T-Cell Responses to Immunodominant LACK Antigen Do Not Play a Critical Role in Determining Susceptibility of BALB/c Mice to Leishmania mexicana

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Although BALB/c mice develop lesions when infected with Leishmania mexicana, the mechanisms which are responsible for susceptibility to this parasite have not been elucidated. In contrast, susceptibility of BALB/c mice to Leishmania major has been shown to depend on the early production of interleukin-4 (IL-4) by T cells which react to the parasitic LACK antigen. Here, we demonstrate that the lesions induced by L. mexicana are delayed compared to those induced by L. major but rapidly develop at later time points. Interestingly, while LACK-tolerant BALB/c-derived IE-LACK transgenic mice were resistant to L. major, they were susceptible to L. mexicana and developed lesions similar to those observed in wild-type BALB/c mice. The latter result was observed despite the fact that (i) LACK was expressed by L. mexicana, (ii) splenocytes from BALB/c mice were able to stimulate LACK-specific T-cell hybridoma cells when incubated with live L. mexicana promastigotes, and (iii) LACK-specific T cells contributed to IL-4 production in L. mexicana-infected BALB/c mice. Thus, in contrast to what was observed for L. major-infected mice, LACK-specific T cells do not play a critical role in determining susceptibility to L. mexicana. Although BALB/c mice are susceptible to both L. major and L. mexicana, the mechanisms which are responsible for susceptibility to these parasites are likely to be different.

Mice have been widely used as an animal model to study human leishmaniasis. In many cases, the outcome of the disease is different depending on the strains which are infected. In the case of mice infected with Leishmania major, it has been shown that CBA, C3H, C57BL/6, and B10.D2 mice develop cutaneous lesions that spontaneously heal, while BALB/c, SWR/J, DBA/2, and A/Jax mice develop nonhealing cutaneous lesions which are susceptible to dissemination (4, 20). Several studies have shown that the early production of interleukin-4 (IL-4), which is conserved among different mammalian receptors for activated C kinase [RACK], a protein determinant derived from the antigen LACK (leishmania receptor for activated C kinase, for its homology with the mammalian receptors for activated C kinase [RACK]), a protein which is conserved among different Leishmania spp. and which accounts for 0.03% of the proteins expressed by Leishmania promastigotes or amastigotes (14, 17, 18). The critical role of the LACK-specific T cells in determining susceptibility to L. major is further demonstrated by the observation that the susceptibility of BALB/c mice which have been made tolerant to LACK are resistant to infection (9, 11).

Although studies have shown that IL-4 is critical for susceptibility to Leishmania mexicana (23–25), it is not known whether LACK-specific T cells play a critical role in the animal model for this infection. To address this issue, we have compared the courses of L. mexicana and L. major infections in wild-type (WT) BALB/c mice and in BALB/c-derived IE-LACK transgenic mice which are tolerant to LACK as the result of its constitutive expression in the thymus. In contrast to what was observed for L. major-infected mice, we found that LACK-specific T cells do not seem to play a critical role in determining the susceptibility of BALB/c mice to L. mexicana infection.

BALB/c male mice (8 to 12 weeks old) were purchased from Banting & Kingman Universal Limited (Hull, United Kingdom). IE-LACK transgenic mice have been previously described (9). L. mexicana (strain MHOM/BZ/82/BEL21) and L. major (strain WHO/IR/−173) promastigotes were grown in RPMI medium (Life Technologies, Merelbeke, Belgium) supplemented with 10% fetal calf serum (Life Technologies), penicillin G (100 U/ml), and streptomycin (100 μg/ml). Promastigote lysates were prepared by 10 cycles of freezing (in liquid nitrogen) and thawing (in a water bath at 37°C), and their endotoxin contents were lower than 0.20 pg/106 promastigotes as determined by the Limulus amebocyte lysate-Coatest endotoxin assay (Chromogenix, Mölndal, Sweden). The LACK-derived peptide, previously identified as an immunodominant epitope in BALB/c mice (amino acids 161 to 172, of sequence SLEHPIVVSGSW) (17), was obtained from Chiron Mimotopes (Paris, France) and from P. Gourlet (Laboratory of Bio-
chemistry, Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium). The LACK-specific T-cell hybridoma LMR16.2, producing IL-2 upon recognition of the immunodominant L. major LACK epitope in an I-A^d major histocompatibility complex class II-restricted fashion, was used as previously described (17). IL-2 and IL-4 levels were determined by enzyme-linked immunosorbent assays using commercially available kits (duoset from Genzyme Diagnostics, Cambridge, Mass.). The limit of detection was 5 pg/ml for both cytokines.

Diseases induced by L. mexicana and L. major in BALB/c mice. Although BALB/c mice are known to be susceptible to both L. major and L. mexicana (1), the diseases induced by these two species have not been compared under the same experimental conditions. In our study, L. mexicana or L. major promastigotes were injected into the left footpad of BALB/c mice. Each mouse received 10^7 stationary-phase promastigotes in a final volume of 25 μl of RPMI medium. The contralateral right footpad received an identical volume of RPMI medium, without parasites, as an internal control. Footpad thicknesses were measured with a metric caliper, and the difference between both measurements corresponded to lesion size. As shown in Fig. 1, while L. mexicana induced slowly progressive primary lesions which reached 10 to 11 mm after 34 weeks, L. major induced the rapid development of lesions which were 4 to 5 mm thick at 4 weeks postinfection (wpi). The cutaneous lesions induced by L. major became ulcerated more frequently and earlier than those induced by L. mexicana. Likewise, L. major, but not L. mexicana, induced spontaneous losses (mutilations) of necrotic footpad lesions which could not be measured after 14 weeks (Fig. 2A). While both L. mexicana and L. major induced secondary lesions which appeared in the contralateral posterior or anterior footpads, the eyelids, the nostrils, the ears, and the base of the tail, these lesions developed later in mice infected with L. mexicana than in those infected with L. major (Fig. 2B). Thus, while BALB/c mice were susceptible to both L. major and L. mexicana infections, the courses of disease induced by these two parasites were markedly different.

Stimulation of a LACK-specific T-cell hybridoma by BALB/c splenocytes incubated with L. mexicana live promastigotes. To determine if antigen-presenting cells could process the L. mexicana LACK antigen and present it to T cells, splenocytes from BALB/c mice were incubated in 96-well plates (Nunc, Roskilde, Denmark) with the LACK-specific T-cell hybridoma LMR16.2 and L. mexicana or L. major live or lysed promastigotes. In some wells, splenocytes (5 × 10^5/well) were incubated with either live L. mexicana or L. major promastigotes for 6 h at 37°C. Other splenocyte-containing wells received L. mexicana or L. major lysate (at the corresponding parasite/splenocyte ratios used for live parasites) or the LACK-derived peptide (at concentrations ranging from 0.2 to 25.6 μM). The LMR16.2 hybridoma cells (10^5/well) were added to each well. Twenty hours later, cellular supernatants were harvested for subsequent measurement of IL-2 levels.

LACK peptide (data not shown) and both L. major and L. mexicana promastigotes (Fig. 3) induced the LMR16.2 hybridoma cells to secrete IL-2 in a dose-dependent manner. The fact that splenocytes incubated with L. mexicana promastigotes could stimulate the LMR16.2 T-cell hybridoma recognizing specifically the L. major LACK antigen suggested that the

FIG. 1. Sizes of primary footpad lesions of BALB/c mice infected with L. mexicana or L. major. Results are from one representative experiment performed with 25 (L. mexicana) and 15 (L. major) animals and are expressed as means ± SEM. **, P < 0.001 (Student’s t test, comparing L. mexicana and L. major infections). Three other experiments using 15, 16, and 12 animals infected with L. mexicana displayed similar results. This was also the case for another experiment including 10 mice infected with L. major.

FIG. 2. Frequencies of ulcerated cutaneous primary lesions and mutilations of footpads (A) and cutaneous secondary lesions (B) during the course of L. mexicana and L. major infections in BALB/c mice. Results are from the same experiment described for Fig. 1. nd, not determined.
antigenic determinant which was recognized by these LACK-
specific cells was conserved between the L. mexicana and L. major LACK proteins.

Interestingly, while the L. mexicana and L. major lysed promastigotes did not differ in the ability to stimulate LMR16.2 hybridoma cells (Fig. 3B). L. mexicana live promastigotes induced less IL-2 than L. major live promastigotes (Fig. 3A).

Though slight, such differences were significant and might be related to differences in the proportion of metacyclic-like promastigotes (the parasitic form infecting cells) within the L. major and L. mexicana stationary-phase populations (22) or to differences in the intracellular development of both parasites, interfering with antigen presentation (19). Indeed, the tight binding of L. mexicana amastigotes to the membrane vacuole of infected macrophages, which is not seen with L. major amastigotes (2), and the sequestration of major histocompatibility complex class II molecules at this point of the membrane (rendering difficult their egress from the parasitophorous vac-
A (8 μg/well; Sigma) or L. mexicana extracts (10^6 lysed parasites/well). Supernatants of unstimulated or stimulated cells were collected for subsequent determination of IL-4 levels. Concanavalin A stimulated splenocytes to produce IL-4 (100 to 390 pg/ml) at each time point for both mouse groups. Neither splenocytes from naive animals nor those from mice which had been infected 2 or 6 weeks earlier produced IL-4 in response to L. mexicana lysates. This agrees with previous studies which have shown that IL-4 transcripts are undetectable in the LNs and the spleens of L. mexicana-infected mice 1 and 7 days after infection (7). In contrast, when mice were analyzed 14 weeks after infection, splenocytes from both IE-LACK transgenic and WT BALB/c mice secreted IL-4 in response to parasite extracts. However, splenocytes from IE-LACK transgenic mice secreted significantly less IL-4 than those from WT BALB/c mice. (Means ± standard errors of the means [SEM] for stimulated cells were as follows: IE-LACK mice, 56 ± 9 pg/ml; WT mice, 179 ± 65 pg/ml [P < 0.05 by Student’s t test, n = 3]. The results for unstimulated cells were as follows: IE-LACK mice, 11 ± 8 pg/ml; WT mice, 29 ± 15 pg/ml.) Such a difference suggests that LACK-specific T cells contributed to the production of IL-4 in L. mexicana-infected WT mice.

In conclusion, despite BALB/c splenocytes being able to stimulate LACK-specific T cells, which contribute to the production of IL-4 in L. mexicana-infected mice, and despite the fact that IL-4 was shown to be required for susceptibility to L. mexicana (23), IE-LACK transgenic mice tolerant of LACK antigen were as susceptible as WT BALB/c mice to L. mexicana infection. This indicates that LACK-specific T cells do not play a critical role in determining susceptibility to L. mexicana. This result is in striking contrast to those obtained in the case of BALB/c mice infected with L. major. It is tempting to hypothesize that such a difference preventing the development of an early counterprotective antiparasite Th2 response contributes to the slower development of lesions in L. mexicana-infected animals. It also suggests that the mechanisms which determine susceptibility to these two species are markedly different.

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REFERENCES


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<th>Group</th>
<th>Parasitic load* (10^6/mg) (mean ± SEM)</th>
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<th>Secondary lesions* (no. present/total) (%)</th>
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<tr>
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* Determinations of parasitic loads at each time point were performed for two or three animals infected at the same time as those from the experiment described for Fig. 4.
* Data on ulcerations and secondary lesions were derived from the mice from the experiment described for Fig. 4.
* No statistical differences were seen between IE-LACK transgenic and WT BALB/c mice.

FIG. 4. Sizes of primary footpad lesions during the course of L. mexicana (A) or L. major (B) infection in IE-LACK (LACK-Tg-BALB/c) and WT BALB/c mice. Results are expressed as means ± SEM. Twelve male mice per group were inoculated. **, P < 0.001 (Student’s t test, comparing IE-LACK and WT animals).


