Clin Chem Lab Med 2003; 41(8):1020–1025 © 2003 by Walter de Gruyter · Berlin · New York

Review

Trace Elements, Innate Immune Response and Parasites

Maria Teresa Rivera[1], Andrea Pereira De Souza[1], Tania Cremonini Araujo-Jorge[1], Solange Lisboa De Castro[1] and Jean Vanderpas[2]*

[1] Lab. Biologia Cellular, DUBC, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil
[2] Unité d'épidémiologie Faculté de Médecine, Faculté Universitaire Notre-Dame de la Paix, Namur, Belgium

Micronutrient deficiencies and infectious disease often coexist and show complex interactions leading to mutually reinforced detrimental clinical effects. Such a combination is predominantly observed in underprivileged people of developing countries, particularly in rural regions. Several micronutrients such as trace elements (zinc, iron, selenium) modulate immune function and influence the susceptibility of the host to infection. Nevertheless, the effect of individual micronutrients on components of innate immunity is difficult to design and interpret. Micronutrient deficiency, in general, has a widespread effect on nearly all components of the innate immune response. Chagas' disease is a pertinent model to study interaction of nutrition, immunity and infection, as it implies many components of innate immunity. An important question is whether alterations on micronutrient intake modify the course of infection. Some interactions of trace elements with innate immunity and acute inflammatory response are reviewed in this article with a special focus on selenium deficiency and *Trypanosoma cruzi* infection. Clin Chem Lab Med 2003; 41(8):1020–1025

Key words: Zinc; Iron; Selenium; Parasite; *Trypanosoma cruzi*.

Abbreviations: DFO, desferrioxamine; GPx, glutathione peroxidase; GPx1, glutathione peroxidase type 1; IFγ, interferon-γ; IL, interleukin; IRE, iron-responsive element; LPS, lipopolysaccharide (endotoxin); NFκB, nuclear factor κB; NK, natural killer; *P. falciparum*, *Plasmodium falciparum*; PM, peritoneal macrophages; ROS, reactive oxygen species; Th1, T-helper lymphocyte type 1; Th2, T-helper lymphocyte type 2; TNF-α, tumor necrosis factor-α; *T. cruzi, Trypanosoma cruzi*.

1. General Background

Seven essential trace elements are required in human nutrition (1), among them zinc, iron and selenium influence the susceptibility of the host to infection and the course and outcome of different diseases (2, 3). The general knowledge about the interaction of parasite infection with these trace elements during the initial phase of immune response – innate immunity and acute inflammatory phase – is reviewed in the context of the following observation (4): mice with severe selenium deficiency since birth and mice with adequate selenium supply since birth were infected with a sub-lethal dose of *Trypanosoma cruzi* (*T. cruzi*). Only selenium-deficient mice died during the acute phase inflammatory response, while selenium-sufficient mice survived. The level of parasitemia was similar in both groups.

Our results present some analogy with those of Beck and collaborators. They have shown that in a selenium-deficient experimental model (mice), harmless Coxsackie B viruses (CVB3/0 strain) become virulent (5). Moreover, it was shown that mutations occurred in the genome to give a cardiovirulent form of the virus that caused myocarditis. When the mutated virus from these selenium-deficient mice was inoculated into mice with adequate selenium supply, it still induced heart damage, showing the persistence of the mutation in the virus. A similar study on mice that were unable to synthesize glutathione peroxidase type 1 (GPx1 knockout mice) showed that this enzyme is essential for the prevention of oxidative damage to the RNA-viral genome that results in the myocarditic mutations (6). This animal model is relevant for the pathogenesis of Keshan disease, an endemic cardiomyopathy of young adults in rural parts of the People's Republic of China with extremely poor selenium supply (7).

Due to the much more complex genomic structure of parasites, it seems unlikely that a specific genetic mutation could result in increased toxicity of *T. cruzi*.

In relation to *T. cruzi*, we hypothesize that an imbalance during the acute phase inflammatory response results in multiple organ failure and death associated with severe selenium deficiency. Indeed, during sepsis there is a delicate balance between pro-inflammatory stress and anti-inflammatory factors. An excessive inflammatory "storm" or a lack of counter-regulation could both result in uncontrolled immune response and death.

Innate or natural immunity is non-specific for a particular foreign organism. Besides cutaneous and digestive barriers, such immune reactions involve activation of macrophages, natural killer (NK) lymphocytes, and the complement system. Vascular adaptation promotes immune cell accumulation at the site of infection and acute inflammation. Lipopolysaccharide endotoxins (LPSs) of Gram negative bacteria or peptidoglycans of the membrane of bacteria or parasites are known promoters of this innate immunity. The macrophages

*E-mail of the corresponding author:
Jean.Vanderpas @skynet.be

and NK cells recognize these poorly specific promoters through receptors of the Toll receptor family (8).

Acute phase inflammatory response is associated with this activation of innate immunity and is multiplied (positive feedback) by some components of acquired immunity (antibodies and T cells). The general concept is that cytokines produced by macrophages, NK cells and T cells induce a cascade of reactions, which stimulate the synthesis and secretion of some liver proteins (positive markers of acute phase inflammation) like C-reactive protein or ferritin, and decrease the synthesis and secretion of other liver proteins (negative markers of acute phase inflammation) like albumin (9, 10).

The regulation of acute phase inflammatory response is crucial: a lack of control is potentially lethal, leading to multi-organ failure. The general concept is that, in addition to the marked cellular reaction at the site of infection, other tissues are in a lethargy state (decreased metabolism, hibernation). An imbalance can result in irreversible tissue damage and death. In the sepsis syndrome, a cytokine "storm" unleashing numerous inflammatory mediators is the likely way to multiple-organ failure and death (11).

The cytokines of phagocyte cell origin, such as tumor necrosis factor-α (TNF-α) and the interleukins IL-1 and IL-6, are predominantly pro-inflammatory. The activation of type 1 helper T cells (Th1) and the secretion of TNF-α, interferon-γ (IFγ) and IL-2 trigger a "cascade storm". Activation of type 2 helper T cells (Th2) has anti-inflammatory properties by secreting other cytokines such as IL-4, IL-5 and IL-10. The balance between pro-inflammatory and anti-inflammatory responses is critical for a controlled reaction to foreign infectious material, with uncontrolled inflammation leading to multiple organ failure (12, 13).

Intracellular parasites (*Plasmodium, Leishmania, Trypanosoma, etc.*) stimulate Th1 immunity, while extracellular ones (*Filaria*) and helminths stimulate Th2. Particularly, IL-5 is the predominant stimulant of hypereosinophilia and immunoglobulin E humoral immunity, which characterize extracellular and helminthic parasite infections (14, 15).

A literature search for the keywords "parasite" and "trace element deficiency" with each one of the seven well-established essential trace elements in humans was carried out in the Medline Database. Since very little relevant data was obtained on cobalt, iodine, manganese or copper deficiencies in parasite infection, these topics are not mentioned further.

The present Review focuses on the most pertinent research results, according to our judgment.

2. Zinc Deficiency, Parasites and Acute Inflammatory Response

2.1 Nematodes

Zinc deficiency causes atrophy of lymphoid tissues (16, 17) and depressed cutaneous delayed-type hypersensitivity reactions (17, 18). In experimental animals, zinc deficiency reduced antibody production, T cell proliferation and cytokine production in response to mitogens or to specific antigens (19–21). In mice infected with nematodes, zinc deficiency impaired both Th1 and Th2 responses (22). These findings, obtained from analysis of thymus or spleen, reflect the essential requirement for zinc by CD4+ helper T- and B cells in systemic lymphoid organs. Such considerations probably apply also to lymphoid tissues in the intestine, although information on the effects of zinc deficiency on the barrier function and on specific immune responses in the gastrointestinal tract is still lacking. Such deficiency may interfere with the recruitment to, and localization of, immune cells in the intestinal epithelium, thus impairing the host's ability to effectively combat parasitic infection.

2.2 Trypanosoma cruzi

Since zinc deficiency has been shown to have a profound effect on the murine immune system, it was of interest to evaluate the effects of such deficiency on host resistance to infection. Balb/C mice were fed either a zinc-deficient or zinc-adequate diet; after 8 days, each group was infected with a sub-lethal inoculum of *T. cruzi*. Twenty-two days post-infection, the cumulative mortality was 80% in the zinc-deficient group and 10% in the zinc-adequate group. Also the parasitemia was 50 times higher in the zinc-deficient group. In all infected groups, food intake and/or body weights were reduced in comparison to their uninfected counterparts. The data show that zinc deficiency and *T. cruzi* interact synergistically to alter both the nutritional and immune status of the host. The experiments also demonstrate the extreme susceptibility of zinc-deficient mice to some pathogens (23).

Using another approach, the same group pointed out an important role for zinc in the biochemical events associated with macrophage uptake and killing of the parasites. They exposed young adult A/J mice to zinc-deficient, zinc-adequate or restricted amounts of a zinc-adequate diet for 28 days. On the basis of weight loss and parakeratosis, the zinc-deficient mice were further divided into moderately and severely zinc-deficient. The peritoneal macrophages (PM) were collected from each group and infected with *T. cruzi*. Both the percentage of PM with associated parasites and the number of parasites per 100 macrophages were significantly lower in the moderately and severely deficient mice than in mice fed restricted or zinc-adequate diets. Furthermore, PM from both zinc-deficient groups killed fewer intracellular parasites than did PM from restricted or zinc-adequate groups. Pre-treatment of PM from zinc-deficient mice with 5 µg zinc for 30 min *in vitro* completely restored both their capacity to take up and kill the parasites. Other trace metals tested, including copper, manganese and nickel, failed to reverse the effects of zinc deficiency (24).

3. Iron Deficiency, Parasites and Acute Inflammatory Response

Most studies are linked with two well-known observations: (i) hookworm infection is a cause of iron loss and iron-deficient anemia (25, 26); (ii) anemia in tropical countries is related to malaria infection and to iron deficiency (27).

The other question, which is central for the present discussion, has received much less attention: Does iron deficiency immunomodulate susceptibility to parasites?

The sparse epidemiological available data do not provide a definitive answer. Some results suggest that iron deficiency does not modify the natural course of malaria (28), while another one suggests a protective effect of iron deficiency (29). On a public health scale, the question is of prime importance: the correction of iron deficiency represents one of the three priorities of the World Health Organization in the Micronutrient Initiative Program, which includes iodine, iron and vitamin A. More data are urgently required to determine whether the correction of iron deficiency has any detrimental effect on parasite infection.

Experimental data clearly suggest that iron is an essential trace element for some parasites, for example *Plasmodium falciparum* (*P. falciparum*), which contains an iron-regulated protein that binds to a mammalian iron-responsive element (IRE), raising the possibility that the malaria parasite expresses transcripts that contain IREs and are iron-dependently regulated (30). Infectious agents must acquire iron from their host to survive, and deficiency in this element has been reported to protect against malaria in humans (28). The susceptibility of *P. falciparum* to iron deprivation was tested *in vitro* by studying the effect of desferrioxamine (DFO), a specific iron-chelating agent, on parasite growth. It was found that DFO inhibits the growth of the parasites at concentrations readily achievable *in vivo*, by a mechanism that may involve interference with the completion schizogony (31).

4. Selenium, Parasites and Acute Inflammatory Response

Selenium deficiency is accompanied by loss of immuno-competence, probably connected with the fact that this trace element is normally found in significant amounts in tissues such as liver, spleen and lymph nodes. Both cellular and humoral responses can be impaired by such deficiency (32).

Selenium supplement, even in subjects with normal serum selenium concentration, has immuno-stimulant effects, including an enhancement of activated cell proliferation (clonal expansion). Enhanced response to antigen stimulation and increased ability to develop cytotoxic lymphocytes and to destroy tumor cells (NK cell activity) were also observed (33).

The mechanism appears to be closely related to the ability of selenium to up-regulate the expression of growth-regulatory cytokine IL-2 receptors on the surface of activated lymphocytes and NK cells, thereby facilitating the interaction with IL-2. This interaction is crucial for clonal expansion and differentiation into cytotoxic T cells (34).

Additionally, cells of the immune system may have an important functional need for selenium. Activated T cells show increased selenophosphate synthetase activity (35), directed toward the synthesis of selenocysteine, the essential building block of selenium-containing proteins, for activation of T cell function and the control of the immune response.

Among more than 25 selenium-containing proteins, many not yet entirely characterized, the 3 or 4 glutathione peroxidases (GPx) and the thioredoxin reductase act enzymatically by detoxifying reactive oxygen species (ROS) (36). These metabolites produced during the inflammatory response enhance pro-inflammatory cytokine production by activation of the transcription factor nuclear factor κB (NFκB), an essential step in the control of cytokine synthesis and secretion (37).

Murine malaria appears to be a useful experimental model for the investigation of the interrelationship between selenium and vitamin E. Vitamin E deficiency protected experimental animals against the parasite, especially when mice are concurrently fed with peroxidizable fat, such as fish or linseed oil; in contrast, selenium deficiency had little or no protective effect against the parasite (38). Endogenous GPx activity in malaria parasites after selenium supplementation has been documented (39).

In *T. cruzi* experimental infection, a few studies pointed to a beneficial effect of selenium supplementation during murine infection (40). Recently, it has been reported that selenium deficiency increases the severity of chronic inflammatory myopathy in *T. cruzi*-infected mice (41).

Chagas' disease, the American trypanosomiasis caused by infection with the flagellate parasite *T. cruzi,* continues to be a major cause of morbidity and mortality, particularly among poor populations in rural and urban areas of Central and South America (42). Most of the seropositive-infected persons remain asymptomatic throughout life, but 20–30% of patients develop the chronic chagasic cardiomyopathy. The variable pathogenesis is not yet well understood (43, 44). In cases with ongoing cardiomyopathy, mononuclear infiltration associated with parasite antigens characterizes the myocarditis, which is associated with microvascular alterations and ventricular remodelling with fibrosis (43).

In endemic areas, the population is also affected by malnutrition (45). Few studies have been performed that specifically pointed to the impact of nutrition on the course of human infection with *T. cruzi* (45). Selenium status has not been studied yet in chagasic patients.

By analogy to the role of selenium deficiency in cardiomyopathy due to Keshan disease in rural China, we decided to study the possible role of this deficiency as

a cofactor in this pathology. We conducted both epidemiological and murine experimental studies.

4.1 Epidemiological study

A community-based case control study was carried out in Brazil to test the hypothesis that chronic Chagas' cardiomyopathy could be related to selenium-deficient status. Selenium status was in the normal range in all groups of chagasic patients according to serum selenium levels and GPx activity. Selenium deficiency was therefore excluded. However, the frequency of chagasic patients with selenium levels in ranges below normal was significantly higher in cases with the most severe form of cardiomyopathy. These results suggest that low selenium level could be a biological marker of a long-standing inflammatory process that may lead to progressive heart damage and dysfunction in chronic chagasic patients (46).

4.2 Experimental study

A study in a murine model was carried out to determine if selenium deficiency in mice could influence the pathological outcome of a *T. cruzi* infection with acute myocarditis. Mice were submitted to either a selenium-deficient or selenium-adequate diet. Eight-week old female C57BL/6 mice were fed selenium-deficient or adequate diets during pregnancy and lactation periods.

Severe selenium deficiency was confirmed by measurement of serum selenium and GPx levels in mothers fed a selenium-deficient diet, while the group fed a selenium-adequate diet presented normal levels of both parameters.

The offspring were fed the same diet as the mother. When 8-week old, mice were inoculated intra-peritoneally with a sub-lethal dose of trypomastigotes of *T. cruzi* (Y strain). The parasitemia and mortality were monitored during 45 days post-infection. Both groups of mice showed similar levels of parasitemia, while mortality differed markedly between them. Mice from the adequate selenium diet group presented a low percentage of mortality (20% in males and none in females). In all the selenium-deficient mice the mortality started very soon after infection (8 days post-infection) and by 20 days post-infection all animals were dead.

The serum levels of specific cardiac CK-MB enzyme, measured on 15 days post-infection, indicated an acute myocarditis in both groups and were higher in selenium-deficient mice.

Despite similar parasitemia curves, mortality parameters clearly indicated that selenium-deficient animals of both sexes were much more susceptible than the corresponding control group. The mechanism contributing to the higher susceptibility of selenium-deficient mice is likely to be related to a higher inflammatory process, interfering with the innate immune response. This latter inference is based on the fact that mice died before 20 days post-infection, meaning in the onset of acute phase, and so without the possibility of a specific immune response (4).

5. Conclusion and Methodological Precautionary Remarks

T. cruzi activates the innate immunological response. During the acute inflammatory phase in an experimental mouse model, there was an overproduction of cytokines (47–49) combined with several immune alterations, such as immunosuppression (decrease of IL-2), lymphocyte polyclonal activation and immunoglobulin overproduction. Parasites survive in some host organs, mainly nervous and cardiac tissues (50, 51). Some of these effects are induced by glycosylphosphatidylinositol mucine-like anchored glycoproteins present on the parasite surface. These molecules modulate the production of inflammatory cytokines (IL-1, IL-6, IL-12 and TNF-α) by macrophages (52). These cytokines increase in parallel with the onset of the parasitemia and decrease down to their normal levels at about 40 days post-infection.

Some studies have shown the important role of NK cells in the initial defence against *T. cruzi* infection; its protective effect is linked to the IFN-γ production by these cells (53). This cytokine is able to activate the nitric oxide pathway responsible for the parasite destruction in infected macrophages (54). Endogenous IL-10 seems crucial for counter-regulating an overshooting pro-inflammatory cytokine response resulting in TNF-α-mediated septic shock (55).

Host depletion in selenium is associated with a reduced immune response (56), and an active immune response is essential to overcome the acute phase of *T. cruzi* infection. The difference found in mortality rate indicates that selenium deficiency interferes with innate and/or specific mechanisms that can operate in the control of infection (the decreasing phase of parasitemia) and disease (the recovery from acute phase). Inflammatory cytokine levels increase significantly with the development of infection in selenium-deficient mice. Death may occur due to a hyper-inflammatory state and a decreased counter-regulatory anti-inflammatory response. This may indicate an immune system that is severely compromised with organ failure.

In conclusion, taking into account the immune mechanism triggered by *T. cruzi* infection and its interaction with selenium's role in the immune system (Figure 1), we suggest the hypothesis depicted in Figure 2. In epidemiological studies it is not possible to get isolated deficiencies of a specific trace element in human populations. Most of the time, these deficiencies occur in populations exposed to many combined nutritional problems and infectious diseases. In this context, it is difficult to analyze the effects of an isolated deficiency of a specific essential trace element. Multi-factorial designs are required to measure the effects of multiple environmental factors and of their interactions.

This difficulty of epidemiological studies has its counterpart in experimental ones. While it is relatively simple to obtain animals deficient for a specific essential trace element, the deficiency will by itself involve stunted growth and general malnutrition. In other

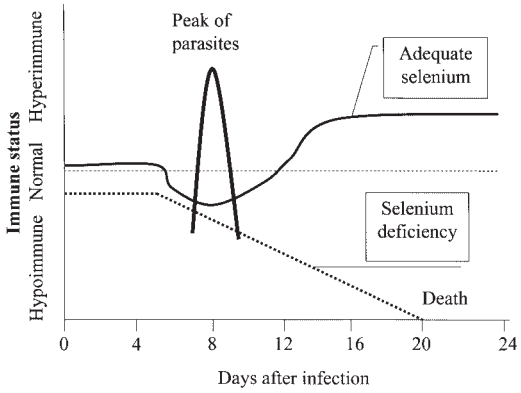

Figure 1 Proposed model of immunological response to parasite infection in mice according to adequate or deficient selenium diet.

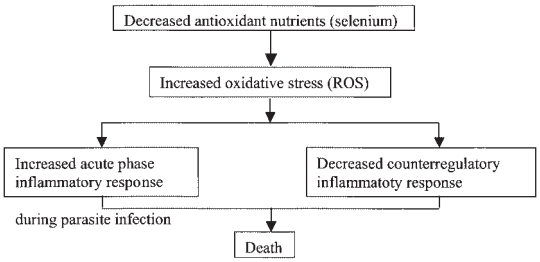

Figure 2 Proposed model of events during *Trypanosoma cruzi* infection in selenium-deficient mice leading to death.

words, in severe deficiency of essential trace elements, there is secondary global malnutrition, and when immune function is studied, it is not possible to discriminate the effects due to the essential element deficiency and those due to global malnutrition.

Experimental diet studies, thus, have their own limits. They represent a first approach to a problem, but the conclusion must be corroborated by subsequent confirmatory experiments. In the case of selenium, some of the enzymes involved in the immune response, like glutathione peroxidases and thioredoxin reductase, have been characterized, and knock-out mice have been developed (at least, for GPx). This progress offers new opportunities to study more specifically the exact mechanism of immune interaction between the trace elements and the parasites.

References

1. Solomons NW, Ruz M. Trace element requirements in humans: an update. J Trace Elem Exp Med 1998; 11:177–95.
2. Erickson KL, Medina EA, Hubbard NE. Micronutrients and innate immunity. J Infect Dis 2000; 182 Suppl:5–10.
3. Bhaskaram P. Micronutrient malnutrition, infection and immunity: an overview. Nutr Rev 2002; 60 Suppl:40–5.
4. De Souza AP, Melo de Oliveira G, Nève J, Vanderpas J, Pirmez CI, de Castro SL, *et al.* Trypanosoma cruzi: host selenium deficiency leads to higher mortality but similar parasitemia in mice. Exp Parasitol 2002; 101:193–9.
5. Beck MA, Kolbeck PC, Rohr LH, Shi Q, Morris VC, Levander OA. Benign human enterovirus becomes virulent in selenium-deficient mice. J Med Virol 1994; 43:166–70.
6. Beck MA, Esworthy RS, Ho YS, Chu FF. Glutathione peroxidase protects mice from viral-induced myocarditis. FASEB J 1998:12:1143–9.
7. Li Y, Wang F, Kang D, Li C. Keshan disease: an endemic cardiomyopathy in China. Hum Pathol 1985; 16:602–9.
8. Modlin RI, Brightbill HD, Godowsky PG. The Toll of innate immunity on microbial pathogens. N Engl J Med 1999; 340:1834–5.
9. Oberholzer A, Oberholzer C, Moldawer LL. Sepsis syndromes: understanding the role of innate and acquired immunity. Shock 2001; 16:83–96.
10. Ertel W, Kremer JP, Kenney J, Steckholzer U, Jarrar D, Trentz O, *et al.* Downregulation of proinflammatory cytokine release in whole blood from septic patients. Blood 1995; 85:1341–7.
11. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348:138–50.
12. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996; 383:787–93.
13. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest 2000; 117:1162–72.
14. Gazzinelli RT, Talvani A, Camargo MM, Santiago HC, Oliveira MA, Vieira LQ, *et al.* Induction of cell-mediated immunity during early stages of infection with intracellular protozoa. Braz J Med Biol Res 1998; 31:89–104.
15. Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, *et al.* Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Annu Rev Immunol 1997; 15:505–33.
16. Keen CL, Gershwin ME. Zinc deficiency and immune function. Annu Rev Nutr 1990; 10:415–31.
17. Shi HN, Scott ME, Stevenson MM, Koski KG. Zinc deficiency impairs T cell function in mice with primary infection of Heligmosomoides polygyrus (Nematoda). Parasite Immunol 1994; 16:339–50.
18. Sempértegui F, Estrella B, Correa E, Aguirre L, Saa B, Torres M, *et al.* Effects of short-term zinc supplementation on cellular immunity, respiratory symptoms and growth of malnourished Equadorian children. Eur J Clin Nutr 1996; 50:42–6.
19. Fraker P. Zinc in the immune system. J Nutr 2000; 130 Suppl:1399–1406.
20. Harbige LS. Nutrition and immunity with emphasis on infection and autoimmune disease. Nutr Health 1996; 10:285–12.
21. Scrimshaw NS, San Giovanni JP. Synergism of nutrition, infection and immunity: an overview. Am J Clin Nutr 1997; 66 Suppl:464–77:
22. Scott ME, Koski KG. Zinc deficiency impairs immune responses against parasitic nematoda infection at intestinal and systemic sites. J Nutr 2000; 130 Suppl:1412–20.
23. Fraker PJ, Caruso R, Kierszenbaum F. Alteration of the immune and nutritional status of mice by synergy between zinc deficiency and infection with Trypanosoma cruzi. J Nutr 1982; 112:1224–9.
24. Wirth JJ, Fraker PJ, Kierszenbaum F. Zinc requirement for macrophage function: effect of zinc deficiency on uptake and killing of a protozoan parasite. Immunology 1989; 68:114–9.
25. Stolzfus RJ, Albonico M, Chwaya HM, Savioli L, Tielsch JM, Schulze KJ, *et al.* Hemoquant determination of hookworm-related blood loss and its role in iron deficiency in African children. Am J Trop Med Hyg 1996; 55:399–404.
26. Wilson WM, Dufour DL, Staten LK, Narac-Nieto M, Reina JC, Spur GB. Gastrointestinal parasitic infection, anthro-

pometric, nutritional status, and physical work capacity in Colombian boys. Am J Hum Biol 1999; 11:763–71.
27. Stoltzfus RJ, Chwaya HM, Albonico M, Schulze KJ, Savioli L, Tielsch JM. Serum ferritin, erythrocyte protoporphyrin and hemoglobin are valid indicators of iron status of school children in a malaria-holoendemic population. J Nutr 1997; 127:293–8.
28. Mockenhaupt FP, May J, Stark K, Falusi AG, Meyer CG, Bienzle U. Serum transferring receptor levels are increased in asymptomatic and mild Plasmodium falciparum-infection. Haematologica 1999; 84:869–73.
29. Beck HP, Felger I, Vounatsou P, Hirt R, Tanner M, Alonso P, *et al.* Effect of iron supplementation and malaria prophylaxis in infants on Plasmodium falciparum genotypes and multiplicity of infection. Trans R Soc Trop Med 1999; 93 Suppl 1:41–5.
30. Loyevsky M, LaVaute T, Allerson CR, Stearman R, Kassim OO, Cooperman S, *et al.* An IRP-like protein from Plasmodium falciparum binds to a mammalian iron-responsive element. Blood 2001; 98:2555–62.
31. Fritsche G, Larcher C, Schennach H, Weiss G. Regulatory interactions between iron and nitric oxide metabolism for immune defense against Plasmodium falciparum infection. J Inf Dis 2001; 183:1388–94.
32. Spallholz JE, Boylan LM, Larsen HS. Advances in understanding selenium's role in the immune system. Ann NY Acad Sci 1990; 587:123–39.
33. Kiremidjian-Schumacher L, Roy M, Wishe HI, Cohen MW, Stotzky G. Supplementation with selenium and human immune cell functions. Biol Trace Elem Res 1994; 41:115–27.
34. Kiremidjian-Schumacher L, Roy M. Selenium and immune function. Z Ernährungswiss 1998; 37 Suppl 1:50–6.
35. Guimaraes MJ, Peterson D, Vicari V, Cocks BG, Copeland NG, Gilbert DJ, *et al.* Identification of a novel SelD homolog from eukaryotes, bacteria and archea: is there an autoregulatory mechanism in selenocysteine metabolism? Proc Natl Acad Sci USA 1996; 93:15068–91.
36. Behne D, Pfiefer H, Rothlein D, Kyriakopoulos A. Cellular and subcellular distribution of selenium and selenoproteins. In: Roussel AM, Favier A, Anderson RA, editors. Trace elements in man and animals 10: proceedings of the 10th International Symposium on Trace Elements in Man and Animals. New York: Plenum Press, 2000:29–33.
37. Grimble RF. Modification of inflammatory aspects of immune function by nutrients. Nutr Res 1998; 18:1297–317.
38. Levander OA. Selenium and sulfur in antioxidant protective systems: relationships with vitamin E and malaria. Proc Soc Exp Biol Med (NY) 1992; 200:255–9.
39. Gamain B, Arnaud J, Favier A, Camus D, Dive D, Slomianny C. Increase in glutathione peroxidase activity in malaria parasites after selenium supplementation. Free Rad Biol Med 1996; 21:559–65.
40. Davis CD, Brooks L, Calisi C, Bennett BJ, McElroy DM. Beneficial effects of selenium supplementation during murine infection with Trypanosoma cruzi. J Parasitol 1998; 84:1274–7.
41. Gomez RM, Solana ME, Levander OA. Host selenium deficiency increases the severity of chronic inflammatory myopathy in Trypanosoma cruzi-inoculated mice. J Parasitol 2002; 88:541–7.
42. WHO. Chagas'disease. Tropical disease research: 13th program report. UNDP/WB/TDR, World Health Organization, Geneva 1997:112–23.
43. Rossi M, Bestetti RB. The challenge of chagasic cardiomyopathy. Cardiology 1995; 86:1–7.
44. Morgan J, Colley DG, Pinto-Diaz JC, Gontijo ED, Bahia-Oliveira R, Correa-Oliveira R, *et al.* Analysis of anti-Trypanosoma cruzi antibody isotype specificities by Western blot in sera from patients with different forms of Chagas' disease. J Parasitol 1998; 84:641–3.
45. Andrade AL, Zincker F. Chronic malnutrition and Trypanosoma cruzi infection in children. J Trop Pediatr 1995; 41:112–5.
46. Rivera MT, De Souza AP, Hasslocher A, Xavier SS, Gomes JAS, Rocha MOC, *et al.* Progressive Chagas' cardiomyopathy is associated with low selenium levels. Am J Trop Med Hyg 2002; 66:706–12.
47. Bambira EA, da Cruz MQ, Campos DS, Lima AO. Some characteristics of the hyperreactivity to bacterial lipopolysaccharide induced by Trypanosoma cruzi infection. Rio de Janeiro: Memoria Instituto Oswaldo Cruz 1984; 79:433–7.
48. Hunter CA, Slifer T, Araujo F. Interleukin-12-mediated resistance to Trypanosoma cruzi is dependent on tumor necrosis factor alpha and gamma interferon. Infect Immun 1996; 64:2381–6.
49. Jacobs F, Dubois C, Carlier Y, Goldman M. Administration of anti-CD3 monoclonal antibody during experimental Chagas' disease induces CD8+ cell-dependent lethal shock. Clin Exp Immunol 1996; 103:233–8.
50. Tarleton RL. Regulation of immunity in Trypanosoma cruzi infection. Exp Parasitol 1991; 73:106–9.
51. Reina-San-Martin B, Degrave W, Rougeot C, Cosson A, Chamond N, Cordeiro-da-Silva A, *et al.* A B-cell mitogen from a pathogenic trypanosome is a eukaryotic proline racemase. Nat Med 2000; 6:865–6.
52. Camargo MM, Almeida IC, Pereira ME, Ferguson MA, Travassos LR, Gazzinelli RT. Glycosylphosphatidylinositol-anchored mucin-like glycoproteins isolated from Trypanosoma cruzi trypomastigotes initiate the synthesis of proinflammatory cytokines by macrophages. J Immunol 1997; 158:5890–901.
53. Cardillo F, Voltarelli JC, Reed SG, Silva JS. Regulation of Trypanosoma cruzi infection in mice by gamma interferon and interleukin 10: role of NK cells. Infect Immun 1996; 64:128–34.
54. Holscher C, Kohler G, Muller U, Mossman H, Schaub GA, Brombacher F. Defective nitric oxide effector functions lead to extreme susceptibility of Trypanosoma cruzi-infected mice deficient in gamma interferon receptor or inducible nitric oxide synthase. Infect Immun 1998; 66:1208–15.
55. Reed SG, Brownell CE, Russo DM, Silva JS, Grabstein KH, Morrissey PJ. IL-10 mediates susceptibility to Trypanosoma cruzi infection J Immunol 1994; 145:4342–7.
56. Beck MA. Nutritionally induced oxidative stress: effect on viral disease. Am J Clin Nutr 2000; 71 Suppl:1676–9.

Received 11 February 2003, revised 28 May 2003, accepted 28 May 2003

Corresponding author: Dr. Jean Vanderpas, Unité d'épidémiologie, Faculté de Médecine, Faculté Universitaire Notre Dame de la Paix, Namur, Belgium
Fax: +32 65 84 10 41, E-mail: Jean.Vanderpas@skynet.be