Biochemical Association Between Essential Trace Elements and Susceptibility to *Leishmania major* in BALB/c and C57BL/6 Mice

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Several enzymes that contribute to immune system responses require zinc and copper as trace elements for their activity. We examined zinc and copper levels in two susceptible Balb/c mouse lines and resistant C57bl/6 mice infected with *Leishmania major* MRHO/IR/75/ER, a prevalent strain that causes cutaneous leishmaniasis in Iran. Serum Zn and Cu were determined by flame atomic absorption spectrophotometry. Higher Cu levels were found in infected C57bl/6 mice and higher Zn levels were found in infected Balb/c mice. Also, Cu/Zn ratios were increased in both the Balb/c and the C57bl/6 mice. We conclude that concentrations of essential trace elements vary during cutaneous leishmaniasis infection and that this variation is associated with susceptibility/resistance to *Leishmania major* in Balb/c and C57bl/6 mice. We detected Zn deficiency in the plasma of infected Balb/c mice; possibly, therapeutic administration of Zn would be useful for treating this form of leishmaniasis. Increases in Cu level might increase resistance to leishmaniasis. Based on our findings, the Cu/Zn ratio could be a useful marker for the pathophysiology of leishmaniasis.

Key-Words: Balb/c, C57bl/6, CL, Iran, *Leishmania major*, treatment, Cu, Zn.

Leishmaniasis is the name given to a large spectrum of diseases caused by a protozoan parasite of the genus *Leishmania* [1] in mammalian-host macrophages, where they differentiate and multiply as amastigotes [2]. The various *Leishmania* species induce different clinical presentations, ranging from localized infection to disseminated visceral disease [3]. Human cutaneous leishmaniasis (CL), caused by *L. major* [4], is still a major health problem throughout much of the world, including Iran [5]. It is generally accepted that pathogenicity and virulence of CL is dependent on the interaction between the individual genotypes of the parasite and its host [6]. Human CL has been studied by using inbred strains of mice in which the various disease phenotypes resemble the spectrum of clinical manifestations [7]. Experimental infection of Balb/c and C57bl/6 mice by *L. major* constitutes one of the most studied models of parasitic disease [8]. Resistant C57bl/6 mice infected with *L. major* develop a Th1 response with IFN-γ production, macrophage activation, parasite mortality, and resolution of the lesion [9]. In contrast, susceptible Balb/c mice develop a Th2 response and show macrophage deactivation, leading to parasite dissemination and severe progressive disease [10].

Trace metals, including zinc and copper, are directly involved in metabolic processes critical to cell differentiation and replication [11]. Many immunological functions depend on these processes; Cu and Zn are believed to be essential for the functioning of immunocompetent cells [12], and changes in their levels are part of defense strategies of organisms [13], being essential for cell membrane stability [14], apoptosis [15], host metabolism [16] and enzyme activities [17].

Received on 10 August 2008; revised 6 December 2008.
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The Brazilian Journal of Infectious Diseases 2009;13(2):83-85.

Material and Methods

Female inbred Balb/c and C57bl/6 mice (supplied by the Karaj Laboratory Animal unit, Pasteur Institute of Iran) were used in this study and assigned to four groups (n = five mice/group); these include *L. major* infected and uninfected Balb/c mice and *L. major* infected and uninfected C57bl/6 (test) mice. The experiments with animals were approved by the ECPII (Ethical Committee of the Pasteur Institute of Iran). The *L. major* MRHO/IR/75/ER used in this study was the standard strain; the parasites were cultured in RPMI 1640 medium supplemented with fetal bovine serum, L-glutamine and glucose (all from Sigma) [18,19]. Promastigotes were harvested from culture media, counted and used to infect test groups of mice. Serum Cu and Zn were determined by flame atomic absorption spectrophotometry (FAAS, Thermo Jarrel Ash, Germany) [20]. Analyses were made with the Student's *t* test, using Graph Pad Prism Software (Graph Pad, San Diego, CA, USA).

Results

No significant differences in serum Cu were observed among Balb/c groups. However, an increase in serum Cu of infected C57bl/6 mice was observed when compared to control mice of the same strain ($p<0.05$, Figure 1). Zinc deficiency in infected Balb/c mice was observed when compared to control Balb/c mice ($p<0.05$), whereas no significant differences were observed in C57bl/6 mice (Figure 2). The Cu/Zn ratios were similar in the two groups of mice; there was a significant increase in infected Balb/c and C57bl/6 mice ($p<0.01$) (Figure 3).

Figure 1. Copper concentration in control and infected Balb/c and C57bl/6 mice. Serum Cu was determined by flame atomic absorption spectrophotometry (*p<0.05, n = 5 mice/group). Test = infected.

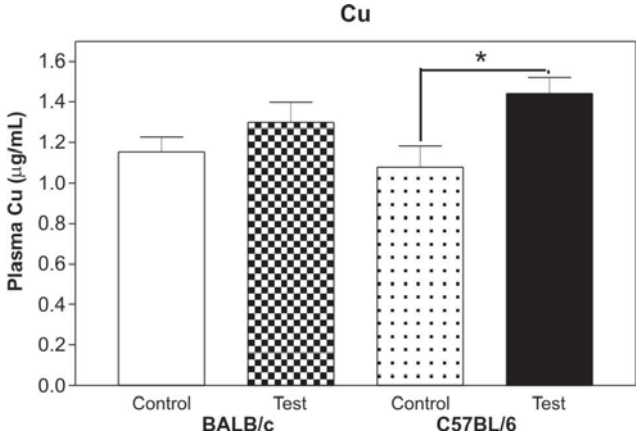


Figure 2. Zinc concentration in control and infected Balb/c and C57bl/6 mice. Serum Zn was determined by flame atomic absorption spectrophotometry (*p<0.05, n = 5 mice/group).

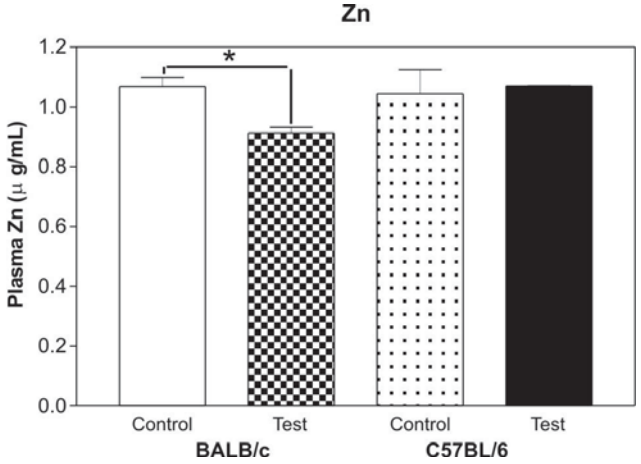
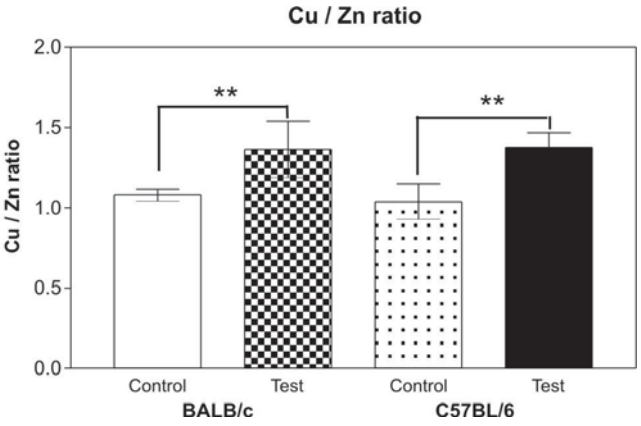


Figure 3. Serum Cu/Zn ratios in control and infected Balb/c and C57bl/6 mice (**p<0.01).



Discussion

We compared serum Cu and Zn levels in resistant C57bl/6 with those of susceptible Balb/c mice, both infected with *L. major*. The principle findings were a higher serum Cu concentration in infected than in control C57bl/6 mice and a lower serum Zn concentration in infected than control Balb/c mice. These changes usually involve increased serum Cu and decreased serum Zn concentrations [21], which normally results in impaired immunological functions [22]. The Zn decline in infected Balb/c mice might be responsible for the inability of the host to clear the parasite and could be attributable to inflammation associated with this disease, resulting from impaired production of various cytokines [23] and enzymes [24]. Zinc supplementation in the diet of human patients has resulted in modest elevation of Zn plasma levels and subsequent improvement of immunological deficiency. In contrast, Sharifi et al. [25] and Khalil et al. [26] reported a weak association of Zn in CL and visceral leishmaniasis in regions of Iran and Sudan respectively. Therefore, high Cu and low Zn values might be a cause, rather than a consequence of CL. It is concluded that serum Zn and Cu concentrations are altered by the leishmania parasites during infection, which should be considered as a parameter for treatment and vaccine strategies.

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