Trace Element Modulation of Immune Responses and Susceptibility to Infection

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Nutritional regulation of host resistance and susceptibility to infection has been suspected by generations of clinicians and investigators. It is only recently, however, that the critical evidence supporting the role of dietary factors in various aspects of immune responses has been collected and analyzed (1,2). Epidemiological data show that the mortality rate among infants in developing countries increases progressively with worsening nutritional status; most of the deaths are due to infection. The duration and severity of both diarrheal illness and measles are greater among the undernourished. Opportunistic infections more easily take hold and cause disease in malnourished individuals. These clinical observations have been strengthened by experiments in laboratory animals. Nutritional deficiency in the controlled environment of the laboratory consistently results in lymphoid involution and dysfunction, the most prominent effect being evident on cell-mediated immunity (CMI).

One crucial question that arises from these observations relates to the role of single nutrients in modulating immunity and infection. Progressive decrease in the intake of trace elements and other nutrients results in impaired immunity and increased risk of infection (Fig. 1). Many vitamins and trace elements are now established as critical factors in the generation, maintenance, and amplification of immune responses. The topic has been the subject of several workshops and monographs (3,4). Among the important confounding problems that have emerged from these exercises are dose dependency of immune responses, threshold of critical nutrient deficiency, interactions among trace elements, and variations in methodology of measurements of both trace element status and immunocompetence. These important issues are summarized and critically analyzed in this review.

ZINC

Zinc was first labeled as an essential nutrient for growth and well-being of rats in 1934 by Todd et al. (5). However, its importance in human nutrition has been recognized only during the last two decades. Being a part of many metalloenzymes, zinc is important for nearly all aspects of cellular metabolism (6). Thymidine
kinase, RNA polymerase, DNA polymerase, and ribonuclease are some of the zinc-dependent enzymes that are crucial catalysts involved in the replication and transcription of DNA during cell division. Thus zinc plays a fundamental role in growth and tissue maintenance. Zinc deficiency through its adverse effect on zinc-dependent enzymes results in growth failure, diffuse symmetrical dermatological manifestations, diarrhea, loss of hair, mental disturbance, hypogonadism in males, and intercurrent infections with opportunistic organisms. Repeated infections could be secondary to depression of immune function and other host defense mechanisms. The impact of zinc deficiency on immune function has been studied in three circumstances: (a) laboratory animals fed diets low in zinc; (b) zinc deficiency as part of generalized malnutrition in patients with chronic medical or surgical problems (disease states in which zinc deficiency has been reported include preterm low-birth-weight infants, sickle cell anemia, uremia, neoplastic diseases, gastrointestinal disorders, alcoholism, Down's syndrome, and a number of other conditions); and (c) acrodermatitis enteropathica, an inherited zinc-deficient state due to diminished ability of the intestine to absorb zinc.

**Lymphoid Tissues**

Zinc deficiency produces a reversible atrophy of lymphoid tissues including the thymus, spleen, and lymph nodes. In zinc-deficient mice and rats, there is a reduction in weight of lymphoid tissues in excess of total body weight reduction (7-9). The effect is most pronounced on the thymus (Table 1) (7); nonlymphoid organs are not affected significantly. The lymphoid atrophy involves cortical areas of the thymus and the thymus-dependent areas of the spleen and lymph nodes. There is depletion of small lymphocytes from these regions. Golden et al. (10) showed thymic atrophy in eight children with protein-energy malnutrition (PEM) and zinc deficiency. Repletion with calories and protein did not bring about im-
The lymphoid atrophy of zinc deficiency could be related to many factors. Firstly, zinc deficiency reduces cellular multiplication and thus decreases the number of T- and B-lymphocytes being produced during the normal resting phase as well as during antigen stimulation. Secondly, zinc deficiency results in low levels of serum thymic inductive factors, and consequently the maturation and release of thymocytes is impaired. Thirdly, zinc deficiency is associated with an elevation of free-cortisol concentration, which may have a lympholytic effect. Finally, it is possible that zinc deficiency causes shifts in pools of lymphocytes in various tissue compartments and in lymphocyte traffic to various organs, as has been shown in PEM (2).

Cell-Mediated Immunity

The cell-mediated immune response involves a complex interaction of T-lymphocyte subsets including helper cells, memory cells, suppressor cells, and cytotoxic or effector cells, as well as inflammatory cells. After interaction with antigen, sensitized lymphocytes undergo transformation into a primitive blast form with increased metabolic and synthetic activities including DNA synthesis and mitosis of effector cells. They also release lymphokines responsible for amplification of the immune response.

Total lymphocyte count and subsets

Zinc deficiency has been shown to result in a reduced lymphocyte count. Acar et al. (11) studied CMI in 22 Hodgkin's disease patients and found a reduction in the absolute number of lymphocytes and in T-lymphocytes which correlated with serum zinc levels. They further showed that the blastogenic response of lymphocytes
to phytohemagglutinin improved on addition of zinc to cultures. The availability of monoclonal antibodies has now allowed the enumeration of lymphocyte subsets (Fig. 2). There is a significant reduction in the proportion of T4+ helper cells with improvement in the number after zinc therapy. Low thymopoietin levels associated with zinc deficiency increase after zinc treatment, and there is an increase in T-lymphocyte number (12). In laboratory animals fed a low-zinc diet, there is a marked reduction in serum thymic factor activity (13). This effect is considerably greater than may be explained by associated inanition and cachexia. Reduction in thymic inductive factors may be responsible in part for the decreased maturation of T-cell precursors and impaired release from the thymus.

**Lymphocyte proliferation response**

The in vitro proliferation response of lymphocytes to mitogens and antigens is markedly depressed in zinc deficiency (Fig. 3). This has been observed in experimental animals (8) and man (14-15). Pekarek and co-workers (14) studied lymphocytes of a decerebrated patient with zinc deficiency before and after zinc supplementation. They showed a significant depression of ³H-thymidine incorporation into DNA upon phytohemagglutinin-induced stimulation of lymphocytes.
The stimulation response reverted back to normal within 3 weeks of zinc supplementation. In eight patients with acrodermatitis enteropathica and low serum zinc, the lymphocyte stimulation response to phytohemagglutinin was reduced; zinc therapy resulted in an increased serum zinc concentration and enhanced lymphocyte proliferation (15). Hildebrandt and co-workers (16) studied the effect of maternal dietary zinc on the immunological development of suckling mice and found a correlation between the amount of zinc fed and the degree of mitogenic response observed in the pups.

**Delayed cutaneous hypersensitivity**

Delayed cutaneous hypersensitivity (DCH) to recall antigens or chemical sensitizing agents is impaired in zinc deficiency. Golden et al. (17) in a study of 10 children with PEM performed skin testing with *Candida* antigen on both arms of each child. One arm was treated with local application of an ointment containing 1% zinc sulfate and the control arm with the same ointment base without zinc sulfate. It was reported that the arm with zinc sulfate application showed a much larger response, which was suggested to be due to local absorption of zinc and its positive effect on cell-mediated immunity. The amount of difference between the test and the control arm correlated negatively with the plasma zinc concentration. A close look at the size of the reported reactions shows, however, that even on the control arm two subjects showed a reaction of about 5 mm induration which cannot be considered negative, and another three showed a reaction of about 3 to 4 mm induration, which would not be classified as "anergy." Others have not been able to confirm these findings (18) (Table 2). This may in fact be due to the lower prevalence of zinc deficiency in association with PEM observed in most parts of the world compared with the Jamaican report (17). Bjorksten et al. (19) also found low serum zinc levels and depressed DCH in patients with Down's syndrome. With dietary therapy there was an increase in serum zinc levels from 11.7 to 24.3 μM/liter, and the DCH response also improved significantly. They suggested that the increased susceptibility to infection in Down's syndrome may in part be due to zinc deficiency.

In laboratory animals fed a low zinc diet, the sensitization response to 2,4-dinitrofluorobenzene is reduced compared with zinc-replete, *ad libitum*, and pair-fed controls (4,20).
TABLE 2. Effect on local application of zinc sulfate ointment, systemic zinc therapy, or general protein-calorie supplements on DCH response to Candida

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of responders</th>
<th>Size of induration (mm) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local zinc sulfate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before therapy</td>
<td>1/20</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>After therapy</td>
<td>2/20</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td>Systemic zinc therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before therapy</td>
<td>1/20</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>After therapy</td>
<td>6/20</td>
<td>4.1 ± 1.6</td>
</tr>
<tr>
<td>Protein-calorie supplements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before therapy</td>
<td>5/50</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>After therapy</td>
<td>29/50</td>
<td>5.3 ± 1.7</td>
</tr>
</tbody>
</table>

Cytotoxicity

Cytotoxic activity of lymphocytes from zinc-deficient animals is markedly depressed very early in the course of nutrient deficiency. Frost and co-workers (21) found only 3% cytotoxic activity as compared to 55% in the control as early as 2 weeks of zinc deficiency; supplementation with zinc restored the activity to 43%. Chandra and Au (7) reported a reduced generation of spleen cell cytotoxicity following in vivo sensitization with tumor cells in zinc-deficient animals. However, the response was normal if sensitization with tumor cells was induced in vitro. Similar findings have been reported by Fernandez et al. (22). Antibody-dependent cell-mediated immune response is normal in zinc-deficient animals (7, 22).

Natural killer cell activity

Natural killer cell activity, one aspect of the immune response, is important for protection against the early phase of infection and against tumors. This activity is reduced in zinc-deficient animals (Fig. 4; Table 3).

Humoral Immunity

The limited number of studies on the impact of zinc deficiency on humoral immunity have produced inconsistent and controversial results. Cunningham-Rundles and co-workers (12) found extreme depression of IgG levels in three patients with combined zinc deficiency in PEM. In experimental animals the response to B-cell-specific mitogen, LPS, has been found to be unaffected or reduced (18, 23), whereas that to T-cell-specific mitogens is depressed (8). Zwinkle and Fraker (24) found a depression of plaque-forming cell (PFC) response to both T-cell-dependent and T-cell-independent antigens in zinc-deficient mice and restoration of the response after zinc supplementation. The impaired response is also correctable by the addition of T-helper cells; these findings are consistent with human observations discussed above. Zinc deprivation during pregnancy depresses the offspring's im-
TABLE 3. NK cell activity against YAC-1 targets

<table>
<thead>
<tr>
<th>Ratio of effector cells to target cells</th>
<th>Cytotoxicity (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn(+)</td>
<td>Zn(-)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>27 ± 4</td>
<td>34 ± 5</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>21 ± 3</td>
<td>27 ± 5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11 ± 3</td>
<td>14 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Zinc supplementation after birth.

A possible beneficial effect of zinc deficiency is its ability to reduce the production and titer of harmful autoantibodies. For example, New Zealand black/white mouse hybrids fed a zinc-deficient diet have delayed onset of autoimmune hemolytic anemia and glomerulonephritis with consequent longer survival. The potential application of this to human autoimmune disorders is under study.

Zinc as Mitogen

Zinc exerts a pharmacological effect on lymphocytes. In healthy individuals and patients with common variable immunodeficiency, the addition of zinc in vitro to lymphocyte cultures increases the stimulation response to mitogens. This effect is maximal on unseparated mononuclear cells from peripheral blood or on nylon-wool-filtered cells to which supernatant derived from monocyte cultures has been added (Table 4).

Neutrophil Function

Deficiency as well as excess of zinc has been associated with neutrophil dysfunction. Chvapil et al. (25) have provided a comprehensive description of the effect of zinc on granulocytes, the interaction of zinc with other cations, and changes in plasma zinc concentration during neutrophil activity. Their study showed that zinc has no effect on the opsonization process. However, in the presence of Mg$^{2+}$ ions zinc inhibits phagocytosis and bacterial killing, whereas in the absence of Mg$^{2+}$ low concentrations of zinc stimulate and high concentrations of zinc

TABLE 4. DNA synthesis in response to concanavalin A and zinc in vitro

<table>
<thead>
<tr>
<th>Human cell population</th>
<th>Saline (cpm)</th>
<th>Con A (cpm)</th>
<th>Zinc (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mononuclear cells (unseparated)</td>
<td>435 ± 111</td>
<td>65,478 ± 6,567</td>
<td>41,234 ± 3,498</td>
</tr>
<tr>
<td>E-rosette separated</td>
<td>397 ± 88</td>
<td>20,723 ± 3,873</td>
<td>1,686 ± 472</td>
</tr>
<tr>
<td>Nylon-wool filtered</td>
<td>755 ± 179</td>
<td>14,550 ± 3,245</td>
<td>3,073 ± 1,106</td>
</tr>
<tr>
<td>Nylon-wool filtered plus monocyte supernatant</td>
<td>551 ± 109</td>
<td>51,765 ± 7,041</td>
<td>33,872 ± 5,730</td>
</tr>
</tbody>
</table>
TABLE 5. Effect of increasing zinc concentration in vitro on neutrophil function

<table>
<thead>
<tr>
<th>Zinc conc. (μM)</th>
<th>Oxygen consumption (%)</th>
<th>Bacterial killing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>20</td>
<td>64</td>
<td>81</td>
</tr>
<tr>
<td>40</td>
<td>39</td>
<td>73</td>
</tr>
<tr>
<td>60</td>
<td>27</td>
<td>61</td>
</tr>
</tbody>
</table>

inhibit these activities of neutrophils. Our data on the effect of increasing zinc concentration in culture medium on oxygen consumption and bactericidal activity of neutrophils are shown in Table 5 and are reported elsewhere (26). Impairment of neutrophil chemotaxis has been reported in zinc-deficient patients with acrodermatitis enteropathica (27). This was corrected after zinc therapy. The depressive effect of high concentrations of zinc on phagocytosis and microbicidal activity could be due to blocking the membrane receptor(s), changing the membrane fluidity, an effect on the cellular microskeleton, or by an antagonistic effect on Ca²⁺ transport across the membrane (26). In addition, changes in membrane lipoproteins may be important (26). The effects of increased quantities of zinc result also in decreased platelet aggregation, poor histamine release by mast cells, and diminished oxygen consumption by neutrophils in association with diminished phagocytosis and bactericidal activities.

Risk of Infection

Depression of immunity may largely be responsible for increased susceptibility to infections and poor wound healing seen so often in acrodermatitis enteropathica and other zinc-deficient states. Mice and rats deprived of zinc are more susceptible to challenges with Listeria monocytogenes, Salmonella typhimurium, Francisella tularensis, and Trypanosoma cruzi (Tables 6 to 8).

COPPER

Copper is an active component of several enzyme systems including cytochrome c oxidase and superoxide dismutase. Copper is essential for the prevention of

TABLE 6. Response to infection with Listeria monocytogenes

<table>
<thead>
<tr>
<th>Day postinfection</th>
<th>Mortality (%)</th>
<th>Log₁₀ organisms/spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn(−)</td>
<td>Zn(+)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>40</td>
</tr>
</tbody>
</table>
IMMUNITY AND INFECTION

TABLE 7. Response to infection with Coxsackie B virus

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mortality at 2 weeks</th>
<th>Cardiac necrosis</th>
<th>Hepatic necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc-deficient</td>
<td>35</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>15</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ad libitum, Zn +</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Deficiency of copper is associated with an increased incidence of infection, as is seen in patients with Menkes' kinky-hair disease, an inherited disease with defective copper metabolism and low serum copper concentration (28). Pneumonia and other infections are a frequent cause of death in this condition. Animals deficient in copper also show increased susceptibility to bacterial pathogens such as *Salmonella* and *Listeria* but not to the same extent for Coxsackie virus infection (Table 9).

The effect of copper deficiency on immunity has been mainly studied in laboratory animals, whereas in humans the data on this aspect of copper are limited. In laboratory animals the function of the reticuloendothelial system is depressed and the microbicidal activity of granulocytes is reduced. This has been attributed to the role of copper in superoxide dismutase and cytochrome c oxidase enzyme systems. Vyas and Chandra (29) have shown an impaired antibody response to heterologous red blood cells and low levels of thymic hormone activity in copper-deficient animals when compared with pair-fed controls (Table 10). This depression of antibody response correlates with the levels of ceruloplasmin (30).

SELENIUM

Selenium, as a part of the glutathione peroxidase system, and in conjunction with vitamin E, which also is an antioxidant, protects against lipid peroxidation of cell membranes in human and animal tissues. Deficiencies of selenium and vitamin

TABLE 8. Response to challenge with Salmonella typhimurium

<table>
<thead>
<tr>
<th>Condition</th>
<th>Average time (range) to death (days)</th>
<th>Mortality at 4 weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc-deficient</td>
<td>19 (9–31)</td>
<td>40</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>17 (11–28)</td>
<td>45</td>
</tr>
<tr>
<td>Ad libitum</td>
<td>23 (11–29)</td>
<td>20</td>
</tr>
</tbody>
</table>
E are well recognized in domestic animals, and there are reports that these animals are more susceptible to infectious diseases than those who have adequate intake of both nutrients. Marsh and associates (31) have shown that in chicks fed a diet deficient in selenium and vitamin E the humoral immune response was depressed. In swine with experimentally induced dysentery, the presence of concomitant selenium and vitamin E deficiency resulted in increased severity and frequency of clinical signs and the extent of enteric lesions. Desowitz and Barnwell (32) showed an enhancement of vaccine-induced immunity against malaria in Swiss Webster mice by addition of selenium (2.5 g/liter) in drinking water, and on further challenge these mice had lower malarial counts and shorter duration of parasitemia than those who were immunized but did not receive selenium. Sheffy and Schultz (33) have shown improvement of CMI with selenium–vitamin E supplementation. Phagocytic cells such as blood neutrophils, pulmonary alveolar macrophages, and peritoneal macrophages, through their high cytosolic glutathione peroxidase content, destroy peroxides produced during the high metabolic activity associated with ingestion and killing of infectious agents. Serfass and Ganther (34) have demonstrated decreased glutathione peroxidase activity of phagocytic cells of Se-deficient animals, and this was shown to be associated with decreased microbicidal activity.

**TABLE 9. Response to infection in rats fed copper-deficient diet**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Copper-deficient</th>
<th>Pair-fed</th>
<th>Ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to death (days)</td>
<td>10 ± 2</td>
<td>16 ± 4</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>70</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td><strong>Coxsackie B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Cardiomyopathy (%)</td>
<td>25</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Hepatic necrosis (%)</td>
<td>25</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE 10. Thymic activity**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Deficient animal</th>
<th>Pair-fed control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Zinc</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>Selenium</td>
<td>16</td>
<td>32</td>
</tr>
</tbody>
</table>

*The results are expressed as the median reciprocal titer. Thymic hormone activity was estimated by rosetting in the presence of azathioprine of splenocytes obtained from thymectomized mice.*
Our studies on rats deficient in both selenium and vitamin E showed that the lymphocyte proliferation response was reduced but there was little effect on macrophage function.

In humans there are a few nutritional circumstances associated with selenium–vitamin E deficiency: (a) ingestion of commercial formulas high in polyunsaturated fats and low in vitamin E and selenium, especially in low-birth-weight infants; (b) in population areas where the selenium content of the soil is poor, e.g., Keshan region of China and some areas of New Zealand; (c) prolonged intravenous hyperalimentation with no selenium; and (d) kwashiorkor.

The role of selenium in prevention of infectious illness in human beings is at present not clear. However, cardiomyopathy seen in Keshan disease may be due to activation of viruses in a selenium-deficient immunologically incompetent host.

MAGNESIUM

Through its effect on ribosomes magnesium is involved in protein synthesis and is important for a number of biochemical and physiological processes in the body. In the alternate complement pathway, the participation of properdin requires magnesium ions. Magnesium deficiency has been recognized in both animals and man. However, the role of magnesium in immune function has been mainly derived from cell culture techniques or laboratory animals.

Magnesium deficiency produces depression of levels of all immunoglobulins except IgE, which is increased (35). The number of antibody-forming cells is reduced, and thus the primary (IgM) and secondary (IgG) responses to antigenic stimuli are depressed. The titers of agglutinating and lysing antibodies are depressed. The classic complement pathway has been described to be insensitive to magnesium deficiency in man (36). However, the alternate pathway of complement activation specifically requires the participation of magnesium ions. Magnesium is essential for the immune-related activities of lymphocytes including growth and transformation in response to mitogens (37), binding reactions (38), and T-lymphocyte-mediated cytolysis in which magnesium is effective in the first step of interaction between the effector T-cell and the target cells. Prolonged deficiency of magnesium in rats has been shown to be associated with the development of thymic lymphoma and myeloid leukemia. Chemotaxis, opsonization, and microbicidal activities all require magnesium ions for optimal function. There is incomplete knowledge about the role of magnesium in healthy human beings and immunological changes in diseases associated with alteration in magnesium metabolism.

COBALT

Cobalt has been linked to antibody synthesis and phagocytic activity of neutrophils and macrophages. The presence of divalent cobalt along with antigen–antibody complexes induces spread of macrophages on a glass surface. Increased phagocyte uptake of albumin-coated paraffin oil particles by human neutrophils and rabbit alveolar macrophages in the presence of ionic cobalt was reported by Stossel (39).
Deficiency of transcobalamin II, the carrier protein for vitamin B$_{12}$, is associated with low IgG levels.

**IODINE**

Iodine plays an important role in the microbicidal activity of polymorphonuclear leukocytes. The conversion of iodide to iodine by neutrophil peroxidases in the presence of hydrogen peroxide results in the formation of radicals capable of oxidizing and killing microbial organisms. DuRubertis (40) has suggested that such an oxidative system might be operative in vivo in activated neutrophils, where thyroid hormone supplies the iodide molecule. Farid et al. (41) demonstrated decreased microbicidal activity of neutrophils from hypothyroid patients of Hashimoto's disease; improved function followed treatment. There are a number of reports on the enhanced deiodination of thyroid hormones during phagocytosis in neutrophils and macrophages. On the other hand, in chronic granulomatous disease with deficiency of H$_2$O$_2$ formation, the deiodination of thyroid hormones is poor during and after phagocytic stimulation. Similarly, PEM results in poor iodination and decreased bactericidal capacity (42).

**OTHER TRACE ELEMENTS**

Arsenic has been shown to inhibit the production of interferon and antibodies. Boron deficiency in chicks results in a syndrome that resembles human arthritis. Intraperitoneal injections of boron analogs in rats and mice inhibited experimentally induced inflammatory arthritis. It also enhanced cyclic AMP activity.

A modest increase in intake of cadmium protects against experimental infection, whereas the effect of toxic doses is to depress the immune system and increase the susceptibility to infections. We have postulated that this may in part be due to the zinc-depleting effect of cadmium. Prolonged feeding of cadmium can lead to diminished formation of neutralizing antibody, decreased number of rosette-forming B-cells in the spleen and bone marrow, and reduced IgM and IgG formation by splenic lymphocytes after primary and booster inoculations with sheep red blood cells. The mitogenic response to splenic lymphocytes is decreased. Cell-mediated immunity and phagocyte function are depressed following exposure to cadmium.

Similar results are observed with other heavy metal exposure, e.g., lead, gold, and silver. These metals also produce contact dermatitis. Silicon exposure through inhalation depresses alveolar macrophage activity, antibody synthesis, and T-cell stimulation by concanavalin A.

**EFFECTS OF INFECTION ON TRACE ELEMENT STATUS**

During infection, reduced dietary intake due to anorexia and decreased absorption can result in a deficiency state. Also, increased losses through urine, diarrheal stools, and sweat can aggravate the problem. Increased metabolic activity would enhance needs. Finally, the chemical mediators produced by inflammatory cells
such as interleukin 1 induce transfer of trace elements from one body compartment to another. Reduced serum levels of iron, zinc, and copper are well known phenomena during acute infection (3). Flynn (43) has reported elevated levels of zinc in the liver and spleen of antigen-treated mice. Partially purified interleukin 1 produced similar alterations in trace elements.

CONCLUDING REMARKS

Trace elements are biological elements present in microamounts in humans. Their physiological function depends on their being a part of metalloenzymes vital for cell viability, function, and proliferation (44,45), which are all essential for the immune system, and also as an active component of hormones, e.g., thymic factors. Their serum and tissue levels are maintained within a physiological range through the regulation of absorption, distribution, metabolism, and excretion. Carrier proteins, storage sites, interaction with other elements, and excretion are some of the regulators of trace element levels in body fluids.

Both deficiency and excess of trace elements have been recognized. Although dietary requirements of most of these elements are met by a balanced diet, there are certain population groups and specific disease states which are likely to be associated with deficiency of one or more of these essential elements. The role of trace elements in maintenance of immune function and their causal role in secondary immunodeficiency is increasingly being recognized. There is growing research concerning the role of zinc, copper, selenium, and other elements in immunity and the mechanisms that underlie such roles. The problem of interaction of trace elements and immunity is a complex one because of the frequently associated other nutritional deficiencies, the presence of clinical or subclinical infections which in themselves have a significant effect on immunity, and finally the altered metabolism due to the underlying disease.

We have reviewed the effects of trace element deficiency and excess on immunological function and susceptibility to infection. However, there are still many unsolved problems, and further studies will hopefully elucidate the issues that defy clear answers today.

REFERENCES

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**DISCUSSION**

*Dr. Heresi:* Dr. Chandra has given an excellent overview of the current knowledge on some trace elements and immune response. With respect to zinc, he showed depression of the humoral and cellular immunity, especially in animal-based studies. The data on skin reaction are interesting. Golden described in marasmic and marasmic kwashiorkor infants a normal skin response to *Candida* antigen after a local application of zinc sulfate to the skin. Dr. Chandra, could you speculate on the difference between these two studies?

*Dr. Chandra:* My only comment is that the prevalence of zinc deficiency in populations around the world may be quite different. This may explain some of the differences in results of zinc therapy in children with malnutrition, the number of responders, and the extent of restoration of immunocompetence. We have not had Golden's good experience with local application of zinc sulfate to the skin. Dr. Chandra, could you speculate on the difference between these two studies?

*Dr. Golden:* As the author of the other half of this controversy I would like to comment. We laid down hemolysates and plasma from these cases when we did the experiment, and recently we have set up assays for other elements. The particular children we reported had normal superoxide dismutase levels in their red cells, normal glutathione peroxidase levels, and normal plasma ferritin levels; and so it seems that at that particular time we were looking at a batch of children who had normal copper, selenium, and iron status. The zinc seems to have been the limiting factor for hypersensitivity response in that group of children, and therefore we got a response to zinc. I think that in other groups of children other nutrients are deficient. We have seen children, as we reported at the Lund meeting, in whom local zinc inhibited the reaction; it may well be that in these particular individuals some other trace nutrient was limiting, such as copper, and when we added zinc we got inhibition instead of stimulation. I think this type of variation explains perfectly the difference in our results—I see no contradiction in what Dr. Chandra and I reported.

*Dr. Chandra:* Also, of course, the so-called "zinc sulfate" ointment may contain other elements. Zinc sulfate, as available in the market, is contaminated with many other substances that may exert an effect on the immune response.

*Dr. Golden:* The zinc sulfate ointment I used was specifically formulated—it was not purchased in the marketplace.

*Dr. Heresi:* I have some *in vivo* data from the Institute of Nutrition and Food Technology in Chile. We evaluated, with Dr. Castillo and Dr. Uauy at INTA, the effect of zinc on
nutritional recovery and immunological parameters in 16 moderately marasmic infants. These infants were supplemented with 2 mg of zinc per kilogram per day for 90 days, and a comparable group received a placebo. No cases of zinc plasma levels under 70 μg/dl were included in the study. The immunological data are as follows. The skin reaction to PPD significantly changed in the zinc-supplemented group after supplementation compared to the placebo group. The lymphocyte reaction to PHA was similar in both groups, but the supplemented group had significantly less infection, especially pyoderma. With respect to copper, Dr. Chandra has reported animal-based studies which showed that other trace elements regulate the immune response. We have studied copper deficiency and immune response in marasmic infants during rehabilitation in nutritional recovery centers in which the infants were fed a milk-based diet. Castillo et al. in previous studies showed increased lower respiratory infections in those infants. The study was done in hypocupremic marasmic infants 6 to 18 months of age after 1 to 2 months of nutrition rehabilitation in the centers. At the time of the study, the weight/length ratio was normal in all infants. Hypocupremia was defined as a serum copper level below 80 μg/dl. Every patient received copper supplementation during 1 month and then the immune response was reevaluated. In addition, the infants received iron, folate, and vitamins A, B, C, and D. Serum copper and ceruloplasmin levels increased to normal after supplementation in all infants. Hypocupremic infants had normal serum immunoglobulin levels and normal delayed cutaneous hypersensitivity reactions to PPD and Candida. The ability of hypocupremic polymorphonuclear leukocytes to phagocytize Staphylococcus aureus was measured using autologous and normal homologous plasma. The mean phagocytic indexes were low before copper therapy and significantly increased after copper supplementation. The lymphocyte transformation using autologous plasma was depressed in most of the infants. The stimulation index significantly increased after copper supplementation. We concluded that copper deficiency in infants impairs the phagocytic function independently of serum factors and that it decreases the proliferative response of lymphocytes to PHA.

I have some questions for Dr. Chandra. (a) Did you give the copper-deficient animals copper and then reevaluate the immune response? (b) Can you comment on the studies related to infection in zinc deficiency and some other trace elements? (c) Can you explain the implication of immune depression without an increase in infectious disease?

Dr. Chandra: Indeed, we did a copper repletion experiment and showed that restoration of immune response does occur, although it is not as rapid as in zinc-deficient animals given zinc. Your second question relates to the incidence of infections in individual trace element deficiencies. You have shown that zinc administration in marasmic infants results in a reduction of infection. Apart from those with acrodermatitis enteropathica and Menkes’ syndrome, both rare entities, there are not many patients who have single nutrient deficiencies for any length of time. Thus it is impossible to know if the incidence or severity of infection is enhanced. We have to resort to animal data to answer that question. What is the importance of depressed immune response? Perhaps this is the first stage which precedes increased susceptibility to infection, especially when given enough stress. I want to emphasize that mild nutrient deficiencies may not be of much consequence in a clean environment as prevails in the western industrialized countries.

Dr. Zoppi: You have extensively studied the interrelationship between nutrition and immune response, and I would like to ask if there are any conditions characterized by deficiency of zinc or other trace minerals without protein-calorie malnutrition.

Dr. Chandra: Most malnourished children have associated trace element deficiencies. The effect on growth may involve a number of mechanisms, e.g., changes in appetite or
malabsorption or interactions with the absorption of other essential nutrients. Zinc deficiency is often complicated with protein-energy malnutrition and deficiencies of other nutrients, each of which contributes to some depression in immunity.

Dr. Gebre Mehdin: I would like to show some data related to trace element status in infection-prone children. Tragically enough, we lost 2 of 27 children last year. This is the pattern of trace elements in serum including magnesium—we leave out calcium for the time being—and the one element that is impressive is zinc, whose level drops significantly. The weights and heights of these children were not affected very much. One would be tempted to give these children zinc or call them zinc-deficient, but if you look at other parameters you can note much more dramatic changes, i.e., in serum levels of albumin or prealbumin, and we have rarely seen such low levels of RBP in any other patient group. In humans it is often very difficult to say which is the egg and which is the hen.

Dr. Chandra: Your data are very interesting. They show that in addition to zinc deficiency there is protein-energy malnutrition. Both prealbumin and RBP are short-half-life proteins that are depressed in malnutrition. It would be tempting to suggest that a prophylactic, small amount of zinc in a cohort of patients with nonsupplemented controls may give an answer to the question whether zinc deficiency is responsible for those infections. When data are collected during infection, it complicates the issue and the interpretation of nutrient levels becomes difficult. There are ongoing studies in which apparently healthy normal children are being supplemented with zinc to see if (a) you can promote more growth and (b) it makes any difference to any of the physiological functions, including neurobehavioral development and immune responses. We must question whether the extra amount of zinc will correct an underlying subclinical deficiency or do something extra which is beyond the physiological range.

Dr. Oster: The same picture is found in cancer patients as in malnourished children: low iron, high copper, somewhat low zinc, but seldom zinc deficiency. These are patients with cancer of the ear, nose, and throat who have a very well defined cancer of epithelial origin. We cannot explain the trace element picture. There seem to be interactions between copper, zinc, and selenium in these patients. The ferritin taken as a tumor marker correlates negatively with serum selenium. The interactions between the trace elements may be important but may be not specifically restricted to a patient group.

Dr. Chandra: Published data indicate a correlation of the zinc/copper ratio with response to chemotherapy and irradiation and with prognosis in terms of metastases in cancer.

Dr. Mertz: With regard to the decreasing plasma zinc levels during an infection, a couple of years ago we heard that the Rowett Institute was conducting experiments in which they prevented the zinc decrease by supplementing swine during infection. The preliminary results at that time were that most of the swine died, and the tentative conclusion was that there is a dual role for zinc: The element is important for preventing an infection, but it can be dangerous, even at reasonable levels, during the course of the infection.

Dr. Chandra: The main explanation for reduced plasma zinc concentration in infection is the release of endogenous mediators produced by inflammatory cells. This is also seen with iron and other trace elements. I want to take the heretical view that the shift in trace element levels occurs not because low plasma concentrations prevent septicemia but that higher concentrations are needed in tissues. We should look at that portion of the equation. A lot of action is taking place in tissues, e.g., antibody synthesis, proliferation of lymphocytes, and so on. The sequestration of elements may have a purpose. Let me draw an analogy with substrates during infection; there is a shift from albumin synthesis, a relatively unimportant event to antibody synthesis, a vital protective mechanism.
Dr. Prasad: I want to comment about the Middle Eastern dwarfs you referred to. Our patients did not have protein-calorie malnutrition; they had a deficiency of zinc and iron only. The design of our repletion experiments was such that we gave one group the unsupplemented diet, the second group received zinc, and the third group received iron. Based on these experiments we were able to conclude that zinc deficiency was responsible for growth retardation and hypogonadism. The question is that there appears to be an overlap between immunological effects of zinc, copper, and perhaps selenium. Can you separate these effects and indicate what you consider to be specific for zinc and what is specific for copper and selenium?

Dr. Chandra: In many ways selenium and zinc behave similarly. Their major impact on host resistance seems to be on cell-mediated immunity dependent on T-cells, including thymic hormonal inductive factors. It is viruses such as Coxsackie B virus which play greater havoc in these deficiencies than do Salmonella or Listeria. In copper deficiency, I think the effect on the phagocytic system may be equally important, as shown by Dr. Heresi in her studies on marasmic infants with hypocupremia and in other studies on copper-deficient rats; in deprived rodents the reticuloendothelial system showed dysfunction. Therefore I think there are differences although there are few overlapping results. Secondly, I want to refer to the mechanisms of zinc regulation of growth. I do not doubt that zinc deficiency is associated with growth failure, and therefore if you give zinc it will restore growth velocity. However, this effect may be achieved through a number of mechanisms including reduction in total food intake and malabsorption of a number of nutrients. Stunting is a feature of both long-standing zinc deficiency and chronic protein-calorie malnutrition.

Dr. Thompson: I think that, like us, Dr. Prasad believes that plasma zinc is so highly dependent on other variables, in this case albumin, that plasma levels do not tell us much. Dr. Chandra, how important is the ambient zinc concentration in vitro when the cells are taken out of the circulating plasma and incubated in autologous serum, pooled AB plasma, or fetal calf serum and medium 199?

Dr. Chandra: There is very good evidence that the zinc content of the medium is an important consideration in in vitro work. Both Fernandez et al. and we published data showing that when you inject zinc-deficient animals with tumor cells and estimate spleen cell cytotoxicity, it is reduced. However, if you do the same experiment by taking the spleen cells out first and exposing them in the test tube to the tumor cells, you do not find any reduction in cytotoxic ability presumably because we were all using zinc-containing media. Thus it was the microenvironment of the zinc-deficient animal which had led to poor response.

Dr. Golden: There is a common misconception that plasma zinc concentration is dependent on albumin levels. A simple calculation—taking a molecular weight of albumin of 69,000 and circulating levels of about 35 g/liter—gives a molality of albumin in plasma of about 500 μM. The molality of zinc in plasma is about 15μM, so that the albumin/zinc molar ratio with albumin concentration at the lower limit of normal is such that only one out of about 30 albumin molecules has a zinc atom associated with it. It is very difficult to see how a reduction in albumin can lead to a loss of available binding sites for zinc, given the resulting molar ratios.

My question concerns the infection. How do the data described by the Murray group, which show that after you feed malnourished people they have a very much higher prevalence of infection, fit into the trace element story?

Dr. Chandra: Albumin is only one of the proteins to which zinc is bound; most zinc-binding proteins move in the same direction. Thus your estimate of 1:20 may not be accurate.
So far as the data from Somalia are concerned, I want to emphasize that these investigators looked at the seasonal prevalence of clinical infections, e.g., enlarged lymph nodes, tuberculosis of the spine (Pott’s disease), etc. My interpretation of their data is that by improving nutrition, if indeed it did improve, there was no change in the incidence of disease but perhaps in its expression, particularly those features that depend on delayed hypersensitivity.