

Effects of Zinc Deficiency on Th1 and Th2 Cytokine Shifts

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Nutritional deficiency of zinc is widespread throughout developing countries, and zinc-deficient persons have increased susceptibility to a variety of pathogens. Zinc deficiency in an experimental human model caused an imbalance between Th1 and Th2 functions. Production of interferon- γ and interleukin (IL)-2 (products of Th1) were decreased, whereas production of IL-4, IL-6, and IL-10 (products of Th2) were not affected during zinc deficiency. Zinc deficiency decreased natural killer cell lytic activity and percentage of precursors of cytolytic T cells. In HuT-78, a Th0 cell line, zinc deficiency decreased gene expression of thymidine kinase, delayed cell cycle, and decreased cell growth. Gene expression of IL-2 and IL-2 receptors (both α and β) and binding of NF- κ B to DNA were decreased by zinc deficiency in HuT-78. Decreased production of IL-2 in zinc deficiency may be due to decreased activation of NF- κ B and subsequent decreased gene expression of IL-2 and IL-2 receptors.

Although the essentiality of zinc for animals has been recognized since 1934, it was not until the early 1960s that zinc was recognized as essential for humans [1, 2]. Zinc deficiency in humans was first documented in Middle Eastern dwarfs in 1963 [2] and has since been documented worldwide. It is now estimated that in populations in the developing world who subsist mainly on cereal proteins containing high amounts of phytate that about 2 billion persons may have zinc deficiency of varying severity. Growth retardation, susceptibility to infections, and cognitive impairment have been related to zinc deficiency in the developing world [3]. It is clear that zinc deficiency is a major public health problem worldwide with substantial consequences.

During studies in the Middle East, my group and I observed that most zinc-deficient dwarfs did not survive beyond the age of 25 years. The cause of death appeared to be related to infections but their exact nature was not clear. Parasitic infections were common. Viral and bacterial infections, however, were undocumented. The possibility that zinc deficiency may have played a role in immune dysfunctions in the zinc-deficient dwarfs was considered but we were unable to gather meaningful data on immune functions in these Middle Eastern patients because of inadequate facilities.

An extreme example of the effects of zinc deficiency in humans

has been observed in patients with acrodermatitis enteropathica, a genetic disorder of zinc malabsorption [4, 5]. This condition is characterized by mucocutaneous lesions, diarrhea, failure to thrive, and frequent severe infections with fungi, viruses, and bacteria. Affected subjects have thymic atrophy, anergy, reduced lymphocyte proliferation response to mitogens, a selective decrease in T4⁺ helper cells, and deficient thymic hormone activity. All of these changes are correctable by zinc supplementation. A less severe cellular immune defect has been reported in patients who become zinc deficient while receiving total parenteral nutrition without zinc. These abnormalities, which include lymphopenia, decreased ratios of CD4-to-CD8 cells, decreased natural killer (NK) activity, and increased monocyte cytotoxicity, are readily corrected by proper zinc supplementation [6].

In the early 1980s, further evidence of a relationship between zinc deficiency and immune dysfunctions in humans was reported in uremic patients on hemodialysis and in the elderly. The skin sensitivity test was restored to normal in 3 of 4 anergic hemodialyzed patients who were treated with zinc [7]. In a study in the elderly, the group given zinc had a higher percentage of circulating T lymphocytes, an increased frequency and magnitude of delayed hypersensitivity skin reactions to purified proteins, and greater IgG antibody response to tetanus toxoid [8]. Although these results suggest that some defects in cellular immunity in these two groups were related to zinc deficiency, the findings were not definitive since zinc status was not evaluated prior to the treatment.

Here I will summarize various studies done by my group related to zinc deficiency. We have studied immune functions in an experimental human model and in patients with sickle cell disease (SCD). I will also present results of pertinent studies in a cell culture model in which we utilized HuT-78 cells, a human Th0 malignant lymphoblastoid cell line.

The experimental protocol was reviewed and approved by the Human and Animal Investigation Committee of Wayne State University. Informed consent was obtained from all subjects.

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Development of an Experimental Human Model of Zinc Deficiency

We developed an experimental model that allowed us to study specific effects of mild zinc deficiency on human immune functions [9]. A semipurified diet based on texturized soy protein was developed for consumption by human volunteers. This diet supplied calories, protein, macro- and microelements, and vitamins according to recommended dietary allowances (RDA; National Academy of Sciences, Food and Nutrition Board), except for zinc, which was varied as desired.

Male volunteers aged 20–45 years were selected for these studies. Before the study, each subject's history was obtained and each had a thorough physical examination and routine laboratory tests (including complete blood count, liver function, sequential multiple analyzer-12, and serum electrolytes). All were normal. The volunteers were ambulatory and were encouraged to do moderate daily exercise throughout the study period.

The subjects were given a hospital diet containing animal protein daily for 4 weeks. This diet averaged 12 mg of zinc/day, consistent with the RDA. After that they received a semipurified soy protein-based experimental diet that supplied 3.0–5.0 mg of zinc/day. The details for preparation of the experimental diet have been published [9]. This regime was continued for 28 weeks, after which the subjects received 27 mg of zinc supplement/day for 12 weeks while still consuming the experimental diet. Throughout the study, the amounts of all nutrients, including protein, amino acids, vitamins, and minerals (both macro- and microelements), were kept constant, meeting the RDA, except for zinc, which was varied as outlined above. By this technique, we were able to induce a specific zinc deficiency in the volunteers.

Peripheral blood cells (lymphocytes, granulocytes, and platelets) for zinc assay were isolated by a modification of a previously published method [10]. Special care was taken to remove red cells from the granulocytes, platelets from the granulocytes and lymphocytes, and trapped plasma from the platelets. Extreme care was taken to avoid exogenous zinc contamination throughout the assay procedure. Zinc was assayed in the samples by means of an atomic-absorption spectrophotometer [10].

When zinc deficiency was very mild (5.0 mg of zinc intake during the zinc-restricted period), the plasma zinc concentration remained more or less within the normal range for 12 weeks and decreased only after 4–5 months of zinc restriction [11]. However, zinc concentrations in lymphocytes, granulocytes, and platelets decreased within 8–12 weeks, suggesting that the assay of cellular zinc may provide a sensitive criterion for diagnosing mild zinc deficiency [11].

In subjects who received 3.0 mg of dietary zinc during the zinc-restricted period, the plasma zinc concentration remained $>100 \mu\text{g/dL}$ for 2 months on the zinc-restricted diet. In the third month, a significant decrease in the plasma zinc concentration was observed [12]. In these volunteers, the zinc in platelets decreased within 1 month, and zinc concentrations in lympho-

cytes and granulocytes decreased within 2 months after institution of the zinc-restricted diet [12]. The maximum decline in cellular zinc concentrations was observed at the end of 6 months of restricted dietary zinc intake.

Serum Thymulin Activity

We [12] assayed serum thymulin activity in mildly zinc-deficient human subjects. Thymulin is a thymus-specific hormone and it requires the presence of zinc for its biologic activity to be expressed [13, 14]. Thymulin binds to high-affinity receptors on T cells, induces several T cell markers, and promotes T cell function, including allogenic cytotoxicity, suppressor functions, and interleukin (IL)-2 production. The serum level of biologically active thymulin was evaluated by a rosette assay as described [13, 14]. The assay analyzes the conversion of relatively azathioprine-resistant splenic cells of adult thymectomized mice to θ -positive rosette-forming cells that are more sensitive to azathioprine.

As a result of mild zinc deficiency, the activity of thymulin in the serum was significantly decreased and was corrected by both in vivo and in vitro zinc supplementation. The in vitro supplementation studies indicated that the inactive thymulin peptide was present in serum in zinc-deficient subjects and was activated by addition of zinc [12]. Thus, the assay of serum thymulin activity with or without zinc addition in vitro may be used as a sensitive criterion for the diagnosis of mild zinc deficiency in humans.

Production of Cytokines by Peripheral Blood Mononuclear Cells (PBMC)

The separation of Th cells into Th1 and Th2 according to their functions in cell-mediated (Th1) and humoral immunity (Th2) is becoming very useful in the understanding of the immune mechanisms in humans [15–17]. IL-2 and interferon (IFN)- γ are considered to be the products of Th1 cells, whereas IL-4, -6, and -10 are products of Th2 cells. IFN- γ down-regulates the Th2 clone, and IL-10 may down-regulate the Th1 clone [15–17]. An imbalance between Th1 and Th2 responses in persons with human immunodeficiency virus infection has been implicated in the immune dysregulation in these patients and it has been proposed that resistance to infection and/or progression to AIDS is dependent on a Th1 $>$ Th2 dominance [15–17]. Th1 cells promote macrophage activation and production of complement fixing and opsonizing antibodies [15–17]. IFN- γ is the major component of Th1 response panel, and it up-regulates major histocompatibility complex class I antigen expression.

In our earlier studies in the experimental model of human zinc deficiency, we assayed IL-2 activity in normal volunteers once a month during baseline and in zinc depletion and repletion phases [12]. PBMC obtained by ficoll-hypaque separation were used for production of IL-2 by culture for 48 h with 1%

Table 1. Effects of dietary zinc restriction in human volunteers on zinc concentrations and immunologic functions.

| | Daily zinc intake | |
|--|--|--|
| | 2–3 mg | 3–5 mg |
| Plasma zinc ($\mu\text{g}/\text{dL}$) | Decreased in 12 weeks | Remained normal until 20 weeks |
| Lymphocyte zinc ($\mu\text{g}/10^{10}$ cells) | Decreased in 8 weeks | Decreased in 20 weeks; no earlier measurements |
| Immunologic effects | | |
| Cytokine production | | |
| Th1 | IL-2 decreased within 8–12 weeks | Production of IL-2 and interferon- γ decreased in 20 weeks; no earlier measurements |
| Th2 | Not measured | IL-4, -6, and -10 remained normal when assayed at 20 weeks |
| Serum thymulin activity | Decreased within 8–12 weeks | Not assayed |
| NK cell lytic activity | Decreased within 16–20 weeks | Not assayed |
| T cell subpopulation | | |
| CD4 ⁺ /CD8 ⁺ | Decreased in 20 weeks; no earlier measurements | Decreased in 20 weeks; no earlier assays |
| CD4 ⁺ CD45RA ⁺ /CD4 ⁺ CD45R0 ⁺ | Not assayed | Decreased in 20 weeks; no earlier assays |
| CD8 ⁺ CD73 ⁺ (CD8 ⁺ precytolytic T cells) | Not assayed | Decreased in 20 weeks; no earlier assays |
| Lymphocyte ecto 5' nucleotidase (CD73 ⁺) | Not assayed | Decreased within 8 weeks |

NOTE. Data from [9, 11, 12, 18, 19, 20]. IL, interleukin.

phytohemagglutinin-M, a T cell mitogen, at 10^6 cells/mL in a 2-mL final volume. The IL-2 content of supernatants harvested after 48 h of culture was measured in a proliferation assay by using a murine IL-2-dependent T cell line, CTLL-20, and a standard reference IL-2 preparation was arbitrarily assigned an IL-2 activity of 100 U/mL. The IL-2 activity declined following zinc restriction and was corrected by zinc repletion [12].

In a more recent study [18], we assayed IL-2, IFN- γ , and IL-4, -6, and -10 by commercial ELISA kits (Quantikine; R&D Systems, Minneapolis). Whereas IL-2 and IFN- γ production declined during zinc restriction, IL-4, -6, and -10 production were not affected by zinc status [18]. Both IL-2 and IFN- γ production were corrected by zinc repletion. These results suggest that zinc deficiency in humans mainly affects the cytokine production of Th1 cells and that the cell-mediated immune dysfunction in human zinc deficiency may be due to an imbalance between Th1 and Th2 cells. Inasmuch as we induced only a mild deficiency of zinc in our volunteers by dietary means, it appears that the immunologic effects on Th cells were sensitive to zinc restriction.

Th cells produce IL-2, which in addition to triggering T cell proliferation, augments NK activity. NK cell activity plays an important role in host resistance to tumors and microbial infections. We measured NK cell lytic activity in a 4-h chromium release assay against standard target cell line k562. The techniques of this assay have been described [19].

In one of our studies in the experimental human model of zinc deficiency, male volunteers received a zinc-restricted diet for 20 weeks followed by zinc repletion with 30 mg/day for 14 weeks. Plasma zinc concentration decreased significantly by week 20 of the zinc-restricted diet and returned to normal after 14 weeks of zinc repletion. NK cell activity also decreased by week 20, but with zinc repletion it returned to the normal range [19].

Our studies in an experimental human model showed that the percentage of CD8⁺CD73⁺ lymphocytes that are predominantly precursors to cytotoxic T lymphocytes (CTL) decreased during zinc-restricted periods. It has been reported that the

presence of CD73 molecule on CTL is required for antigen recognition, the proliferative process, and for the generation of cytolytic process [20]. IL-2 is required not only for NK cell lytic activity but also CTL.

T cell subpopulation studies revealed that the CD4⁺ to CD8⁺ ratio was significantly related to zinc status [18, 21]. A decrease in this ratio was observed during zinc deficiency but was corrected by zinc supplementation. A borderline significant effect of zinc status on the ratio of CD4⁺CD45RA⁺ to CD4⁺CD45R0⁺ cells was seen in the volunteers. The newly produced CD4⁺ T lymphocytes express CD45 isoforms, designated CD45RA⁺, and once these cells encounter a specific antigen, they become "memory" T lymphocytes, and express a small isoform of cleaved CD45, designated CD45 R0⁺ cells [22]. Zinc is required for the regeneration of new CD4⁺ T cells. Inasmuch as zinc is essential for the activity of thymulin, a thymic hormone, it is possible that zinc may be intrinsically involved in the development of hematopoietic stem cells to T lymphocytes in the thymic microenvironment [13, 14].

Our studies show that even a mild deficiency of zinc in humans may be accompanied by an imbalance of functions of Th1 and Th2 cells, decreased serum thymulin activity, decreased recruitment of T naive cells, decreased percentage of T cytolytic cells, and decreased NK cell lytic activity. These immunologic consequences of zinc deficiency may be responsible for decreased cell-mediated immune functions in zinc-deficient subjects.

Indicators of Human Zinc Deficiency

In our studies in human subjects in whom we induced zinc deficiency by dietary means, the zinc concentrations in lymphocytes and granulocytes appeared to be better indicators of a mild deficiency of zinc than the plasma zinc concentration (table 1). Assay of lymphocyte ecto 5' nucleotidase (CD73 is a marker of this enzyme); IL-2 production and serum thymulin activity were also sensitive indicators of mild deficiency of zinc in our studies (table 1).

Plasma zinc concentration is the most widely used assay for diagnosing zinc deficiency. Although it is adequate in developing countries where zinc deficiency is more pronounced and prevalent, it is not suitable for diagnosing zinc deficiency in areas where one would expect to find only a marginal or mild nutritional zinc deficiency. In the United States, a mild nutritional zinc deficiency is seen in elderly subjects and in some younger adults who restrict calories and animal protein intake for various reasons. Requirement for zinc is increased in pregnant women and in adolescents due to growth and, if the zinc intake is not appropriate, one may see a mild zinc deficiency in these persons in developed countries.

Studies in Persons with SCD

Zinc deficiency is relatively common in adults with SCD, affecting about 60%–70% of adult subjects in our center [23–25]. In SCD patients, our diagnosis of zinc deficiency is based on zinc levels in lymphocytes and granulocytes [12, 20, 23–25]. Increased hemolysis in SCD patients releases a considerable amount of zinc, which circulates in the plasma pool. This results in an increase in glomerular filtration of zinc, but its reabsorption is hampered by the renal tubular damage caused by repeated vasoocclusive episodes [26]. The resultant hyperzincuria and a high protein turnover due to increased hemolysis increases the daily requirement for zinc significantly in SCD patients; however, this requirement is not met by the usual dietary intake. The role of intestinal absorption in the homeostasis of zinc in SCD remains to be investigated. Zinc deficiency has been also documented in SCD patients aged 3–18 years. Patients classified as having “poor” growth had lower serum zinc concentrations than those with “normal” growth [27]. Other observers have also reported zinc deficiency in SCD patients [28–31]. We previously showed that growth delays, hypogonadism in males, abnormal dark adaptation, and hyperammonemia in SCD patients are related to zinc deficiency and corrected by zinc supplementation [24, 32–34].

Immune Functions and SCD

We evaluated cell-mediated immunity in 26 patients with SCD by using skin tests for delayed hypersensitivity reaction [35]. Patients with impaired delayed hypersensitivity reactions had lower zinc levels in plasma, erythrocytes, and neutrophils than persons with normal delayed hypersensitivity skin reactions or controls. The activity of nucleoside phosphorylase, an enzyme essential for T lymphocyte function, was significantly lower in anergic zinc-deficient SCD patients. Three anergic patients entered a trial of oral zinc supplementation (zinc acetate, 45 mg/day) and were reevaluated 6 months later. All 3 subjects showed an improvement following zinc supplementation in delayed hypersensitivity skin reactions and in increased neutrophil zinc levels and nucleosidephosphorylase activity. We concluded

that zinc deficiency in patients with SCD is associated with impaired delayed hypersensitivity skin reactions.

To assess the relationship of zinc and NK cell activity, we studied NK cell activity in adults with SCD [19]. NK cell lytic activity was significantly lower in persons with SCD who were zinc deficient than in controls (5.1 ± 2.9 vs. 11.7 ± 5.0 lytic U/ 10^6 cells, respectively, $P \leq .0003$). Inasmuch as IL-2 is known to augment NK activity, we believe that the decreased NK activity in SCD patients is due to decreased IL-2 production as a result of zinc deficiency [12, 18].

We assayed IL-2 production by PBMC after PHA stimulation for 48 h in 10 SCD patients with normal plasma zinc concentration and in 11 SCD patients who were hypozincemic [36]. IL-2 was measured in a proliferation assay by use of a murine IL-2-dependent T cell line CTLL-20. The hypozincemic SCD subjects had significantly decreased IL-2 production when compared with normozincemic SCD subjects and controls ($P < .01$). IL-2 is an activator of NK cells, is required for the expansion and maintenance of thymocyte and peripheral T cell populations, and is essential for many T cell-dependent *in vivo* immune functions, including generation of antiviral- and antitumor-specific CTL and delayed-type hypersensitivity responses.

We assayed serum thymulin (a thymic hormone) activity in 6 zinc-deficient adults with SCD before and after zinc supplementation. The diagnosis of zinc deficiency was based on the assay of zinc in lymphocytes, granulocytes, and platelets. Serum thymulin activity was decreased due to zinc deficiency but was corrected by both *in vivo* and *in vitro* zinc supplementation, suggesting that this parameter is a sensitive indicator of zinc deficiency [12]. SCD patients before zinc supplementation had increased levels of absolute lymphocytes and of $slg^+ T101^-$ and slg^- cells and a decreased ratio of T4/T8 cells compared with the normal controls. After zinc supplementation, we observed a significant increase in slg^+ and T4⁺ cells [12]. The number of T101⁻ slg^- cells decreased significantly after zinc supplementation. Inasmuch as thymulin induces intra- and extrathymic T cell differentiation, our data provide a possible mechanism for the role of zinc on T cell functions.

T lymphocytes from normal human controls and SCD patients were isolated from peripheral blood and cultured for 72 h following PHA stimulation. The ratio for the fraction of cells in DNA synthesis (S phase) over the fraction in G₂ phase was significantly higher in SCD patients than in the controls (mean \pm SD) (4.01 ± 0.78 vs. 2.78 ± 0.76 ; $P < .02$). After *in vivo* zinc supplementation, the S/G₂ ratio was normalized [37]. We concluded that the cell cycle of T lymphocytes is altered in SCD patients and that this effect is zinc dependent [37].

We used flow cytometry to assess the changes in T cell populations resulting from zinc deficiency in subjects with SCD and in healthy human volunteers [20]. Zinc deficiency was associated with significant decreases in cellular zinc concentrations, CD4⁺/CD8⁺ ratio, and percentage of CD73⁺ cells in the CD8⁺ population. The decrease in the percentage of CD73⁺

cells in the CD8⁺ subset was significantly correlated with lymphocyte zinc concentration and was accompanied by essentially no change in percentage of CD11b⁺ cells in the CD8⁺ subset. Daily oral zinc supplementation of 9 zinc-deficient volunteers (25 mg of elemental zinc) and of 7 zinc-deficient SCD subjects (50 mg of elemental zinc) resulted in an increase in absolute lymphocytes, a significant increase in the CD4⁺/CD8⁺ ratio, and an increase in the percentage of CD73⁺ cells in the CD8⁺ subset. In the zinc-supplemented subjects, the increase in the percentage of CD73⁺ cells was accompanied by a significant decrease in the percentage of CD11b⁺ cells in the CD8⁺ subset. Changes in the CD4⁺/CD8⁺ and CD73⁺/CD11b⁻ cell ratios in the CD8⁺ subset after treatment indicate that important changes take place in the T cell subpopulation as a result of zinc deficiency in SCD patients.

Zinc deficiency adversely affects Th1 functions, cell-mediated immunity, and IL-2 production in zinc-deficient subjects. We hypothesized that zinc supplementation would improve Th1 function and decrease incidence of infections in persons with SCD [25] and tested this hypothesis in 32 SCD subjects, whom we divided into three groups (A, B, and C). Groups A ($n = 11$) and B ($n = 10$) were zinc deficient on the basis of cellular zinc criteria. Group C ($n = 11$) comprised zinc-sufficient subjects. We observed group A subjects for 1 year (baseline) before they were given zinc acetate (50–75 mg elemental zinc orally/day) for 3 years. Group B subjects were observed for 1 year (baseline), received placebo for 1 year, and then were switched to zinc supplementation (50–75 mg elemental zinc orally/day) for 2 years. Group C subjects were zinc sufficient and did not receive any intervention. Prolonged zinc supplementation resulted in an increase in lymphocyte and granulocyte zinc ($P = .0001$), increased IL-2 production ($P = .0001$), decreased incidence of documented bacteriologically positive infections ($P = .0026$), decreased number of hospitalizations, and decreased number of vasoocclusive pain crises ($P = .0001$). The predominant pathogens isolated were staphylococcus and streptococcus involving the respiratory tract and aerobic gram-negative bacteria, particularly *Escherichia coli*, involving the urinary tract. Confirmation of our observations will require prospective studies of zinc supplementation in a larger group of SCD patients.

Effect of Zinc on IL-2 Expression in HuT-78 Cells

For IL-2 expression studies, we utilized HuT-78, a human malignant T lymphoblastoid cell line (ATCC). In our laboratory this Th0 cell line is very responsive to zinc status. Details of techniques involved in developing zinc-deficient cells have been published [38]. In short, we chelated fetal bovine serum (FBS) and reconstituted FBS with all essential minerals except zinc. In the zinc-deficient culture media, the zinc concentration was 1 μM . The zinc-sufficient media was prepared by adding zinc chloride to the zinc-deficient media, thus making the zinc concentration 15 μM .

The growth of the cells in zinc-deficient medium was significantly decreased, although the cells remained viable. The zinc concentration of cells were reduced by about 50% in 4 days in the zinc-deficient medium. In zinc-deficient and zinc-sufficient media, the cell doubling time (mean \pm SD) of HuT-78 cells were 59 ± 8 h and 32.6 ± 6 h, respectively [38]. Thymidine kinase (TK) activity and the relative accumulation of TK mRNA were significantly decreased in zinc-deficient cells during the gastrointestinal cell cycle phase in comparison with zinc-sufficient cells. Nuclear run-on experiments and actinomycin-D studies showed that the transcription of TK mRNA was affected adversely by zinc deficiency. Cell cycle studies showed that more zinc-deficient cells remained in S phase and did not undergo mitosis in comparison with zinc-sufficient cells. We concluded that zinc is a T cell-specific growth factor and that a decreased gene expression of DNA-synthesizing enzyme TK in zinc-deficient HuT-78 cells in the G1 phase affected adversely the DNA synthesis in S phase and delayed cell cycle [38].

The production of IL-2 is a key event in the activation of T lymphocytes. IL-2 triggers peripheral T lymphocytes to enter the S phase of the cell cycle and divide [39]. This is probably due to the suppressive effect of IL-2 on cell cycle inhibitors, which interferes with the activity of cyclin-dependent kinases at checkpoints of the cell cycle [39].

A relatively short piece of DNA, a 275-bp-long segment in the promoter area, integrates numerous signaling pathways leading to IL-2 synthesis and the activation and proliferation of T lymphocytes [39]. Both murine and human IL-2 promoters contain one binding site for genuine Rel/NF- κ B factors. The sequence of this site, GGGATTCAC, is identical for both promoters. NF- κ B factors are involved in the acute-phase response, inflammatory processes, and the specific immune response. They are rapidly induced by a variety of stimuli and factors activating T cells. Nearly every stimulus leading to T cell activation also activates NF- κ B. Such stimuli include triggering of T cells by antigens, exposure of T cells to antibodies directed against T cell surface proteins, and treatment of lymphocytes by phorbol esters, calcium ionophores, lectins, and cytokines (e.g., TNF- α and IL-1).

Relationship of Zinc to IL-2 Expression and HuT-78 Cells

I will summarize our recent findings (unpublished data) regarding IL-2 expression in HuT-78 cells. After the zinc-deficient and -sufficient HuT-78 cells were stimulated with PHA/PMA for 6 h, the supernatant of the culture media was assayed for IL-2 and total soluble (s) IL-2 receptor (R) by methods previously established in our laboratory. The IL-2 production showed 40% decrease in zinc-deficient cells in comparison with zinc-sufficient cells. The relative abundance of IL-2 mRNA was assayed by Northern blot analysis by established techniques. A 50% decrease in relative abundance of IL-2 mRNA was

observed in zinc-deficient cells after stimulation for 6 h in contrast with the zinc-sufficient cells. The IL-2 mRNA stability was not affected by zinc deficiency as determined by use of actinomycin D, an mRNA synthesis inhibitor. We also used cycloheximide, a protein synthesis inhibitor, in our experiments. Our results showed that the effect of zinc was on the gene expression of IL-2 and not at the translational level.

sIL-2R α and total sIL-2R were also significantly decreased (70%–80%) as a result of zinc deficiency in HuT-78 cells in comparison with zinc-sufficient cells. By Western blot analysis, IL-2R α was decreased 70% in zinc-deficient cells compared with zinc-sufficient cells. The IL-2R α mRNA was decreased 40% in zinc-deficient cells in comparison with zinc-sufficient cells. Inasmuch as NF- κ B, a zinc finger transcription factor protein, appears to be involved in the promoter area of IL-2 and IL-2R α genes, we assayed the binding of NF- κ B to DNA after 3 h of stimulation with PHA/PMA in zinc-deficient and -sufficient cells. The nuclear binding of NF- κ B was significantly decreased in HuT-78 cells as a result of zinc deficiency. The effect of zinc deficiency on NF- κ B activation was also determined by gene transfection studies by luciferase assay. Zinc deficiency adversely affected the NF- κ B enhancer in HuT-78 cells.

Our studies in a cell culture model show that zinc is involved in the gene expression of IL-2 and IL-2R α . The activation of NF- κ B (a zinc finger protein), a transcription factor involved in the promoter area of IL-2 and IL-2R α genes, also appears to be zinc dependent. The effect of zinc deficiency on TK and IL-2 and IL-2R α production provide basic mechanisms for the role of zinc on T cell proliferation and activation.

Effect of Zinc on Infectious Diseases

Several studies have demonstrated the benefits of zinc supplementation on infectious diseases in human populations. In double-blind placebo-controlled trials of zinc supplementation, zinc reduced the incidence and duration of acute and chronic diarrhea and acute lower respiratory tract infections in infants and children [40–42]. Zinc also reduced the incidence of clinical disease caused by *Plasmodium falciparum* [40]. Zinc supplementation of sickle cell anemia patients in a placebo-controlled trial resulted in decreased incidence of *Staphylococcus aureus* pneumonia, *Streptococcus pneumoniae* tonsillitis, and *E. coli* urinary tract infections [25].

Effects of Micronutrients Other than Zinc on Immunity

Decreased lymphocyte proliferation in iron deficiency has been reported. Reduced protein kinase C (PKC) activity and poor translocation of PKC from cytosol to membrane results in aberrant signal transduction, which in turn might be responsible for the impaired lymphocyte proliferation associated with iron deficiency in mice [43]. Marginal vitamin A status in older Lewis rats was associated with more CD8⁺ T cells, lower CD4⁺/CD8⁺

ratios, decreased production of IL-2 by PBMC and splenocytes (due to fewer T cells), and an increase in NK T cells [44]. These changes may reflect an increase in extrathymic T cell differentiation as a result of decreased T cell export from the thymus in older rats [44]. Extrathymically derived T cells often differ from thymus-derived T cells in phenotype and function. Extrathymically derived T cells express a restricted T cell receptor repertoire, less CD3, preferentially express CD8 versus CD4, and display markers usually associated with NK cells. Vitamin A supplementation protects against measles in some developing countries. Vitamin E may protect against some T cell-mediated diseases (e.g., tetanus and hepatitis) but is ineffective for diphtheria, which is also a T cell-mediated disease. However, the mechanism of vitamin E action on T cells is not well understood. In selenium-deficient mice, a benign strain of coxsackievirus B₃ undergoes mutation to become a virulent strain and causes myocarditis. The mechanism of selenium action in this transformation of coxsackievirus is not known.

Conclusions

Our studies show that serum thymulin (a thymic hormone) activity is decreased in zinc-deficient humans. Thymulin is a nonapeptide and contains zinc at the active site. Decreased thymulin activity may account for decreased export of naive T cells from the thymus in zinc-deficient persons as indicated by a decreased ratio of CD4⁺CD45RA⁺/CD4⁺CD45RO⁺ cells. In human studies, we found that the production of IL-2 and IFN- γ (products of Th1 cells) are decreased and the production of IL-4, -6, and -10 (products of Th2 cells) are not affected in zinc deficiency. These data indicate an imbalance between Th1 and Th2 functions in zinc deficiency. The decreased activities of NK cell lytic activity and T cell lytic activity in our human studies were most probably due to decreased production of IL-2.

Zinc influences the activity of multiple enzymes at the basic levels of replication and transcription. The activity of TK, an enzyme involved in DNA replication, is decreased in zinc deficiency. Zinc is required for the gene expression of TK. Gene expression of IL-2 and IL-2 receptors (both α and β) also require zinc, and in zinc deficiency the lymphocyte cell proliferation is decreased.

NF- κ B, a zinc finger protein, is present in the promoter area of IL-2 and IL-2 receptors (α and β) genes, and we have observed that in zinc-deficient cells, the activation, translocation, and binding of NF- κ B to DNA are affected adversely (unpublished data). We hypothesize that the effect of zinc on NF- κ B binding to DNA may be a critical factor in gene expression of IL-2 and IL-2 receptors. IFN- γ and IL-12 (a product of macrophages) together potentiate killing of parasites by macrophages. Inasmuch as IFN- γ is zinc dependent, one may hypothesize that the beneficial effect of zinc supplementation on parasitic infections in humans may be due to increased production of IFN- γ . We conclude that zinc has specific effects on

T cell proliferation and functions that are not duplicated by other micronutrients.

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