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Immune Responses Associated with Susceptibility of C57BL/10 Mice to *Leishmania amazonensis*

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Leishmaniae are protozoans which, depending upon both the host and parasite species, can cause either a healing or nonhealing infection. While C57BL/10 mice are able to heal following infection with *Leishmania major*, they fail to heal following infection with *Leishmania amazonensis*. In order to address the role of Th1 and Th2 cell responses in the outcome of these infections in C57BL/10 mice, gamma interferon (IFN-γ) and interleukin-4 (IL-4) production was assessed. While cells from *L. major*-infected C57BL/10 mice produced high levels of IFN-γ, cells from *L. amazonensis*-infected animals produced little or no IFN-γ. On the other hand, IL-4 was produced only by cells from *L. amazonensis*-infected C57BL/10 mice, but this production was restricted to the first few weeks of infection. Later in infection, when lesions were evident, no IL-4 was detected. Treatment of BALB/c mice with a monoclonal antibody (11B11) directed against IL-4 induced a dramatic reduction in *L. amazonensis* lesions. This reduction was associated with a decrease in IL-4 levels and an increase in IFN-γ production. However, only a slight reduction in lesion sizes and parasite numbers was observed when anti-IL-4-treated C57BL/10 mice were infected with *L. amazonensis*. These results suggest that IL-4 may have an important role in mediating susceptibility to *L. amazonensis* in BALB/c mice, as previously demonstrated for *L. major*. More importantly, however, the data suggest that susceptibility to *L. amazonensis* in C57BL/10 mice is due to the absence of a Th1 cell response, rather than to the presence of a Th2 cell response.

Leishmaniasis encompasses a large spectrum of clinical syndromes which, depending upon the parasite species and the host immune response, can have various outcomes. These clinical syndromes include single cutaneous lesions, which may or may not spontaneously heal, and more severe cases associated with metastasis to other cutaneous or mucocutaneous sites, as well as visceralization of the parasites leading to a fatal infection if not properly treated. Our understanding of the factors that lead to this diversity of clinical symptoms has, in large part, come from studies using the mouse model. As in humans, murine cutaneous leishmaniasis can also have distinct outcomes, depending on both the mouse strain and the parasite (19, 31). Some animals develop a small lesion which, after a variable length of time, heals, leaving few to no parasites at the site of infection. Other mouse strains show progressive growth of lesions that eventually leads to death of the animals from generalized metastasis. Between these two extremes, some mouse strains develop only small lesions and although the lesions have elevated parasite numbers, infection is not fatal. For example, BALB/c mice develop uncontrolled disease when infected with either *Leishmania major* or *Leishmania amazonensis*, while *Leishmania braziliensis* and *Leishmania panamensis* induce only negligible lesions in any mouse strain (10, 27). Other mice show various levels of susceptibility, depending on the parasite species. C3H/HeN mice are able to heal following *L. major* infection but develop chronic disease when infected with *Leishmania mexicana* (17). Similarly, C57BL/6 and C57BL/10 mice heal following infection with *L. major* but when infected with *L. amazonensis*, fail to heal and C57BL/6 mice develop metastatic lesions (3).

It is now well established that one of the main factors controlling murine *L. major* infections is the nature of the T-cell response (40). In particular, differential stimulation of $CD4^+$ Th subsets seems to be of primary importance in determining disease progression (18, 38, 39). $CD4^+$ T-cell subsets are defined by the type of cytokines produced following stimulation: $CD4^+$ Th1 cells produce interleukin-2 (IL-2) and gamma interferon (IFN-γ), while Th2 cells produce IL-4, IL-5, and IL-10 (13, 24, 25). The ability to control *L. major* infection in resistant mice, such as C3H/HeN and C57BL/6 mice, is correlated with the induction of $CD4^+$ Th1 cells. Thus, cells from these mice produce IFN-γ, but little to no IL-4, when stimulated in vitro (37), and mRNA levels for IFN-γ are elevated during infection (18). In contrast, *L. major* infection in BALB/c mice induces a Th2 response, characterized by elevated levels of IL-4 and IL-4 mRNA (18, 37). The critical role for Th2 cells in mediating the susceptibility in these mice is demonstrated by the fact that IL-4 depletion, using anti-IL-4 monoclonal antibodies administered in vivo, reverses susceptibility (9, 35). However, all these observations were made with mice with a distinct genetic background infected with the same parasite species, namely, *L. major*. In this study, we address the role of $CD4^+$ T-cell subsets in mediating the outcome of infection of C57BL/10 mice with two different parasite species, *L. major* and *L. amazonensis*. A direct comparison of the cytokine responses in C57BL/10 mice infected with either *L. amazonensis* or *L. major* suggests that while the resistance to *L. major* observed in these mice is mediated by Th1 cells, the susceptibility to *L. amazonensis* is not exclusively controlled by Th2 cells. This work suggests that mechanisms other than the induction of a Th2 type response can mediate susceptibility to leishmaniasis.

MATERIALS AND METHODS

Mice. Female C57BL/10J mice (4 to 6 weeks old) were obtained from The Jackson Laboratory, Bar Harbor, Maine.

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Female BALB/c and female athymic BALB/c *nu/nu* mice were obtained from The National Cancer Institute, Bethesda, Md. Animals were maintained in a specific-pathogen-free environment. The animal colony was screened regularly for the presence of murine pathogens and consistently tested negative.

Parasites and antigens. *L. amazonensis* (MHOM/BR/00/LTB0016) and a clone of *L. major* (MHOM/IL/80/Friedlin) promastigotes were grown to stationary phase (5-day-old culture) at 27°C in Grace's insect medium (GIBCO Laboratories, Grand Island, N.Y.) with 20% fetal bovine serum (HyClone Laboratories, Inc., Logan, Utah), 2 mM L-glutamine, 100 U of penicillin-6-potassium per ml, and 100 μg of streptomycin sulfate per ml.

Leishmanial antigen was obtained from stationary-phase promastigotes washed four times in phosphate-buffered saline (PBS) and adjusted to a concentration of 10^8 organisms per ml. Parasite suspensions were submitted to four cycles of freezing at −70°C followed by thawing at 37°C. The preparations were visually inspected for the presence of intact parasites, aliquoted, and stored frozen at −20°C. Antigens were thawed immediately before use in cell cultures.

Infection. Mice were injected in the footpad with 10^6 *L. major* or *L. amazonensis* stationary-phase (5-day-old) promastigotes. Purified metacyclic promastigotes were not used in these studies, since no reproducible method to obtain *L. amazonensis* metacyclic forms exists. Lesion size was measured during the course of infection with a dial micrometer (L. S. Starrett Co., Athol, Mass.) and expressed as the difference in size between the infected footpad and the contralateral uninfected footpad.

In vivo IL-4 depletion. Mice (five per group) were injected intraperitoneally with 5 mg of 11B11 (rat anti-murine IL-4 immunoglobulin G1 [29]) 1 day before infection. In some experiments, control animals were injected with GL113 (rat anti-*Escherichia coli* β-galactosidase immunoglobulin G1). Both antibodies were obtained by 45% ammonium sulfate precipitation of both fetal bovine serum-free cell culture supernatant and ascitic fluid obtained from pristane-primed BALB/c *nu/nu* mice injected with 11B11 cells.

Parasite quantitation. The number of parasites in the footpad was estimated by limiting dilution assay. Infected footpads were harvested in cold PBS containing 200 U of penicillin-6-potassium per ml and 200 μg of streptomycin sulfate per ml (PBS-pen/strep), after removal of the skin. Footpads were ground in glass tissue grinders and resuspended in 10 ml of PBS-pen/strep. After centrifugation at 50 × *g* for 1 min, the supernatant was transferred to another tube and centrifuged at 1,700 × *g* for 15 min. The pellet was resuspended in 10 ml of Grace's medium containing 20% fetal bovine serum, 200 U of penicillin-6-potassium per ml, and 200 μg of streptomycin sulfate per ml. The parasite suspension was then serially diluted in 10-fold dilutions in quadruplicate in 200-μl portions of medium in 96-well plates. Plates were incubated for 4 days at 27°C, and then the wells were inspected for parasite growth. Results were expressed as −log parasite titer.

Analysis of cytokine production. Single-cell suspensions were prepared from lymph nodes and spleens harvested at various time points after infection. Cells were adjusted to a concentration of 5×10^6/ml in Dulbecco's minimal essential medium containing 4.5 mg of glucose (GIBCO Laboratories) per ml, 10% fetal bovine serum (HyClone Laboratories Inc.), 2 mM L-glutamine, 100 U of penicillin-6-potassium per ml, 100 μg of streptomycin sulfate per ml, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 50 μM 2-mercaptoethanol and plated at 1 ml per well in 24-well tissue culture plates. Cells were stimulated with freeze-thawed antigen (5×10^6 parasite equivalents per ml), and supernatants were harvested after 72 h.

IFN-γ levels were measured by enzyme-linked immunosorbent assay (ELISA) as previously described (38). IL-4 was measured either in a biological assay by means of its ability to promote the growth of CT.4S cells (6), obtained from W. E. Paul, or by ELISA (9).

Statistical analysis. The significance of differences between the groups was determined by the Student's *t* test.

RESULTS

Course of *L. major* and *L. amazonensis* infection in mice. Mice were injected with 10^6 *L. major* or *L. amazonensis* promastigotes, and lesion progression was monitored (Fig. 1). The lesions in C57BL/10 mice infected with *L. major* were at their peak size by 4 weeks and had healed by 8 weeks of infection. In contrast, C57BL/10 mice infected with *L. amazonensis* promastigotes exhibited a quite distinct pattern of disease. For example, *L. amazonensis* lesions appeared later than *L. major* lesions, but once established, they rapidly increased in size, reaching a maximum which persisted without showing signs of resolving. Since parasite dose can have a profound influence on the outcome of infection (7), it was possible that the susceptibility of C57BL/10 mice to *L. amazonensis* was due to a higher number of infective promastigotes in the parasite inoculum. However, this possibility appears not to be the case, since the *L. amazonensis* parasite burden in C57BL/10 mice at 2 weeks was, in fact, less than that measured in *L. major*-infected mice (Fig. 1, insert). Thus, while C57BL/10 mice are resistant to *L. major*, they are susceptible to *L. amazonensis*. However, the nature of this susceptibility can be distinguished from that observed in BALB/c mice infected with either *L. major* or *L. amazonensis*. For example, while lesions in C57BL/10 mice infected with *L. amazonensis* failed to regress, the growth of lesions was controlled, growing to a size of approximately 2.5 to 3 mm. In contrast, both leishmanial species produced rapidly developing lesions in BALB/c mice which continuously increased in size and showed no signs of recovery (19) (see Fig. 3, solid triangles).

Cytokine patterns in C57BL/10 mice following infection with *L. major* or *L. amazonensis*. Since susceptibility to infection by *L. major* in BALB/c mice is associated with the development of $CD4^+$ Th2 type responses, it was possible that Th2 cells might predominate in C57BL/10 mice infected with *L. amazonensis*. To address this question, C57BL/10 mice infected with *L. amazonensis* or *L. major* were sacrificed at various times after infection, and in vitro cytokine production by cells from both the spleen and the lymph node draining the lesion site was measured. As expected, cells from mice infected with *L. major* produced high levels of IFN-γ at most time points. In contrast, IFN-γ production was deficient in *L. amazonensis*-infected animals. Except for the day 2 response, lymph node cells from *L. amazonensis*-infected animals always produced less IFN-γ than cells from *L. major*-infected mice (Fig. 2a). In fact, at several time points, lymph node cells from *L. amazonensis*-infected animals produced no detectable IFN-γ. Similar responses were observed with spleen cells (Fig. 2b). Thus, IFN-γ was consistently produced by spleen cells from *L. major*-infected mice, while spleen cells from *L. amazonensis*-infected ani-

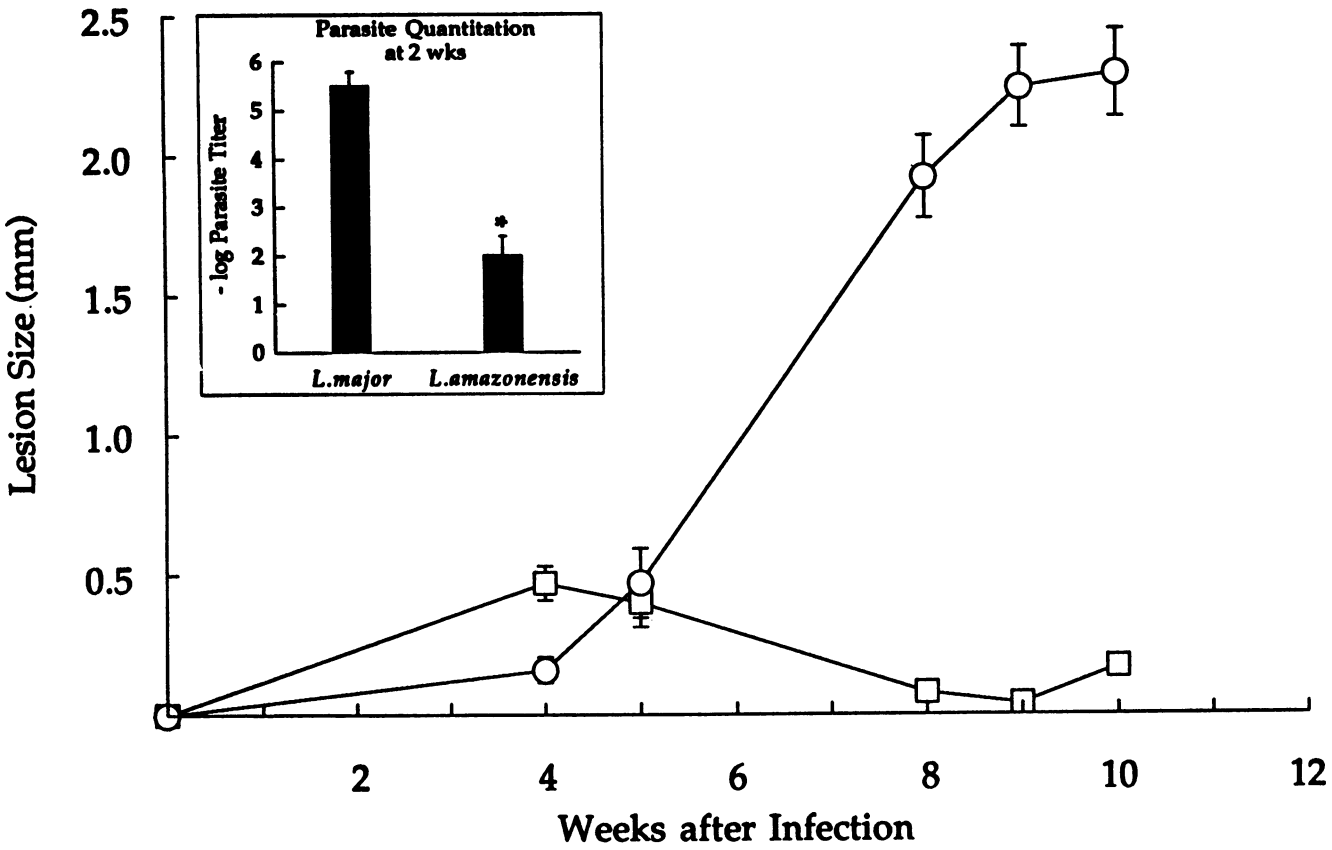

FIG. 1. *L. amazonensis* and *L. major* course of infection in C57BL/10 mice. Mice were injected in the left hind footpad with 10^6 stationary-phase promastigotes. Each point represents the mean difference in size ± standard error of the mean between infected and uninfected footpads for seven mice per group at each time point. Symbols: ○, *L. amazonensis*-infected mice; □, *L. major*-infected mice. (Insert) Parasite quantitation in footpad lesions after 2 weeks of infection. Each bar represents mean ± standard error of the mean for six animals per group. The asterisk indicates a statistically significant difference ($P \leq 0.05$) compared with the other group.

mals either failed to produce IFN-γ or produced significantly less than cells from *L. major*-infected mice. In contrast to IFN-γ, lymph node cells from *L. amazonensis*-infected C57BL/10 mice produced significant levels of IL-4, while the production of this cytokine by cells from *L. major*-infected mice was undetectable (Fig. 2c). However, IL-4 was observed only during the first 3 weeks of infection with *L. amazonensis*. At later time points, when these mice showed increased lesions, no IL-4 was produced by lymph node cells. In addition, no IL-4 was ever detected in the supernatants from spleen cells from either group.

In order to determine whether differences in the *L. major* and *L. amazonensis* antigen preparations might explain our results, spleen and lymph node cells were also stimulated with heterologous freeze-thawed antigens. Although showing an optimum response with the homologous antigen preparation, significant cross-reaction was observed between cells from *L. amazonensis*- and *L. major*-infected animals. A representative experiment is shown in Table 1. These data suggest that our results are not related to differences in the antigen preparation used to stimulate the cells in vitro.

Course of *L. amazonensis* infection in anti-IL-4-treated BALB/c and C57BL/10 mice. Treatment of BALB/c mice with an anti-IL-4 monoclonal antibody abrogates susceptibility to *L. major*, resulting in a healing or at least controlled infection (18, 35). Associated with this alteration in the outcome of infection is a change in the predominant cytokine patterns observed in these animals. Thus, in IL-4-depleted animals, Th2 cells fail to develop and Th1 cells predominate (9). Since our data show that IL-4 is also produced by cells from C57BL/10 mice infected with *L. amazonensis*, albeit at low levels and only in the draining lymph node, we decided to investigate the role of this cytokine in the induction of susceptibility to *L. amazonensis*. BALB/c and C57BL/10 mice were treated with anti-IL-4 monoclonal antibody (11B11) 1 day prior to infection, and the development of lesions was observed. Similar to the results with *L. major*, treatment with 11B11 reversed the course of *L. amazonensis* infection in BALB/c mice (Fig. 3). Thus, IL-4-depleted BALB/c animals developed smaller lesions; lesion growth peaked at 5 weeks of infection and was controlled during the course of observation. In contrast, untreated control animals developed lesions that progressively increased in size without showing any signs of recovery. It should be noted, however, that in spite of the small lesion size and the substantial reduction in parasite number, significant numbers of parasites ($>10^5$ per footpad) were still recovered from 11B11-treated BALB/c mice 12 weeks after infection (Fig. 3, insert).

Associated with the alteration in the course of *L. amazonensis* infection in the BALB/c mice were different cytokine profiles for the control and experimental groups (Table 2). Both spleen and lymph node cells from control animals

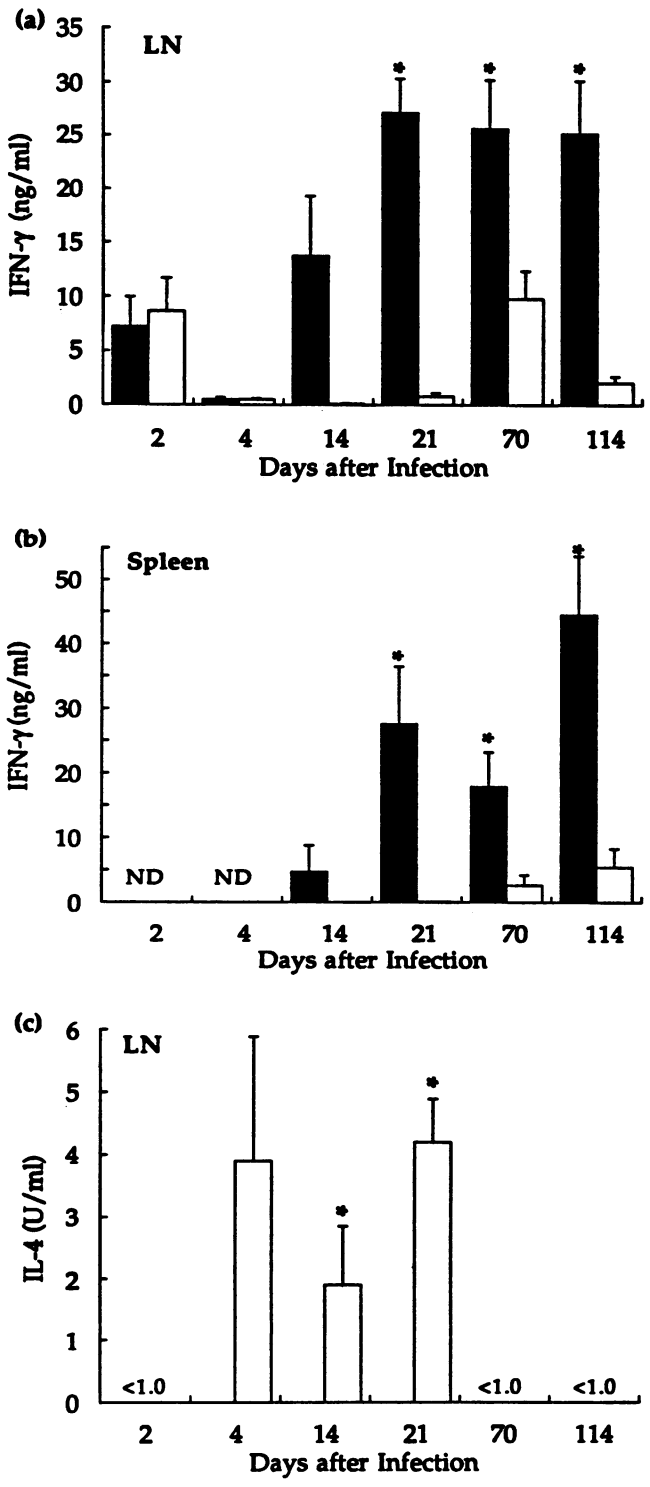

FIG. 2. Cytokine production by cells from C57BL/10 mice infected with *L. amazonensis* or *L. major*. Mice were injected in the left hind footpad with 10^6 stationary-phase promastigotes. Draining lymph nodes (LN) (a and c) and spleens (b) were harvested at the various time points, and cells were stimulated with homologous freeze-thawed antigen (10^6 parasite equivalents per ml of medium) for 3 days. Supernatants were harvested and assayed for cytokine production as described in Materials and Methods. Each bar represents the mean ± standard error of the mean for pooled lymph nodes and individual spleens from different experiments (for day 2, $n = 6$; for day 4, $n = 3$; for days 14 and 21, $n = 4$; 3 or 4 mice per group). The bars for days 70 and 114 after infection represent means ± standard errors of the means for seven and five mice, respectively. ND, not determined. Symbols: □, *L. amazonensis*-infected animals; ■, *L. major*-infected animals. Asterisks indicate statistically significant differences ($P \leq 0.05$) compared with the other infection at the same time point.

produced substantial amounts of IL-4, but only small amounts of IFN-γ. However, spleen cells from anti-IL-4-treated animals failed to produce any IL-4, and there was an 80% reduction in IL-4 levels in lymph node cell cultures. At the same time, in both the spleen and the lymph node, there was a corresponding increase in the production of IFN-γ.

IL-4 depletion had a less dramatic effect on the course of infection with *L. amazonensis* in C57BL/10 mice (Fig. 4). During the first 8 weeks, no difference between treated and untreated animals was observed. Interestingly, however, after 9 weeks, the development of lesions in anti-IL-4 treated mice stopped, while lesions of control animals continued to increase in size for another 3 to 4 weeks. Thus, the maximum lesion size obtained in IL-4-depleted C57BL/10 mice was significantly smaller than that seen in control animals. Nevertheless, IL-4-depleted animals were still unable to heal and maintained measurable lesions throughout the experiment. Furthermore, although parasite quantitation in lesions from anti-IL-4-depleted mice showed a significant decrease in the number of organisms relative to untreated controls, high numbers of parasites were still present in these lesions, especially when compared with those from healed *L. major*-infected C57BL/10 mice (Fig. 4, insert).

Cytokine responses in anti-IL-4-treated and control C57BL/10 *L. amazonensis*-infected animals were not different (Table 3). Although IFN-γ levels were slightly higher in lymph node cell cultures from anti-IL-4 treated mice, they were, in fact, lower in spleen cell cultures. In any case, a comparison of IFN-γ production by either spleen or lymph node cells from *L. major*-infected animals indicates that anti-IL-4-treated *L. amazonensis*-infected mice still exhibited a deficit in their ability to produce this cytokine. Finally, as shown above (Fig. 2), no IL-4 was detected in culture supernatants from in vitro-stimulated cells from any of the C57BL/10 groups at this late time point.

DISCUSSION

In murine models of cutaneous leishmaniasis, animals can be classified as either resistant or susceptible to the disease on the basis of the course of infection. BALB/c mice are susceptible to many *Leishmania* species. In contrast, several other mouse strains show graded degrees of resistance, depending upon the leishmanial species used for infection. For example, C57BL/6 and C3H/HeN mice are resistant to *L. major*, as indicated by their ability to heal, while infections in these same mice with either *L. mexicana* or *L. amazonensis* are associated with chronic lesions and, in some cases, late metastatic spread of the organisms (3, 17). In fact, immunogenetic analysis of resistance and susceptibility to *L. major* and *L. mexicana* suggest that different genes influence the outcome of their infection (23, 32, 33). Although considered by some researchers (34) as resistant to *L. amazonensis* infection, in our experiments, C57BL/10 mice infected with *L. amazonensis* were never able to heal from the infection and showed relatively high numbers of parasites at the lesion site even after 18 weeks of infection. In contrast, animals infected with *L. major* for the same

TABLE 1. In vitro cytokine production by lymph node cells from C57BL/10 mice infected for 3 weeks with *L. major* or *L. amazonensis* stimulated with homologous and heterologous antigen[a]

Species	Amt (ng/ml) of IFN-γ[b]			Amt (U/ml) of IL-4[b]		
	Bkg	LM	LA	Bkg	LM	LA
L. amazonensis	0.1 ± 0.1	1.4 ± 0.6	0.8 ± 0.3	<1.0	2.5 ± 1.0	4.5 ± 0.6
L. major	6.2 ± 0.5	27.1 ± 3.2	20.5 ± 2.0	<1.0	<1.0	<1.0

[a] Data are the means ± standard error of the means of three to four experiments in which lymph node cells from three to four mice were pooled.
[b] Bkg, no antigen; LM, *L. major* freeze-thawed antigen; LA, *L. amazonensis* freeze-thawed antigen.

period of time had little or no recoverable parasites at the lesion site. In fact, it has been shown that C57BL/10 mice infected at the base of the tail with *L. amazonensis* and observed for over 18 months never recovered from infection (12). Nevertheless, it should be pointed out that the susceptibility pattern in C57BL/10 mice is quite different from that observed in BALB/c mice. *L. amazonensis* infection in BALB/c mice is associated with progressive increase in lesion size and metastasis to other cutaneous sites, as well as visceralization of the parasite (2, 5), while lesions in *L. amazonensis*-infected C57BL/10 mice did not increase after reaching a size of 2 to 3 mm and the mice did not develop metastatic lesions during the observation period.

In murine models, susceptibility to *L. major* infection has been attributed to the induction of a Th2 type response associated with the production of increased levels of IL-4 (15, 18). It was of interest then to determine whether *L. amazonensis* infection in C57BL/10 mice was also related to the induction of a Th2 response. We found that IL-4 was produced by cells from *L. amazonensis*-infected C57BL/10 mice and that anti-IL-4 treatment substantially reduced lesion size. Nevertheless, for several reasons, we do not

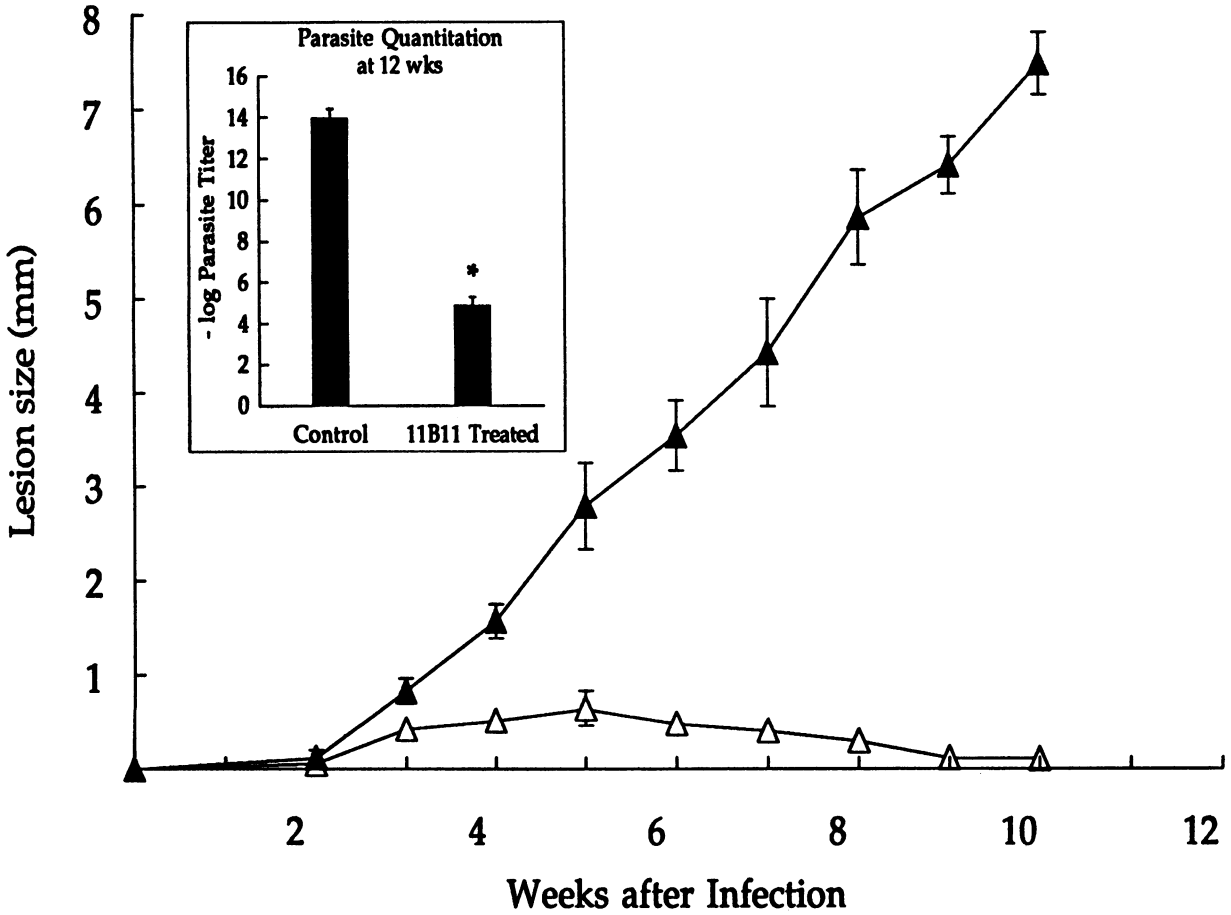

FIG. 3. Effect of anti-IL-4 treatment on *L. amazonensis* course of infection in BALB/c mice. Animals were injected with anti-IL-4 monoclonal antibody 1 day prior to infection, and lesion size was monitored weekly. Each point represents the mean difference in size ± standard error of the mean between infected and contralateral uninfected footpads for five mice per group in a representative experiment. Infection of mice injected with control antibody (GL113) was comparable to that of uninjected mice. Symbols: ▲, control animals; △, 11B11-treated animals. (Insert) Parasite quantitation in footpad lesions after 12 weeks of infection. The asterisk indicates a statistically significant difference ($P \leq 0.05$) compared with the control group.

TABLE 2. Effect of anti-IL-4 treatment on cytokine production in BALB/c mice infected for 12 weeks with *L. amazonensis*[a]

Source of cells	Treatment	Amt (ng/ml) of IFN-γ	Amt (U/ml) of IL-4
Lymph nodes	None (control)	0.21 ± 0.16	6.90 ± 2.80
	11B11	9.65 ± 2.67	1.48 ± 0.47
Spleen	None (control)	1.71 ± 0.47	10.06 ± 2.60
	11B11	7.04 ± 2.65	<1.0

[a] Data are means ± standard error of the means, with five animals per group.

TABLE 3. Effect of anti-IL-4 treatment on cytokine production in C57BL/10 mice infected for 18 weeks with *L. amazonensis*[a]

Species	Source of cells	Treatment	Amt (ng/ml) of IFN-γ	Amt (U/ml) of IL-4
L. amazonensis	LN	None (control)	0.16 ± 0.04	<1.0
		11B11	2.37 ± 0.38	<1.0
	Spleen	None (control)	2.02 ± 0.35	<1.0
		11B11	0.56 ± 0.44	<1.0
L. major	LN	None (control)	25.55 ± 6.59	<1.0
	Spleen	None (control)	14.67 ± 5.99	<1.0

[a] Data are the means ± standard error of the means, with five animals per group.

believe that this response is fundamental to the susceptibility of C57BL/10 mice to *L. amazonensis* infection. For example, IL-4 was detected only at the beginning of the infection. Later in infection, when lesions had developed, no production of this cytokine was ever observed. Furthermore, the levels observed were somewhat low compared with the levels that have been demonstrated for *L. major*-infected BALB/c mice (38). In addition, IL-4 was not produced by spleen cells at any time point during the infection, suggesting that a systemic Th2 type response was never established. A final and perhaps more important observation was the fact that anti-IL-4 treatment was not associated with healing or with any enhancement of IFN-γ production. Taken together, these data suggest that IL-4 production early after infection may not be the major factor controlling susceptibility to *L. amazonensis* in C57BL/10 mice. These results are similar to those seen in B10.D2 mice infected with *Leishmania dono-*

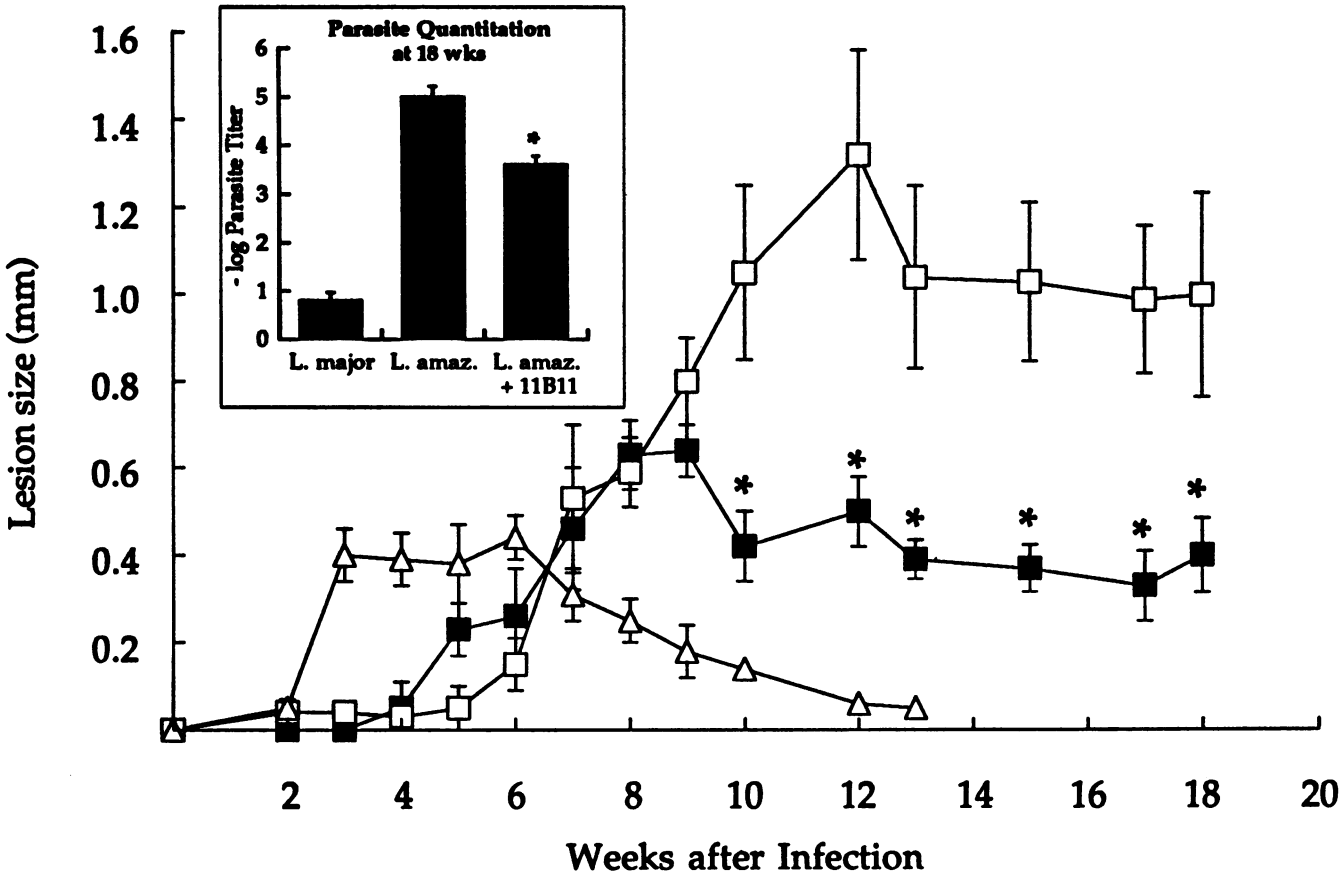

FIG. 4. Effect of anti-IL-4 treatment on *L. amazonensis* course of infection in C57BL/10 mice. Animals were injected with anti-IL-4 monoclonal antibody 1 day prior to infection, and lesion size was monitored weekly. Each point represents the mean difference in size ± standard error of the mean between the infected and contralateral noninfected footpads for five mice per group in a representative experiment. Infection of mice injected with control antibody (GL113) was comparable to uninjected mice. Symbols: □, *L. amazonensis*-infected control animals; ■, *L. amazonensis*-infected animals treated with 11B11; △, *L. major*-infected animals. Asterisks indicate statistically significant differences ($P \leq 0.05$) compared with the control at the same time point. (Insert) Parasite quantitation in footpad lesions after 18 weeks of infection. L. amaz., *L. amazonensis*.

vani, which although exhibiting a susceptible phenotype, failed to produce IL-4 or IL-5 during infection (20). In contrast to C57BL/10 mice, however, IL-4 appears to be critical in the susceptibility of BALB/c mice to *L. amazonensis*. Anti-IL-4 treatment dramatically reduced lesion size in *L. amazonensis*-infected BALB/c mice, similar to results reported with *L. major* (9, 29). It should be pointed out, however, that although lesions were quite small in anti-IL-4-treated *L. amazonensis*-infected BALB/c mice, these animals still maintained a significant number of parasites in their lesions (Fig. 3, insert). In fact, the number of parasites was similar ($\sim 10^5$) to that seen in *L. amazonensis*-infected C57BL/10 mice. Thus, the development of a Th2 type response may be an important component in the extreme susceptibility of BALB/c to *Leishmania* infection but may be less important in maintaining the chronic, nonfatal infections associated with *L. amazonensis* infections in C57BL/10 mice.

T-cell-mediated events have a profound influence on the outcome of leishmaniasis. However, the initiation of a T-cell response is subsequent to infection of macrophages by these parasites. The interaction between the parasite and the macrophage is likely to be a critical event both in determining the type of immune response which develops and in influencing the ability of macrophages to be activated to eliminate the parasites. Macrophages produce a large repertoire of cytokines following *Leishmania* infection that may both positively and negatively regulate these events (26). One of the most important may be tumor necrosis factor α (TNF-α), which is required for the induction of nitric oxide production responsible for parasite elimination (15, 16). Thus, differences in the levels of TNF-α produced by macrophages following infection with different *Leishmania* species might dictate the efficiency of macrophage activation. Recent experiments from this laboratory showing that *L. amazonensis* induces less TNF-α production from macrophages than *L. major* supports this hypothesis (43). Moreover, these observations may explain our previous findings that several *L. amazonensis* strains are relatively resistant to activated macrophage killing (41). Currently, experiments in our laboratory are directed at investigating the relationship between TNF production and the production of cytokines inhibitory for macrophage activation, such as IL-10 and transforming growth factor β (TGF-β). IL-10 has been shown to inhibit macrophage activation in a variety of systems, although at present, its role in modulating macrophage activity in leishmaniasis is unclear (14, 30, 42). On the other hand, TGF-β is produced by macrophages following *Leishmania* infection and in vivo injection of this cytokine significantly augments leishmanial lesion development (4). While the mechanisms mediating this effect are unclear, in vitro studies indicate that TGF-β blocks the capacity of lymphokine supernatants containing a mixture of macrophage-activating factors to induce macrophage leishmanicidal activity (28). Since TGF-β has been shown to inhibit TNF-α production, enhanced production of TGF-β in *L. amazonensis*-infected macrophages would be consistent with our data (8).

Another striking difference between the infection of C57BL/10 mice with *L. amazonensis* and *L. major* is the inflammatory response associated with infection. It was previously reported that *L. amazonensis* infection in either susceptible or resistant mice is associated with the absence of a T-cell infiltration into the lesions during the first 12 weeks of infection (22). Interestingly, we also found a dramatic difference in the total number of cells in the draining lymph nodes of *L. amazonensis*- and *L. major*-infected mice during the first several weeks after injection of the parasites. For example, C57BL/10 mice infected for 2 weeks with *L. amazonensis* had an average of 5.2×10^6 cells per lymph node, while lymph nodes from *L. major*-infected mice contained 19.2×10^6 cells. Since TNF-α is an important chemotactic factor that appears to be required for granuloma formation in several infections (1, 21), it is possible that lower levels of TNF-α produced by *L. amazonensis*-infected macrophages may contribute to these observations.

Cutaneous leishmaniasis is often self-healing in humans and induces a protective cellular immune response which is generally long-lasting (for a review, see reference 44). However, complicated forms of disease with chronic nonhealing lesions, recurrence of infection, and general dissemination of the parasite are not uncommon. In these cases, a decreased ability to mount a cellular response to the parasite has been observed (11, 36). Also, the appearance of cutaneous lesions long after exposure of the patient to the parasite has been reported (44). We have made similar observations following infection of C57BL/10 mice with *L. amazonensis*. These mice developed chronic lesions that were delayed in their onset and did not heal spontaneously, and their cellular immune responses as measured by their ability to produce cytokines, were impaired. Thus, infection of C57BL/10 mice by *L. amazonensis* may be an excellent model to study chronic forms of human cutaneous leishmaniasis. In fact, the course of *L. amazonensis* infection in C57BL/10 may more closely mimic the human disease than the more commonly studied BALB/c model.

The results described here show that induction of a Th2 type response is not always associated with susceptibility to murine cutaneous leishmaniasis. Our data indicate that in addition to host genetics, parasite-associated factors can play a critical role in the outcome of this disease. Identification of these factors may not only be important to understanding the human disease but may also be critical in designing vaccine and therapeutic strategies.

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