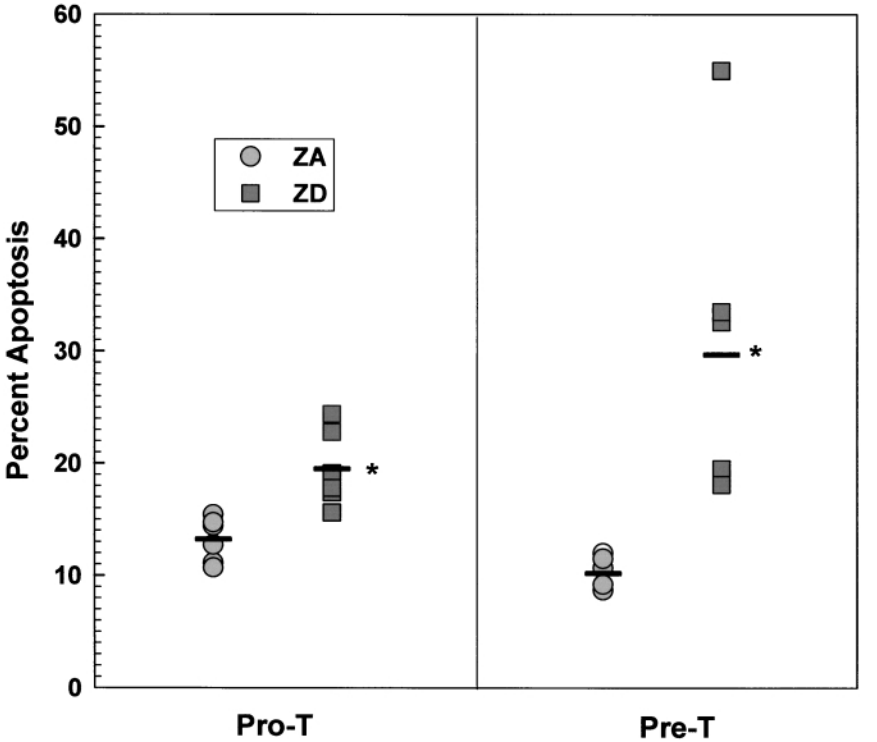


Figure 4 The degree of apoptosis among early T cells is shown for the thymuses harvested from zinc-adequate (ZA) and zinc-deficient (ZD) young adult mice. Quantitation of apoptosis was determined by DNA analysis using the flow cytometer.

in an analogous manner, the rate of apoptosis was accelerated 50% and 100% above that of pre-T cells from ZA mice. No enhanced apoptosis was noted in mature cytolytic (CD4-CD8$^+$) or helper T cells (CD4$^+$CD8$^-$) from ZD mice, with a modest increase noted in cell death in pro-T cells (CD3$^+$CD4$^-$CD8$^-$) (41). Clearly this degree of heightened apoptosis among pre-T and -B cells in ZD mice would substantially disrupt lymphopoiesis over time and would greatly reduce the ability of ZD mice to replenish their peripheral immune system with lymphocytes. Thus, the mechanistic explanation for lymphopenia and thymic atrophy in ZD is due, at least in part, to high losses of precursor lymphoids caused by apoptosis.

It has also been apparent that zinc status can cause DNA strand breaks in cells (2). Low concentrations of zinc alone also have been shown to induce DNA strand breaks and apoptosis in vitro in thymocytes, glioma cells, and human fibroblasts (33, 34, 49). However, the latter studies employed zinc chelators, which could create different distributions and losses of zinc within a cell than those generated

in a host with suboptimal zinc status. In this regard, the work of Ho et al. (34) is of interest because the impact of culture medium low in zinc was compared to results obtained with media containing a zinc chelator. Human lung fibroblasts from each culture contained DNA strand breaks. However, DNA microarrays showed substantial differences in the changes in gene expression between the two culture conditions, although they both induced DNA repair enzymes and increased p53 expression (34). In confirmation of these in vitro studies, it is interesting that DNA strand breaks were also noted in the livers of ZD rhesus monkeys and rat embryos (35, 60). It is fair to say that it is very difficult to mimic a true form of zinc deficiency in vitro given the changes created in metabolism, hormones, cytokines, etc., by the deficiency. Furthermore, these studies make the important point that the method of generating a culture low in zinc can also affect the outcome. Regardless, it is clear that zinc status can impact apoptosis and DNA integrity in a variety of cells.

CHANGES IN HEMATOPOIETIC COMPARTMENTS

Erythropoiesis

Data from the hematopoietic studies of ZD mice verified that the anemia that accompanies zinc deficiency in humans also has its origins in the marrow, where losses occurred of up to 60% in cells of the erythroid lineage. These results reaffirm the observations made by clinicians (38, 58, 59). However, a substantial proportion of this population was actively dividing in the marrow and to our amazement they not only continued to cycle but exhibited small increases in S and G_2/M (39). The important observation has been made in recent literature that in the presence of glucocorticoids the progenitor cells of the erythroid lineage can, in fact, cycle, but fail to differentiate and mature (65). Thus, this rodent model of ZD is helping to elucidate new understandings regarding the regulation of hematopoiesis.

Role of Mixed Progenitor Cells in Adaptation to Zinc Deficiency: A Possible Fail-Safe

Stem cells that are key to the regeneration of the immune system have been shown to survive moderate irradiation and pharmacological doses of Gc (61). Indeed, early progenitors also appear to survive in ZD mice (38). Less was known of the survival of the very early progenitors or pluripotent cells. It is possible that they too have moderate expression of Bcl-2, as do pre-pro B cells (Figure 1) (31). There are signs of protective or adaptive increases in these progenitors among ZD mice; they increased about 50%, going from 6% in ZA mice to 9.3% in SZD mice (38). These cells have the ability to develop into a variety of lineages. A large proportion of these cells also showed enhanced cycling with increases of cells in S and G_2/M noted for both MZD (33%) and SZD mice (56%) (39). The actual lineages or degree of commitment of the progenitor cells is also of great interest. It would

be interesting to know if a high proportion of these progenitors exhibit so-called lineage plasticity, becoming myeloid cells to a greater degree as ZD advances (61). Such a shift would also contribute to the reprogramming of the immune system by changing the distribution and production of lymphocytes and myeloid cells as the deficiency advances. Lineage development in the marrow is, of course, affected by the cytokines produced by stromal cells that could also be greatly affected by ZD. It will be important to know if production of interleukin-7 that is essential to development of pre-B and -T cells has declined, and if production of granulocytic-monocytic cytokines that promote myeloid cell development is increased during ZD (45).

Myelopoiesis

In considering the effects of ZD on hematopoietic processes, it is important to remember that cells of the myeloid lineages represent the first line of immune defense or innate immunity. Myeloid progenitors develop into neutrophils or granulocytes and monocytes within the marrow. A marked increase was noted in the myeloid compartments of the marrow of ZD mice (38). In the case of granulocytic cells that are the precursors of neutrophils, a nearly 40% increase was noted in MZD and a 60% increase in these lineages in SZD mice. Moreover, an 80% increase in monocytic cells was noted in MZD and SZD mice. It is also important to know that in seven different studies the number of nucleated cells in the marrow never declined in ZD (38, 39, 41, 42). Thus, these represent not only proportional increases within the marrow, but actual or absolute increases in number of cells of the myeloid lineage developing there. Only a small proportion of young granulocytic cells are cycling, yet a 50% increase was noted in cells in S and G_2/M phase (39). For the monocytes, there was also 40% increase in the proportion of cells in S and G_2/M. Taken together, these findings over the course of three analogous experiments indicate that cells of the myeloid lineages increase both in proportion and absolute numbers, with substantial increases in actively proliferating subsets in both MZD and SZD mice (Figure 5). This, of course, is in stark contrast to lymphopoiesis, which is rapidly downregulated by ZD.

Reprogramming of the Immune System

The dramatic shift shown in Figure 5, whereby far fewer lymphocytes are produced compared to myelocytic cells in the marrow, indicates an active reprogramming of the immune system as ZD advances. The meaning of this reprogramming of hematopoietic processes is not entirely clear, but it is potentially an adaptation put into place to protect the first line of immune defense at all costs. Retention of phagocytic capacity not only protects innate host defense, but provides clearance for the large numbers of apoptotic cells being generated in the marrow, thymus, and other tissues as a result of limitations in zinc (41, 60). Thus surveillance, seminal attacks on pathogens, clearance of apoptotic and aging cells, wound healing, etc., would be maintained if production of myeloid cells were protected during ZD.

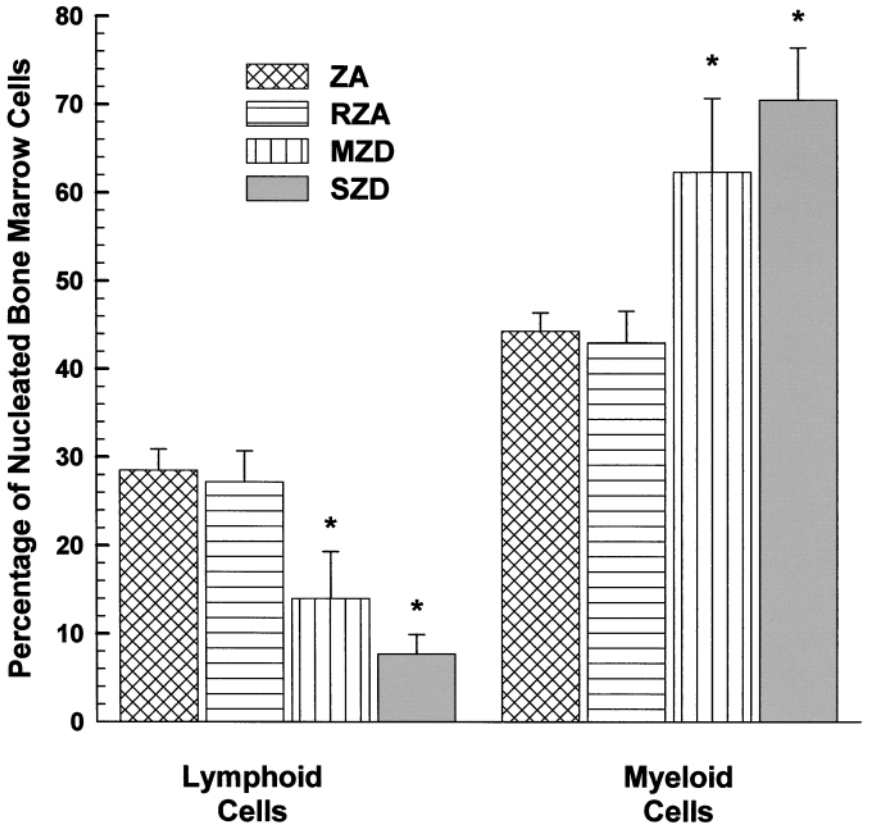


Figure 5 During a 30-day dietary study of the effects of zinc-adequate (ZA), restricted zinc-adequate (RZA), marginally zinc-deficient (MZD), and severely zinc-deficient (SZD) on hematopoiesis, there was a significant decline in the proportion and number of cells in the lymphoid compartment created by suboptimal zinc. Conversely, the myeloid compartments that contain granulocytic and monocytic cells exhibited a substantial increase through a reprogramming of the immune system, which includes significant changes in hematopoietic processes in the marrow.

As the body moves from well fed to the starved state, many metabolic changes take place, including increased use of fats and ketone bodies as energy sources. As ZD becomes limiting, the immune system may also be obliged to make significant changes. Perhaps the choice is to maintain the very basic, and very vital, first line of immune defense that is at work at all times providing ongoing protection. Though also key to defense against an invasive infection, lymphocytes are nevertheless the second line of defense, called into action after the first line is overrun. The overwhelming majority of lymphocytes survive only days or weeks at most and never perform a single useful task. Maintaining and producing cells of the

lymphoid lineage may become nutritionally costly as ZD advances and zinc becomes limiting. If so, it appears that chronic production of glucocorticoids is initiated perhaps for the very purpose of downregulating lymphopoiesis by accelerating the death of T and B cell precursors, eventually leading to the shift in production of myeloid and lymphoid cell noted in Figure 5.

In regard to the above theory, it is important to note that implantation of mice with tablets that released corticosteroids at concentrations analogous to that observed in ZD mice also promoted myelopoiesis in the marrow at the expense of lymphopoiesis, which rapidly declines (46). Moreover, in ZD mice, higher proportions of neutrophils are observed in the face of declining lymphocytes in the peripheral blood, which suggests greater numbers of myeloid cells are, indeed, released from the marrow into the periphery (24). Although some early literature implied higher neutrophil counts in ZD humans, oddly many investigators noted the decline in lymphocytes in the peripheral blood of deficient humans, but did not provide data on numbers of neutrophils or monocytes. Of particular interest is our recent finding that the incubation of human neutrophils with natural glucocorticoid at 0.1 μM or concentrations lower than that found during ZD actually prolongs their half life by several hours (J.W. Frentzel & P.J. Fraker, unpublished). This is again in contrast to early lymphoid cells and lymphocytes themselves that undergo apoptosis in the presence of glucocorticoids (25–27). This suggests a shift in strategy within the immune system as it adapts to stress conditions. Thus, it would be important to determine if neutrophils increase in moderately or acutely ZD humans and if they maintain normal functional capacity.

Evidence of Gene-Directed Adaptations of the Immune System to Zinc Deficiency

A 20% difference in body weight was accompanied by 50% to 80% losses in the cellularity of the thymus and spleen, with concomitant losses in antibody and cell-mediated responses noted in ZD mice (24). These early observations made it seem as if ZD simply caused a rapid and unorganized dismantling of the immune system. However, we began to rethink this supposition upon finding that myelopoiesis and progenitor cells were protected and accumulating in ZD mice (38). Perhaps there was, in fact, a more organized, gene-directed adaptation of the immune system to suboptimal zinc than had been originally thought. Indeed, as discussed earlier, the splenocytes from ZD mice gave an enhanced proliferative response to the mitogen ConA, producing as much as 50% more interleukin-2, and antigen-activated B cells produced 20% to 50% more antibody per cell than splenocytes from ZA mice (13). The mixed lymphocyte response to foreign target cells was 50% to 70% enhanced among lymphocytes from ZD mice, as were the proliferative responses to several mitogens such as pokeweed mitogen and lipopolysaccharide (13). The residual lymphocytes in ZD mice appear to be hyperactive and perhaps more potent. This raises the question as to whether there is an immunological way of doing more with less as part of reprogramming of the lymphocytic system to adapt to ZD. Using

a variety of markers available in 1991 we noted no differences in the phenotypic distribution of T and B cells in the spleens of ZD mice (40). However, there was also more expression of Ig, Ia, and MHC-I, which actively participate in response to antigens, suggesting a greater degree of activation.

Immunologists and nutritionists may not have given enough consideration to the fact that the cells of the immune system may try to adapt to environmental stresses such as extreme changes in temperature, reduced availability of food, hypoxia, and trauma. Recently, cytolytic lymphocytes (CTL) were maintained in a normal (20% O_2) and a hypoxic state (2.5%). Although development was delayed, CTL from hypoxic conditions exhibited enhanced lytic capacity that was more sustained than CTL-maintained normal oxygen (10). Short day lengths that occur during harsh winters cause greater migration of cells to the skin, perhaps in anticipation of injury and infection, in Siberian hamsters that had enhanced delayed-type hypersensitivity responses (8). These examples of immune response to stress represent the interesting tradeoffs in functions recently noted in ZD.

Changes in Gene Expression: Further Evidence of Reprogramming

New data denoting variance in changes in gene expression among cells of the immune system also indicate reprogramming is occurring in response to an environment low in zinc. Thymocytes prepared from mildly ZD mice prior to any shift in lineage composition created interesting, though modest, changes among the 1200 genes subjected to microarray analysis (51). Of particular interest was enhanced expression of a DNA repair enzyme, perhaps to prepare for the DNA strand breaks and apoptosis that can occur as the deficiency advances (2, 51). Similarly, there was enhanced expression of LCK, a lymphocyte-specific tyrosine kinase that requires zinc for linkage to the CD4 receptor of the T cell. The latter enzyme was also elevated in splenocytes during zinc deficiency, perhaps as a compensatory mechanism for key signaling events (47). Yet, culturing Th0 and Th1 cell lines in low zinc followed by mitogenic stimulation led to a reduced message expression for interleukin II and interferon-γ that would adversely affect their functional capacity (3). These initial experiments reveal that much remains to be done to understand how changes in zinc status modulate gene expression in the various cell lineages at the molecular level.

Given the survival of cells of the myeloid lineage in the marrow of ZD adult mice, it is interesting to note that HL-60 cells, a myeloid-like human precursor cell line, also survived in cultures with low concentrations of zinc (3). Moreover, when they were stimulated they exhibited enhanced expression of message for tumor necrosis factor (α), interleukin I (β), and interleukin 8. Thus, it provides an in vitro facsimile of the divergent effect suboptimal zinc seems to have on lymphoid versus myeloid cells.

Certainly survival of cells of the immune system during ZD will be affected by their ability to maintain zinc homeostasis. Another important group of experiments

examined the effect of low zinc conditions on a human monocytic cell line (THP-1) and peripheral blood monocytes, albeit with the use of a chelator. Both normal monocytes and the cell line had reduced mRNA expression for metallotheionein and the transporter Zip 1 (14). However, there was increased expression for Zip 2 messages, which suggests myeloid cells are attempting to increase their uptake of zinc during low zinc conditions. Clearly, a better understanding of the changes in expression of zinc transporters that control the uptake and efflux of zinc is needed to determine their role in cell survival during ZD.

The above experiments are moving the field in the right direction. It is also clear that we have barely scratched the surface in identifying the changes in expression of enzymes, kinases, transcription factors, cytokines, chaperones, death genes, transporters, etc., that will affect whether or not a cell survives ZD.

IMMUNOTHERAPEUTIC AND NUTRITIONAL INTERVENTIONS

In the case of acute forms of zinc deficiency the mechanisms responsible for the lymphopenia and thymic atrophy that are the hallmarks of this deficiency have been identified. It also is now clear that suboptimal zinc initiates significant neuroendocrine changes to include the chronic production of glucocorticoids, which, in turn, accelerates the death of pre-B and pre-T cells. If we begin with this information and ask how we might reduce the impact of ZD on lymphopoietic processes, a number of interventions are possible. The literature indicates that interleukin-7 is key to promoting proliferation of early T and B cells that experienced high losses in the marrow and thymus during ZD (45). Short-term bone marrow cultures were set up and corticosterone (0.1–1 μM) at concentrations observed in the ZD mice was added, which induced 25% to 30% apoptosis among pro-B cells and an astonishing 40% to 60% apoptosis in pre-B cells and immature B cells (45). As in ZD mice, corticosteroid also reduced proliferation and cell cycle status among these early B cells by 50% to 60%, as determined by flow cytometry. Remarkably, interleukin-7 at very reasonable concentrations (0.1–1 ng/ml) was able to reduce corticosteroid-induced apoptosis by 30% to 40% among pro-B and pre-B cells (45). Moreover, it completely restored the cycling capacity of pro-B cells with modest efficacy for pre-B cells. Insulin-like growth factor might also have potential; it increases weight as well as promotes lymphopoiesis in mice deficient in B cells (61). Regrettably, the ability to effectively deliver an array of cytokines in situ is a cloud that has hung over immunotherapy for two decades and may impact the ability to offset the effects of ZD.

Of additional consideration would be to block the apoptotic signals sent by the endogenously produced glucocorticoids. In the presence of steroid, the receptor-ligand translocates from the cytoplasm to the nucleus, where changes in gene expression vary from cell type to cell type, but can include induction of apoptosis (Figure 2). Antagonists of the glucocorticoid receptor such as RU486 readily block

ligand binding and prevent glucocorticoid-induced apoptosis (27). In a mouse model, this same family of drugs prevented glucocorticoid-induced apoptosis of early immune cells in vitro (27). It is possible that the administration of these antagonists will provide substantial protection in the case of acute forms of zinc deficiency and/or protein calorie malnutrition.

Inexpensive Nutritional Supplements for Stabilization of AIDS

Nutritional management of chronically ill patients is modest compared to what could and should be done. A powerful example of the value of zinc supplementation already has been judiciously applied to a specific disease. Low serum zinc is frequently found in HIV-1^+ patients and is associated with a higher rate of mortality (4, 5). Those HIV-1^+ subjects were supplemented with modest levels of zinc for several months. It reduced mortality by 30% for each 1 mg of zinc provided (4). Disease progression was slowed and $CD4^+$ counts increased. This is a remarkable example of the value of appropriate nutritional supplementation for a disease state in which immune defense is compromised. To have an impact on the care of malnourished or chronically ill patients, a regimen of immunotherapy, drugs, and nutritional supplementation may have to be developed on a disease-by-disease basis not unlike the aforementioned regimen for AIDS patients.

CONCLUSIONS AND PERSPECTIVES

The impact of ZD on cell- and antibody-mediated responses has been reasonably well defined. In addition, demonstrating that accelerated apoptosis among precursor lymphocytes is the mechanism underlying reduced production of lymphocytes provides important insight into the effects of ZD on hematopoietic processes. Recently investigators have shifted toward molecular studies to identify the mechanisms and changes in gene expression created by suboptimal zinc. Such studies will greatly facilitate the identification of nutritional and immunotherapeutic interventions. For this to occur, however, there must be a substantial increase in the number of human nutritional studies with immunological components. This must be coupled with better education of clinicians and dieticians to insure improved nutritional-immunological management of the chronically ill in the future.

On an optimistic note, interest in nutritional immunology has resurged significantly. The number of quality contributions to the literature in this area has increased substantially in the last few years. The applications to nutrition and health are readily evident. Moreover, this field has matured enough that it can also make substantial and compelling contributions to our understanding of immunology. It is now evident that ZD constitutes a stress on the immune system, and it provides a well-characterized model for learning how the immune system reprograms itself to adapt and respond to this deficiency frequently found in nature.

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LITERATURE CITED

1. Allen JI, Perri RT, McClain CJ, Kay NE. 1983. Alterations in human natural killer cell activity and monocyte cytotoxicity induced by zinc deficiency. *J. Lab. Clin. Med.* 102:577–89
2. Ames B. 2001. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutat. Res.* 475:7–20
3. Bao B, Prasad AS, Beck FW, Godmere M. 2003. Zinc modulates mRNA levels of IL-2 and IFN-{gamma} positively in HUT-78 and D1.1 cells and negatively mRNA levels of TNF-{alpha}, IL-1 {beta}, and IL-8 in HL-60 cells. *Am. J. Physiol. Endocrinol. Metab.* 10:1152–59
4. Baum M, Campa A, Lai S, Lai H, Page J. 2003. Zinc status in human immunodeficiency virus type 1 infection and illicit drug use. *Clin. Infect. Dis.* 37:117–23
5. Baum M, Shor-Posner G, Compa A. 2000. Zinc status in human immunodeficiency virus infection. *J. Nutr.* 130:1421–23S
6. Beck MA. 1997. Increased virulence of coxsackie virus B3 in mice due to vitamin E or selenium deficiency. *J. Nutr.* 127:966–70S
7. Beck MA, Levander OA, Handy J. 2003. Selenium deficiency and viral infection. *J. Nutr.* 133:1463–67S
8. Bilbo S, Dhabhar F, Viswanathan K, Saul A, Yellon S, Nelson R. 2002. Short day lengths augment stress-induced leukocyte trafficking and stress induced enhancement of skin immune function. *Proc. Natl. Acad. Sci. USA* 99:4067–72
9. Black R. 2000. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries. *Am. J. Clin. Nutr.* 72:1516–22
10. Caldwell G, Kojima H, Lukasher D, Armstrong J, Farber M, et al. 2001. Differential effects of physiologically relevant hypoxic conditions on T lymphocyte development and effector functions. *J. Immunol.* 167:6140–49
11. Caulfield L, Zavaleta N, Figucio A. 1999. Adding zinc to prenatal iron and folate supplements improves maternal and neonatal zinc status in a Peruvian population. *Am. J. Clin. Nutr.* 69:1257–63
12. Cohen J, Duke R. 1992. Apoptosis and programmed cell death in immunity. *Annu. Rev. Immunol.* 10:267–304
13. Cook-Mills J, Fraker PJ. 1993. Functional capacity of residual lymphocytes from zinc deficient adult mice. *Br. J. Nutr.* 69:835–48
14. Cousins RJ, Blanchard RK, Moore JB, Cui L, Green CL, et al. 2003. Regulation of zinc metabolism and genomic outcomes. *J. Nutr.* 133:1521–26S
15. DePasquale-Jardieu P, Fraker PJ. 1979. The role of corticosterone in the loss in immune function in the zinc-deficient A/J mouse. *J. Nutr.* 109:1847–55
16. DePasquale-Jardieu P, Fraker PJ. 1980. Further characterization of the role of corticosterone in the loss of humoral immunity

in zinc-deficient A/J mice as determined by adrenalectomy. *J. Immunol.* 124:2650–55
17. Dutta S, Procaccino F, Aamodt R. 1999. Zinc metabolism in patients with exocrine pancreatic insufficiency. *J. Am. Coll. Nutr.* 17:556–63
18. Endre L, Katona Z, Gyurkovits K. 1975. Zinc deficiency and cellular immune deficiencies in *Acrodermatitis enteropathica*. *Lancet* 1:1196–2001
19. Fernandes G, Nair N, Onoe K, Tanaka T, Floyd R, Good R. 1979. Impairment of cell mediated immunity function in dietary zinc deficiency in mice. *Proc. Natl. Acad. Sci. USA* 76:457–61
20. Finamore A, Roselli M, Meredino N, Nobili F, Vignolini F, Mengheri E. 2003. Zinc deficiency suppresses the development of oral tolerance in rats. *J. Nutr.* 133:191–98
21. Fong L, Li J, Farker J, Magee P. 1996. Cell proliferation and esophageal carcinogenesis in the zinc deficient rat. *Carcinogenesis* 17:1841–48
22. Fraker PJ, Caruso R, Kierszenbaum F. 1982. Alteration of the immune and nutritional status of mice by synergy between zinc deficiency and infection with *Trypanosoma cruzi*. *J. Nutr.* 112:1224–29
23. Fraker PJ, DePasquale-Jardieu P, Zwickl CM, Lueke R. 1978. Regeneration of T-cell helper function in zinc-deficient adult mice. *Proc. Nat. Acad. Sci.* 75:5660–66
24. Fraker PJ, King L, Laakko T, Vollmer T. 2000. The dynamic link between the integrity of the immune system and zinc status. *J. Nutr.* 130:13995–4065
25. Fraker PJ, Osati-Ashtiani F, Wagner M, King LL. 1995. Possible roles for glucocorticoids and apoptosis in the suppression of lymphopoiesis during zinc deficiency. *J. Am. Coll. Nutr.* 14:11–17
26. Garvy B, King L, Telford W, Morford L, Fraker PJ. 1993. Chronic levels of corticosterone reduces the number of cycling cells of the B-lineage in murine bone marrow and induces apoptosis. *Immunology* 80: 587–92
27. Garvy B, Telford W, King L, Fraker PJ. 1993. Glucocorticoids and irradiation induced apoptosis in normal murine bone marrow B-lineage lymphocytes as determined by flow cytometry. *Immunology* 79: 270–77
28. Golden M, Golden B, Harland P, Jackson A. 1978. Zinc and immunocompetence in protein energy malnutrition. *Lancet* i:1226–28
29. Gross R, Hansel H, Schultiuk S, Shrimpton R, Matulessi P, et al. 1998. Moderate zinc and vitamin A deficiency in breast milk of mothers from East-Jaharta. *Eur. J. Clin. Nutr.* 52:884–90
30. Hambidge M. 2000. Human zinc deficiency. *J. Nutr.* 130:1344–49S
31. Hardy R, Hayakawa K. 2001. B-cell developmental pathways. *Annu. Rev. Immunol.* 19:495–521
32. Hengartner M. 2000. The biochemistry of apoptosis. *Nature* 407:770–76
33. Ho E, Ames B. 2002. Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. *Proc. Natl. Acad. Sci. USA* 17:1841–48
34. Ho E, Courtemanche C, Ames B. 2003. Zinc deficiency induces oxidative DNA damage and increases p53 expression in human lung fibroblasts. *J. Nutr.* 133:2543–48
35. Keen C. 1993. Maternal dietary zinc influences DNA strand break and 8-hydroxy-2-deoxyguanosine levels in infant rhesus monkey liver. *Proc. Soc. Exp. Biol. Med.* 203:416–66
36. Keen C, Gershwin M. 1990. Zinc deficiency and immune function. *Annu. Rev. Nutr.* 10:415–31
37. King J. 2000. Specific nutrient requirements. In *Nutrition and Immunology*, ed. M Gershwin, J German, C Keen, T Otowa, pp. 65–74. Totowa, NJ: Humana
38. King LE, Fraker PJ. 2002. Zinc deficiency in mice alters myelopoiesis and hematopoiesis. *J. Nutr.* 132:3301–7
39. King LE, Fraker PJ. 2000. Variations in cell cycle status of lymphopoietic and

myelopoietic cells created by zinc deficiency. *J. Infect. Dis.* 182:16–22
40. King LE, Fraker PJ. 1991. Flow cytometric analysis of the phenotypic distribution of splenic lymphocytes in zinc deficient adult mouse. *J. Nutr.* 121:1433–38
41. King LE, Osati-Ashtiani F, Fraker PJ. 2002. A distinct role for apoptosis in the loss of precursor lymphocytes during zinc deficiency. *J. Nutr.* 132:974–79
42. King LE, Osati-Ashtiani F, Fraker PJ. 1995. Depletion of cells of the B-lineage in the bone marrow of zinc deficient mouse. *Immunology* 85:69–73
43. Krebs N, Sontag M, Accuiso F, Hambidge M. 1998. Low plasma zinc concentration in young infants with cystic fibrosis. *J. Pediatr.* 133:761–64
44. Kuvibidila S, Yu L, Ode D, Warrier RP. 1993. The immune response in protein-energy malnutrition and single nutrient deficiencies. In *Human Nutrition: A Comprehensive Treatise*, ed. DM Klurfeld, 8:121–57. New York: Plenum
45. Laakko T, Fraker PJ. 2002. Interleukin 7 mediated protection of pro and pre B-cells from the adverse effects of corticosterone. *Cell Immunol.* 220:39–50
46. Laakko T, Fraker PJ. 2002. Rapid changes in lymphopoietic and granulopoietic compartments of the marrow caused by stress levels of corticosterone. *Immunology* 105:1–15
47. Lepage L, Giesbrecht J, Taylor C. 1999. Expression of T lymphocyte p56(lck), a zinc-finger signal transduction protein, is elevated by dietary zinc deficiency and diet restriction in mice. *J. Nutr.* 129:620–27
48. Luecke RW, Simonel CE, Fraker PJ. 1978. The effect of restricted dietary intake on the antibody-mediated response of the zinc-deficient A/J mouse. *J. Nutr.* 108:881–87
49. McCabe M, Jiang J, Orrenius S. 1993. Chelation of intracellular zinc triggers apoptosis in mature thymocytes. *Lab. Invest.* 69:101–10
50. Merino R, Ding L, Veis D, Korsmeyer S, Nunez G. 1994. Development regulation of the Bcl-2 protein and susceptibility to cell death in B-lymphocytes. *EMBO J.* 13:683–91
51. Moore JB, Blanchard RK, McCormack WT, Cousins RJ. 2001. cDNA array analysis identifies thymic LCK as upregulated in moderate murine zinc deficiency before T-lymphocyte population changes. *J. Nutr.* 131(12):3189–96
52. Murton SA, Tan ST, Prickett TC, Frampton C, Donald RA. 1998. Hormone responses to stress in patients with major burns. *Br. J. Plast. Surg.* 51(5):388–92
53. Offner PJ, Moore EE, Ciesla D. 2002. The adrenal response after severe trauma. *Am. J. Surg.* 184(6):649–53
54. Oleski J, Westphal M, Starr S, Shore S, Gordon D, et al. 1979. Corrections with zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. *Am. J. Dis. Child.* 133:915–21
55. Ortiz R, Cortes L, Gonzalez-Marquez H, Gomez JL, Gonzalez C, Cortes E. 2001. Flow cytometric analysis of spontaneous and dexamethasone-induced apoptosis in thymocytes from severely malnourished rats. *Br. J. Nutr.* 86:545–48
56. Osati F, King L, Fraker PJ. 1998. Variance in the resistance of murine early B-cells to a deficiency in zinc. *Immunology* 94:94–100
57. Osendarp S, van Raaij J, Darmstadt G, Baqui A, Fuchs G. 2001. Zinc supplementation during pregnancy and effects on growth and morbidity in low birth weight infants. *Lancet* 357:1080–85
58. Prasad AS. 1985. Clinical and biochemical manifestation of zinc deficiency in human subjects. *J. Pharmacol.* 16:344–52
59. Prasad AS, Miale A, Faud Z, Schubert A, Sandstead A. 1963. Zinc metabolism in patients with the syndrome of iron deficiencies, anemia, hypogonadisin and dwarfism. *J. Clin. Med.* 61:537–45
60. Rogers J, Taubeneck M, Daston G, Sulik K, Zucher R, et al. 1995. Zinc deficiency causes apoptosis but not cell cycle alterations in organogenesis-stage rat embryos:

effect of varying duration of deficiency. *Teratology* 52:149–59

61. Rolink A, Melchers F. 2001. Hematopoietic stem cells: lymphopoiesis and the problem of commitment versus plasticity. In *Stem Cell Biology*, ed. D Marshak, R Gardner, D Gottlich, pp. 307–27. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press
62. Shi H, Scott M, Stevenson M, Koski K. 1998. Energy restriction and zinc deficiency impair the functions of murine T cells and antigen presenting cells during gastrointestinal nematode infection. *J. Nutr.* 28:20–27
63. Smith H, Latham M, Azaburke J, Butler W, Phillips L, et al. 1981. Blood plasma levels of cortisol, insulin, growth hormone and somatomedin in children with marasmus, kwashiorkor, and intermediate forms of protein-energy malnutrition. *Proc. Soc. Exp. Biol. Med.* 167:607–11
64. Tapazoglou E, Prasad AS, Hill G, Brewer G, Kaplan J. 1985. Decreased natural killer cell activity in patients with zinc deficiency with sickle cell disease. *J. Lab. Clin. Med.* 105:19–22
65. Wessely O, Deiner E, Beug H, Von Linden M. 1987. The glucocorticoid receptor is a key regulator of the decision between self-renewal and differentiation in erythroid progenitors. *EMBO J.* 16:267–80
66. Wing EG, Magee DM, Barczynski LK. 1988. Acute starvation in mice reduces number of T cells and suppresses the development of T cell mediated immunity. *Immunology* 63:677–82
67. Zemel-Babett S, Kawchali D, Fung E, Obene K, Stallings V. 2002. Effect of zinc supplementation on growth and body composition in children with sickle cell disease. *Am. J. Clin. Nutr.* 75:300–7

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