The Effect of BCG-Vaccine upon Experimental Visceral Leishmaniasis in Hamsters*

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ABSTRACT

Stimulation with Bacillus Calmette-Guerin (BCG) vaccine has been reported to enhance resistance of mice against Leishmania donovani infection. Such infection is usually lethal in hamsters, thus providing a more stringent animal model to assess the effect of BCG upon visceral leishmaniasis. Animals received two IP injections (2–8 × 10^7 BCG) pre or post IC challenge with 4 × 10^6 amastigotes. Controls received BCG alone (with no infection) or were untreated (NT). Pretreated animals exhibited significantly fewer (P < 0.05) hepatic or splenic amastigotes than NT animals at days 7, 14, and 28 post challenge, but most BCG treated hamsters died earlier than NT. Post treated hamsters showed no significant reduction in parasite burdens, or in median time to death as compared to NT group. Hamsters which received BCG but were not infected appeared healthy during the study. The reason for increased susceptibility of BCG-treated hamsters to disease is not clear, but observed pathologic complications of L. donovani infected hamsters appear to be exacerbated by BCG stimulation.

Introduction

Visceral leishmaniasis is an often fatal disease of humans caused by the kinetoplastid protozoan parasite Leishmania donovani. It has been found that stimulation with Bacillus Calmette-Guerin (BCG) enhanced resistance of mice against L. donovani. Since the mouse recovers spontaneously from infection from the parasite, the effect of BCG on visceral leishmaniasis in hamsters was investigated. The hamster is highly susceptible to L. donovani, and death of this host is the usual result of infection.6

Materials and Methods

Male random-bred (LVG:LAK) hamsters, 12 weeks old, were infected by intracardial injection of 4.0 × 10^6 amastigote stages of L. donovani (strain 2S) in ground hamster spleen. The infection course was monitored by killing five to
seven animals in each treatment group in chloroform vapor on postinfection days 7, 14, and 28. Spleen and liver parasite burdens were determined from Giemsa-stained slides by Stauber’s method. Briefly, the parasite burden is estimated by multiplying the weight of the organ \( \times 200,000 \times \) the ratio of amastigotes to mononuclear cell nuclei. Additional hamsters in each treatment group were set aside to determine survival time.

Bacillus Calmette-Guerin was obtained as lyophilized preparation, reconstituted with sterile water, and diluted to the desired concentration with Earle’s balanced salt solution immediately prior to injection. Stimulation with BCG consisted of two intraperitoneal injections (each with \( 2-8 \times 10^7 \) BCG) 9 and 28 days before \( L. \) donovani infection (group Pre-BCG) or on days 7 and 16 after infection (group Post-BCG). An additional group of 10 animals received BCG treatment equivalent to group Pre-BCG, but was not infected with \( L. \) donovani.

The Mann-Whitney U test was used for statistical evaluation of results with a probability of \( P < 0.05 \) considered as significant.

**Results**

Animals which received BCG treatment before infection exhibited significantly fewer \( L. \) donovani amastigotes in spleen and liver than untreated controls on days 7, 14, and 28 after infection, but most BCG treated hamsters died earlier (\( P < 0.05 \)) than untreated animals (table I). The earliest hamster death in group Pre-BCG was on day 24, and five animals in that group were dead when the first untreated hamster died (postinfection day 38). Postinfection BCG treatment had no apparent effect on amastigote numbers in organs. The difference in median time to death in group Post-BCG was not statistically significant from untreated animals. Liver and spleen weights were significantly (\( P < 0.05 \)) larger in Pre-BCG groups as compared to untreated animals; thus, the decrease in parasite burden was reflective of a lower parasite density and not a result of smaller organ size. There was no difference between organ weights in Post-BCG and untreated animals. Hamsters which received BCG but no \( L. \) donovani infection exhibited no adverse reaction and appeared healthy during the time course of the study.

**Discussion**

Previous studies of BCG effects on leishmaniasis have produced conflicting results. Grimaldi et al found no beneficial effect of intraperitoneal BCG treatment on established \( L. \) mexicana infection in mice, but intravenous BCG treatment before infection significantly enhanced resistance against \( L. \) tropica as measured by decreased severity of cutaneous lesions, reduced mortality, and failure of parasites to visceralize in highly susceptible BALB/C mice. Intravenous

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BCG stimulation prior to *L. donovani* infection also conferred protection in mice.\(^4\) That treatment resulted in decreased parasite burdens in both spleen and liver, while postinfection BCG treatment decreased splenic amastigote numbers without apparent effect on the liver parasite burden. It was also noted that treatment was less effective when the intraperitoneal route was used for BCG stimulation. Treatment of mice with cortisone or cyclophosphamide partially abrogated the protective effect of BCG.\(^5\)

The importance of route of administration has been noted as an explanation for contrasting results in studies of BCG effects on *Trypanosoma cruzi* infection in mice. Intravenous administration conferred *in vivo* resistance,\(^1\) while intraperitoneal BCG stimulation did not.\(^3\) Intraperitoneal BCG stimulation before or after intravascular *L. donovani* infection was the design used in the present study. While a study of intravascular BCG administration in hamsters may be useful, it should be noted that intraperitoneal stimulation did produce discernible, although divergent, effects on *L. donovani* infection. Lower parasite burdens were seen in hamsters which received BCG treatment prior to infection, but animals succumbed to infection earlier than untreated controls. Stimulation with BCG after *L. donovani* infection was established in hamsters had no apparent therapeutic effect.

The reason for increased susceptibility of BCG-treated hamsters to disease (i.e., earlier death than untreated, *L. donovani*-infected animals), despite exhibiting reduced parasite burdens in organs, is not clear. Stauber emphasized the extreme degree of susceptibility of hamsters to visceral leishmaniasis.\(^6\) Pathogenic complications associated with ultimate death of the *L. donovani*-infected hamster are not completely defined but, based on the present result, appear to be exacerbated by BCG stimulation.

A role for immunological enhancement by BCG has long been recognized, but evidence also points to BCG-induced immune suppression under certain conditions.\(^7\) Possible immune suppression may have contributed to our divergent results; however, because BCG stimulation without *L. donovani* infection produced no discernible adverse effect in hamsters, it would appear that other factors may also be involved. Clearly, the immune mechanisms induced by BCG and other immunomodulating agents are complex and deserve further study, especially with regard to specific or non-specific immunotherapy in infectious or neoplastic diseases.

References