

Invited Review

Micronutrient Supplementation and Infection: A Double-Edged Sword?

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Single-nutrient supplementation targeted at specific population groups has become an increasingly popular strategy to combat micronutrient malnutrition. This population-based approach is a pragmatic response to limited resources and assumes that, within a targeted group, the diagnosis of the nutrient deficiency is secure, its prevalence is clinically significant, and the benefits of supplementation outweigh the risks. The introduction of routine vitamin A supplementation to children in developing countries has encouraged this approach and the development of further micronutrient supplementation programs. The population-based approach, by definition, ignores individual variation in response caused by either genetic differences or clinical conditions, such as the presence of ongoing infection.

The acute inflammatory response is a systemic adaptation that optimizes the immune response to an infection. Cytokines, such as interleukins (IL) 1 and 2 and tumour necrosis factor are released by leukocytes, macrophages, and mast cells in response to tissue injury and act on an array of cells to increase vascular permeability, to activate complement, and to promote chemotaxis. Plasma protein concentrations may increase, as with the positive acute-phase proteins C-reactive protein, serum amyloid A, α -1-antitrypsin, fibrinogen, and ferritin, or they may decrease, as with the negative acute-phase proteins albumin, prealbumin, and retinol-binding protein.

Inflammation also is associated with dramatic changes in plasma micronutrient concentrations. Changes in the

micronutrient milieu may be the host's attempt to optimize immune function and to deprive invading organisms of essential micronutrients for replication. Iron and zinc are important in the immune response, and different plasma concentrations are associated with either immunostimulatory or suppressant effects. Intracellular pathogens compete with host cells for available micronutrients. Either micronutrient deficiency or supplementation can impair metabolic adaptation to maintain an optimal microenvironment during inflammation. Immune dysfunction may result from this or from genetic variations in cellular mechanisms that control micronutrient flux and competition.

In this review, we first describe the key roles of iron and zinc in immune function and the effect of inflammation on their plasma concentrations. We next discuss the insights gained from clinical studies of children in developing countries in whom the metabolic response of plasma iron and zinc to inflammation has been disturbed by either deficiency or supplementation. Finally, we outline some newly characterized cellular mechanisms involved in transporting and in competing for these ions and demonstrate their clinical relevance. In reviewing the literature on iron or zinc supplementation and infection, we emphasize the variability of the immune response to supplementation and attempt to identify groups in which this variability is high.

IRON AND THE ACUTE INFLAMMATORY RESPONSE

Serum iron decreases and catabolism of transferrin increases during inflammation, resulting in decreased transferrin concentrations with decreased iron saturation (1). Decreased release of iron from reticuloendothelial ferritin stores and decreased intestinal iron absorption

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augments this hypoferremic response. The observation that ferritin synthesis in human volunteers increased 4 to 8 hours after injection of a bacterial endotoxin underlies the tissue block in iron release (2). Polymorphonuclear leukocytes release lactoferrin during inflammation, which binds iron that is then taken up by macrophages. Iron deprivation limits bacterial growth and has long been proposed as an important adaptive mechanism to limit the growth of invading microorganisms (3).

Hemoglobin and intracellular ferritin in the liver, bone marrow, and spleen account for more than 99% of total body iron. At physiologic pH, plasma iron is tightly bound to transferrin in the ferric form. Uptake and internalization of transferrin by endocytosis is followed by its dissociation at lower intracellular pH and storage of iron in cytoplasmic ferritin molecules. Free iron catalyzes the production of reactive oxygen species that damage both host and intracellular microbe cell membranes through lipid peroxidation. The hydroxyl radical is produced by either the Fenton or the Haber-Weiss reactions (4), and its generation represents an important intracellular killing mechanism. Invading organisms compete for available iron by secreting small, soluble iron chelators (siderophores) that are involved in both extracellular competition and transport across the microbial cell membrane. Mycobacteria are a good example of this, secreting salicylic and citric acids, mycobactins, and exochelins (5).

IRON DEFICIENCY, SUPPLEMENTATION, AND IMMUNE FUNCTION

Studies of iron deficiency and immune function in vivo are frequently confounded by coexisting nutritional deficiencies, poor socioeconomic conditions, and differing diagnostic criteria, which make them difficult to interpret and compare. Twenty-eight percent of 153 Peruvian infants had elevated C-reactive protein concentrations or leucocytosis associated with elevated ferritin concentrations, resulting in an underestimation of iron deficiency by 12% (6). Impaired T-cell proliferation, impaired delayed-type hypersensitivity, and decreased bactericidal activity of polymorphonuclear leukocytes have been consistently reported in iron deficiency (7). Neutrophils and macrophages require iron for phagocytosis and for generation of toxic oxygen intermediates to kill bacteria. Nitroblue tetrazolium reduction and hydrogen peroxide production are decreased in neutrophils and macrophages, respectively, if they become iron deficient (8). In contrast, exposure of neutrophils to excessive iron decreases phagocytosis, possibly because of increased free-radical production and consequent lipid-peroxidation damage of the phagosome membrane (9). Activated lymphocytes require iron to proliferate during the immune response (10). The DNA synthetic enzyme ribonucleotide reductase is iron dependent (11). How-

ever lymphocyte proliferation also decreases when transferrin saturation reaches 100% (12).

Although iron deficiency is associated with an immune defect in vitro, studies of the effect of iron supplementation on morbidity from infection have often been contradictory and offer little evidence for benefit. Observational evidence that iron supplementation leads to increased morbidity or mortality from infection has been reported. Low transferrin levels were associated with increased risk of death in septic children with kwashiorkor who were given supplemental oral iron (the dosage regimen is unclear) (13), and it was proposed that administering iron to these children before transferrin concentrations had time to recover may have led to dangerously high levels of free iron in the circulation. Prophylactic intramuscular injections of iron dextran to a population of Polynesian newborns in New Zealand caused increased Gram-negative neonatal sepsis that decreased after stopping supplementation (14), and adult iron-deficient Somali nomads given 900 mg of ferrous sulphate for 30 days had increased episodes of infection compared with controls (15). Chronically iron-overloaded patients, such as those with hemochromatosis, chronic renal failure, or hereditary hemolytic anemia, who require repeated blood transfusions provide further insights. Phagocytic function (16) was impaired in patients undergoing hemodialysis, as was natural killer cell function in patients with thalassemia (17). The iron chelator desferrioxamine could reverse both effects. However, in a large multicenter study of 988 adult patients undergoing hemodialysis and monitored for 6 months, serum ferritin concentrations and iron treatment were not found to be risk factors for infection (18). Iron-overloaded patients are susceptible to infection with *Yersinia*, a bacterium that does not produce iron-binding chelators and is more likely to be invasive in the presence of free iron (19).

A large body of clinical and laboratory data suggests that although iron is essential for immune function and that an immune defect is associated with iron deficiency, supplementation with iron does not decrease infectious disease morbidity and mortality and may even increase it in certain circumstances.

ZINC AND THE ACUTE INFLAMMATORY RESPONSE

Zinc is predominately (95%) found in muscle, bone, skin, and hair. Only 5% is labile and accessible in the liver and plasma. Plasma zinc is 99% bound to albumin (80%), α -2 macroglobulin (15%), and other low-molecular-weight proteins. Metallothioneins are a group of intracellular monomeric polypeptides that bind zinc and act as homeostatic modulators of zinc availability. Serum concentrations of zinc decrease sharply in inflammation. Two doses of the bacterial endotoxin lipopoly-

saccharide caused an increase in IL-6 and tumor necrosis factor- α associated with decreased serum zinc concentrations in adult volunteers; cytokine-directed internal redistribution of zinc was proposed (20). This hypozincemic response is accounted for largely by redistribution to the liver and reticuloendothelial system and may favor the immune system in producing acute-phase proteins (21). Calprotectin, an acute-phase zinc-binding protein produced by polymorphonuclear leukocytes and monocytes may play a role in sequestering extracellular zinc away from invading microorganisms (22). Adding zinc reversed calprotectin-inhibited growth of *Candida albicans*.

Lower serum concentrations of zinc in inflammation and infection may reflect a natural protective mechanism, because lower concentrations of zinc are associated with optimal phagocytic function and decreased microbial virulence (23). Optimal phagocytosis of latex particles correlates with decreased concentrations of zinc in human serum and polymorphonuclear cells (24). Zinc is important in microbial virulence, and near physiologic concentrations of zinc are necessary for toxin production by *Clostridium perfringens* (25) and *Pseudomonas aeruginosa* (26).

ZINC DEFICIENCY, SUPPLEMENTATION, AND IMMUNE FUNCTION

Zinc plays a central role in the immune system, and deficiency affects immune function at many levels, both innate and specific. Deficiency is associated with a down-regulation of basic biologic functions at the cellular level, including DNA synthesis, RNA transcription, and cell division and activation. Cell-mediated immunity is profoundly affected in zinc deficiency. Lymphopenia, lymphoid atrophy, defects in specific T- and B-lymphocyte function, and impaired phagocytosis have all been described (27).

Zinc deficiency rarely occurs alone and has no pathognomonic clinical features. Zinc supplementation of malnourished children improves immune function. Initial observations included enlargement of thymic shadows in children with marasmus who were given 10 days of zinc supplementation (28). Children with marasmus who were given supplemental zinc for 105 days had significantly increased conversion of delayed hypersensitivity skin reactions, enhanced lymphoproliferative response to phytohemagglutinin, and increased salivary immunoglobulin A concentrations compared with nonsupplemented infants (29). Zinc supplementation of malnourished Ecuadorian children with 10 mg/d for 60 days was associated with significantly larger delayed-type hypersensitivity skin reactions than those of controls and significantly decreased incidence of fever, cough, and upper respiratory tract secretions (30). One dose of 20 mg zinc to Indian children with acute diarrhea resulted in signifi-

cant decreases in the duration and severity of diarrhea (31), and supplementation of 10 mg/d 6 days each week for 6 months to rural, Ethiopian children with stunted growth resulted in significantly decreased infectious disease morbidity (32).

Supplementation of zinc increases plasma concentrations to differing extents. In vitro evidence points to the free zinc ion concentration of the culture medium relative to the protein concentration as a determinant of effects on monocytes and lymphocytes (33). In cultured human peripheral blood mononuclear cells, excess zinc (0.25 mmol/L) induced the release of IL-1 β and IL-6 and tumor necrosis factor- α (34). Paradoxically, although zinc-induced monokines stimulated T-cell proliferation, higher concentrations of zinc inhibited IL-1-dependent T-cell stimulation (35). Different leukocyte subtypes respond differently to similar zinc concentrations. Granulocyte phagocytosis was impaired by zinc in the presence of Mg²⁺ in a concentration-dependant manner (36). In animal experiments, supplemental zinc increased serum concentrations and was associated with decreased mobilization of polymorphonuclear cells and macrophages into the peritoneal cavity and with reduced phagocytosis (37). In adult men, administration of 150 mg of elemental zinc twice a week for 6 weeks was associated with decreased lymphocyte stimulation responses to phytohemagglutinin and with decreased chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes (38).

Zinc supplementation during sepsis has caused clinical problems, particularly in the presence of a compromised immune system. Infants with marasmus who were fed a zinc-fortified liquid diet (15 mg/L) had decreased phagocytic and fungicidal monocytic activity and significantly increased number and duration of episodes of impetigo (39). High-dose zinc supplementation (6 mg/kg daily) given early in rehabilitation to severely malnourished Bangladeshi children led to increased sepsis and mortality compared with low-dose supplementation (1.5 mg/kg daily) (40). Figure 1 shows the effect of 15 days of zinc supplementation and concurrent infection on plasma zinc concentrations in these children. On day 15, the high-dose zinc group had plasma zinc concentrations in the presence of infection that were equivalent to those of the low-dose zinc group free of infection. This study highlighted the difficulties in assessing the zinc requirements of the severely malnourished child; the wide variability in dosage regimens currently used, that is "physiologic" versus "pharmacologic" supplementation; and the detrimental effects of inappropriate dosage regimens.

Zinc is essential to immune function, and its deficiency is associated with an immune defect. However, although zinc supplementation of zinc-deficient children has successfully decreased infectious disease morbidity, clinical and laboratory data indicate potential detrimental effects in certain groups.

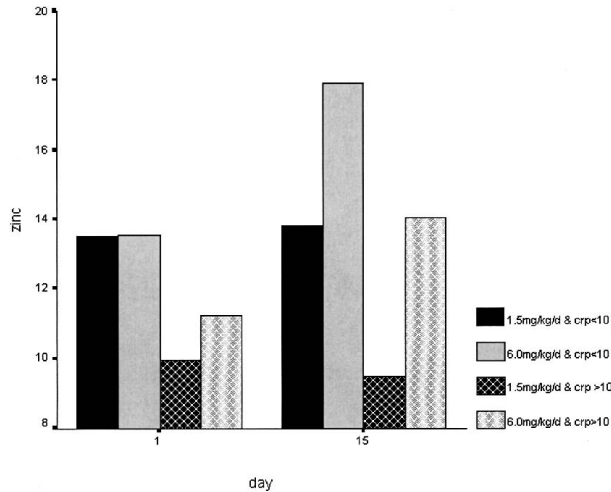


FIG. 1. Mean plasma zinc concentrations ($\mu\text{mol/L}$) on days 1 and 15 for 141 severely malnourished Bangladeshi children, aged 6 to 36 months, given either 1.5 or 6.0 mg/kg daily zinc in the absence or presence of infection. C-reactive protein concentrations greater than 10 mg/dL were deemed indicative of infection.

MECHANISMS OF FLUX AND COMPETITION

Micronutrient flux and transport across cell membranes are prominent during inflammation. The cellular mechanisms that maintain the micronutrient environment and those underlying host/microbe competition for divalent cations are beginning to be characterized. Micronutrient gene interactions and direct effects of inflammatory mediators on micronutrient metabolism have been described and offer further insights into the homeostatic mechanisms of micronutrient metabolism and the effects of infection and inflammation. Zinc promotes mRNA stability and thus regulates gene expression (41). Zinc stimulation of peripheral blood mononuclear cells also induces tumor necrosis factor- α mRNA transcription (42). Tumor necrosis factor- α increases ferritin synthesis (43) and IL-1 increases the expression of metallothionein 1 and 2 in the liver, bone marrow, and thymus, which accompanies the increased uptake of zinc in these organs (44).

The identification of specific genetic polymorphisms that determine infectious disease susceptibility suggests that genes controlling micronutrient flux may explain some of the variability of response to supplementation. For instance, the methods by which macrophages compete with intracellular microbes for divalent cations recently have been identified. Natural resistance-associated macrophage protein 1 (NRAMP1) expression determines mouse susceptibility to *Leishmania donovani*, *Salmonella typhimurium*, *Mycobacterium bovis*, and *lepraemurium* infection. Expressed exclusively on the phagosome membrane of macrophages, this phosphoglycoprotein modulates growth of these diverse intracellular microbes by altering the intravacuolar micro-

environment in which they grow. Divalent cation transporter 1 (DCT1), a rat homologue of NRAMP, has been shown to be a divalent cation transporter of iron, zinc, copper, and manganese in *Xenopus* oocytes (45). A mycobacterial NRAMP homologue, MRAMP mRNA, induced a pH-dependant 20-fold increase in zinc and iron uptake when injected into *Xenopus laevis* oocytes (46).

Divalent cations are essential for microbial survival as cofactors for both superoxide dismutase and catalase redox enzymes, which neutralize the reactive oxygen intermediates integral to phagosomal killing, and for enzymes required for DNA synthesis. Infection with mycobacteria results in their residence within the phagolysosome of the macrophage. The phagolysosome matures and becomes increasingly acidic, allowing the host's killing systems to operate. However *Mycobacterium tuberculosis* arrests this maturation. The NRAMP1 is recruited early to the membrane of the phagolysosome and may modulate the divalent cation concentration of the phagolysosome, inhibiting growth of the parasite. In response, the *Mycobacterium* uses its own NRAMP homologues to compete for these cations.

Substitution of glycine with aspartic acid at position 169 of the fourth transmembrane domain of NRAMP1 is associated with mycobacterial survival and growth within macrophages (47). Adding iron to macrophages from BCG susceptible mice (expressing the aspartic acid substitution in NRAMP 1) stimulated mycobacterial growth, whereas addition of low levels of iron to BCG-resistant mice macrophages inhibited growth (Fig 2) (48). However, adding hydroxyl radical scavengers abrogated this latter effect.

Both NRAMP and MRAMP seem to use intraphagosomal protons to modulate intraphagosomal cation concentration, and the intraphagosomal pH of mycobacteria-infected macrophages may be crucial in determining which predominates. Other divalent cations also may be involved (Fig. 3).

Evidence is emerging that NRAMP1 is a predisposing factor in intracellular infections in humans. Susceptibil-

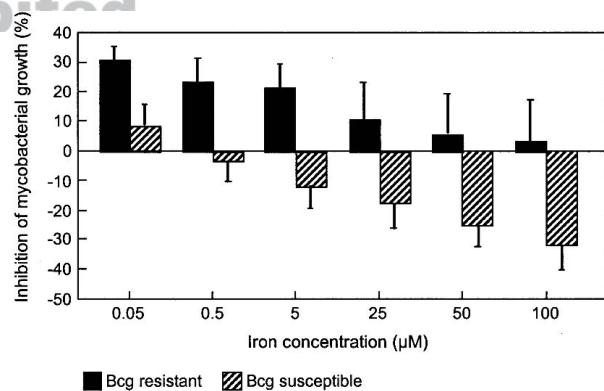


FIG. 2. Effect of iron on the growth of *Mycobacterium avium* in macrophages from BCG resistant (BCG^r) and susceptible (BCG^s) mice. Reproduced with permission (48).

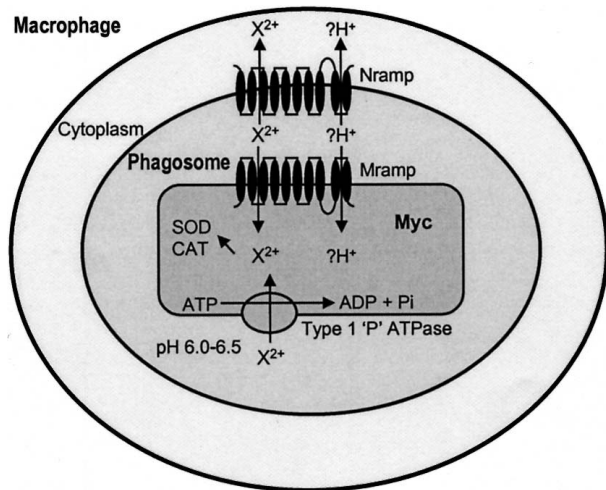


FIG. 3. Divalent cation (X^{2+}) competition between intraphagosomal mycobacteria (myc) and macrophage. Natural resistance-associated macrophage protein 1 (NRAMP) functions to modulate intraphagosomal cation concentration either by exporting them from the phagolysosome and depriving the mycobacteria (as seen here, Agranoff et al. (54), with permission) or by transporting them into the phagolysosome to catalyse hydroxyl radical production (45). The mycobacteria counters with a mycobacterial NRAMP homologue, MRAMP, and type 1 P-type adenosine diphosphatase transporting ions into the mycobacteria for use in superoxide dismutase (SOD) and catalase (CAT) reactive oxygen intermediate detoxification systems.

ity to leprosy in Vietnamese families was linked to the NRAMP1 gene (49), and polymorphic variants were also associated with susceptibility to *Mycobacterium tuberculosis* in a case control study in The Gambia, West Africa (50).

CONCLUSIONS

Divalent cation flux, around and within cells of the immune system, plays a critical role in their optimal function and defense against invading microbes. Iron and zinc deficiencies and excesses have been associated with immunomodulatory effects, and micronutrient supplementation has had beneficial and detrimental effects on immune function. Zinc supplementation of children who are zinc deficient improves immune function, whereas iron supplementation of iron-deficient children does not. There is no evidence that zinc supplements cause harm when given to nonseptic immunocompetent children. In general, the immune response to an iron or zinc supplement of a child who is uninfected but micronutrient deficient is predictable.

However, many children targeted for supplementation have multiple micronutrient deficiencies, coexisting macronutrient malnutrition, and associated immunoincompetence, which may influence their response to supplementation. In the septic, severely malnourished or immunocompromised child, the immune response to

supplementation is less predictable. Study of the relationship between iron and zinc supplementation and infection in these children highlights this and indicates factors influencing this variability. The fine balance between benefit and harm reflects the perilous nature of their immunocompromise, and an inappropriate micronutrient supplement may have adverse effects that are not likely to occur in the immunocompetent child. In a healthy, micronutrient-deficient child, the net effect of the supplement is beneficial, because a partially compromised immune system seems to benefit from micronutrient supplementation in the absence of competing microbes. In the septic, severely malnourished, or immunocompromised child, the competing microbes may benefit immediately and before the severely compromised immune system can use the supplement to aid recovery (Fig 4).

Although this review has concentrated on the interaction between micronutrient supplements and infection, the dosage administered (41) and interactions between micronutrients may contribute to the variability of response. Zinc supplementation studies of malnourished children are striking in the diversity of supplementation regimens used, reflecting difficulties in accurately assessing the zinc status and requirements of these children. Micronutrients interact, and interactions among zinc, iron, and copper are well recognized (51,52). Inflammation and zinc supplementation can induce metallothionein synthesis, principally in the liver but also in other tissues. Metallothionein binds copper and zinc with different affinities, influencing absorption and bioavailability of both at a cellular level. A review of these interactions is beyond the scope of this paper, but the interactions certainly could influence response to supplementation. Zinc deficiency rarely occurs in isolation.

The effect of a supplement given during an infection on the course of that infection may depend on the mag-

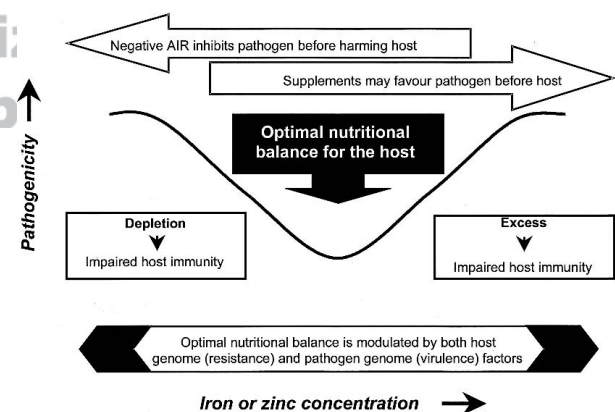


FIG. 4. Schematic representation of how the host/pathogen relationship of the septic, malnourished child is influenced by iron and zinc status, the acute inflammatory response (AIR), and host genome (resistance) and pathogen genome (virulence) factors. The effect of iron or zinc supplementation will depend on all these factors.

nitude of the micronutrient deficiency, dose of the supplement, presence of other nutritional deficiencies and interactions with other micronutrients, level of immunocompromise, type and pathogenicity of infection, and genetic variability in divalent cation transport mechanisms. With increasing recognition of specific polymorphisms that influence micronutrient flux and with demonstration of their influence on susceptibility to tuberculosis and leprosy, predicting response to supplementation may improve. The NRAMPI is localized exclusively to the phagolysosomal membrane of the macrophage, and so far NRAMPI has been shown to be important only in infections characterized by chronic intracellular residence. As mechanisms of divalent cation flux and competition are characterized, other polymorphisms in host and microbe may be shown to affect susceptibility to infection.

Variability in response and potential detrimental effects must be considered in micronutrient supplementation strategies. Recognition and characterization of subgroups within populations that might not benefit from iron or zinc supplementation would be an important first step in better micronutrient targeting. These subgroups should include septic, severely malnourished, or immunocompromised children. The World Health Organization has recently advised on micronutrient supplementation regimens for severely malnourished children and warned that iron supplements should not be given in the initial phase of treatment (53). This warning should be extended to high-dose zinc supplements. Further research is urgently required into the benefits or otherwise of iron or zinc supplementation during sepsis, into the mechanisms of divalent cation transport, and into micronutrient gene interactions in host cells and microbes. Improved supplement targeting requires better understanding of the immune response to supplementation during infection and recognition that variability to response exists and is poorly understood.

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