Trace Elements and Host Defense: Recent Advances and Continuing Challenges[1,2]

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ABSTRACT Although it is widely recognized that essential trace elements are required for the differentiation, activation and performance of numerous functions of immune cells, the specific roles of these inorganic micronutrients in these processes remain largely undefined. New insights about the participation of zinc, iron and copper in the selection, maturation and early activation events of the immune cells have been gained by judicious use of available tools in analytical cell biology, molecular genetics and array technology. Also, randomly controlled clinical and community trials demonstrate that zinc supplementation can enhance immunocompetence and decrease the incidence and severity of some infections in individuals with diagnosed or suspected mild zinc deficiency. These exciting results provide an impetus to evaluate the potential benefits of supplementation programs for individuals and groups with suboptimal trace element status as a cost-effective means of reducing the risk of infectious diseases. J. Nutr. 133: 1443S–1447S, 2003.

KEY WORDS: • *immunity* • *zinc* • *copper* • *iron* • *cytokines* • *cell activation* • *nuclear factor-κB*

Research during the last quarter of the 20th century clearly established the importance of adequate trace element nutrition for protection of animals and humans against infections. The standard paradigm was to catalog the impact of induced and genetic trace element deficiencies on the numbers and effector activities of the various classes of leukocytes in blood, marrow and lymphoid organs. Iron, zinc, copper and selenium have received the majority of attention. The data overwhelmingly support the following general conclusions: *1*) an inadequate supply of these essential micronutrients is associated with suppression of numerous activities of cells in both the innate and acquired branches of the immune system; *2*) the observed alterations can reflect either decreased activities of individual cells, a reduction in the total number of effector cells in one or more tissues or a combination of fewer cells with each cell having diminished capacity; *3*) the extent of the impairment in the immune system due to trace element deficiency can be sufficient to increase the risk of morbidity and mortality due to viral, microbial and parasitic infections; and *4*) reversal of the trace element deficiency restores immunocompetence. Moreover, there is increasing evidence that some cell types and effector activities appear to be particularly sensitive to marginal and moderate deficiencies of the trace elements. Recent reviews should be consulted for specific details about the relationship between the nutritional status of trace elements and the function of the immune system (1–7).

The availability of powerful tools in analytical cell biology and molecular genetics has facilitated efforts to begin to identify the specific cellular and molecular functions of trace elements in the maturation, activation and functions of host defense cells. In particular, deficiencies of a number of trace elements alter the secretion of extracellular factors that modulate the activities of immune cells and other types of cells that participate in the host response to irritants and infectious agents. Moreover, recent demonstrations that supplementation of individuals with mild zinc deficiency decreases the prevalence or severity of several common infectious diseases provides optimism that improvement of the trace element status of individuals with primary and secondary deficiencies represents an efficacious means for increasing immunocompetence. These topics are further considered below with a focus on recent discoveries for zinc, iron and copper.

Before proceeding, two items merit comment. The first is that much remains to be learned about the general effects of "ultratrace" elements on immunity as exemplified by recent observations for boron. Supplementation of a low-boron diet with physiologic levels of the trace element increases total serum antibody concentrations in rats in response to admin-

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istration of the human typhoid vaccine and decreases paw swelling in rats with antigen-induced arthritis (8). In addition, supplementation of a low-boron diet with as little as 2 mg of boron/kg decreases paw swelling in rats with antigen-induced arthritis. This attenuated response is associated with decreased numbers of neutrophils and increased numbers of natural killer cells and cytotoxic T-cells in the blood of boron-supplemented rats compared with those in similarly treated rats that are fed the basal diet (0.1 mg of boron/kg diet). Likewise, supplementation of a low-boron diet with as little as 5 mg of boron/kg decreases inflammation in pigs after the animals receive intradermal injection of phytohemagglutinin but does not alter mitogenic reactivity of lymphocytes or serum antibody titers in response to injection of sheep red blood cells (9). Investigation of the influences of other ultratrace metals on host responses to immunologic changes is likely to generate additional insights about the requirements and immunomodulatory roles of the inorganic micronutrients in host defense.

A general overview of the roles of trace elements and immunity is incomplete without mention of the exciting discoveries by Beck and Levander. These investigators have shown that changes in the intracellular environment that are induced by alterations in micronutrient status can directly influence viral virulence and in addition can cause immune system dysfunction. The sentinel discovery is that selenium deficiency promotes the mutation of an avirulent strain of Coxsackie virus in the heart to a strain that causes myocarditis when the virus is passed from the selenium-deficient to a nutritionally adequate host (10). Because this modification of the viral genome also is observed in mice that are either deficient in vitamin E, overloaded with iron or lacking the glutathione peroxidase gene, it appears that the more oxidative intracellular environment that results from deficient or excess levels of some of the micronutrients promotes the genomic alteration that enhances virulence. The recent discovery that selenium deficiency also increases lung pathology in mice that are infected with influenza virus demonstrates that this effect is not limited to Coxsackie virus (11). The presentation by M.E. Beck at this conference provides further details about these studies and considers the implications of trace element deficiency and excess on the emergence of infectious diseases (12).

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

Immune cells, like all other types of cells, require an adequate supply of trace elements for the structure and function of metalloproteins that participate in housekeeping processes such as energy production (e.g., iron for cytochromes *a, b* and *c*, NADH and succinate dehydrogenases; copper for cytochrome *c* oxidase in the mitochondrial electron-transport chain) and protection against reactive oxygen species (e.g., copper and zinc for superoxide dismutase, selenium for glutathione peroxidases 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 necessitates the availability of sufficient iron and zinc for 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 likely are required for the activity of a number of enzymes that directly participate in host defense processes. The evident example of such a role is the need for heme iron for myeloperoxidase-dependent generation of hypochlorous acid, which is a microbiocidal factor. Undoubtedly there will be additional discoveries of metalloenzymes that are required for the normal development and reactivity of immune cells.

Trace element status can affect plasma levels of hormones that regulate the development and function of host defense cells. The most detailed insights in this area have been provided by the elegant studies of Fraker and associates. Initial studies that used the murine model suggested that the thymic atrophy and lymphopenia that is associated with zinc deficiency in rodents resulted from chronic elevation of plasma corticosterone (4). 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 (13) and of pre–T-cells in the thymus (14). In conjunction with the decline in lymphoid compartments, zinc deficiency is associated with an increase in myelopoiesis and the number of neutrophils present in the periphery. This suggests that the expansion of the innate arm of the immune system at the expense of the acquired arm may represent an adaptive switch when zinc becomes limiting. Zinc deficiency also is associated with loss of thymulin activity (15). Thymulin is a zinc-binding nonapeptide secreted by thymic epithelial cells that requires zinc to promote extrathymic maturation of T-lymphocytes.

Trace element status also affects the synthesis and secretion of cytokines and chemokines that modulate the activities of immune and other cells. Studies by Prasad and associates demonstrate that mild zinc deficiency in humans induces an imbalance in ex vivo cytokine secretion by peripheral blood mononuclear cells despite maintenance of normal numbers of total leukocytes and B- and T-lymphocytes (16). Dietary zinc restriction is associated with decreased secretion of interferon-γ, tumor necrosis factor-α and interleukin (IL)-2,[4] without changes in concentrations of IL-4, -6 and -10, when blood mononuclear cells are treated with phytohemagglutinin. The affected and nonaffected cytokines are secreted by T-helper (TH)1 and TH2 cells, respectively, and phytohemagglutin primarily induces a response by TH1 cells. The adverse impact of zinc deficiency on TH2 cell responsiveness also is evident when the stimulus elicits the response by this subpopulation of cells. Splenocytes isolated from zinc-deficient mice infected with an enteric nematode secrete less IL-4 and -5 after exposure to parasite antigen than identically treated cells from zinc-adequate mice (17). 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.

Absorptive epithelial cells in the small intestine participate in immunosurveillance: these cells process and present dietary antigens on their basolateral surface and synthesize numerous cytokines, chemokines and growth factors that modulate the activities of gut immune cells. Recent studies with differentiated cultures of Caco-2 human intestinal cells show that elevated cellular iron status decreases the amounts of transforming growth factor-β1 and monocyte chemotactic protein-1 mRNA in uninfected cells (18). In contrast, the amounts of transforming growth factor-β1, monocyte chemotactic protein-1, IL-8 and tumor necrosis factor-α mRNA are higher in iron-loaded cells infected with *Salmonella enteritidis* than in infected cells with normal iron status. The impact of enterocyte iron load on the response of gut immune system to irritants and infectious agents merits investigation especially in light of the widespread use of iron supplements.

[4] Abbreviations used: HIV, human immunodeficiency virus; IL, interleukin; LCK, lymphocyte kinase; NF, nuclear factor; PKC, protein kinase C; TH, T-helper.

Recent studies suggest that intracellular zinc, copper and iron participate in the activation of immune cells. For example, deficiency of each of these trace metals decreases the synthesis and secretion of IL-2 (6,19,20). Transcriptional activity of the IL-2 gene is suppressed in zinc-deficient (21) and copper-deficient (19,22) T-cells treated with phytohemagglutinin and phorbol myristate acetate. In light of the central role of the transcription factor nuclear factor (NF)-κB in cytokine gene expression in T-lymphocytes and other immune cells, the impact of inadequate zinc and copper status on NF-κB was examined to determine whether the decreased transcription of the IL-2 gene is associated with impaired activity of this redox-sensitive transcription factor. NF-κB is a homo- or heterodimeric complex that is localized in the cytoplasm of quiescent cells by its association with the inhibitory protein I-κB (23). Binding of stimuli to surface receptors leads to the phosphorylation of several seryl residues in I-κB with its subsequent ubiquitination, dissociation from the NF-κB complex and degradation. The NF-κB dimer is translocated to the nucleus where it interacts with other nuclear proteins and recognition sequences in the promoter region of target genes. Similar to many transcription factors, NF-κB has several zinc-binding fingers that are required for binding of the activated dimer to DNA. Although it is possible that cellular zinc deficiency decreases zinc content and, therefore, the DNA-binding activity of the transcription factor, low zinc status is associated with decreased transfer of NF-κB to the nucleus, decreased levels of phosphorylated and ubiquitinated I-κB and decreased activity of I-κB kinase, which phosphorylates I-κB in stimulated cells (21). Moreover, these changes are sufficiently robust to affect transcriptional activity of NF-κB–driven reporter gene expression in zinc-deficient transfected cells. These results suggest that the signaling defect in zinc-deficient T-cells is upstream from I-κB kinase. Zinc-dependent activities in lymphocytes that are candidates for defective activation of the NF-κB pathway include phospholipase C, protein kinase C (PKC) and the zinc-finger signal transduction protein lymphocyte kinase (LCK). LCK is required for T-cell–receptor-linked signal transduction processes that activate peripheral lymphocytes and the selection and maturation of lymphocytes in thymus. Expression of LCK in both splenic lymphocytes (24) and thymocytes (25) is increased in response to moderate zinc deficiency in mice, whereas the expression and activities of splenic phospholipase C and PKC are independent of zinc status (24).

In marked contrast to the findings with zinc-deficient cells, preliminary results from our laboratory suggest that copper deficiency does not alter nuclear translocation and transcriptional activity of NF-κB in either Jurkat T-cells or U937 monocytic cells (26,27). However, NF-AT–dependent reporter gene expression in Jurkat cells is decreased by copper deficiency (26). The possible participation of copper in calcineurin-dependent activation of cytoplasmic NF-AT (28) requires investigation. Reports that copper is required for the expression, activity and membrane binding of PKC in several nonimmune cell types (29,30) and phosphoinositide-3-kinase in human skin fibroblasts and HeLa cells (31) provide additional direction for examining roles for copper in the activation of immune cells.

Iron deficiency also decreases total activity of PKC in activated T-cells and the transfer of PKC from the cytoplasm to the *cis* face of the plasma membrane (32). The membrane-bound enzyme phosphorylates numerous target proteins that participate in signaling processes. The defect in translocation of PKC from the cytoplasm to the plasma membrane is associated with impaired hydrolysis of phosphatidylinositol-4,5-bisphosphate for the formation of inositol phosphates (33). These data suggest that suppressed effector functions in iron-deficient T-cells result in part because an adequate pool of iron is needed for early events that are associated with cell activation including the generation of second messengers.

Trace metal supplementation for reduction of infectious disease morbidity

The widespread occurrence of marginal and moderate deficiencies of iron and zinc in humans has served as the impetus to determine whether supplementation with these trace metals alone or as an adjuvant has the potential to prevent, attenuate and treat infectious diseases. However, it also is 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 that viruses and infectious microorganisms also require the same trace elements for their survival and replication as the host. In addition, trace metal overload, like deficiency, suppresses immune cell function and increases the risks of morbidity and mortality due to infectious disease. For example, the bactericidal and tumoricidal activities of phagocytic cells, natural killer cell cytotoxicity, the phenotypic profile of T-cell subsets, the proliferative capacity and activities of CD4 cells and the reactivity of T-cells to mitogens are all adversely affected by hereditary hemochromatosis and conditions that require frequent blood transfusions (33,34). Several clinical reports suggest that high iron status can enhance the rate of progression of human immunodeficiency virus (HIV) disease and decrease survival (35,36). With regard to excess zinc, supplementation of healthy adult men with 300 mg of zinc/d for 6 wk impairs chemotaxis and phagocytic activity of neutrophils against bacteria and lymphocytic response to mitogenic stimulation (37). Similarly, phagocytic and fungicidal activities of monocytes are impaired, and the incidence and duration of episodes of impetigo are increased when marasmic infants are supplemented with zinc (1.9 mg of zinc $\cdot$ kg^{-1} $\cdot$ d^{-1}) during a 105-d nutritional rehabilitation program (38). It is unclear whether these problems result from the direct toxicity of high zinc levels on immune cells or from a zinc-induced secondary copper deficiency. Copper overload can also affect the immune system. The LEC rat is characterized by excessive accumulation of hepatic copper, impaired biliary copper excretion and low plasma ceruloplasmin activity due to a deletion in the ATP7B copper-transporting gene that is homologous to the human Wilson disease gene (39). Lymphoid abnormalities in this model include thymic and splenic hypoplasia, decreased concentrations of serum immunoglobulin G and the selective arrest of CD4 cell maturation (40). It is unknown whether lymphoid cells in these animals or in humans with Wilson disease accumulate toxic levels of copper.

During the past several years, a number of investigators have reported encouraging findings about the apparent benefits of zinc supplementation for select target populations. For example, individuals with HIV are at high risk of developing zinc and other micronutrient deficiencies as a result of decreased appetite, malabsorption and increased losses of endogenous zinc due to diarrhea and recurrent infections. Progression to stage IV of the disease is associated with decreased plasma zinc, inactivation of thymulin due to the dissociation of bound zinc and reduced numbers of CD4 T-cells (15). The potential benefit of oral zinc supplementation for individuals with HIV to restore adequate zinc status and perhaps improve immune-system competence was challenged

by results from an observational study with HIV-infected homosexual men. Results show that increased intake of zinc is associated with a greater risk of progression to acquired immunodeficiency syndrome and increased mortality (41,42). It is proposed that the additional complement of zinc may favor HIV replication by increasing the availability of the metal for zinc-dependent viral proteins such as integrase, nucleocapsid protein and TAT (twin arginine translocation) protein. However, information on zinc intake is limited to self-reported levels at the beginning of the study. The inverse relationship between reported intake and CD4 cell count suggests that those with a more advanced stage of the disease are likely to use supplements. In sharp contrast to the above outcome, the beneficial effect of oral zinc as an adjuvant for the treatment of the disease was demonstrated in a randomized controlled study. For 30 d, patients with stage III and IV HIV who used zidovudine either received a zinc supplement (45 mg of zinc/d) or no supplement (15). In addition to increased plasma zinc levels, thymulin activity and absolute numbers of CD4 cells, zinc-supplemented individuals maintained weight, had lower titers of HIV and experienced fewer opportunistic infections for 2 y. The reader is encouraged to consult several comprehensive reviews for additional information on the relationship between zinc nutrition and HIV infection (43,44).

Mild zinc deficiency is common in children and adults with adult sickle-cell disease (45). Results from a pilot study show that long-term supplementation of zinc-deficient adult sickle-cell patients with 50–75 mg of zinc/d (taken orally) significantly increases the zinc content of lymphocytes and granulocytes and induces a severalfold increase in IL-2 secretion by cultures of peripheral blood mononuclear cells treated with phytohemagglutinin. Also, the incidence of confirmed bacterial infections in respiratory and urinary tracts and clinical infections that likely were of viral origin significantly decreased in zinc-treated groups. It is noteworthy that there were no changes in any of these outcome measures for sickle-cell patients with normal levels of leukocyte zinc at the beginning of the trial who did not receive supplemental zinc. These positive results encourage researchers to hold trials with larger numbers of sickle-cell patients and to evaluate oral zinc supplementation with regard to enhancement of immunocompetence in other groups with diseases that induce secondary zinc deficiency, e.g., chronic renal disease.

A major success regarding the beneficial impact of improving trace element nutritional status and the immune system during the past several years concerns the demonstration that zinc supplementation of children (0.5–5 y of age) significantly decreases the incidence and duration of diarrhea and the incidence of pneumonia (46–49). Details about these important results are provided by Robert Black in another presentation at the conference (49).

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LITERATURE CITED

1. Bonham, M., O'Connor, J. M., Hannigan, B. M. & Strain, J. J. (2002) The immune system as a physiological indicator for marginal copper status? Br. J. Nutr. 87: 393–403.

2. Brock, J. H. & Mulero, V. (2000) Cellular and molecular aspects of iron and immune function. Proc. Nutr. Soc. 59: 537–540.

3. Failla, M. L. & Hopkins, R. G. (1997) Is low copper status immnosuppressive? Nutr. Rev. 56: S59–S64.

4. Fraker, P. J., King, L. E., Laakko, T. & Vollmer, T. L. (2000) The dynamic link between the integrity of the immune system and zinc status. J. Nutr. 130: 1399S–1406S.

5. McKenzie, R. C., Rafferty, T. S. & Beckett, G. J. (1998) Selenium: an essential element for immune function. Immunol. Today 19: 342–345.

6. Shankar, A. H. & Prasad, A. S. (1998) Zinc and immune function: the biologic basis of altered resistance to infection. Am. J. Clin. Nutr. 68: 447S–463S.

7. Spears, J. W. (2000) Micronutrients and immune function in cattle. Proc. Nutr. Soc. 59: 587–594.

8. Hunt, C. D. & Idso, J. P. (1999) Dietary boron as a physiological regulator of the normal inflammatory response: a review and current research progress. J. Trace Elem. Exp. Med. 12: 221–233.

9. Armstrong, T. A., Spears, J. W. & Lloyd, K. E. (2001) Inflammatory response, growth, and thyroid hormone concentrations are affected by long-term boron supplementation in gilts. J. Anim. Sci. 79: 1549–1556.

10. Beck, M. A. (2001) Antioxidants and viral infections: host immune response on viral pathogenicity. J. Am. Coll. Nutr. 20: 384S–388S.

11. Beck, M. A., Nelson, H. K., Shi, Q., Van Dael, P., Schiffrin, E. J., Blum, S., Barclay, D. & Levander, O. A. (2001) Selenium deficiency increases the pathology of an influenza virus infection. FASEB J. 15: 1481–1483.

12. Beck, M. A., Levander, O. & Handy, J. (2003) Selenium deficiency and viral infection. J. Nutr. 133: 1463S–1467S.

13. Fraker, P. J. & King, L. E. (2001) A distinct role for apoptosis in the changes in lymphopoiesis and myelopoiesis created by deficiencies in zinc. FASEB J. 15: 2572–2578.

14. King, L. E., Osati-Ashtiani, F. & Fraker, P. J. (2002) Apoptosis plays a distinct role in the loss of precursor lymphocytes during zinc deficiency in mice. J. Nutr. 132: 974–979.

15. Mocchegiani, E. & Muzzioli, M. (2000) Therapeutic application of zinc in human immunodeficiency virus against opportunistic infections. J. Nutr. 130: 1424S–1431S.

16. Beck, F. W., Prasad, A. S., Kaplan, J., Fitzgerald, J. T. & Brewer, G. J. (1997) Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans. Am. J. Physiol. 272: E1002–E1007.

17. Scott, M. E. & Koski, K. G. (2000) Zinc impairs immune responses against parasitic nematode infections at intestinal and systemic sites. J. Nutr. 130: 1412S–1420S.

18. Foster, S. L., Richardson, S. H. & Failla, M. L. (2001) Elevated iron status increases bacterial invasion and survival and alters cytokine/chokine mRNA expression in Caco-2 human intestinal cells. J. Nutr. 131: 1452–1458.

19. Hopkins, R. G. & Failla, M. L. (1997) Cu deficiency reduces interleukin-2 production and mRNA levels in human T-lymphocytes. J. Nutr. 127: 257–262.

20. Galan, P., Thibault, H., Preziosi, P. & Hercberg, S. (1992) Interleukin 2 production in iron-deficient children. Biol. Trace Elem. Res. 32: 421–426.

21. Prasad, A. S., Bao, B., Beck, F. W. J. & Sarkar, F. H. (2001) Zinc activates NF-κB in HUT-78 cells. J. Lab. Clin. Med. 138: 250–256.

22. Hopkins, R. G. & Failla, M. L. (1999) Transcriptional regulation of interleukin-2 gene expression is impaired by copper deficiency in Jurkat human T lymphocytes. J. Nutr. 129: 596–601.

23. Janssen-Heininger, Y. M. W., Poynter, M. E. & Baeuerle, P. A. (2000) Recent advances towards understanding redox mechanisms in the activation of nuclear factor κB. Free Radic. Biol. Med. 28: 1317–1327.

24. Lepage, L. M., Giesbrecht, J. A. C. & Taylor, C. G. (1999) Expression of T lymphocyte p56lck, a zinc-finger signal transduction protein, is elevated by dietary zinc deficiency and caloric deficiency in mice. J. Nutr. 129: 620–627.

25. Moore, J. B., Blanchard, R. K., McCormack, W. T. & Cousins, R. J. (2001) cDNA array analysis identifies thymic LCK as upregulated in moderate murine zinc deficiency before T-lymphocyte population changes. J. Nutr. 131: 3189–3196.

26. Hopkins, R. G. & Failla, M. L. (2001) Decreased synthesis of interleukin-2 (IL-2) in copper deficient T-cells mediated by inhibition of the transcription factor NF-AT. FASEB J. 15: A272 (abs. 237.8).

27. Huang, Z. L., Hopkins, R. G. & Failla, M. L. (2001) Copper deficiency decreases TNF-α expression despite normal activities of NF-κB and AP-1 in human U937 monocytic cells. FASEB J. 15: A1097 (abs. 842.2).

28. Crabtree, G. R. (2001) Calcium, calcineurin, and the control of transcription. J. Biol. Chem. 276: 2313–2316.

29. Johnson, W. T. & Dufault, S. N. (1991) Copper deficiency alters protein kinase C mediation of thrombin-induced dense granule secretion from rat platelets. J. Nutr. Biochem. 2: 663–670.

30. Davis, C. D. & Johnson, W. T. (2002) Dietary copper affects azomethane-induced intestinal tumor formation and protein kinase C isozyme protein and mRNA expression. J. Nutr. 132: 1018–1025.

31. Ostrakhovitch, E. A., Lordnejad, M. R., Schliess, F., Sies, H. & Klotz, L. O. (2002) Copper ions strongly activate the phosphoinositide-3-kinase/AKT pathway independent of the generation of reactive oxygen species. Arch. Biochem. Biophys. 397: 232–239.

32. Kuvibidila, S. R., Kitchens, D. & Baliga, B. S. (1999) In vivo and in vitro iron deficiency reduces protein kinase C activity and translocation in murine splenic and purified T cells. J. Cell. Biochem. 74: 468–478.

33. Kuvibidila, S. R., Baliga, B. S., Warrier, R. P. & Suskind, R. M. (1998) Iron deficiency reduces the hydrolysis of cell membrane phosphatidyl inositol-4,5-bisphosphate during splenic lymphocyte activation in C57BL/6 mice. J. Nutr. 128: 1077–1083.

34. Walker, E. M. & Walker, S. M. (2000) Effects of iron overload on the immune system. Ann. Clin. Lab. Sci. 30: 354–365.

35. Cunningham-Rundles, S., Giardina, P. J., Grady, R. W., Califano, C., McKenzie, P. & deSousa, M. (2000) Effect of transfusional iron overload on immune response. J. Infect. Dis. 183: S115–S121.

36. Gordeuk, V. R., Delanghe, J. R., Langlois, M. R. & Boelaert, J. R. (2001) Iron status and the outcome of HIV infection: an overview. J. Clin. Virol. 20: 111–115.

37. Chandra, R. K. (1984) Excessive intake of zinc impairs immune response. J. Am. Med. Assoc. 252: 1443–1446.

38. Schlesinger, L., Arevalo, M., Arredondo, S., Lonnerdal, B. & Stekel, A. (1993) Zinc supplementation impairs monocyte function. Acta Paediatr. 82: 734–738.

39. Wu, J., Forbes, J. R., Chen, H. S. & Cox, D. W. (1994) The LEC rat has a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene. Nat. Genet. 7: 541–544.

40. Agui, T., Oka, M., Yamada, T., Saki, T., Izumi, K., Ishida, Y., Himeno, K. & Matsumoto, K. (1990) Mutational arrest from $CD4^+8^+$ to $CD4^+8^-$ thymocytes in a mutant strain (LEC) of rat. J. Exp. Med. 172: 1615–1624.

41. Tang, A. M., Graham, N. M. H., Kirby, A. J., McCall, L. D., Willett, W. C. & Saah, A. J. (1993) Dietary micronutrient intake and risk of progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. Am. J. Epidemiol. 138: 937–951.

42. Tang, A. M., Graham, N. M. & Saah, A. J. (1996) Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection. Am. J. Epidemiol. 143: 1244–1256.

43. Kupka, R. & Fawai, W. (2002) Zinc nutrition and HIV infection. Nutr. Rev. 60: 69–79.

44. Siberry, G. K., Ruff, A. J. & Black, R. (2002) Zinc and human immunodeficiency virus infection. Nutr. Res. 22: 527–538.

45. Prasad, A. S., Beck, F. W. J., Kaplan, J., Chandrasekar, P. H., Ortega, J., Fitzgerald, J. T. & Swerdlow, P. (1999) Effect of zinc supplementation on incidence of infections and hospital admissions in sickle cell disease. Am. J. Hematol. 61: 194–202.

46. Bhutta, Z. A., Black, R. E., Brown, K. H., Gardner, J. M., Gore, S., Hidayat, A., Khatun, F., Martorell, R., Ninh, N. X., Penny, M. E., Rosado, J. L., Roy, S. K., Ruel, M., Sazawal, S. & Shankar, A. (1999) Zinc investigators' Collaborative Group: Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. J. Pediatr. 135: 689–697.

47. Strand, T. A., Chandyo, R. K., Bahl, R., Sharma, P. R., Adhikari, R. K., Bhandari, N., Ulvik, R. J., Molbak, K., Bhan, M. K. & Simmerfelt, H. (2002) Effectiveness and efficacy of zinc for the treatment of acute diarrhea in young children. Pediatrics 109: 898–903.

48. Bhandari, N., Bahl, R., Taneja, S., Strand, T., Molbak, K., Ulvik, R. J., Sommerfelt, H. & Bhan, M. K. (2002) Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomized controlled trial in an urban slum. BMJ 324: 1358–1362.

49. Black, R. E. (2003) Zinc deficiency, infectious disease, and mortality in the developing world. J. Nutr. 133: 1485S–1489S.