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Effect of Visceral Leishmaniasis on Congenitally Athymic Mice

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Congenitally athymic mice were more susceptible to challenge with amastigotes of *Leishmania donovani* than were their thymus-intact littermates. This increased susceptibility correlated with a lack of Arthus and delayed-type responses when animals were skin tested with leishmanial antigen.

Cell-mediated immunity is important in protection against infections with a variety of pathogenic microorganisms, such as *Mycobacteria* (8), *Listeria* (10), *Brucella* (6), and *Leishmania* (7). Since the immunological role of the thymus-derived lymphocytes (T-cells) in visceral leishmaniasis is poorly understood, it was of interest to study the course of this disease in congenitally athymic mice.

Congenitally athymic (*nu/nu*) mice and littermates (*nu/+* or +/+), 4 weeks old, were used in all experiments. Athymic (nude) mice were maintained in isolation and received sterilized food, water, and bedding. Experimental manipulations were performed under aseptic conditions. Ten *nu/nu* mice were divided into two groups of five mice each. Mice in group A (Table 1) were inoculated intravenously with 2.0×10^6 amastigotes of *Leishmania donovani* prepared from infected hamster spleen tissue as described by Stauber (16). Briefly, the spleen from an infected animal was aseptically removed and weighed, and the total parasite burden was determined. The spleen was suspended in sterile phosphate-buffered saline, pH 7.2, and ground in a Ten Broeck tissue grinder. The suspension was filtered through three sterile gauze pads to remove remaining spleen tissue. The filtrate containing the amastigotes was used to infect mice. Contaminating spleen tissue was further separated from amastigotes by differential centrifugation. Amastigotes used for preparation of skin test antigen (13) were prepared in a similar manner. After separation from spleen tissue, the parasites were pelleted ($3,000 \times g$, 35 min), and the pellet was resuspended in 0.5% phenol saline (10^9 amastigotes per ml). An equal weight of normal hamster spleen tissue was similarly treated and used as control antigen. Both antigen preparations were lyophilized, stored at −4°C, and suspended in sterile saline for use in skin testing (0.5 mg/0.05 ml).

Mice in group B were similarly treated with sterile phosphate-buffered saline (pH 7.2). Age- and sex-matched littermates (+/*nu* or +/+) which possessed intact and functional thymus glands were similarly divided and treated (groups C and D). At 24 h before necropsy all mice were skin tested (footpad) with leishmanial antigen. Differences in footpad thickness (right and left) were read at 4 and 24 h. At 18 days postinfection all mice were necropsied, and the estimated total parasite burdens of the spleen and liver (Fig. 1) were determined (16). In addition, the footpads of these mice were excised, fixed, embedded in paraffin, sectioned, and stained. These tissue sections were examined with light microscopy for the presence or absence of mononuclear cells, which are characteristic of delayed-type responses.

Table 1 shows the Arthus and delayed-type skin reactions of congenitally athymic nude mice and their littermates after challenge with amastigotes. Athymic mice inoculated with amastigotes did not exhibit an Arthus or delayed-type reaction when skin tested with leishmanial antigen. However, all of the thymic littermates did respond with a positive Arthus reaction, and four of five mice had a positive delayed-type reaction. Control mice of both types that were not infected (groups B and D) were skin test negative for both types of reactions.

Figure 1 shows that athymic mice inoculated with amastigotes had significantly higher spleen parasite burdens (*t* test, $P < 0.01$) compared with thymic littermates, although there was no significant difference in the liver parasite burdens between the two groups.

These data suggest several interesting concepts. The immune response of congenitally athymic mice to visceral leishmaniasis is different from that observed in their thymic littermates. In the absence of functional T-cells the

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TABLE 1. *Hypersensitivity in congenitally athymic mice and their littermates after intravenous inoculation of 2.0 × 10⁶ amastigotes of L. donovani*

Group	Type of mice	No. of mice	Treatment	No. of mice with positive reactions after skin test[a]	
				Arthus (4 h)	Delayed-type (24 h)
A	Nude	5	Amastigotes on day 0	0	0
B	Nude	5	0.2 ml of sterile phosphate-buffered saline on day 0	0	0
C	Thymic littermates	5	Amastigotes on day 0	5	4
D	Thymic littermates	5	0.2 ml of sterile phosphate-buffered saline on day 0	0	0

[a] All mice were skin tested by injection of 500 μg of leishmanial antigen in the right hind footpad and a similar amount of normal hamster spleen tissue in the left hind footpad. Differences in footpad thickness at 4 and 24 h was read by using a Schnell tester. A difference in footpad thickness of 0.4 and 0.2 mm was considered positive Arthus and delayed-type reactions, respectively. In addition, the delayed-type response was confirmed by histological examination.

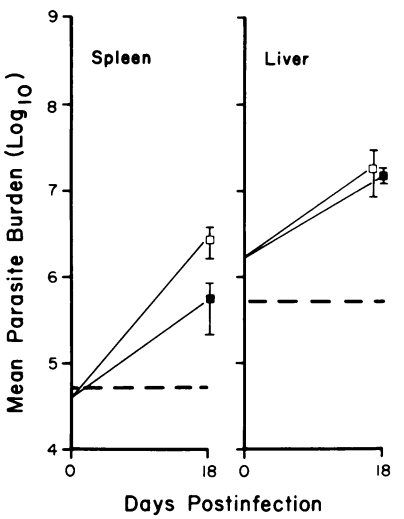

FIG. 1. *Mean spleen and liver parasite burdens of congenitally athymic nude mice (nu/nu)* (□) *and thymic littermates (+/nu or +/+)* (■) *inoculated intravenously with 2.0 × 10⁶ amastigotes of L. donovani on day 0. The dashed lines indicate patency level.*

host is apparently unable to develop a humoral or cellular response to leishmanial antigen. As a consequence, the infection in the spleen progresses unabated. The role of antibody in host defense against visceral leishmaniasis has yet to be established, but the observation that positive Arthus and delayed-type reactions were observed only in thymis littermates suggests that the infection does involve T-dependent antigen recognition. In the light of previous findings (15) these data further suggest a causal relationship between a positive delayed-type response to leishmanial antigen and control of splenic parasite numbers. A lack of a positive correlation between the presence or absence of a thymus and control of liver parasites may be due to the short duration of the study period or possible sequestering of the parasites in this target organ. Of course, one must also consider that the liver is not a classic immunocompetent organ, i.e., does not possess germinal centers or thymus-dependent regions for lymphocyte maturation and production. We previously reported (15) that some mice infected with *L. donovani* for long periods of time would undergo thymic atrophy, resulting in a loss of their delayed-type response to leishmanial antigen, leaving intact their Arthus-type response. The course of the disease in these mice included very high parasite burdens of both the liver and spleen, eventual wasting, and death. In other studies (Smrkovski, Larson, and Reed, Am. J. Trop. Med. Hyg., in press) using drug-induced immunosuppression, we found similar evidence of a causal relationship between T-cell responsiveness and control of *L. donovani* infections. In addition, we have previously shown that BCG will drastically alter the course of visceral leishmaniasis in BALB/c mice (14). The importance of thymus-derived lymphocytes in other types of leishmanial infections in mice (11, 12), guinea pigs (1, 2), and humans (4, 5, 9) has also been reported. In contrast, congenitally athymic mice infected with *Trypanosoma rhodensiense* (3) or *Plasmodium berghei* (17) showed increased survival time over their thymic littermates, indicating that at least in these models thymic lymphocyte function may be abbrogated by intrinsic factors which have yet to be defined.

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