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Disruption of the Murine Interleukin-4 Gene Inhibits Disease Progression during *Leishmania mexicana* Infection but Does Not Increase Control of *Leishmania donovani* Infection

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The growths of both cutaneous leishmaniasis and visceral leishmaniasis caused by *Leishmania mexicana* and *Leishmania donovani*, respectively, were measured in interleukin-4 (IL-4) knockout mice (IL-4−/−) and compared with those of similarly infected wild-type (IL-4+/+) control mice. While large, nonhealing, cutaneous lesions containing large numbers of parasites developed in IL-4+/+ mice subcutaneously infected with 5×10^6 *L. mexicana* amastigotes in the shaven rump, in IL-4−/− mice no lesions whatsoever developed and parasites were difficult to detect. Systemic spread and metastasis were also noted in IL-4+/+ but not IL-4−/− mice. In contrast, IL-4−/− mice infected intravenously with 10^7 *L. donovani* amastigotes were found to have consistently higher parasite burdens in their livers throughout infection than did their wild-type counterparts. However, these differences were only significant at 15 days postinfection. While the results reported here pertaining to *L. donovani* largely support previous studies, those related to *L. mexicana* provide new observations. The immunological responses of IL-4−/− and IL-4+/+ mice infected with *L. mexicana* were, therefore, examined both in vivo and in vitro. Although neither IL-4−/− nor IL-4+/+ mice infected with *L. mexicana* produced parasite-specific immunoglobulin G2a antibodies, IL-4+/+ mice, unlike IL-4−/− mice, developed significant immunoglobulin G1 antibody titers as infection progressed, indicating a Th2-influenced response in wild-type mice. In addition, IL-4−/− mice, unlike IL-4+/+ mice, developed a significant delayed-type hypersensitivity response, indicating a Th1-influenced response in IL-4−/− mice. Following in vitro stimulation, splenocytes from IL-4+/+ mice infected with *L. mexicana* displayed significantly higher antigen-specific proliferative responses than did IL-4−/− mice. However, gamma interferon production as measured from the supernatants of the in vitro splenocyte cultures of IL-4−/− mice was significantly higher than that from IL-4+/+ mice. This again would indicate a predominantly Th1-influenced response in the absence of a Th2 response in IL-4−/− mice infected with *L. mexicana*. On the other hand, at the same time point, draining lymph node cells from IL-4+/+ mice produced significantly higher quantities of IL-5 than did those from IL-4−/− mice following in vitro antigenic stimulation. These results demonstrate that, whereas IL-4 and/or Th2 lymphocyte expansion is necessary for disease progression and the inhibition of a protective response in cutaneous infection caused by *L. mexicana*, such a role for Th2 cells in visceral leishmaniasis caused by *L. donovani* cannot be demonstrated.

The leishmaniases comprise a number of diseases with a wide spectrum of clinical manifestations. Thus, for example, cutaneous leishmaniasis caused by the *Leishmania mexicana* complex and visceral leishmaniasis caused by *Leishmania donovani* are clinicopathologically quite different diseases of humans in the New and Old Worlds respectively (24). While American cutaneous leishmaniasis resulting from *L. mexicana* infection is mainly a localized disease often associated with chronic infections of the ear, visceral leishmaniasis caused by *L. donovani* is characterized by the dissemination of parasites into the spleen, liver, lymph nodes, and bone marrow (24). Although the clinical outcome of infection is undoubtedly due in part to the parasite species initiating infection, genetically controlled immunoregulatory factors operating within an individual host also influence disease progression (3, 6). Furthermore, studies of murine models of disease have shown that different genetic controls can operate within a single host to control the growth of different species of *Leishmania* even when these species may infect the same tissue sites. Thus, while the visceral growth of *Leishmania major* is totally independent of *Lsh* gene involvement (23), which controls the early growth of visceral *L. donovani* (6), the cutaneous growth of the former parasite has been shown to be under genetic and immunoregulatory controls different from those associated with the cutaneous growth of *L. mexicana* (2, 7, 26). In addition, while the vast majority of mouse strains develop healing lesions when infected subcutaneously with *L. major*, virtually all develop nonhealing lesions full of parasites when infected subcutaneously with *L. mexicana* (6).

Although numerous recent studies have clearly established the immunological mechanisms governing the growth of *L. major* in experimental cutaneous leishmaniasis (20), those immunological mechanisms controlling the visceral growth of species such as *L. donovani* and the cutaneous growth of species such as *L. mexicana* await elucidation. The control of *L. major* lesion growth in genetically resistant mice has been clearly associated with the expansion of the Th1 lymphocyte subset of the $CD4^+$ T-cell population and the production of the cytokines gamma interferon (IFN-γ) and interleukin-2 (IL-2) (12, 28). Conversely, nonhealing responses in susceptible mice have been related to the expansion of the Th2 subset and the production of cytokines such as IL-4 and IL-10 (11, 21). The few experimental studies to date on the immunological control of *L. donovani* (15) and parasites of the *L. mexi-*

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cana complex, which includes *Leishmania amazonensis* (1, 27), have indicated that the inability to generate a Th1 cell response rather than the presence of a Th2 response may be responsible for disease susceptibility.

The recent demonstration that disruption of the murine IL-4 gene blocks Th2 cytokine responses (17) has provided a tool by which to determine the precise role, if any, of Th2 cells in the growth of *Leishmania* species. Therefore, in the present study we have compared the visceral growth of *L. donovani* and the cutaneous growth of *L. mexicana* in 129/Sv × C57BL/6 mice homozygous for the disrupted IL-4 gene (IL-4−/−) with those of their age- and sex-matched wild-type (IL-4+/+) counterparts.

MATERIALS AND METHODS

Mice. IL-4−/− (129/Sv × C57BL/6)F_2 mice were generated as described previously (17). Mice used in these experiments were bred and maintained at the University of Strathclyde. Sex- and age-matched wild-type mice (IL-4+/+) of the same strain combination were used as controls in all the experiments.

Parasites. *L. mexicana* (MNYC/BZ/62/M379) was maintained by serial passage of amastigotes inoculated subcutaneously into the shaven rumps of BALB/c mice. Amastigotes for use in experimental studies were isolated from the lesions and enumerated as described previously (4). *L. donovani* (L82) was maintained by serial passage of amastigotes into hamsters. The parasites were isolated and purified from the spleens of infected hamsters as described elsewhere (14) and were used for infecting mice.

Infection protocols. (i) *L. mexicana*. In a typical experiment, groups of six sex-matched IL-4−/− and control IL-4+/+ mice, 8 to 10 weeks old, were infected with 5×10^6 *L. mexicana* amastigotes by subcutaneous inoculations into their shaven rumps. Disease progression was monitored by the measurement of lesion diameters at 2-week intervals up to 16 weeks postinfection. At regular intervals up to week 24 following infection, serial sections of the skins from incubation sites were analyzed by routine histopathological examination and parasite burdens in the spleen and liver were assessed as described by Stauber (30).

(ii) *L. donovani*. In a typical experiment, groups of 20 8- to 10-week-old IL-4−/− and IL-4+/+ mice were infected intravenously via tail vein injections with 10^7 *L. donovani* amastigotes. At 15, 30, 50, and 110 days postinfection, five mice from each group were sacrificed and parasite burdens in the liver were assessed as described previously (30).

Preparation of soluble leishmanial antigen. Soluble leishmanial antigen was prepared from stationary-phase promastigotes of *L. mexicana*. Promastigotes were washed twice in ice-cold phosphate-buffered saline (PBS) and resuspended in hypotonic buffer consisting of 10 mM Tris-HCl, 2 mM EDTA, pH 7.8, with 50 mM *N-p*-tosyl-L-lysine chlormethyl ketone (TLCK) and 15 mM leupeptin (Sigma, Poole, United Kingdom). Following 15 min of incubation on ice, the promastigotes were disrupted in a Braun homogenizer and centrifuged at 10,000 × *g* for 60 min at 4°C. The supernatant containing soluble leismanial antigen was collected and dialyzed against PBS (pH 7.4) overnight at 4°C, and the protein concentration was determined by a Bradford assay (9).

Isotype-specific enzyme-linked immunosorbent assay (ELISA). Blood was collected with heparinized capillary tubes from tail snips from *L. mexicana*-infected IL-4 knockout and wild-type control mice at various time points after infection. Plasma samples were obtained following centrifugation at 200 × *g*, and these samples were used to determine the titers of Th2-dependent immunoglobulin G1 (IgG1) and Th1-dependent IgG2a *Leishmania*-specific antibodies (29). Briefly, each well of a 96-well microtiter plate was coated with 1 μg of soluble leishmanial antigen in PBS (pH 9.0). Following a 1-hr incubation at 37°C with serially diluted test plasma (starting dilution, 1:100), plates were washed three times and either rat anti-mouse IgG1 horseradish peroxidase conjugate (dilution, 1:8,000; Southern Biotechnology Associates, Birmingham, Ala.) or rat anti-mouse IgG2a horseradish peroxidase conjugate (1:2,000 dilution; Jackson Laboratories) was applied. After a further 1 h of incubation at 37°C and three washes, tetramethyl benzidine in a sodium acetate buffer (pH 5.5) containing H_2O_2 was added. The reaction was stopped after 20 min by adding 10% H_2SO_4, and the A_{450} was measured on a Titertek Multiscan plate reader.

Induction and measurement of DTH responses. Delayed-type hypersensitivity (DTH) responses in IL-4+/+ and IL-4−/− mice were examined at week 16 following subcutaneous infection. Fifty microliters of 10^6 formalin (5%)-fixed, PBS-washed *L. mexicana* promastigotes was inoculated into the right hind footpad of each mouse, and the increase in footpad thickness was measured 48 h later with a dial-gauge micrometer. Left hind footpads were injected with sterile PBS as a negative control.

T-cell proliferation assay. IL-4+/+ and IL-4−/− mice were killed at week 16 following infection with *L. mexicana*. Their spleens and draining inguinal lymph nodes were removed aseptically, and cell suspensions were prepared by gentle teasing in RPMI 1640 supplemented with 10% fetal calf serum (heat inactivated), 2 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.05 mM β-mercaptoethanol (Gibco, Paisley, United Kingdom). The cells were centrifuged at 200 × *g* for 5 min, and erythrocytes were lysed by resuspending spleen cells in 3 ml of Boyle's solution (0.17 M Tris–0.16 M ammonium chloride; BDH Ltd., Dorset, United Kingdom) and incubating at 37°C for 3 min. Boyle's lysis was not carried out for lymph node cells. Following two washes, the viable cells were counted by trypan blue exclusion with an improved Nuebaer hemocytometer and cell suspensions were adjusted to either 5×10^6 cells/ml for spleen cells or 3×10^6 lymph node cells in complete RPMI medium. Aliquots (100 μl) containing either 5×10^5 splenocytes or 3×10^5 lymph node cells were added in triplicate to the wells of 96-well flat-bottomed tissue culture plates (Costar, Cambridge, Mass.) containing 100 μl of soluble *L. mexicana* antigen per well in different concentrations (50, 10, 5, and 0 μg/ml for splenocyte proliferation; 25 and 0 μg/ml for lymph node cell proliferation) or concanavalin A (ConA) (5 μg/ml for splenocyte proliferation; 2.5 μg/ml for lymph node cell proliferation). Following incubation at 37°C in 5% CO_2 for 60 h, 100 μl of the culture supernatant was removed from each well (stored at −70°C for cytokine assays) and replaced with 100 μl of complete medium containing the appropriate amount of antigen or ConA. At the same time, the cells were pulsed with 0.25 μCi of tritiated thymidine (specific activity, 35 Ci/mmol; ICN/Flow, High Wycombe, United Kingdom) and, following a further 12 h of incubation at 37°C, were harvested onto filter paper (ICN/Flow) with a cell harvester (Skatronas, Lier, Norway). Thymidine uptake was measured by liquid scintillation on a β-counter (Pharmacia LKB Biotech., Milton Keynes, United Kingdom) with 1 ml of Optiscint (Pharmacia Biosystems) added to the filter discs in vials (Hughes and Hughes, Somerset, United Kingdom) and counted for 5 min each.

IFN-γ and IL-5 assays. IL-5 production and IFN-γ production by stimulated (antigen or ConA) and nonstimulated cells from *L. mexicana*-infected IL4−/− and IL-4+/+ mice were measured by capture ELISA. The supernatants, at a 1/2 dilution for IFN-γ and undiluted for IL-5, were analyzed in duplicate. The culture supernatants and standards, consisting of murine recombinant IL-5 (0 to 10,000 pg/ml) or IFN-γ (0 to 7,000 pg/ml) (Cambridge Bioscience, Cambridge, United Kingdom), were added in duplicate to the wells of a microtiter plate which had been precoated 12 h previously with capture antibody (rat anti-mouse/human IL-5, clone TRFK-5, or rat anti-mouse IFN-γ, clone R4-6A2; Cambridge Bioscience, Cambridge, United Kingdom) in PBS, pH 9.0, at 4°C. Following incubation at 37°C for 120 min, the wells were washed three times with PBS-Tween (pH 7.4; 0.05% Tween 20) and biotinylated anti-IFN-γ or anti-IL-5 antibodies were added. After a further incubation at 37°C for 60 min, the plates were washed as described above and 100 μl of detection complex (streptavidin-linked alkaline phosphatase, 1:2,000 dilution) was added to each well. The plates were further incubated at 37°C for 45 min in the dark, and the wells were washed three times with PBS-Tween before the addition of 100 μl of *p*-nitrophenylphosphatase prepared in glycine buffer (pH 10.1). After a further 30 min of incubation at 37°C, the A_{405}s were measured on a Titertek Multiskan plate reader. IFN-γ and IL-5 concentrations were determined from the standard curve (regression coefficient $r = 0.99$).

Statistical significance. Student's unpaired *t* test was used to determine the statistical significance of values obtained.

RESULTS

Growth of *L. mexicana* and *L. donovani* in IL-4+/+ and IL-4−/− mice. Following subcutaneous inoculation with 5×10^6 *L. mexicana* amastigotes into the shaven rump, no lesions were found to develop at the site of inoculation or elsewhere in any IL-4−/− mice at any time during the study (Fig. 1). By contrast, all similarly infected wild-type control mice developed progressively growing nonhealing lesions throughout the period of study (Fig. 1). These results were completely reproducible in five separate experiments (results not shown), and there were no sex-influenced differences in the growth of this parasite in these mice. Only occasional parasites could be detected histologically at the site of infection in IL-4−/− mice and no visceralization could be detected (results not shown) up to 24 weeks postinfection with *L. mexicana*. On the other hand, the systemic spread of parasites and the development of metastatic lesions at the extremities were often observed in IL-4+/+ mice as infection progressed. Following intravenous inoculation of 10^7 *L. donovani* amastigotes into IL-4+/+ and IL-4−/− mice, the parasite growth in the liver was monitored from day 15 through day 110 postinfection. Surprisingly, at all time points more *L. donovani* amastigotes were observed in the IL-4+/+ wild-type control mice than in the IL-4-deficient mice. However, the difference in parasite burdens between the

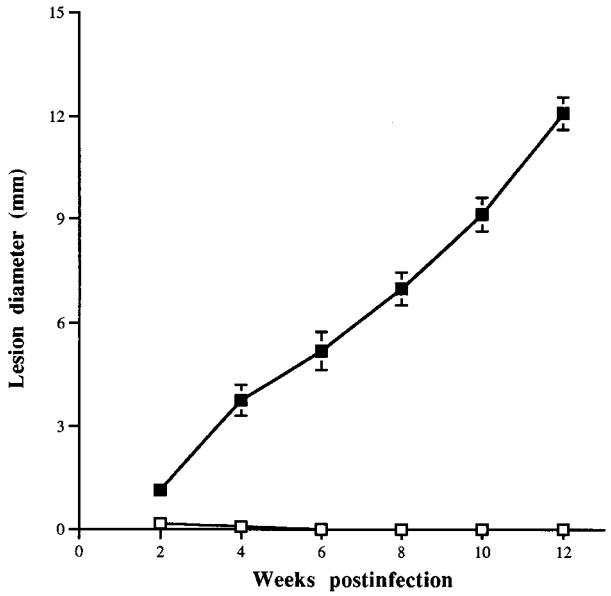


FIG. 1. Mean lesion growths in the shaven rumps of IL-4+/+ (■) and IL-4−/− (□) mice infected with *L. mexicana*. Six animals were used in each group. Bars show standard errors of the mean.

two groups was significant only during the early stage of infection, at day 15 ($P < 0.05$) (Fig. 2).

Antibody response to *L. mexicana* and *L. donovani* in IL-4+/+ and IL-4−/− mice. The specific antibody response measured during *L. mexicana* infection was barely detectable in any of the mice until week 8 postinfection (Fig. 3). At this time point and thereafter, neither IL-4+/+ nor IL-4−/− mice produced any measurable quantities of *L. mexicana*-specific IgG2a. However, IL-4+/+ wild-type control mice produced significant quantities of *L. mexicana*-specific IgG1 in the absence of any antibody production by IL-4−/− mice. At week 16 following infection, although IL-4+/+ mice had significantly higher titers of antigen-specific IgG1 than at week 8 ($P < 0.05$), IL-4−/− mice still had no detectable antibody response (Fig. 3). By contrast, *Leishmania*-specific IgG2a, though not IgG1, was detected in plasma samples from both IL-4+/+ and IL-4−/− mice at day 50 following infection with *L. donovani* (data not shown).

In vitro and in vivo T-cell responsiveness. As IL-4 deficiency did not confer increased resistance to *L. donovani* as previous reports would suggest (15), the following studies were confined to *L. mexicana*-infected mice in which disruption of the IL-4 gene induced resistance. At week 16 postinfection, resistant IL-4−/− mice displayed a significant DTH response ($P < 0.05$) to antigen at 48 h postinoculation with formalin-fixed parasites (Fig. 4). No significant DTH response was detected in IL-4+/+ mice at this time (Fig. 4). Splenocytes from *L. mexicana*-infected susceptible IL-4+/+ mice as well as resistant IL-4−/− mice displayed significant ConA-induced ($P < 0.025$) and *L. mexicana*-specific proliferative responses ($P < 0.025$) (Fig. 5). However, ConA-induced and antigen-specific proliferative responses were significantly greater ($P < 0.025$) in IL-4+/+ mice than in IL-4−/− mice (Fig. 5).

IFN-γ and IL-5 assays. The supernatants from the splenocyte assays described above were analyzed by ELISA for the presence of the Th1-associated cytokine IFN-γ and the Th2-associated cytokine IL-5 (Fig. 6). While spleen cells from *L. mexicana*-infected IL-4−/− mice produced significant quantities of IFN-γ ($P < 0.025$) following stimulation with *L. mexicana* antigen, no IFN-γ above basal level could be detected in the supernatants from antigen-stimulated IL-4+/+ spleen cells (Fig. 6b). No differences in IFN-γ or IL-5 production could be detected from unstimulated splenocyte cultures from either IL-4+/+ or IL-4−/− *L. mexicana*-infected mice (Fig. 6a). Similarly, no increased IL-5 production above basal levels could be detected in the supernatants from antigen-stimulated splenocytes from either IL-4+/+ or IL-4−/− *L. mexicana*-infected mice (Fig. 6b). However, the supernatants from ConA-stimulated splenocytes from *L. mexicana*-infected IL-4+/+ mice contained significantly higher quantities of IL-5 ($P < 0.025$) than did those derived from IL-4−/− mice (Fig. 6c). As splenocytes from *L. mexicana*-infected IL-4+/+ and IL-4−/− mice failed to show significant differences in IL-5 production following in vitro antigenic stimulation, the supernatants from assays with draining lymph node cells were analyzed for the presence of IL-5. The draining lymph node cells from IL-4+/+ mice produced significantly higher quantities of IL-5 ($P < 0.05$) than did those from IL-4−/− mice following in vitro stimulation with ConA and *L. mexicana* antigen (Fig. 7).

DISCUSSION

The present study quite clearly demonstrates that the presence of IL-4 and, perhaps as a consequence, the ability to generate Th2 responses are essential for the rapid lesion growth and nonhealing responses of 129/Sv × C57BL/6 mice following cutaneous infection with *L. mexicana*. This indicates that although *L. mexicana* has been shown to be under host genetic and immunoregulatory controls different from those of *L. major* (2, 26), the nonhealing response to both parasites is ultimately dependent on the downregulatory effect of Th2 products on protective immunity, which has been shown to be mediated through IFN-γ production (1, 12, 27, 28). However, the results also show that the Th2 subset does not play a disease-exacerbating role during visceral *L. donovani* infections, which fully supports previous studies of *L. donovani* (15).

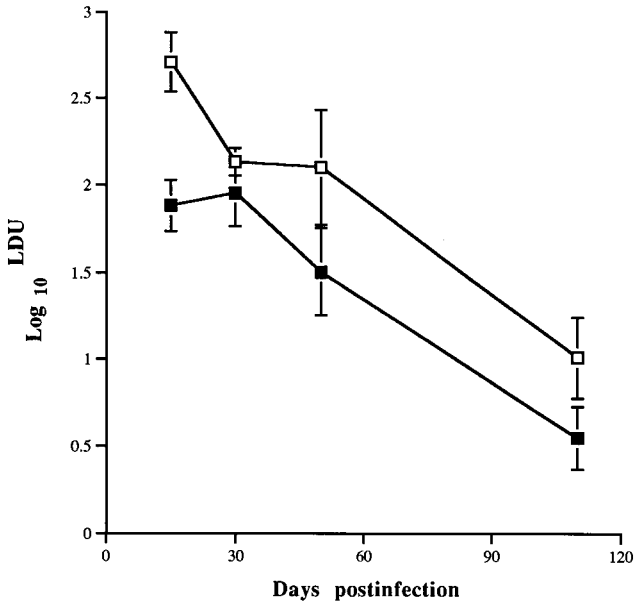
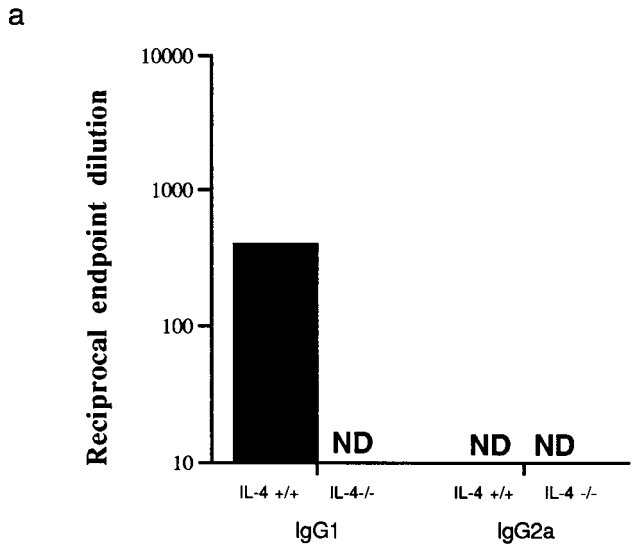


FIG. 2. Liver parasite burdens in IL-4+/+ (■) and IL-4−/− (□) mice infected with *L. donovani*. Data are expressed as mean $\log_{10}$ Leishman Donovan units (LDU) ± standard errors of the mean (bars).



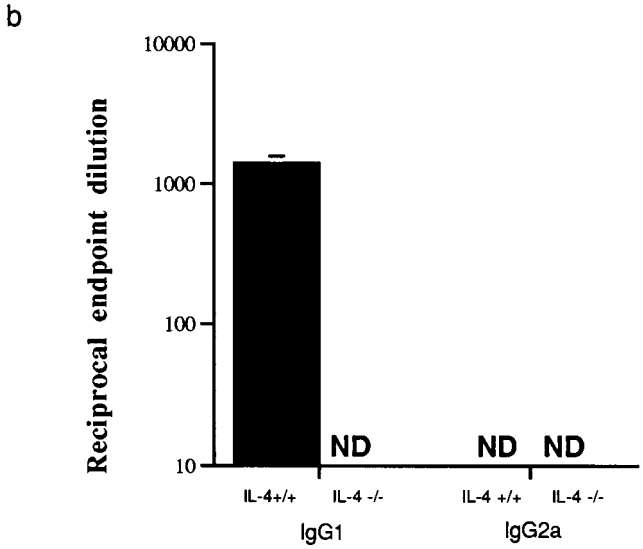


FIG. 3. *L. mexicana*-specific IgG1 and IgG2a production in IL-4+/+ and IL-4−/− mice at week 8 (a) and week 16 (b) postinfection presented as mean reciprocal endpoint dilutions. Bar in panel b shows ± standard error of the mean. ND, no antibody detected at the 1:100 dilution.

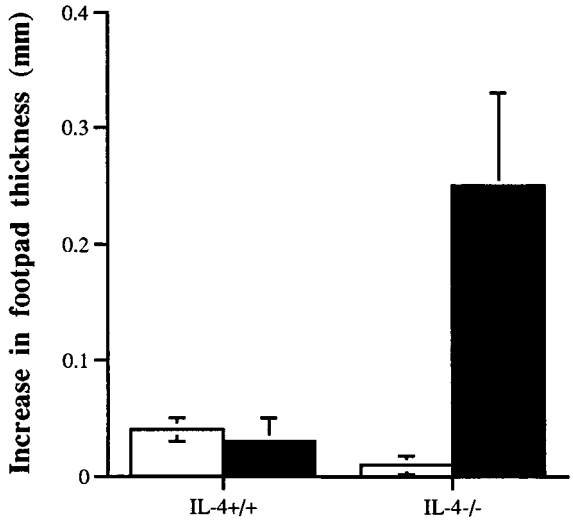


FIG. 4. DTH responses at week 16 postinfection with *L. mexicana* in IL-4+/+ and IL-4−/− mice. Footpad thickness was measured in infected mice (closed columns) and control mice (open columns) 48 h after inoculation with formalin-fixed parasites. Data are expressed as means ± standard errors of the mean (bars).

Although the murine model of *L. donovani* infection has provided valuable information on the genetic control of disease (3), a major disadvantage of this model is that the direct inoculation of parasites intravenously effectively bypasses the peripheral lymphoid tissue. Hepatic antigen-presenting cells are known to preferentially stimulate Th1 but not Th2 cells (22), a phenomenon clearly demonstrated by the successful vaccination of BALB/c mice against cutaneous *L. major* infection by the intravenous but not the subcutaneous route of administration (19). It is perhaps not surprising, therefore, that lymphocytes from mice with even large *L. donovani* burdens failed to produce the Th2 cytokines IL-4 and IL-5 following in vitro stimulation (15). Nevertheless, the lack of a role for Th2 cells in the disease progression of visceral leishmaniasis in mice demonstrated in this and other studies (15) is mirrored in some recent studies with humans. Thus, not only have parasite-reactive Th1 and Th2 clones been isolated from individuals who have recovered from visceral leishmaniasis (18), but both IL-4 and IFN-γ have been shown to be produced by peripheral blood monocytes from these individuals (16). Furthermore, in untreated patients, both IL-10 and IFN-γ mRNAs were greatly upregulated as measured in bone marrow aspirates (13). These observations clearly emphasize the apparent lack of a clear-cut role for Th1 or Th2 cell activation in either disease cure or noncure in visceral leishmaniasis. In fact, IL-4−/− mice were slightly, though significantly, more susceptible to *L. donovani* in the early stages of infection than were their wild-type counterparts, indicating a potentially protective role for IL-4. Un-

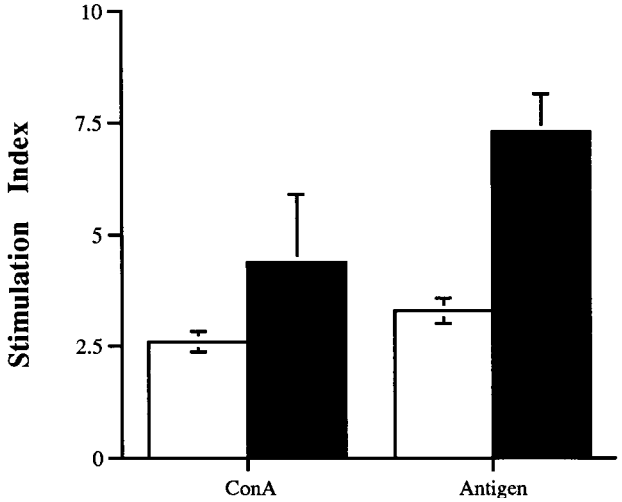


FIG. 5. ConA (5 μg/ml)-induced and *L. mexicana* soluble antigen (50 μg/ml)-induced splenocyte proliferation responses in *L. mexicana*-infected IL-4+/+ mice (closed columns) and IL-4−/− mice (open columns) at week 16 postinfection. Data are expressed as means ± standard errors of the mean (bars).

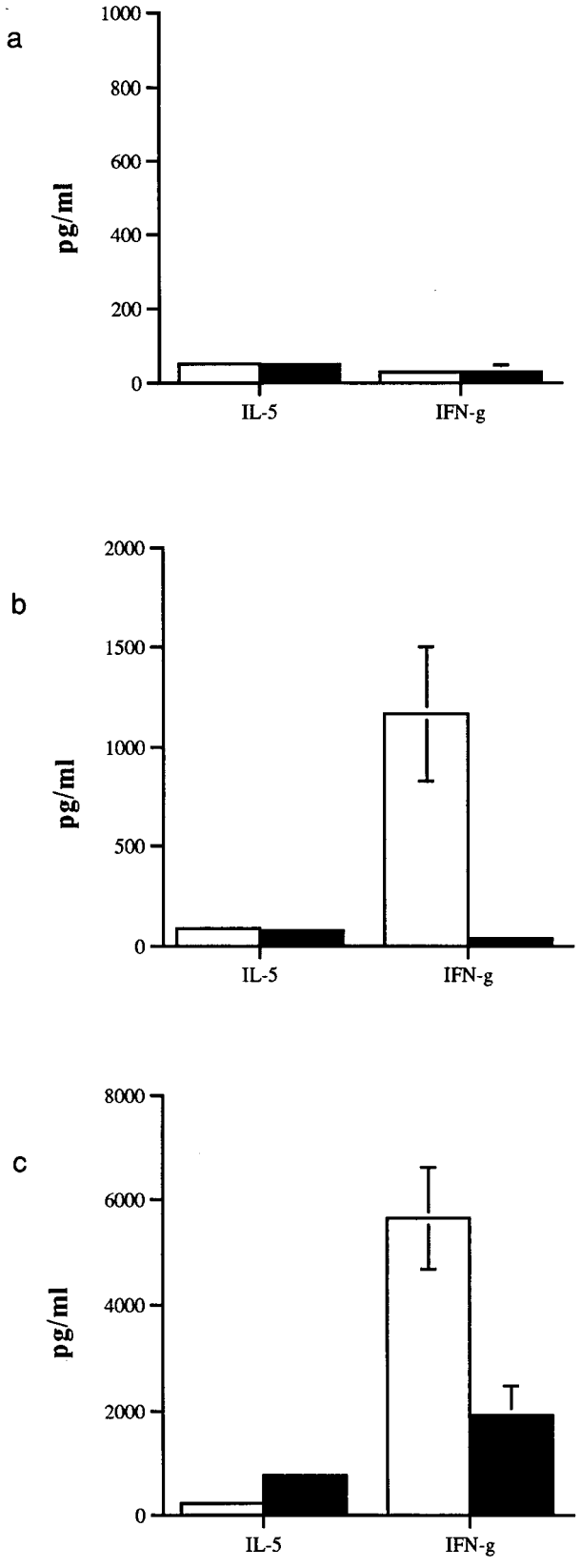


FIG. 6. IL-5 and IFN-γ (IFN-g) production by splenocytes from *L. mexicana*-infected IL-4+/+ mice (closed columns) and IL-4−/− mice (open columns) at week 16 postinfection. Results in the absence of antigen (a), in the presence of *L. mexicana* soluble antigen (50 μg/ml) (b), and in the presence of ConA (5 μg/ml) (c) are shown.

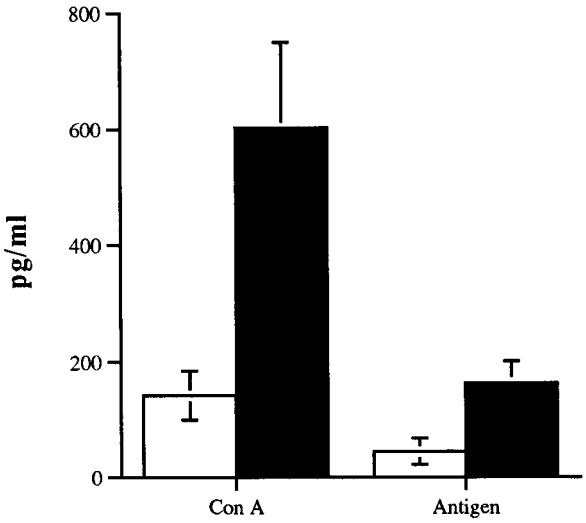


FIG. 7. In vitro IL-5 production by inguinal lymph node cells from *L. mexicana*-infected IL-4+/+ mice (closed columns) and IL-4−/− mice (open columns) at week 16 postinfection. Results in the presence of ConA (2.5 μg/ml) or *L. mexicana* soluble antigen (25 μg/ml) are shown.

der certain circumstances IL-4 may act synergistically with IFN-γ to induce leishmanicidal activity (5, 8).

Previous studies by us and others (1, 27) have clearly demonstrated that acquired protective immunity against the *L. mexicana* complex, as with *L. major*, is ultimately dependent on the generation of a Th1 response and IFN-γ production. However, while many studies indicate a disease-exacerbating role for IL-4 and Th2 cells in cutaneous infections produced by *L. major* (11, 21), the evidence for a similar role for this T-cell subset and its products in cutaneous *L. mexicana* infections is limited. For example, mRNA transcripts for IL-4 and other Th2 cytokines have been shown to be present in both resistant female and susceptible male DBA/2 mice infected with *L. mexicana* (27). In addition, neutralizing IL-4 monoclonal antibodies had little effect on the cutaneous growth of the closely related parasite *L. amazonensis* in C57BL/10 mice (1). However, in the same study neutralizing IL-4 antibodies did limit the growth of this parasite in BALB/c mice (1). This and other studies (2) would suggest that perhaps the susceptibility of BALB/c mice to parasites of the *L. mexicana* complex is under the control of immunoregulatory pathways different from those operating in other mouse strains susceptible to these parasites. Furthermore, while mouse strains such as CBA/Ca, C3H/He, C57BL/6, C57BL/10 ScSn, A, and ATL, as well as BALB/c mice, go on to develop nonhealing lesions when infected subcutaneously with *L. mexicana*, all these strains, unlike the BALB/c mouse, develop small lesions which heal following subcutaneous infection with *L. major* (6). Nevertheless, it is quite evident from the present study with IL-4−/− mice of the 129/Sv × C57BL/6 background, that IL-4 and/or Th2 cells are also of paramount importance in mediating susceptibility to cutaneous *L. mexicana* infection.

In addition to demonstrating cutaneous growth, *L. mexicana* has been shown to disseminate systematically to the viscera and further cutaneous sites (25) to a degree independent of the size of the initial lesion. While parasite dissemination was observed in IL-4+/+ mice, no parasites were detected in any tissue site other than the site of parasite inoculation in IL-4−/− mice. Thus, disruption of the IL-4 gene not only controls the cutaneous growth of *L. mexicana* in 129/Sv × C57BL/6

mice but also inhibits the metastatic spread of the parasite. Total resistance to the growth of *L. mexicana* in IL-4−/− mice was observed in both sexes and not confined to females as observed for the *Scl-2* gene-controlled resistance to *L. mexicana* in the DBA/2 mouse strain (26).

Surprisingly, the classical in vitro correlate of DTH, antigen-specific lymphocyte proliferation, as well as mitogen-induced proliferation, was suppressed in IL-4−/− mice compared with that in IL-4+/+ mice. IL-4, however, is an important T-cell growth factor (21), and the value of the lymphocyte proliferation assay as a measure of protective immunity must be questioned. What is perhaps more relevant to the disease state or outcome of infection is that cytokine production is measured. The present study also clearly demonstrates that in a susceptible mouse strain, the disruption of the IL-4 gene can confer protective immunity by polarizing the immune response in *L. mexicana* to a Th1 type, as characterized by a positive DTH skin test response and IFN-γ production. Conversely, the nonhealing response in wild-type IL-4+/+ mice is associated with IL-4-mediated Th2 cell activation as measured by the production of IL-5 and the IgG1 antibody isotype. It is, perhaps, significant that the correlation between disease severity and the presence of IL-4 demonstrated for mice in this study is mirrored in various forms of human American cutaneous leishmaniasis for which large amounts of mRNA for IL-4 have been associated with disease progression (10).

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