Experimental visceral leishmaniasis in high and low antibody - producer mice (selection IV-A)

Leishmaniose visceral experimental em camundongos bons e maus produtores de anticorpos

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Abstract *Leishmaniasis is a typical parasite infection whose protective immunity depends on macrophage activation. Susceptibility to* **Leishmania donovani** *infection was compared in H (high antibody responder) and L (low antibody responder) mice from selection IV-A. H mice infected intravenously with 10^7 amastigotes of* **L. donovani** *were more susceptible to infection than their L counterparts. This higher susceptibility was characterized by a higher splenic and hepatic parasite burden. An increased splenic index was observed in both lines after sixty days of infection. This splenomegaly was caused, at least partially, by an increase in the number of splenic cells as determined by direct counts of cells from spleen. The results show that selection IV-A is susceptible to visceral leishmaniasis, with the H line being more susceptible than the L line.*

Key-words: Leishmania donovani. *Biozzi mice.*

Resumo *A leishmaniose é uma infecção parasitária cuja imunidade protetora envolve a ativação de macrófagos. Neste trabalho avaliamos a susceptibilidade de camundongos H e L (bons e maus produtores de anticorpos, respectivamente) da seleção IV-A, à infecção com o protozoário* **L. donovani**. *Camundongos H infectados com 10^7 amastigotas por via intravenosa foram mais suscetíveis, apresentando maior carga parasitária tanto no fígado quanto no baço. Após 60 dias de infecção ambas as linhagens apresentaram um aumento no índice esplênico. Esta esplenomegalia foi conseqüência, pelo menos parcialmente, de um aumento no número de células esplênicas. Os resultados indicam que a seleção IV-A é susceptível à infecção com* **L. donovani** *e que dentro desta seleção a linhagem H apresenta maior suscetibilidade do que a linhagem L.*

Palavras-chaves: Leishmania donovani. *Camundongos Biozzi.*

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Leishmaniae are obligate intracellular protozoan parasites of macrophages that cause a spectrum of human diseases, including self-healing skin lesions, diffuse cutaneous and mucosal manifestations, or severe visceral diseases. Important immunological features of visceral leishmaniasis include circulating immune complexes[12], polyclonal B-cell activation with elevated IgM and IgG levels[15], immunosuppression and absence of detectable cell-mediated immunity during acute infection[13].

High (H) and low (L) responder lines of mice provide a useful model to investigate the contribution of humoral and cellular immunity to immune phenomena. H and Lantibody responder lines of mice were produced by bidirectional selective breeding of high and low responder lines of mice to natural multideterminant immunogens administered at optimal doses. The different antigens used were sheep erythrocytes (SE) and pigeon erythrocytes (Selection I), SE only (Selection II), flagellar and somatic antigens of Salmonellae (Selections III and IV respectively), bovine serum albumin, and rabbit gamma globulin (Selection V)[3]. Selection IV-A was derived from F2 interline segregants between HIV and LIV lines in response to SE[9]. This approach has permitted to establish that quantitative antibody production is a polygenic trait regulated by a group of about 10 independent loci[2] [14]. The alleles separated in each line by selective breeding have a nonspecific effect, operating on the quantitative antibody response to many unrelated immunogens[3]. Fundamental immunological differences of H and L lines have been described. The level of serum antibody is markedly higher in H than L lines and T cell-mediated immunity is of similar intensity in both lines[1] [8]. It was also demonstrated that humoral and cell-mediated responses to the same antigens are subjected to independent polygenic regulation[21]. The importance of these immunogenetic characteristics for the general strategy of anti-infectious immunity has been hypothesized[5]. More recently, differences in T cell proliferative responses and cytokine production by H and L lines have been characterized[10] [22].

The resistance of H and L lines to various bacterial and parasitic infections has been studied. These studies demonstrated that H mice are more resistant than L mice to infections in which antibodies play a major protective role[17] [20]. In contrast, L mice are more resistant to infections caused by intracellular pathogens[11]. Low antibody responses in Lmice have been attributed to higher catabolism of immunogens in the macrophages and consequent decreased antigen presentation by these cells. These macrophage catabolic differences have been previously demonstrated in selections I, II and IV-A[16].

Considering that *L. donovani* is an intracellular parasite and that the macrophage function is altered in selection IV-A, the purpose of this study was to evaluate the susceptibility of H and L mice from selection IV-A to infection with *L. donovani*.

MATERIALAND METHODS

Animals. Twenty-nine H male mice and 29 L male mice from selection IV-Aaged 8-10 weeks, developed by the Immunology Section of the Biological Institute of São Paulo and maintained at the Animal Facility of the Department of Microbiology and Immunology, Institute of Biosciences, UNESP, Botucatu, were used.

Parasites. L. donovani strain 1S was maintained *in vivo* by serial passage in golden hamsters. Amastigotes for inoculation into mice were obtained from the spleens of chronically infected hamsters, washed three times in phosphate buffered saline (PBS, pH 7.2) and adjusted to a concentration of 5×10^7 amastigotes/ml.

Infection. Thirty eight mice (19 H and 19 L) were infected by the intravenous route with 10^7 amastigotes. Groups of 8 (4 H and 4 L) infected animals were sacrificed at 15, 20, 30 and 60 days postinoculation and their spleens and livers collected to evaluate the different parameters. Six mice (3 H and 3 L) were sacrificed 24 hours after inoculation to establish the acute parasite population growth rate in the liver. Twenty non-infected mice (10 H and 10 L) were used as normal controls.

Hepatic and splenic parasite burden. Impression smears from livers and spleens were stained with Giemsa to evaluate parasite burden. The number of amastigotes per host cell nucleus was determined by counting 1000 host cells as previously described[6]. The relative and total numbers of parasites per organ, named Leishman-Donovan Units (LDU) and total Leishman-Donovan Units (total LDU). respectively, were calculated according to the formula:

$$LDU = \frac{\text{number of parasites}}{1000 \text{ host cell nucleus}}$$

Total LDU = LDU x organ weight (mg) x 2.10^5

Splenic index. This index was determined according to the formula:

$$\text{Splenic index} = \frac{\text{spleen weight}}{\text{whole body weight}} \times 100$$

Splenic cell number. Spleens from normal and infected mice were removed and cell suspensions prepared by teasing the material through a stainless steel sieve in PBS. The total number of cells was counted in a Neubauer chamber.

Statistical analysis. The Kruskall-Wallis non-parametric test was performed to determine the statistical significance of the data (p < 0.05 was considered significant).

RESULTS

Acute growth rate of L. donovani in H and L mice from selection IV-A. Three and four mice from the H and L lines were killed after 1 and 15 days of infection, respectively. Liver parasite counts on the first day showed no difference between H and L mice, with 138 LDU being the mean count for both lines. After 15 days of infection the mean parasite burden was 1803 and 1500 LDU for H and Lmice, respectively. These results, regardless of the later course of infection, characterized H and L mice from selection IV-A as intermediate in terms of acute susceptibility when compared with inbred lines of mice experimentally infected with *L. donovani*[6].

Splenic and hepatic parasite burden. To establish the extent of parasitism in the liver and spleen, imprints from these organs were microscopically assessed after 15, 20, 30 and 60 days of infection. As can be observed in Figure 1a for the liver and 1b for the spleen, the parasitic burden was always higher in the H than in the L line. However, the difference between H and L was statistically significant only after 20 and 30 days of infection. After 60 days of infection, the last period evaluated, the parasitic burden was similar in liver and spleen of both lines.

Splenic index. The splenic index in normal H mice was higher than in normal L mice. This difference was preserved in infected animals and was statistically significant during all periods analyzed (Figure 2a). When evaluated in each
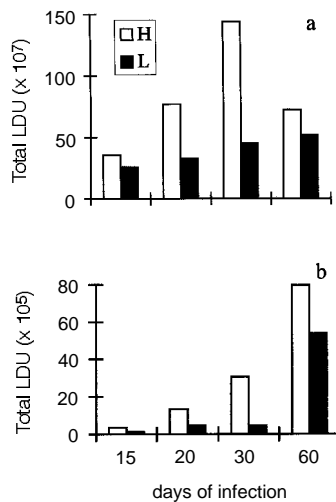

Figure 1 - Parasite burden in mice from selection IV-A infected with **L. donovani**. Giemsa-stained impression smears from liver (a) and spleen (b) were microscopically assessed. Results represent the median for four animals. Statistical analysis (liver): 15 days (d): H = L; 20d: H > L; 30d: H > L; 60d: H = L; (spleen): 15d: H = L; 20d: H > L; 30d: H > L; 60d: H = L

line, the splenic index increased significantly at 30 and 60 days of infection. In the L line the increase in the splenic index was significant only at 60 days of infection.

Quantitation of splenic cells. Spleens from normal and infected H and Lmice were removed and cell suspensions prepared and counted in a Neubauer chamber. The results are presented in Figure 2b. Control H mice had a significantly higher splenic cell number than control L mice and this difference was unchanged during the first 30 days of infection. Also during this period
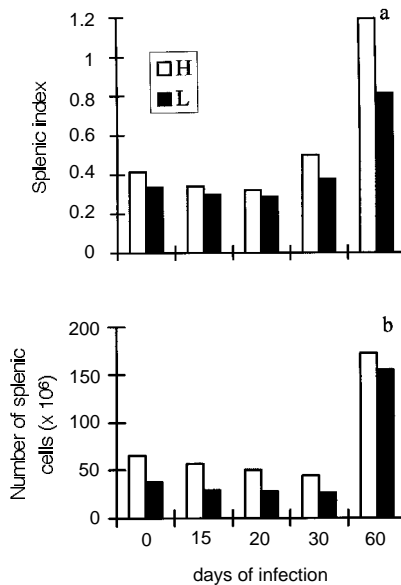

Figure 2 - Splenic index (a) and number of splenic cells (b) in mice from selection IV-A infected with L. donovani. *Results represent the median for four animals. Statistical analysis (splenic index): normal mice: H > L; infected mice: 15 days (d): H > L; 20d: H > L; 30d: H > L; 60d: H > L; (number of splenic cells): normal mice: H > L; infected mice: 15d: H > L; 20d: H > L; 30d: H > L; 60d: H = L*

there was no alteration in total splenic cell number. However, both lines showed a striking increase in total splenic cell number after 60 days of infection. This increase was statistically significant in relation to any of the other periods analyzed in the same line.

DISCUSSION

In this work we used high (H) and low (L) responder lines of mice from selection IV-A as an experimental model for visceral leishmaniasis.

The H mice presented a higher parasite burden in spleen and liver compared to their L counterparts. This result is similar to those obtained when the lines H and L were used as experimental models for other intracellular parasites such as *Leishmania tropica*[4] and *Salmonella typhimurium*[24] and *Brucella suis*[11]. In our model we observed that after 20 and 30 days H mice were more susceptible than L mice since H mice showed a significantly higher number of parasites both in spleen and liver. However, after 60 days of infection the parasite burden

was equivalent in H and Lmice. In addition, after 15 days of infection, the parasite burden in the liver, which has been used as an index to evaluate acute susceptibility in experimental murine leishmaniasis[6], was identical in H and L mice. It is possible that this difference observed on the 20th and 30th day of infection was caused by a delay in developing a specific immune response able to partially restrain the parasite growth in H mice. This possibility finds some support in the fact that macrophages from the H line have a lower metabolic and bactericidal activity[16]. This may consequently delay the ability of these macrophages to present antigens to Th cells or to kill the intracellular leishmania amastigotes.

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Another parameter evaluated was the splenic index. Interestingly, normal H mice already presented a significantly higher splenic index compared to normal L mice and this difference was maintained during infection. In each line there was also an increase in the splenic index from the 30th day of infection on. This increase was highly significant after 60 days of infection. Splenic hipertrophy is a classical description both in human and experimental kala-azar[18] and has been attributed to both monocyte recruitment[23] and polyclonal activation[7]. In our study we found a very pronounced increase in the splenic cell number at 60 days of infection in both mouse lines. This result shows that both lines from the IV-A selection, experimentally infected with *L. donovani*, reproduce splenomegaly, which is the hallmark for visceral leishmaniasis.

In view of the significant difference between the H and L lines from selection IV-A in terms of parasite burden after 20 and 30 days of infection, the distinct immunological characteristics already described for these two lines and the fact that they represent extreme phenotypes found in a natural heterogeneous (outbred) population, we believe that this model could be used to study the macrophage-*Leishmania* interaction and the kinetics of the specific immune response to this parasite.

ACKNOWLEDGMENTS

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