Changes in immunoglobulin levels in zinc-deficient mice infected with Trypanosoma musculi

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CHANGES IN IMMUNOGLOBULIN LEVELS IN ZINC-DEFICIENT MICE INFECTED WITH TRYPANOSOMA MUSCULI

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A metabolic imbalance technique was used to study the effects of zinc deficiency on immunoglobulin levels in mice infected with Trypanosoma musculi or immunized with parasite products. Zinc-deficient mice developed higher numbers of parasitemia earlier and exhibited prolonged infection. Irrespective of the diet, higher IgG1, IgG2b, and IgM levels, lower IgG2a and IgA levels, and uniform IgG3 levels were exhibited primarily by mice infected with T musculi and to a lesser extent by mice immunized with parasite products. Zinc-deficient mice showed smaller increases in IgG1 and IgM, but larger gains in IgG2b compared with mice on full-complement and pair-fed diets. However, IgG2a decreased significantly in zinc-deficient mice. (J Natl Med Assoc. 1994;86:613-619.)

Key words • zinc deficiency • Trypanosoma musculi infection • immunoglobulin levels

Zinc deficiency constitutes one of the most widespread and persistent worldwide problems, exceeded in frequency of occurrence only by protein calorie and iron deficiencies. Some of the symptoms of the deficiency include anorexia, alopecia, retarded sexual development, stunted growth, gastrointestinal malfunction, and skin diseases. In addition, some immune dysfunction identified as lymphopenia, poor wound healing, atrophied thymuses, and reduced capacity to respond to skin sensitization were exhibited consistently by these initial zinc-deficient subjects.2,3 Rat studies have shown that zinc uptake occurs partly by a regulated carrier-mediated diffusion mechanism that responds homeostatically to dietary zinc supply. Evidence from transport kinetics has confirmed the presence of both passive and saturable processes.4 Even though the actual site of zinc absorption is unclear, it has been estimated that approximately 20% to 30% of ingested dietary zinc is absorbed.5 Further, the presence of this trace element in various tissues, fluids, and body secretions is highly variable and can be affected by development, growth, hormones, stress, and inherited disorders in zinc metabolism. In plasma, 65% of portal zinc is associated with albumin and a portion of the remainder with other proteins, notably alpha-2-macroglobulin and transferrin.6 Once absorption has occurred, substantial amounts of zinc are taken up by the liver7 and subsequently redistributed to other tissues, mainly bone and muscles.8 Pekarek et al9 observed increased susceptibility to experimental infection with Francisella tularensis (tularemia) in zinc-deficient rats. McMurray and Yetley10 reported that zinc-deficient guinea pigs vaccinated with Mycobacterium bovis had reduced total serum protein and albumin levels, and weakened ability to control mycobacterial population at the site of vaccination or in the lymph nodes that drain the site. Carlomagno et al11 also showed thymic atrophy, reduced delayed type hypersensitivity responses to Listeria monocytogenes antigens, and impaired lymphocyte response of spleen cells to phytohemagglutinin in zinc-deficient rats. In rats, zinc deficiency greatly

From the Department of Biology, Howard University, Washington, DC. Data in tabular format are available from the author on request. Requests for reprints should be addressed to Dr Clarence M. Lee, Office of the Dean, College of Arts and Sciences, Locke Hall #101, Howard University, Washington, DC 20059.
increased the trapping of *Escherichia coli* by liver, lungs, and kidneys during gram-negative sepsis. The present study was carried out to analyze the effect of zinc deficiency on the immunoglobulin levels in mice infected with *Trypanosoma musculi*.

**MATERIALS AND METHODS**

**Experimental Hosts**

One thousand six hundred eighty Swiss Webster female albino mice (Charles River Laboratories, Portage, Michigan), each weighing 12 g, were used. The mice were quarantined for 7 days prior to their designation into one of the following three dietary groups: full-complement, zinc-deficient, and pair-fed. The mice were housed in stainless steel cages with wire-mesh bottoms, their quarters were kept on a 12-hour light and dark cycle, and mean temperature and humidity were maintained at 24°C and 55%, respectively. All diets were purchased commercially from ICN Nutritional Biochemicals (Cleveland, Ohio). The mice were fed appropriate diets from metal feeding containers and were allowed to eat and drink at will. The water did not contain any detectable amount of zinc when analyzed by flame atomic absorption spectrophotometry. Glass bottles, sipper tubes, and silicone stoppers were used to avoid any metal contamination of the water. The mice in the pair-fed group were fed the control diet daily in amounts equal to the food consumed by their zinc-deficient paired mates in 24 hours. The average daily food intake was determined by subtracting the amount of food remaining in the feeding containers from the amount given the previous day.

**Parasitic Cells**

The experimental parasite was *T musculi*, which has been maintained in the laboratory by syringe passage in mice for more than 27 years. The mice were infected intraperitoneally with 1 × 10³ living trypanosomes in 0.25 mL sterile physiological saline (PS).

**Parasitic Derivatives**

Blood containing dividing trypanosomes was washed with PS by centrifugation two times and resuspended in culture medium. Medium containing 2 × 10⁸ parasites per millimeter was incubated for 24 hours in a water bath at 27.5°C. After incubation, the trypanosomes were separated by centrifugation, and supernatant containing metabolic products of the parasite was filtered through a fritted glass funnel of fine porosity and was stored at −20°C until used. The separated trypanosomes were washed in PS and trituted by sonication to form the homogenate.

**Experimental Inoculations**

Twenty-eight days after initiation of a dietary regimen, each of the three major groups (full-complement, zinc-deficient, and pair-fed) were divided into five subgroups:

- uninoculated controls,
- mice inoculated intraperitoneally with 0.25 mL of PS,
- mice inoculated with 0.25 mL of PS containing 1 × 10³ living trypanosomes,
- mice inoculated intraperitoneally with 0.25 mL of metabolic products (MP), and
- mice inoculated intraperitoneally with 0.25 mL of homogenate.

Mice were infected once 28 days after initiation, whereas the PS and *T musculi* derivatives were injected at 3-day intervals between 28 and 80 days postinoculation. Mice were killed on the fifth and seventh days until day 80 for various analyses. Sera were collected and stored at −20°C until used.

**Parasitemia**

Wet blood films of tail blood were prepared to determine the presence of trypanosomes, which were counted using hemacytometer.

**Measurement of Changes in Immunoglobulin Levels**

Levels of IgG₁, IgG₂a, IgG₂b, IgG₃, IgA, and IgM were measured using radial immunodiffusion assay as described by Mancini et al. Serial dilutions of mouse gammaglobulin standards, J-401 (Bio-Whittaker Corp, Walkersville, Maryland), were used to quantitate each serum globulin level. Regression analysis program curves were used to compute and tabulate the concentrations of the immunoglobulin levels in serum samples.

**Determination of Zinc Levels in Plasma and Liver**

Zinc levels in plasma and liver were analyzed using Perkin-Elmer Model 603 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp, Norwalk, Connecticut). Plasma was diluted fivefold with double-deionized water, and the diluted samples were analyzed directly for zinc. Plasma concentrations of zinc were measured against zinc standard solutions (Harleco Manufacturers, Gibbstown, New Jersey) diluted with 5% (vol/vol) glycerol in double-deionized water.
steady decrease in the plasma zinc levels was observed in zinc-deficient mice between days 15 and 80.

Statistical Evaluation

Data were analyzed using two-way analysis of variance with replication Model I and Duncan’s multiple range test at the 5% significant level. Data results in tabular form are available by contacting Clarence M. Lee, PhD, Office of the Dean, College of Arts and Sciences, Locke Hall #101, Howard University, Washington, DC 20059.

RESULTS

Parasitemia

The Figure charts hemacytometer counts of trypanosomes in the blood of mice after infection with \(10^3\) parasites for the three dietary groups. Throughout the infection, mice fed a zinc-deficient diet exhibited greater numbers of parasites than those fed a full-complement and pair-fed diet. In the full-complement and pair-fed groups, trypanosomes appeared in the blood after 4 days of infection. The zinc-deficient mice became positive on day 3.

On average, the parasitemias of zinc-deficient hosts were about four times greater than those given full-complement or pair-fed diets. Average parasitemias were comparable in the full-complement and pair-fed groups. Peak parasitemias occurred on day 12 in mice fed the full-complement and pair-fed diets and day 15 in the zinc-deficient mice. Parasites were no longer visible in the blood of full-complement and pair-fed mice after days 26 and 29, respectively, but persisted in the zinc-deficient mice until day 38.

Changes in IgG1 Levels

From day 3 to day 31, IgG1 levels remained uniform (range: \(189 \pm 1\) mg/dL to \(210 \pm 4\) mg/dL) in all three dietary groups. After inoculation, the IgG1 concentrations for the infected and MP-inoculated mice were much higher than controls. The zinc-deficient infected and MP-inoculated mice showed much smaller increases compared with the full-complement and pair-fed groups. At the height of parasitemia (day 45: 15 plus 30 days after initiation of feeding zinc-deficient diet), there was about a ninefold increase in IgG1 levels in the full-complement infected mice over the noninfected mice fed the same diet. Statistically significant differences between these two groups were observed from day 38 and persisted until day 59. For the full-complement MP-inoculated versus un inoculated mice, significant differences were observed from day 38 to day 59. However, there were no significant differences in the IgG1 levels of mice on full-complement diet inoculated with homogenate or PS compared with controls.

In the zinc-deficient dietary group, higher IgG1 levels (sevenfold increase on day 45) were observed for the infected mice compared with the noninfected controls. From day 38 to day 59, significant increases were noticed in the zinc-deficient MP-inoculated over the uninoculated group. There were no significant differences in IgG1 levels in zinc-deficient homogenate- or PS-inoculated mice compared with the controls. Pair-fed infected mice showed higher IgG1 concentrations than the noninfected mice between days 38 and 59. The greater differences in IgG1 concentration occurred at day 45 when eightfold greater values were recorded for the infected pair-fed over the noninfected mice. Homogenate- or PS-inoculated mice showed no significant differences compared with controls throughout the observational period.

Changes in IgG2a Levels

Prior to inoculation, the average IgG2a levels in all mice ranged from \(47 \pm 0.4\) mg/dL to \(52 \pm 0.6\) mg/dL. After inoculation, full-complement infected mice showed significant decrease (\(23 \pm 0.3\) mg/dL to \(29 \pm 1; 53\%\) decrease at day 59) compared with noninfected mice from day 38 to day 59. Metabolic products-inoculated mice showed significant differences on days 45 and 59 and homogenate-inoculated mice on days 38 and 45.

Zinc-deficient infected mice exhibited IgG2a de-
creases of 16.6 ± 0.0 mg/dL to 32.7 ± 0.3 mg/dL over the noninfected mice from days 38 to 80. Metabolic products-inoculated mice showed a significant decline between days 38 and 80. Pair-fed infected mice also showed a significant decrease of 25.4 ± 0.3 mg/dL to 28.6 ± 0.6 mg/dL compared with noninfected mice. Metabolic products-inoculated mice showed significant decreases on days 52, 59, and 73. Homogenate-inoculated mice revealed lower values from days 38 to 52 and again on day 73.

### Changes in IgG_{2b} Levels

The IgG_{2b} levels for infected and MP-inoculated mice were higher compared with noninfected mice. Significantly higher IgG_{2b} levels were recorded for zinc-deficient infected mice. Full-complement infected and MP-inoculated mice showed higher IgG_{2b} values over noninfected mice. The increases in IgG_{2b} levels were higher in the zinc-deficient infected groups between day 45 and day 59. A significant increase in the levels of IgG_{2b} was observed for the pair-fed infected mice compared with the noninfected mice. Metabolic products-inoculated mice also showed an increase on days 45 to 59 and homogenate-inoculated mice on days 52 and 59.

### Changes in IgG_{3} Levels

Except in zinc-deficient infected mice, overall IgG_{3} levels were uniform (80 ± 0.5 mg/dL to 101 ± 0.6 mg/dL) throughout the observational period. An increase in IgG_{3} levels for infected mice was observed on day 45 and day 52.

### Changes in IgM Levels

In all three dietary groups, IgM levels remained fairly uniform (8.0 ± 0.2 mg/dL to 11.0 ± 0.8 mg/dL) from day 3 to day 38. There was a significant increase in IgM level in infected mice; however, zinc-deficient mice showed less increase compared with mice on full-complement and pair-fed diets. Differences in IgM concentrations between zinc-deficient infected and noninfected mice became significant by day 38 and continued until day 59. At day 45, a fourfold increase was reached for the zinc-deficient infected mice compared with the noninfected mice. The levels of IgM for the full-complement infected mice were still double at day 59 compared with noninfected mice. Pair-fed infected mice also showed a significant increase in IgM concentration from day 38 to day 59 with a ninefold increase at day 45. No significant difference was observed for pair-fed MP-inoculated and homogenate-inoculated mice.

### Changes in IgA Levels

From day 3 to 31, similar levels of IgA (about 40 mg/dL) were observed in all dietary groups. After day 31, the infected mice showed lower values compared with noninfected mice. Full-complement infected mice showed a maximum decrease in IgA (35%) on day 45. There was no significant difference in IgA levels in zinc-deficient mice compared with full-complement and pair-fed mice. However, on day 59, the MP- and homogenate-inoculated mice had higher IgA levels than noninfected mice.

### DISCUSSION

Although zinc deficiency has complicated health problems worldwide, correlation of the pathogenesis of disease states and the resulting immunodeficiency tend to confound both nutritionists and health-care providers. An understanding of the contribution of zinc to various immunological and biochemical responses of host could contribute significantly to the treatment of malnutrition and maladies affecting a large proportion of the world's population. This investigation not only adds to the evidence relevant to zinc that has accumulated within the past decade, but also has explored the relationship between a nutritional imbalance and an infection with a protozoan organism (T. musculi). The relationship with the parasite derivatives also was examined.

In this study, a gradual decline in plasma and liver zinc levels was observed in the zinc-deficient mice throughout the experimental period. The reduced levels of plasma and liver zinc tend to correlate with dietary zinc intake. Zinc deficiency occurred after 28 days on the particular experimental diet. In addition, the typical signs of zinc deficiency, as demonstrated by reduced food intake, alopecia, and lesions on feet and tail, also were observed. Similar zinc-deficient symptoms were noticed in rats and guinea pigs.

Food intake and body weight gains increased substantially in mice infected with T. musculi or immunized with a parasite product. However, zinc-deficient mice gained less weight than mice on full-complement or pair-fed diets (data not shown). It has been suggested that during parasitic infection, growth factors are released or parasitic infection stress influences the action of the pituitary or thyroid glands, resulting in stimulation of growth.

Several reports show depressed immune capacity results in impaired humoral and cell-mediated responses in the zinc-deficient animals. Mac-Askill et al. and Whitelaw et al. showed that acute
fatal infection of *Trypanosoma brucei* and *Trypanosoma congolense* resulted in inability of the host to achieve effective levels of circulating antibodies against rapidly replicating trypanosome clones. It has been hypothesized that the degree of parasitemia correlated to the levels of immunoglobulins and the production of antibodies controlled the severity of disease process.

Alterations in immunoglobulin levels have been noted during zinc deficiency in humans, rhesus monkeys, and guinea pigs. Verna et al. have shown an impairment in both cellular and humoral immune responses due to zinc deficiency. It was suggested that the changes in immunoglobulin production may be due partly to decreased activity of DNA polymerase, RNA polymerase, and thymidine kinase, the enzymes that are zinc dependent and are required for rapid proliferation of immunocytes and protein synthesis. It also has been suggested that the immunosuppression seen during zinc deficiency may be due to an impairment in T-cell helper function. In addition, it has been shown that even marginal zinc deprivation causes major reduction in immune response when the deprivation occurs during development. This intriguing relationship between zinc deficiency during development and immune capacity has been demonstrated by Beach et al. It was noted that moderately deprived mice exhibited significant maturation delays in immunoglobulin production. Neonatal development problems in immunoglobulin production persisted even in the second and third generation of offspring. It was suggested that the impairment in intergestational activities could have resulted from either reduced protein synthesis or abnormal protein breakdown.

Increases and decreases in the various immunoglobulin classes have been observed during trypanosomal infections. Luckins observed increases in IgG and IgM in zebu cattle infected with *T congolense* and *Trypanosoma vivax*. Lumsden et al. demonstrated elevations in IgM levels in African trypanosome-infected humans. It is suggested that the increased levels of immunoglobulins could result from B-cell mitogenic factors produced by the trypanosomes themselves or by the induction of IgG production of helper T cells acting nonspecifically. Cross showed that there might be elaborated antigenic variation expressed by the different trypanosomal clones. The antigenic variation elaborated through the changes in the structure of the glycoproteins present on the surface coat of the trypanosomes could have contributed to the immunological responses of the host. Navalkar et al. showed higher levels of IgA, IgG1, IgG2, and IgM in mice infected with *Mycobacterium leprae* and *Mycobacterium lepraeum*.

Although the increases and decreases do not occur in the same classes of immunoglobulins as shown in this study, it is still evident that like *T musculi*, the disease agents influence the changes that occur in immunoglobulin production. The changes in the immunoglobulin production thus are modified significantly by both zinc deficiency and trypanosomiasis, and in turn, the host's immunological and defense capabilities are altered greatly. Clinton et al. suggested that the depression of humoral response during trypanosomal infection might be due to the varieties of biological factors supplied by the proliferating parasites. In addition, it was also suggested that interferon, in some way, could have modified both T and B cells. St Charles et al. reported that immunosuppression during trypanosomal infection was partly due to a break in the link of cooperation between T and B cells or by induction of suppressor cells. Other mechanisms suggested for the immunosuppression involve an exhaustion of B-cell clones, lack of helper T cells, or unavailability of sufficient T cells.

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