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Endogenous Interleukin-12 Is Critical for Controlling the Late but Not the Early Stage of *Leishmania mexicana* Infection in C57BL/6 Mice

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The role of interleukin-12 (IL-12) has been clearly established in the resistance of C57BL/6 (B6) mice to *Leishmania major* infection, but its involvement in the control of *Leishmania mexicana* infection remains to be determined. Here, we show the following. (i) *L. mexicana*, in contrast to *L. major*, induces the development of nonhealing lesions in B6 mice. (ii) Cells expressing IL-12p40, gamma interferon (IFN-γ), NOS2, and CD40L are numerous in the footpad lesion and/or the draining popliteal lymph node of animals infected for up to 14 weeks with *L. mexicana*. (iii) B6 mice, either IL-12p40 deficient or treated with IL-12p40-neutralizing antibodies, display a dramatic enhancement of primary and secondary lesions leading to death 10 weeks after inoculation with *L. mexicana*. (iv) Splenocytes harvested 4 and 8 weeks after infection of IL-12p40$^{-/-}$ B6 mice with *L. mexicana* are unable to produce IFN-γ, but secrete IL-4, IL-10, and IL-18. Thus, the early control of *L. mexicana* infection by B6 mice is independent of IL-12, whereas IL-12 and Th1 responses play a key role in controlling the late stages of *L. mexicana* infection. However, they fail to resolve lesions, in contrast to *L. major* infection, emphasizing the different outcomes induced by these two *Leishmania* species in B6 mice.

The various clinical forms of human leishmaniases have been partially reproduced in different inbred strains of mice. For example, CBA, C3H, C57BL/6 (B6), and B10.D2 mice infected with *Leishmania major* develop cutaneous lesions that spontaneously resolve, whereas BALB/c, SWR/J, DBA/2, and A/J mice harbor nonhealing primary cutaneous lesions prone to dissemination (6). Such experimental models have allowed definition of some of the immunological factors involved in resistance and susceptibility to *L. major* infection (29).

Interleukin-12 (IL-12), a heterodimeric cytokine composed of p35 and p40 subunits, through its binding to IL-12 receptor (IL-12R) (23) and WSX-1 (40), plays a key role in the induction and the long-term maintenance of the T-cell-dependent resistance of B6 mice to *L. major* infection (20, 27, 32, 34, 38). Macrophages and dendritic cells produce high levels of IL-12 when infected in vitro with *L. major* amastigotes (37). Gamma interferon (IFN-γ) produced by Th1 cells mediates protection by activating the NOS2 gene in macrophages and the subsequent production of the leishmanicidal NO. This cascade depends on CD40-CD40L interaction (8, 18), as well as other factors (26), and each element is essential to lesion resolution in resistant mice.

Although *Leishmania mexicana* induces cutaneous or diffuse lesions in American patients (3, 11, 35), the immunological mechanisms determining susceptibility and resistance to this *Leishmania* species have been less studied. In vitro experiments suggest that neither macrophages nor dendritic cells produce IL-12 when infected with *L. mexicana* amastigotes (5, 39), and susceptibility to *L. mexicana* seems to be due to a prostaglandin-dependent inability to produce IL-12 (28, 30).

To extend our previous studies of this infection model (1, 2), we investigated the role of IL-12 in B6 mice infected with *L. mexicana*. The courses of *L. mexicana* and *L. major* infections were compared in wild-type (WT), IL-12p40-deficient, and B6 mice treated with IL-12p40-neutralizing antibodies. We also analyzed the density of cells producing IL-12p40, IFN-γ, and NOS2 and expressing CD40L in the footpad lesion and/or the draining lymph node (LN) of *L. mexicana*-infected WT animals, and the capacity of splenocytes of IL-12p40$^{-/-}$ B6 mice infected with *L. mexicana* to secrete IFN-γ, IL-4, IL-10, and IL-18. The data indicate that IL-12 is not required to control the early phase of infection, but is critical for long-term stabilization of *L. mexicana* lesions, although insufficient to totally cure the disease, in contrast with *L. major* infection.

MATERIALS AND METHODS

Parasites. Promastigotes of *L. mexicana* (strain MHOM/BZ/82/BEL21) and *L. major* (strain WHOM/IR/−173) were cultured in RPMI medium (Life Technologies, Gaithersburg, Md.), supplemented with 10% fetal calf serum (FCS; Life Technologies), penicillin G (100 U/ml), and streptomycin (100 μg/ml). Parasites in the stationary phase, harvested after 8 to 10 days of culture, were centrifuged (2,500 × *g*, 10 min, 4°C) and then washed three times in RPMI before being counted and used for inoculation of animals. *L. mexicana* promastigotes lysed by 10 cycles of freezing in liquid nitrogen and thawing in a water bath at 37°C were also used for cell stimulation studies.

Mice and *Leishmania* infection. C57BL/6 male mice were purchased from Banting & Kingman Universal, Ltd. (Hull, United Kingdom). Male and female IL-12p40$^{-/-}$ C57BL/6 mice were prepared as previously described (13). In another experiment, each week, C57BL/6 mice received intraperitoneally (i.p.) anti-IL-12p40 monoclonal antibody (MAb; clone C17.8, rat immunoglobulin G2a [IgG2a]; kindly given by V. Flamand, Free University of Brussels [ULB],

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Brussels, Belgium) or unrelated rat IgG2a (clone IR418, kindly provided by H. Bazin, UCL, Brussels, Belgium). The maintenance and care of mice complied with the guidelines of the ULB Ethics Committee for the Humane Use of Laboratory Animals. Mice (8 to 12 weeks old) were infected subcutaneously, as reported previously (1, 2), in the rear left hind footpad with 10^7 stationary-phase promastigotes of *L. mexicana* or *L. major* in a final volume of 25 μl (in RPMI medium). The controlateral right footpad received an identical volume of RPMI medium without parasites as an internal control.

Lesion monitoring, tissue processing, and quantification of *L. mexicana* amastigotes. The thickness of infected and uninfected footpads was regularly measured with a Vernier calliper, and the difference between both measurements corresponded to lesion size. Mice were also regularly examined to detect cutaneous ulcers and secondary lesions.

At selected time points, some infected mice were killed by ether inhalation. Footpad lesions cut tangentially to the bone ground and popliteal homolateral draining LN were collected for histological and/or immunohistochemical studies.

The parasite burdens in footpad lesions of IL-12p40$^{-/-}$ and WT C57BL/6 infected animals were determined after tissue homogenization by staining released amastigotes with acridine orange (Sigma, Brussels, Belgium), as described previously (2).

Histological and immunohistochemical studies. Footpad tissues and LN were either snap-frozen in liquid nitrogen or fixed in 10% formalin, immediately after the sacrifice of animals. Tissue sections (5 μm) of fixed material embedded in paraffin were stained with hematoxylin-eosin-safranin to study their microarchitecture by light microscopic examination.

All immunohistochemical procedures used for in situ analysis of IL-12p40, CD40L, IFN-γ, and NOS2 in frozen tissues have been described in detail elsewhere (9). Briefly, 6-μm frozen sections were thaw mounted on glass slides and kept overnight at room temperature in a box with humidified atmosphere. The next day, sections were air dried at room temperature and fixed for 10 min in fresh acetone containing 0.02% hydrogen peroxide to inhibit endogenous peroxidase activity. Air-dried sections were washed once with phosphate-buffered saline (PBS)–0.05% Tween 20 and incubated overnight at 4°C with the antibody (rat anti-IL-12p40 C15.6 labeled with biotin from Pharmingen; mouse anti-IFN-γ DB-1–biotin from U-Cytech, Utrecht, The Netherlands; rabbit anti-NOS2–biotin from Calbiochem; hamster anti-CD40L MR-1 labeled with alkaline phosphatase (AP) from R. J. Noelle, Lebanon, N.H.) at the previously determined optimal dilution in PBS–0.1% bovine serum albumin. Sections incubated with the AP-labeled antibody were washed with PBS and revealed with Fast Blue BB Base giving dark blue precipitates. Horseradish peroxidase-conjugated streptavidin was applied for 1 h at room temperature on sections previously incubated with biotin-labeled antibodies, and aminoethylcarbazole was used as a chromogen, giving a bright red translucent precipitate. Enzymatic reactions were stopped by washing in PBS and slides were mounted with glycerol-gelatine. Immunohistochemical stains were evaluated by light microscopy, and positive cells were counted in the whole-section area to determine their number per square millimeter by using the Image analysis Vidas system (Zeiss, Weesp, The Netherlands)

Cell stimulation and quantification of cytokines. Single-splenocyte suspensions of IL-12p40$^{-/-}$ and WT mice were treated for 1 min with a lysis buffer (9 parts 0.16 M ammonium chloride and 1 part 0.17 M Tris-HCl [pH 7.5]). The erythrocyte-free cells were then washed twice in RPMI 1640 medium (containing 5% FCS), and their viability was checked by the trypan blue exclusion test (viable cells, >95%), before being resuspended and distributed in 24-well plates (Nunc, Roskilde, Denmark; 3×10^6 cells per 800 μl per well). Cell stimulations were performed with lysed *L. mexicana* promastigotes (10^6 parasites per well). After incubation at 37°C for 48 h in a 5% CO_2-humidified atmosphere, supernatants of unstimulated or stimulated cells were collected by centrifugation at $280 \times g$ for 10 min and kept frozen at −70°C for subsequent determination of cytokine levels.

Cytokine quantification was performed by enzyme-linked immunosorbent assay (ELISA) in cell supernatants by using commercially available kits (IL-18, M1800; IFN-γ, IL-4, and IL-12, duo set; IL-10, Intertest 10x [all from Genzyme Diagnostics, Cambridge, Mass.]). The IL-12 assay detected monomeric and dimeric p40, as well as p70 subunits. All ELISAs were performed according to the manufacturer's instructions, with a detection limit of 5 pg/ml for each of them.

RESULTS

***L. mexicana*, in contrast to *L. major*, induces nonhealing cutaneous lesions in B6 mice.** As shown in Fig. 1, lesions in B6 mice inoculated with *L. mexicana* and *L. major* displayed similar maximal sizes (of about 1.4 mm in diameter), at weeks postinfection (wpi) 8 and 4, respectively. The *L. major* lesions resolved progressively from wpi 4 and were totally cured by wpi 24. In contrast, the lesions induced by *L. mexicana* never resolved, and their size, although decreasing in some mice, remained nearly constant for the entire observation period. Data on amastigote counts in footpad lesions and draining LN of *L. mexicana*-infected animals are reported elsewhere (2)

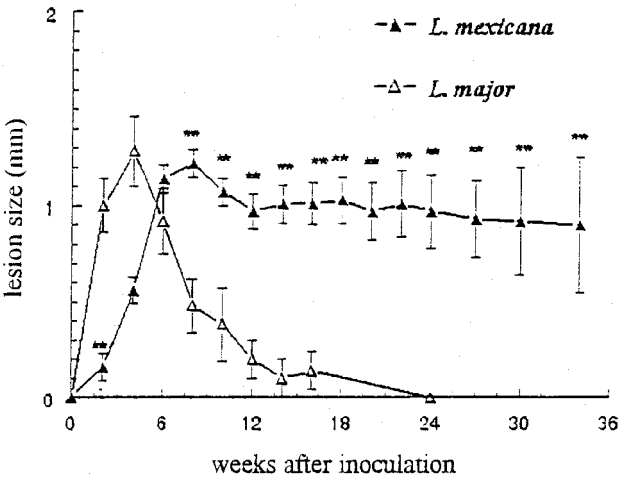

FIG. 1. Size of primary footpad lesions in B6 mice infected with *L. mexicana* or *L. major*. Results are expressed as means ± standard errors obtained from 18 animals infected with *L. mexicana* and 13 animals infected with *L. major*. **, $P < 0.001$ (Student's *t* test for comparison of *L. mexicana* and *L. major* infections).

Lesions and/or LN of B6 mice infected with *L. mexicana* contain cells expressing IL-12p40, CD40L, IFN-γ, and NOS2. Immunostaining of footpad sections with the MAb C15.6 showed some large cells containing intracellular IL-12p40 throughout the dermis and near the vessels of B6 mice 24 weeks after infection with *L. mexicana.* Such cells were not detected in footpad tissues collected before infection or at wpi 14 (data not shown). Popliteal LN of infected mice harbored numerous IL-12p40-containing cells on wpi 14 and 24, which are rarely found in uninfected animals (Table 1). Positive cells in infected mice were found either isolated or in clusters of two to three cells, mainly in the medulla and occasionally in the cortex, but not in the subcapsular sinus of LN. *L. mexicana* infection also strongly increased the frequencies of CD40L-,

TABLE 1. Number of cells expressing IL-12p40, CD40L, IFN-γ, and NOS2 in LN draining footpad lesions in B6 mice infected with *L. mexicana*

wpi	No. of positive cells/mm^2 in LN[a]			
	IL-12p40	CD40L	IFN-γ	NOS2
0	0.1 ± 0.1	1.9 ± 1.7	0.0 ± 0.0	3.3 ± 0.6
14	5.2 ± 0.9*	15.0 ± 2.7*	9.4 ± 2.1*	30.0 ± 10.7*
24	7.2 ± 0.7*	12.9 ± 2.4*	11.3 ± 3.1*	38.8 ± 7.6*

[a] Results are expressed as means ± standard errors obtained from four animals, with four sections being examined per mouse. *, $P < 0.05$ (Student's *t* test, comparing data from infected animals [wpi 14 and 24] to uninfected ones [wpi 0]).

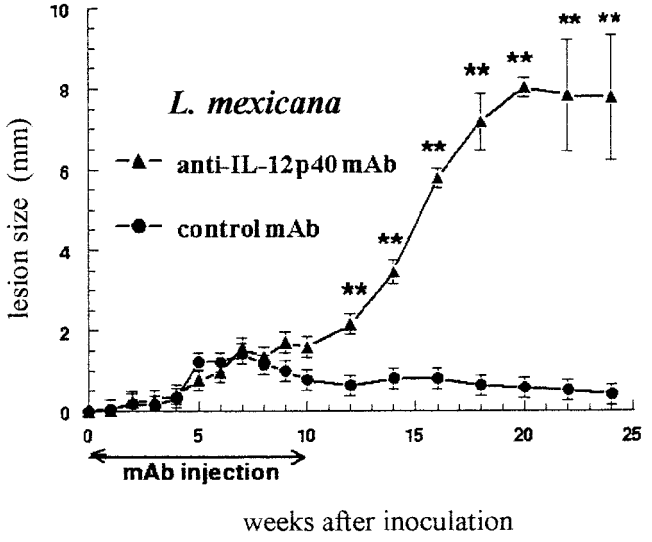

FIG. 2. Size of primary footpad lesions in B6 mice receiving anti-IL-12p40 antibodies and infected with *L. mexicana*. Results are expressed as means ± standard errors ($n = 5$ in both animal groups). **, $P < 0.001$ (Student's t test for comparisons between the two groups).

IFN-γ-, and NOS2-expressing cells by 6- to 12-fold in LN of B6 mice at wpi 14 and 24 compared to uninfected mice (Table 1).

IL-12 is involved in the late control of *L. mexicana* infection in B6 mice. The role of IL-12 produced in response to *L. mexicana* infection was investigated in two types of experiments, by using B6 mice either deficient in IL-12 production by targeted inactivation of IL-12p40 genes or treated with anti-IL-12p40 MAb (clone C17.8). Control mice were WT B6 mice or B6 mice receiving the isotype-matched control antibody IR418, respectively. C17.8 or IR418 antibodies were injected intraperitoneally (i.p.) (1 mg in 0.1 ml of PBS) 1 h before parasite inoculation and weekly for 10 weeks. All mice were inoculated with 10^7 *L. mexicana* promastigotes. In addition, as positive controls, IL-12p40$^{-/-}$ or WT mice were similarly infected with *L. major*.

As shown in Fig. 2 and 3A, during the first 10 wpi with *L. mexicana*, the lesion sizes remained similar in both C17.8 antibody-treated and IL-12p40$^{-/-}$ B6 mice and their respective IL-12-sufficient controls. Thereafter, IL-12p40-deficient mice exhibited a dramatic increase in lesion sizes, comparable to that previously observed in BALB/c mice inoculated with the same strain of parasite (1). Moreover, all of the IL-12p40-deficient animals displayed multiple secondary cutaneous lesions that were never observed in control B6 mice, which instead showed stabilized or slightly reduced lesion sizes from wpi 10 onward. While all control mice survived *L. mexicana* infection, all IL-12p40$^{-/-}$ mice and three out of the five mice receiving the anti-IL-12p40 antibody died between wpi 22 and 25. As expected, the IL-12p40$^{-/-}$ mice infected with *L. major* showed a dramatic increase in lesion sizes from wpi 6 (Fig. 3C), and all mice developed ulcerative lesions leading to mutilations and died at wpi 15, while all WT animals survived.

The early control of *L. mexicana* infection in IL-12p40$^{-/-}$ B6 mice is associated with Th2 cytokine release in the presence of IL-18. Some IL-12p40$^{-/-}$ and WT animals infected with *L. mexicana* were killed on wpi 4 or 8 to collect footpad lesions for parasite quantification and spleen cells for cytokine analysis. As shown in Fig. 3B, footpad parasite burdens remained roughly similar in IL-12p40$^{-/-}$ and WT mice at wpi 4 and 8 ($n = 2$, $P > 0.05$). As shown in Table 2, the analysis of culture supernatants of splenocytes, incubated for 48 h in the presence or absence of parasite lysate, confirmed the IL-12p40 depletion in gene-deleted mice. The IFN-γ response to *L. mexicana* was also undetectable, whereas the secretion of IL-4 and IL-10 was higher than that in cells from WT mice similarly infected. Interestingly, despite the absence of IFN-γ, IL-18, an IFN-γ-amplifying factor (22), was present at comparable levels in all samples from both groups of mice (Table 2).

DISCUSSION

This study demonstrates that IL-12 is critical for long-term stabilization of *L. mexicana* lesions, but insufficient to totally cure the infection, in contrast with *L. major* infection. Interestingly, the early control of *L. mexicana* infection appears to be independent of IL-12. Indeed, *L. mexicana*-infected B6 mice depleted of IL-12 reversed their disease phenotype from a localized and stabilized lesion to an uncontrolled, disseminated, and lethal form of leishmaniasis, similar to that in susceptible BALB/c mice (1). The activation of the IL-12–IFN-γ–NOS2 cascade, leading to the release of leishmanicidal NO, likely contributes to the control of this late phase of *L. mexicana* infection, as indicated by the increased frequencies of LN cells expressing these mediators at wpi 14 and 24. This is consistent with previous reports on the capacity of macrophages infected in vitro with *L. mexicana* to release NO (39) and with the NO production by cells from *L. mexicana*-infected mice (28). Moreover, the abrogation of IFN-γ production in *L. mexicana*-infected IL-12 p40$^{-/-}$ mice, which bear exacerbated lesions, suggests a causal relationship between these mediators and lesion stabilization. The concomitant expression of CD40L suggests that CD40-CD40L interactions are necessary to trigger IL-12 production in mice infected with *L. mexicana,* as previously reported for the closely related parasite *Leishmania amazonensis* (33). The present results obtained with *L. mexicana* agree with those previously reported for *L. major*, confirming that IL-12, IFN-γ, and NOS2 are elements essential to maintain long-term control of *Leishmania* replication (20, 27).

However, besides these similarities in the events occurring during the late phases of both infection models, *L. mexicana* lesions never heal and parasites persist (2), in contrast with the complete cure that occurs in *L. major* infection. Different mechanisms could account for this late persistence of *L. mexicana*-containing lesions. First, the stimulation and maintenance of IL-12 production and/or the responsiveness to IL-12 might be different between these two parasite species. Indeed, lesions of *L. major*-infected resistant mice treated with anti-IL-12 antibodies resolve once the treatment is terminated (15), unlike our observations with *L. mexicana* infection. The latter species, in addition to a reduced capacity to stimulate IL-12 production (30), might also downregulate the expression of the IL-12R β2 chain, as previously observed in infection of resistant mice with *L. amazonensis* (17). Second, the Fas/FasL-dependent cytotoxicity of IFN-γ-activated Th1 cells against infected macrophages might be less efficient against *L. mexicana* than against *L. major* (10, 16). Third, although not yet

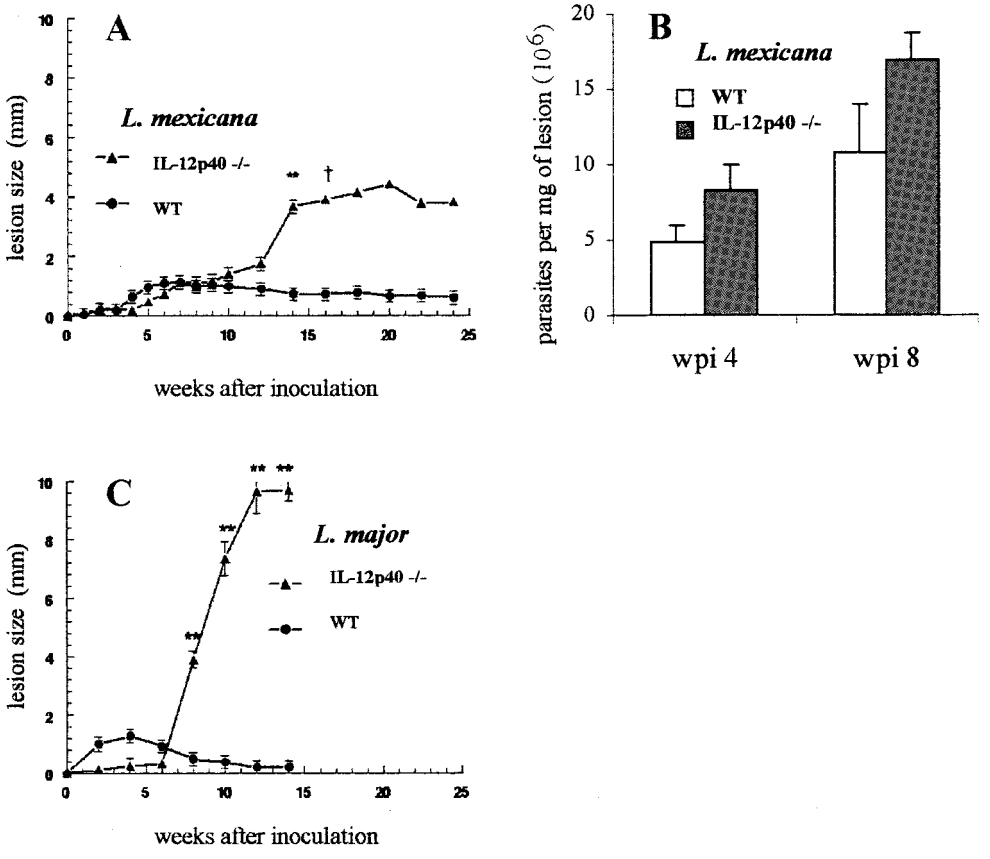

FIG. 3. Size of primary footpad lesions in IL-12p40$^{-/-}$ B6 mice infected with *L. mexicana* (A) or *L. major* (C) and parasite burdens in lesions of *L. mexicana*-infected animals (B). Results are expressed as means ± standard errors. Four IL-12p40$^{-/-}$ mice and seven WT mice were inoculated with *L. mexicana* or *L. major*, and two animals of each group were used for determination of parasite burdens. **, $P < 0.001$ (Student's *t* test for comparison of IL-12p40$^{-/-}$ and WT mice). †, results from wpi 16 onward are from one mouse.

explored, a possible higher release of antagonistic factors of IL-12 and IFN-γ production, such as IL-10 (19) or IL-12p40 monomer or dimer (14), might reduce the Th1 response in *L. mexicana* infection. Fourth, another possibility relates to a reduced rate of lymphocyte apoptosis within the *L. mexicana* lesion through tumor necrosis factor (TNF)-TNF receptor (TNFR) and Fas-FasL interactions (12, 36). Whether or not similar mechanisms may operate in *L. mexicana*-infected mice remains to be addressed.

Another interesting result from our experiments with IL-

TABLE 2. Cytokine production by splenocytes from IL-12p40$^{-/-}$ and WT B6 mice infected with *L. mexicana*

Mice	wpi	Stimulus[a]	Cytokine production (pg/10^6 cells)[b]				
			IL-12p40	IFN-γ	IL-18	IL-4	IL-10
IL-12p40$^{-/-}$	4	−	<5	<5	203 ± 31	<5	<5
	4	*L. mexicana*	<5	<5	389 ± 53	32 ± 10	20 ± 2*
	8	−	<5	<5	118 ± 4	<5	8 ± 1
	8	*L. mexicana*	<5	<5	206 ± 18	23 ± 2	86 ± 26*
WT	4	−	148 ± 32	ND[c]	161 ± 8	<5	<5
	4	*L. mexicana*	177 ± 71	ND	275 ± 82	<5	10 ± 1
	8	−	213 ± 4	<5	131 ± 14	<5	<5
	8	*L. mexicana*	450 ± 21	558 ± 100	173 ± 26	<5	36 ± 1

[a] Cells were incubated for 48 h in the presence (*L. mexicana*) or absence (−) of parasite lysate.
[b] Results are expressed as means ± standard errors obtained from three mice per group. *, $P < 0.05$ (Student's *t* test, comparing data from IL-12p40$^{-/-}$ and WT C57BL/6 mice).
[c] ND, not determined.

12p40-depleted mice is the absence of marked growth of lesions in the first weeks after inoculation of *L. mexicana* or *L. major*. Moreover, in this early step of infection, cells from *L. mexicana*-infected IL-12p40$^{-/-}$ mice secreted only type 2 cytokines, such as IL-4 and IL-10, whereas WT mice produced type 1 cytokines, such as IL-12p40 and IFN-γ. This suggests the involvement of IL-12-independent immunological mechanisms, mediated by CD8 or NK cells, able to control the first phase of *L. mexicana* infection. However, involvement of CD8 cytotoxic T cells is unlikely, since it has been previously reported that *L. mexicana* antigens are preferentially presented in association with MHC class II molecules (4), and class I-deficient mice display *L. mexicana* lesions similar to those of WT mice (25). Moreover, immunohistochemical studies from our laboratory indicated that CD8 T cells are very scarce in *L. mexicana* cutaneous lesions (F. Aguilar Torrentera, unpublished results). A more likely mechanism could be the activation of cytotoxic NK cells, which might target *L. mexicana*-infected macrophages. Indeed, SCID mice, lacking mature T and B cells, but with normal NK cells, also display a latency period before lesion growth when infected with *L. mexicana* (31). Activation of NK cells might be induced by the simultaneous presence of IL-10 and IL-18, since the combination of both cytokines has been shown to be a potent activator of NK proliferation and cytotoxic activity (7). Cells from IL-12p40$^{-/-}$ B6 mice released larger amounts of IL-10 upon stimulation with parasite lysate at wpi 4 and 8 in comparison to WT animals, and significant levels of IL-18 were detected in these culture supernatants. IL-18 is known to play a role in the early control of *L. major* infection (21, 24). Whether such a mechanism also occurs in *L. mexicana*-infected WT B6 mice, in addition to or instead of the classical IL-12–IFN-γ pathway, remains to be determined.

In conclusion, our results show that the early control of *L. mexicana* infection by B6 mice is independent of IL-12, whereas IL-12 and Th1 responses have a key role in the late control of *L. mexicana* infection, although it is insufficient to resolve lesions, as in *L. major* infection. Such results emphasize marked differences between both pathogens in driving the immune response of B6-resistant mice, as previously reported for susceptible BALB/c animals (1).

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REFERENCES

1. **Aguilar Torrentera, F., N. Glaichenhaus, J. D. Laman, and Y. Carlier.** 2001. T-cell responses to immunodominant LACK antigen do not play a critical role in determining susceptibility of BALB/c mice to *Leishmania mexicana*. Infect. Immun. **69:**617–621.
2. **Aguilar Torrentera, F., M. A. Lambot, J. D. Laman, M. Van Meurs, R. Kiss, J. C. Noel, and Y. Carlier.** 2002. Parasitic load and histopathology of cutaneous lesions, lymph node, spleen and liver from BALB/c and C57Bl/6 mice infected with *Leishmania mexicana*. Am. J. Trop. Med. **66:**275–281.
3. **Andrade-Narvaez, F. J., E. Simmonds-Diaz, S. Rico-Aguilar, M. Andrade-Narveez, A. Palomo-Cetina, S. B. Canto-Lara, M. R. Garcia-Miss, M. Madera-Sevilla, and N. Albertos-Alpuche.** 1990. Incidence of localized cutaneous leishmaniasis (Chiclero's ulcer) in Mexico. Trans. R. Soc. Trop. Med. Hyg. **84:**219–220.
4. **Antoine, J. C., E. Prina, T. Lang, and N. Courret.** 1998. The biogenesis and properties of the parasitophorous vacuoles that harbour *Leishmania* in murine macrophages. Trends Microbiol. **6:**392–401.
5. **Bennett, C. L., A. Misslitz, L. Colledge, T. Aebischer, and C. C. Blackburn.** 2001. Silent infection of bone marrow-derived dendritic cells by *Leishmania mexicana* amastigotes. Eur. J. Immunol. **31:**876–883.
6. **Bradley, D. J.** 1987. Genetics of susceptibility and resistance in the vertebrate host, p. 551–581. *In* W. Peters and R. Killick-Kendrick (ed.), The leishmaniases in biology and medicine. Academic Press, London, United Kingdom.
7. **Cai, G., R. A. Kastelein, and C. A. Hunter.** 1999. IL-10 enhances NK cell proliferation, cytotoxicity and production of IFN-gamma when combined with IL-18. Eur. J. Immunol. **29:**2658–2665.
8. **Campbell, K. A., P. J. Ovendale, M. K. Kennedy, W. C. Fanslow, S. G. Reed, and C. R. Maliszewski.** 1996. CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. Immunity **4:**283–289.
9. **Claassen, E., and S. H. M. Jeurissen.** 1997. A step by step guide to in situ immune response analysis of lymphoid tissues by immunohistochemical methods p. 1–12. *In* D. M. Weir, L. A. Herzenberg, L. Herzenberg, and C. Blackwell (ed.), Handbook of experimental immunology. Blackwell Scientific Publications, Oxford, United Kingdom.
10. **Conceicao-Silva, F., M. Hahne, M. Schroter, J. Louis, and J. Tschopp.** 1998. The resolution of lesions induced by *Leishmania major* in mice requires a functional Fas (APO-1, CD95) pathway of cytotoxicity. Eur. J. Immunol. **28:**237–245.
11. **Convit, J., M. Ulrich, C. T. Fernandez, F. J. Tapia, G. Caceres-Dittmar, M. Castes, and A. J. Rondon.** 1993. The clinical and immunological spectrum of American cutaneous leishmaniasis. Trans. R. Soc. Trop. Med. Hyg. **87:**444–448.
12. **Desbarats, J., J. E. Stone, L. Lin, Z. F. Zakeri, G. S. Davis, L. M. Pfeiffer, R. G. Titus, and M. K. Newell.** 2000. Rapid early onset lymphocyte cell death in mice resistant, but not susceptible to *Leishmania major* infection. Apoptosis **5:**189–196.
13. **Galbiati, F., L. Rogge, and L. Adorini.** 2000. IL-12 receptor regulation in IL-12-deficient BALB/c and C57BL/6 mice. Eur. J. Immunol. **30:**29–37.
14. **Gately, M. K., L. M. Renzetti, J. Magram, A. S. Stern, L. Adorini, U. Gubler, and D. H. Presky.** 1998. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu. Rev. Immunol. **16:**495–521.
15. **Hondowicz, B. D., A. Y. Park, M. M. Elloso, and P. Scott.** 2000. Maintenance of IL-12-responsive CD4$^+$ T cells during a Th2 response in *Leishmania major*-infected mice. Eur. J. Immunol. **30:**2007–2014.
16. **Huang, F. P., D. Xu, E. O. Esfandiari, W. Sands, X. Q. Wei, and F. Y. Liew.** 1998. Mice defective in Fas are highly susceptible to *Leishmania major* infection despite elevated IL-12 synthesis, strong Th1 responses, and enhanced nitric oxide production. J. Immunol. **160:**4143–4147.
17. **Jones, D. E., L. U. Buxbaum, and P. Scott.** 2000. IL-4-independent inhibition of IL-12 responsiveness during *Leishmania amazonensis* infection. J. Immunol. **165:**364–372.
18. **Kamanaka, M., P. Yu, T. Yasui, K. Yoshida, T. Kawabe, T. Horii, T. Kishimoto, and H. Kikutani.** 1996. Protective role of CD40 in *Leishmania major* infection at two distinct phases of cell-mediated immunity. Immunity **4:**275–281.
19. **Ma, X., and G. Trinchieri.** 2001. Regulation of interleukin-12 production in antigen-presenting cells. Adv. Immunol. **79:**55–92.
20. **Mattner, F., J. Magram, J. Ferrante, P. Launois, K. Di Padova, R. Behin, M. K. Gately, J. A. Louis, and G. Alber.** 1996. Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. Eur. J. Immunol. **26:**1553–1559.
21. **Monteforte, G. M., K. Takeda, M. Rodriguez-Sosa, S. Akira, J. R. David, and A. R. Satoskar.** 2000. Genetically resistant mice lacking IL-18 gene develop Th1 response and control cutaneous *Leishmania major* infection. J. Immunol. **164:**5890–5893.
22. **Nakahira, M., H. J. Ahn, W. R. Park, P. Gao, M. Tomura, C. S. Park, T. Hamaoka, T. Ohta, M. Kurimoto, and H. Fujiwara.** 2002. Synergy of IL-12 and IL-18 for IFN-gamma gene expression: IL-12-induced STAT4 contributes to IFN-gamma promoter activation by up-regulating the binding activity of IL-18-induced activator protein 1. J. Immunol. **168:**1146–1153.
23. **Nishikomori, R., S. Gurunathan, K. Nishikomori, and W. Strober.** 2001. BALB/c mice bearing a transgenic IL-12 receptor beta 2 gene exhibit a nonhealing phenotype to *Leishmania major* infection despite intact IL-12 signaling. J. Immunol. **166:**6776–6783.
24. **Ohkusu, K., T. Yoshimoto, K. Takeda, T. Ogura, S.-I. Kashiwamura, Y. Iwakura, S. Akira, H. Okamura, and K. Nakanishi.** 2000. Potentiality of

interleukin-18 as a useful reagent for treatment and prevention of *Leishmania major* infection. Infect. Immun. **68:**2449–2456.
25. **Overath, P., and D. Harbecke.** 1993. Course of *Leishmania* infection in beta 2-microglobulin-deficient mice. Immunol. Lett. **37:**13–17.
26. **Padigel, U. M., P. J. Perrin, and J. P. Farrell.** 2001. The development of a Th1-type response and resistance to *Leishmania major* infection in the absence of CD40-CD40L costimulation. J. Immunol. **167:**5874–5879.
27. **Park, A. Y., B. D. Hondowicz, and P. Scott.** 2000. IL-12 is required to maintain a Th1 response during *Leishmania major* infection. J. Immunol. **165:**896–902.
28. **Perez-Santos, J. L., and P. Talamas-Rohana.** 2001. In vitro indomethacin administration upregulates interleukin-12 production and polarizes the immune response towards a Th1 type in susceptible BALB/c mice infected with *Leishmania mexicana*. Parasite Immunol. **23:**599–606.
29. **Reiner, S. L., and R. M. Locksley.** 1995. The regulation of immunity to *Leishmania major*. Annu. Rev. Immunol. **13:**151–177.
30. **Rodriguez-Sosa, M., G. M. Monteforte, and A. R. Satoskar.** 2001. Susceptibility to *Leishmania mexicana* infection is due to the inability to produce IL-12 rather than lack of IL-12 responsiveness. Immunol. Cell Biol. **79:**320–322.
31. **Satoskar, A., F. Brombacher, W. J. Dai, I. McInnes, F. Y. Liew, J. Alexander, and W. Walker.** 1997. SCID mice reconstituted with IL-4-deficient lymphocytes, but not immunocompetent lymphocytes, are resistant to cutaneous leishmaniasis. J. Immunol. **159:**5005–5013.
32. **Scharton-Kersten, T., L. C. Afonso, M. Wysocka, G. Trinchieri, and P. Scott.** 1995. IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development in experimental leishmaniasis. J. Immunol. **154:**5320–5330.
33. **Soong, L., J. C. Xu, I. S. Grewal, P. Kima, J. Sun, B. J. Longley, N. H. Ruddle, D. McMahon-Pratt, and R. A. Flavell.** 1996. Disruption of CD40-CD40 ligand interactions results in an enhanced susceptibility to *Leishmania amazonensis* infection. Immunity **4:**263–273.
34. **Swihart, K., U. Fruth, N. Messmer, K. Hug, R. Behin, S. Huang, G. Del Giudice, M. Aguet, and J. A. Louis.** 1995. Mice from a genetically resistant background lacking the interferon gamma receptor are susceptible to infection with *Leishmania major* but mount a polarized T helper cell 1-type $CD4^+$ T cell response. J. Exp. Med **181:**961–971.
35. **Velasco, O., S. J. Savarino, B. C. Walton, A. A. Gam, and F. A. Neva.** 1989. Diffuse cutaneous leishmaniasis in Mexico. Am. J. Trop. Med. Hyg. **41:**280–288.
36. **Vieira, L. Q., M. Goldschmidt, M. Nashleanas, K. Pfeffer, T. Mak, and P. Scott.** 1996. Mice lacking the TNF receptor p55 fail to resolve lesions caused by infection with *Leishmania major*, but control parasite replication. J. Immunol. **157:**827–835.
37. **von Stebut, E., Y. Belkaid, T. Jakob, D. L. Sacks, and M. C. Udey.** 1998. Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-*Leishmania* immunity. J. Exp. Med. **188:**1547–1552.
38. **Wei, X. Q., I. G. Charles, A. Smith, J. Ure, G. J. Feng, F. P. Huang, D. Xu, W. Muller, S. Moncada, and F. Y. Liew.** 1995. Altered immune responses in mice lacking inducible nitric oxide synthase. Nature **375:**408–411.
39. **Weinheber, N., M. Wolfram, D. Harbecke, and T. Aebischer.** 1998. Phagocytosis of *Leishmania mexicana* amastigotes by macrophages leads to a sustained suppression of IL-12 production. Eur. J. Immunol. **28:**2467–2477.
40. **Yoshida, H., S. Hamano, G. Senaldi, T. Covey, R. Faggioni, S. Mu, M. Xia, A. C. Wakeham, H. Nishina, J. Potter, C. J. Saris, and T. W. Mak.** 2001. WSX-1 is required for the initiation of Th1 responses and resistance to *L. major* infection. Immunity **15:**569–578.

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