

## TRACE ELEMENTS AND IMMUNITY

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Nutrition and health are known to be intimately linked since ancient times but the vital importance of micronutrients on the immune response was clearly established only in the last quarter of the 20<sup>th</sup> century. The understanding of adequate trace element nutrition is a key factor to design a better nutrition that protects animals and humans against infections. The present paradigm to evaluate the status of trace element deficiencies is to characterize some effector activities in leukocytes from various tissues such as blood, bone marrow and lymphoid organs. Copper, zinc and iron nutrition have so far concentrated much attention as tested by recent reviews describing the relationship between trace elements and immune functions (1-4).

### ***Role of trace elements in maturation, activation and function of immune cells***

The availability of new powerful tools in analytical cell biology and molecular genetics has facilitated efforts to identify specific cellular and molecular functions of trace elements in the maturation, activation and functions of host defense mechanisms. Immune cells, like all other types of cells, require an adequate supply of trace elements to express and preserve the structure and function of key metalloproteins that participate in housekeeping processes such as energy production (e.g. iron for cytochromes a, b and c, NADH and succinate dehydrogenases); copper is also fundamental for cytochrome c oxidase in the mitochondrial electron-transport chain) and to protect the cell against highly toxic reactive oxygen species (e.g. copper and zinc for superoxide dismutase and iron for catalase). Moreover, the continuous generation of immune cells in bone marrow and the clonal expansion of lymphocytes in response to antigenic stimulation require the availability of sufficient iron and zinc in the synthesis of deoxyribonucleotide precursors by ribonucleotide reductase and for the various nucleotidyl transferases and zinc-finger proteins that are required for DNA replication and cell division, respectively. In addition, trace elements are also required to maintain the activity of a number of enzymes that directly participate in important defense processes (5). The most evident example of such a role is the need of heme iron in the myeloperoxidase-dependent generation of hypochlorous acid, which is a well known microbiocidal factor (6). Copper and zinc are linked in cytosolic defense against reactive oxygen and nitrogen species (7). Undoubtedly there will be additional discoveries of new metalloenzymes that are required for normal development and reactivity of immune cells.

Trace element status can affect the immune function not only in a direct way but also but also indirectly by modulating plasma levels of hormones that regulate the development and function of host defense cells. Initial studies suggested that the thymic atrophy and lymphopenia associated to zinc deficiency in rodents resulted from chronic elevation of plasma corticosterone. Subsequent research revealed that the increased plasma glucocorticoid level and the decreased cellular zinc content in zinc-deficient animals contributes to a reduction in the B- and T-cell compartments by inducing apoptotic loss of precursor and immature B-cells in bone marrow and of pre-T-cells in the thymus (8). As the deficiency increases, a reprogramming of the immune system occurs. A pathway used by

glucocorticoids to induce apoptosis in thymocytes has recently been proposed (9). Micronutrients may potentially influence some processes of nonspecific immunity by modulating inflammatory cell functions. For instance, the effect of copper deficiency on phagocytic cells, particularly neutrophils and macrophages, is well detailed in the literature. Functions of neutrophils include traveling to the site of infection, adhering to the endothelium and transmigration across the endothelium, where they are involved in phagocytosis and killing of foreign invaders by activation of the respiratory burst (10). Copper deficiency causes a decrease in the number of circulating neutrophils, a condition termed neutropaenia. This condition is observed in copper-deficient animals and humans (11). Cellular copper status, respiratory burst, and candidacidal activity of peritoneal macrophages have been shown to decrease in severely copper deficient rats (12). Iron deficiency in humans also compromises the ability of neutrophils to kill bacteria (13), where by counterpart, free iron is necessary for bacterial growth. However, there is only scarce understanding on the exact mechanisms triggered by an inadequate trace element nutrition to affect the normal macrophage function.

Effects of mineral deficiency on the acquired immune system can be further demonstrated by examining the response of lymphocytes to T cell mitogens (blastogenesis). Impaired proliferative response has been reported in copper- and iron-deficient animals and humans (14,15). Blastogenic activity appears to be a sensitive marker of marginal trace element status and one of the cell-mediated immune parameters that responds to mineral repletion. One of the possible mechanisms by which iron deficiency impairs lymphocyte proliferation is by reducing translocation or activation of protein kinase C (PKC) (16). Further evidence of a role for Fe in lymphocyte signaling pathways comes from a study showing that phosphatidyl inositol-4,5-bisphosphate hydrolysis is reduced in activated spleen cells from Fe-deficient mice (17).

Trace elements status also affects the synthesis and secretion of cytokines and chemokines that modulate the activities of immune and other cells. Beck and associates demonstrated that mild zinc deficiency in humans induces an imbalance in *ex vivo* cytokine secretion by peripheral blood mononuclear cells despite maintenance of normal number of total leukocytes and B- and T-lymphocytes (18). Dietary zinc restriction is associated with decreased secretion of interferon- $\gamma$ , tumor necrosis factor- $\alpha$  and interleukin (IL)-2, without changes in IL-4, -6 and -10 concentrations when blood mononuclear cells are treated with mitogens like phytohemagglutinin. The affected and non-affected cytokines are secreted by T-helper (TH) TH1 and TH2 cells, respectively. These observations suggest that zinc plays an important role in maintaining the proper balance between cell-mediated and humoral immunity by regulating patterns of cytokine secretion. Recent studies have demonstrated that in the Jurkat T-cell line, copper deficiency (induced by the copper chelator 2,3,2-tetramine) decreased IL-2 synthesis in T-lymphocytes by inhibiting transcription of the IL-2 gene (19). Thus, current understanding of how immune response develops in human beings must now be considered in light of changing emphasis on the role of the microenvironment in regulation of immune response and growing knowledge concerning the activity of cytokine patterns in influencing the immune function.

### ***Iron deficiency and immunity: a human model***

Worldwide, an estimated 2 billion people have iron deficiency anemia. Although the problem affects to all-age groups, this is specially prevalent and severe in children and pregnant women in developing countries (20). The whole area of iron and its relationship

with immunity is complex, and has been extensively reviewed by us and other groups (21-23). Studies conducted in animals and humans suggest that iron deficiency affects different immune functions causing thymic atrophy, abnormal delayed hypersensitivity reaction, impaired lymphocyte proliferation, disturbed natural killer cell activity and low IL-1 and IL-2 production.

Studies conducted by our group at INTA have focused the attention on the multifunctional cytokine tumor necrosis factor-alpha (TNF- $\alpha$ ), since this intercellular mediator plays important roles in both immunity and iron metabolism. It is known that TNF- $\alpha$  provides signals for T-B cell interaction and activation of lymphocytes, respectively. On the other hand, TNF- $\alpha$  also inhibits the release of iron from peritoneal macrophages and induces increased transferrin receptor mRNA in fibroblasts; administration of TNF- $\alpha$  also leads to hypoferrremia in animals and humans. We investigated whether iron, by itself or bound to the transport protein transferrin, induces TNF- $\alpha$  secretion by human blood mononuclear cells in healthy subjects (24,25). We found that the addition of an iron salt or transferrin result in a dose-dependent increase of this cytokine. These data agree with the results reported for other minerals and transport proteins (26,27), suggesting that iron controls TNF- $\alpha$  secretion through a process that depends on the form in which iron is supplied. However, the total cytokine response to iron concentration is lower as compared to the potent inducer lipopolysaccharide.

The study of isolated micronutrient deficiencies on individual components of immune system is extremely difficult to design and interpret in human beings, where deficits in other elements and/or the likely concomitant presence of active infections may act as confounding variables. Several investigators have preferentially used laboratory animals or cell lines, but data can not be totally extrapolated to humans. In our studies we have included adult healthy women free of clinical infections coursing iron deficiency due to chronic genital bleeding without neoplasia. The results showed that all hematological parameters were impaired but the copper and zinc levels remained normal. To our knowledge, the loss of iron through this via represents a human model of "pure" iron deficiency. Through this model we recently analyzed the cellular process by which transferrin (Tf) modulates TNF- $\alpha$  production in blood mononuclear cells from adult women either with normal iron status or with iron deficiency (ID) (28). We found that Tf induced secretion and transcription of the cytokine, but not its membrane expression, which levels were significantly lower in ID subjects compared to controls. These data suggest that Tf-induced TNF- $\alpha$  secretion is transcriptionally regulated, and the impaired results in cells from ID subjects indicate that the quality of the immune response is linked to the iron status of mononuclear cells. Presently, the participation of Tf-receptor, as a possible signaling mechanism of TNF- $\alpha$  induction, is being investigated by our group.

To summarize in vitro evidence, iron deficiency depresses certain aspects of cell-mediated immunity as well as cytokine production including the intercellular mediator TNF- $\alpha$ ; humoral immunity is unaffected and the significance of hypoferrremia (as opposed to normal transferrin saturation) on growth of microorganisms is uncertain. The conflicting effects of iron deficiency and iron supplementation "in vitro" on the defensive systems, reveals the urgent necessity to gain information on the "in vivo" situation.

### ***Trace metal supplementation and infectious diseases and morbidity***

Does the amount of dietary trace element influence the risk of infection? The widespread occurrence of deficiencies of copper, zinc or iron in humans has served as the impetus to determine whether supplementation with these trace metals alone or as adjuvant has the potential to prevent, attenuate and eventually treat infectious diseases. However, it is also recognized that the host's needs must be balanced against the possibility that excess amounts of redox-active metals such as iron and copper can induce free-radical-mediated damage, and this would enhance microbial infectious diseases. Selected clinical trials on trace element supplementation and infectious diseases are discussed below.

Isolated human copper deficiency is difficult to find due to other concomitant macro and micronutrient deficiencies. Some years ago, in a pioneer study, our group analyzed the effect of copper therapy on leukocyte phagocytosis in infantile hypocupremia (11). Nineteen hypocupremic infants 5-9 months of age, normal weight/length ratio and free of infections, received during a month a daily fed of an enriched copper cow's milk formula containing 40 ug/Kg copper. Plasma copper and ceruloplasmin, and phagocytic activity of polymorphonuclear leukocytes were measured before and after therapy. After supplementation, copper and ceruloplasmin levels as well as phagocytic index increased to normal values. Further clinical trials of copper supplementation in infected subjects are urgently needed.

Recent excellent clinical trials have demonstrated that zinc supplementation of children significantly decreases the incidence of diarrhea, pneumonia, respiratory infections and mortality (29-31). Since shigellosis is also a major cause of childhood mortality in developing countries, Raqib et al. (32) investigated the effect of zinc supplementation on innate and specific immune and inflammatory responses in *Shigella* infected children aged 18 months, who received zinc (20 mg/d) or placebo for 2 weeks. Lymphocyte proliferation and *Shigella* antigen-specific IgG improved in the zinc supplemented patients. In addition, zinc therapy significantly increased serum zinc and C-reactive protein levels during convalescence. These data suggest that short-term zinc supplementation could be useful, practical, and cost-effective therapy from a public health perspective.

Since iron deficiency anemia is a major worldwide health problem, especially in children from developing countries, the evaluation of balance between the risks and benefits of an oral Fe supplementation is of particular importance before implementing an intervention. Berger and co-workers (33) assessed the impact of 3-months daily oral Fe supplementation on hematological status, cell-mediated immunity and susceptibility to infections in 6-36 months-old children from rural Africa, living in an environment where Fe deficiency and malaria are frequent. Iron supplementation had significant and positive effects on iron status and some immune factors since hemoglobin and total T and Th cells were improved post-therapy. However, no impact on the incidence of malaria was found. These data suggest that control of iron deficiency by oral Fe supplementation in young children has to be conducted, associated with prophylaxis and treatment of malaria and repeated deworming.

Another application of micronutrient intervention is related to vaccine response in older people since mortality associated with influenza is more likely to occur in this population. The report of UK National Diet and Nutritional Survey found that up to 40% of older, institutionalized people had low biochemical indices for certain micronutrients. Since nutritional intervention might be important in improving seroconversion, recently Allsup and associates (34) investigated whether a short period of micronutrient therapy could

improve antibody response to influenza vaccine when administered to older people living in long-term facilities. One hundred sixty-four residents aged 60 and older were randomized to receive a micronutrient supplement or placebo (one tablet twice a day for 8 weeks); influenza vaccine was administered 4 weeks after their commencement. Seroconversion results to each of the 3 antigens contained in 2000/2001 influenza vaccine (H1N1, H3N2, B) showed no differences between supplemented and placebo groups. Thus, short micronutrient supplementation had no beneficial effect on the antibody response to influenza vaccine. Larger trials are needed to investigate the effects of long-term therapy and clinical outcome in poorly nourished institutionalized older people.

### ***Possible future directions***

Micronutrient deficiencies are of clinical and public health magnitude in developing countries and account for significant infectious morbidity. In the zinc status, successful intervention at the level of glucocorticoids action or prevention of apoptotic cell loss could dramatically improve the medical outcome of patients facing nutritional deficiencies. Studies already available suggest micronutrient deficits may have a great negative impact in innate immunity. In supplementation studies, micronutrients can have varied direct or indirect effects on inflammatory cells, but still remain difficulties to link the in vitro observations with the results of in vivo interventions. A better understanding of the molecular and cellular changes patients have in response to inadequate trace elements should lead to enhanced immunotherapeutic interventions and to improve the quality of life for many human beings.

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