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In Vivo Alterations in Cytokine Production following Interleukin-12 (IL-12) and Anti-IL-4 Antibody Treatment of CB6F1 Mice with Chronic Cutaneous Leishmaniasis

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CB6F1 mice exhibit intermediate resistance to infection with *Leishmania major* compared to their highly susceptible (BALB/c) and resistant (C57BL/6) parental strains. Unlike the C57BL/6 and BALB/c strains, which rapidly develop dominant Th1- or Th2-type responses, respectively, after infection, CB6F1 mice develop responses in which both Th1- and Th2-type cytokines are elevated through at least the first month of infection before Th1 responses become dominant as cutaneous lesions gradually heal. We have examined the effects of interleukin-12 (IL-12) and/or anti-IL-4 antibody treatment on cytokine production and the course of disease in CB6F1 mice with chronic *L. major* infections. When administered at 1 month of infection, IL-12 treatment led to a rapid decrease in mRNA levels for IL-4 within parasitized lesions and a moderate increase in gamma interferon (IFN-γ) transcript levels in lymph nodes draining the site of infection. When IL-12 and anti-IL-4 antibody were administered together, they induced a marked decrease in IL-4 and transforming growth factor β mRNA expression within lesions and a more dramatic increase in lymph node IFN-γ transcript levels within 4 days after treatment. In comparison, similar treatment of infected BALB/c mice led to only a moderate increase in IFN-γ transcripts but no decrease in mRNA levels for Th2-type cytokines. Treatment of CB6F1 mice with either IL-12 or anti-IL-4 antibody had no significant effect on the subsequent course of infection, whereas combined IL-12 plus anti-IL-4 treatment resulted in a decrease in lesion size and parasite numbers and a shift towards a Th1-dominant response. These results suggest that the immediate effects of cytokine or anti-cytokine therapy may be predictive of the long-term effects on the course of infection and that down-regulation of Th-2 type cytokines may be critical to the development of a Th1-dominant response.

Leishmania major infections in inbred strains of mice have been utilized extensively as models for the study of $CD4^+$ cell differentiation and regulation. A majority of inbred mouse strains, including C57BL/6, develop infections which spontaneously heal, while a few strains, such as BALB/c, develop a severe form of progressive disease (5, 12). Disease outcome is determined by the type of immune response generated during infection; resistant mice develop a T helper type 1 (Th1) response characterized by the production of high amounts of gamma interferon (IFN-γ), while susceptible, nonhealing strains develop nonprotective Th2-type responses characterized by the elevated production of cytokines such as interleukin-4 (IL-4) but not IFN-γ (9, 10, 23). Several laboratories have shown that treatment of mice with cytokines or anti-cytokine antibodies, including antibody to IL-4, can reverse the expected disease phenotype of both resistant or susceptible strains of mice (4, 21). These treatments are most effective if given at the time of infection and presumably act to direct the differentiation of naive T helper cells to either Th1 or Th2 effector cells (4, 21). It has proven more difficult to switch an established Th2-type response to a Th1-type response in mice with established infection with either cytokine or anti-cytokine therapy. However, we have shown that BALB/c mice treated with IL-12, in combination with an anti leishmanial drug, sodium stibogluconate, will develop Th1-type responses and heal, even when treatment is delayed until the third or fourth week of infection (16).

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IL-12 has been shown to enhance IFN-γ production by NK cells and activated T cells and to promote Th1 cell development (27). When administered at the time of parasite inoculation, IL-12 promotes the development of a Th1-type response and healing of an *L. major* infection in normally susceptible BALB/c mice (11, 26). However, when administered after 1 week of infection, IL-12 fails to induce healing or significantly alter established patterns of cytokine production (26). IL-12 treatment initiated at the start of infection in BALB/c mice has been shown to suppress IL-4 production, an effect which appears to be independent of IFN-γ production (28). In contrast, IL-12 treatment of BALB/c mice at 2 weeks of infection enhanced, rather than suppressed, IL-4 production, an effect which was also IFN-γ independent (28), suggesting that populations of differentiated Th2-type cells which predominate in infected BALB/c mice are resistant to IL-12-mediated suppression of IL-4 production. We have examined the effects of IL-12 therapy in another murine model of cutaneous leishmaniasis, namely, *L. major*-infected CB6F1 mice, which are intermediate in resistance between the parental BALB/c and C57BL/6 strains. When CB6F1 mice are given inoculations in the footpad, they develop chronic infections which eventually heal over a period of 4 to 6 months (18). Importantly, cells from these mice produce high levels of both Th1- and Th2-type cytokines through at least the first month of infection (18). The development of a mixed Th1/Th2 response might enable CB6F1 mice to respond differently to therapy than does the susceptible parental BALB/c strain. Here we show that both IL-12 treatment and anti-IL-4 monoclonal antibody (MAb) treatment of CB6F1 mice induce rapid changes in the levels of cytokine mRNA transcripts within infected

lesions and draining lymph nodes and that combined IL-12 and anti-IL-4 treatment can induce a Th1-dominant response and rapid healing in chronically infected mice.

MATERIALS AND METHODS

Parasites and animals. Female BALB/cByJ (BALB/c) and (BALB/c × C57BL/6)F_1 (CB6F1) mice were obtained from The Jackson Laboratory (Bar Harbor, Maine) and were 5 to 7 weeks of age at the time of infection. The clone of *L. major* (WHO MHOM/IL/80/Friedlin) used in this study was maintained in Grace's insect cell culture medium (GIBCO Laboratories, Grand Island, N.Y.) containing 20% fetal bovine serum, 2 mM L-glutamine, 100 mg of streptomycin per ml, and 100 U of penicillin G-potassium per ml. Soluble leishmanial antigen (SLA) was prepared as previously described and was used at a concentration of 50 μg/ml (24).

Infections and treatment protocol. Mice were inoculated in one hind footpad with 5×10^5 metacyclic promastigotes selected from stationary-phase cultures with *Arachis hypogaea* agglutinin (Sigma Chemical Co., St. Louis, Mo.) as previously described (20). Lesion size was measured with a dial caliper (L. S. Starrett Co., Athol, Mass.) and expressed as the difference in thickness between the infected footpad and contralateral footpad. The degree of infection in parasitized footpad lesions was determined by limiting dilution analysis of homogenized tissue, as described elsewhere (4). At 4 weeks of infection, individual groups of mice received the following treatments: recombinant murine IL-12 as a single intraperitoneal injection of 0.75 μg in phosphate-buffered saline (PBS), 5 mg of anti-IL-4 MAb 11B11 intraperitoneally, or both IL-12 (0.75 μg) and 11B11 (5 mg) intraperitoneally. In certain experiments, control mice received 5 mg of normal rat immunoglobulin G (IgG) intraperitoneally.

Collection of tissues. Infected mice were sacrificed at designated times after treatment and tissues were collected. Footpad lesion tissues or popliteal lymph node tissues draining the site of footpad infections were homogenized in RNAzol (Biotecx Laboratories, Houston, Tex.) for preparation of total RNA. Total tissue RNA from noninfected mice was prepared in a similar manner. In some studies, single-cell suspensions of popliteal lymph nodes from infected mice were prepared as previously described.

Reverse transcription-PCR (RT-PCR). Analysis of mRNA levels in infected tissues was performed as previously described (18). Nucleotide sequences of primers and probes for IFN-γ, IL-4, IL-10, hypoxanthine phosphoribosyltransferase (HPRT), and the small subunit rRNA for *L. major* were as previously published (7, 16, 18, 29). Primer and probe sequences for TGF-β1 were provided by Tom Wynn, National Institutes of Health, and were as follows: sense, CTC CCACTCCCGTGGCTTCTA; antisense, AGTTCCACATGTTGCTCCACAC TTG; probe, CTACTATGCTAAAGAGGTCACCCCGG. Different numbers of PCR cycles were selected to give a positive signal for each cytokine within lesion and lymph node tissue and to fit on the linear portion of positive cDNA standard curves. PCR products were applied to Hybond-N+ membranes using a slot blot apparatus after denaturation in 0.4 M NaOH–25 mM EDTA and neutralization in 1 M Tris, pH 8.0. Membranes were then hybridized with oligonucleotide probes, and specific hybridization was detected by using an enhanced chemiluminesence system (Amersham, Arlington Heights, Ill.). Chemiluminescent signals were analyzed according to published procedures, and the percent increase or decrease in tissue mRNA levels was compared between groups of treated and nontreated infected mice.

Antibody isotype assays. Levels of total serum IgE as well as parasite-specific IgG1 and IgG2a were analyzed by enzyme-linked immunosorbent assay (ELISA) as previously described (18).

ELISPOT assays. Single cell suspensions were prepared from lymph nodes, and serial dilutions of these cells were cultured in duplicate in 96-well tissue culture plates coated with MAb to either IFN-γ (R46A2) or IL-4 (11B11) starting at 10^6 cells per 100 μl in RPMI 1640 (GIBCO) containing 5% fetal bovine serum, antibiotics and SLA (20 μg). After overnight incubation at 37°C, plates were washed with PBS and incubated for 1 h with biotinylated BVD6-24G2 (anti-IL-4) or a rabbit antiserum against IFN-γ. After washing with PBS, plates were incubated for an additional hour with streptavidin-conjugated alkaline phosphatase or an alkaline phosphatase-conjugated donkey anti-rabbit antibody (Jackson ImmunoResearch, West Grove, Pa.). Color was developed with 5-bromo-4-chloro-3-indolylphosphate toluidinium in 0.1 M 2-amino-2 methyl-1-propanol buffer (Sigma) in 0.6% SeaPlaque agarose (FMC Bioproducts). Following solidification of the agarose, individual spots were counted with an dissecting microscope.

Statistical analysis. Data were analyzed for statistical significance by the unpaired Student's *t* test. Results were considered significant at a P *of* <0.05.

RESULTS

Acute effects of IL-12 and/or anti-IL-4 antibody treatment in CB6F1 mice with chronic *L. major* infections. We have previously shown that CB6F1 mice inoculated with *L. major* produce elevated levels of both Th1- and Th2-type cytokines through at least the first month of infection (18). Since we were interested in modifying an established immune response following injection of recombinant IL-12 or anti-IL-4 antibody, we first determined that mRNA expression for various cytokines was increased within infected lesion tissue prior to treatment. Cytokine mRNA expression was assessed in footpad lesion tissues of CB6F1 mice infected for 4 weeks with *L. major* and compared to mRNA expression in footpad tissues from normal mice. As can be seen in Fig. 1, mRNA levels for IFN-γ, IL-4, IL-10, and transforming growth factor β (TGF-β) were all increased within infected lesions. When compared to normal mice, mRNA levels for IFN-γ were increased by about 12-fold, while mRNA levels for IL-4 and IL-10 were increased by approximately 6- and 13-fold, respectively. The fold increase in TGF-β mRNA expression could not be calculated since mRNA for this cytokine was not detected in normal footpad tissue at the level of PCR amplification used in this study.

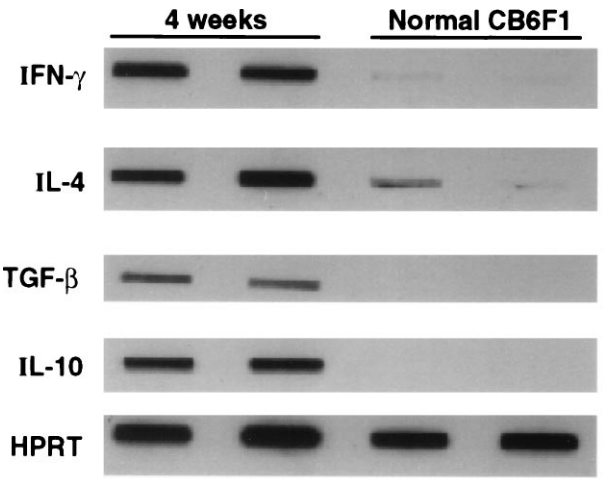

FIG. 1. RT-PCR analysis of IFN-γ, IL-4, TGF-β, and IL-10 gene expression in footpad tissue from normal CB6F1 mice and CB6F1 mice infected for 4 weeks with *L. major*. Total RNA was reverse transcribed, amplified by PCR using gene-specific primers, and blotted onto nylon filters as described in Materials and Methods. The level of HPRT mRNA in each sample served as a control for mRNA integrity and content.

To assess the short-term effects of IL-12 and/or anti-IL-4 treatment on the immune response, groups of 4-week-infected mice received a single intraperitoneal injection of IL-12 (0.75 μg) and/or 11B11 MAb (5 mg) and were sacrificed 4 days after treatment to assess the effects on lesion cytokine production. Figure 2 illustrates the effects of treatment on lesion transcript levels in groups of mice from one experiment, while Fig. 3 shows the average alteration in transcript levels from three separate experiments. IL-12, when given alone, had little effect on mRNA expression for IFN-γ, IL-10, or TGF-β within footpad lesions but did induce a significant decrease (70%) in IL-4 mRNA expression within parasitized lesion tissue (Fig. 3A). Anti-IL-4 treatment, alone, resulted in a moderate decrease in lesion mRNA levels for IL-4 but had no effect on IFN-γ and only marginal effects on IL-10 and TGF-β mRNA expression (Fig. 3A). When IL-12 and anti-IL-4 antibody were administered together, they induced a rapid and significant decrease in both IL-4 and TGF-β mRNA expression. Although IFN-γ mRNA levels were not altered within lesions, message levels for *L. major* rRNA were decreased by 65%, suggesting that this combined treatment resulted in a reduction in lesion parasite numbers.

The effect of IL-12 and/or anti-IL-4 treatment on cytokine message levels in draining lymph nodes was also examined.

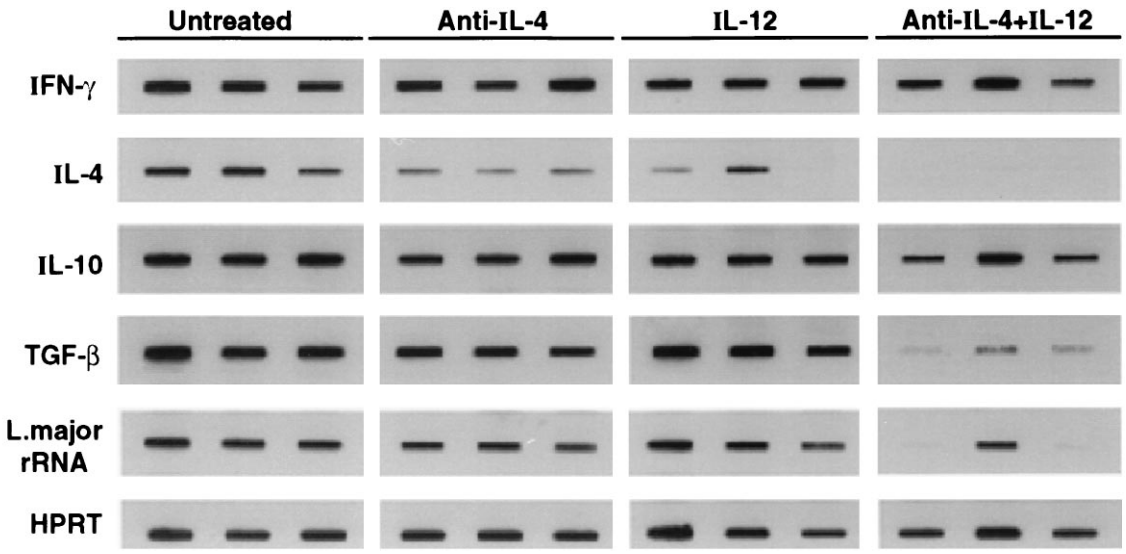

FIG. 2. RT-PCR analysis of IFN-γ, IL-4, IL-10, TGF-β, *L. major* rRNA, and HPRT gene expression in footpad lesions of infected CB6F1 mice. Groups of mice infected for 4 weeks with *L. major* were treated with a single injection of either IL-12 (0.7 mg) or anti-IL-4 MAb (5 mg) or with both IL-12 and anti-IL-4 MAb. Gene expression in parasitized lesions was determined 4 days after treatment.

Although neither treatment suppressed IL-4 or TGF-β message levels to the degree observed in footpad lesion tissue, these treatments had a marked effect on IFN-γ transcripts which was not noted in infected lesions (Fig. 3B). IL-12, alone, induced a twofold increase in IFN-γ transcript levels, while mRNA levels in lymph nodes of mice treated with IL-12 plus anti-IL-4 MAb were increased more than fourfold (Fig. 3B). The production of IFN-γ protein by spleen cells from these same mice was also examined by ELISA, and compared with results for untreated control mice, IL-12 induced a 3.5-fold increase in IFN-γ production while spleen cells from mice treated with IL-12 plus anti-IL-4 MAb produced 11-fold more IFN-γ (data not shown).

Effect of IL-12 and/or anti-IL-4 antibody treatment in BALB/c mice. In contrast to CB6F1 mice, which develop a mixed Th1/Th2-type response, susceptible BALB/c mice develop a highly polarized Th2-type response during infection with *L. major*. We and others have reported that IL-12 treatment of BALB/c mice during the chronic stage of infection will not induce healing (16, 26, 28). It has also been shown that treatment with IL-12 at the initiation of infection will induce IFN-γ and suppress IL-4, whereas treatment of mice at day 14 of infection induced both IFN-γ and IL-4 production by cells in lymph nodes draining the site of infection (28). We examined the effects of IL-12 and/or anti-IL-4 treatment on lesion cytokine production in 4-week-infected mice as outlined above. In contrast to CB6F1 mice, neither IL-12 treatment alone nor IL-12 in combination with anti-IL-4 MAb suppressed IL-4 or TGF-β within infected lesions or draining lymph nodes (Fig. 4). IFN-γ mRNA levels were slightly increased in footpad and

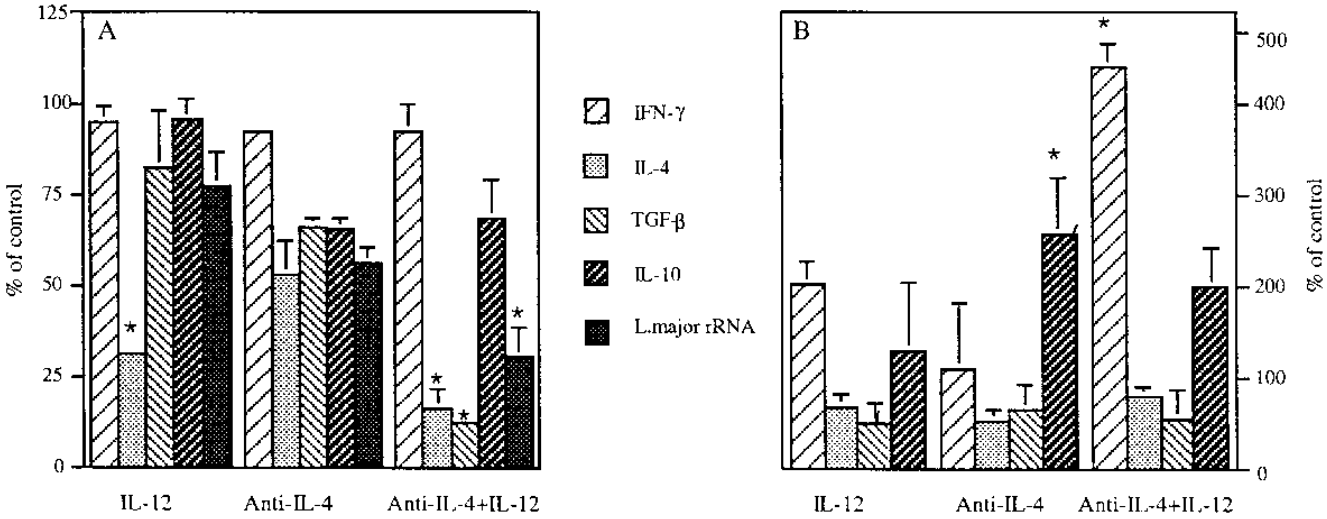

FIG. 3. Percent change in mRNA levels within footpad lesions (A) and draining lymph nodes (B) from infected CB6F1 mice. Mice were treated with IL-12, anti-IL-4 MAb, or both IL-12 and anti-IL-4 MAb at 4 weeks of infection as described in the legend to Fig. 2. Gene expression was determined on day 4 following treatment. Values represent percent change in mRNA levels in treated mice relative to mRNA levels in untreated control mice, which were designated as 100%. Each value is the mean (± standard error [SE]) change in mRNA levels of 10 mice from three separate experiments. *, $P < 0.05$ compared to untreated control mice.

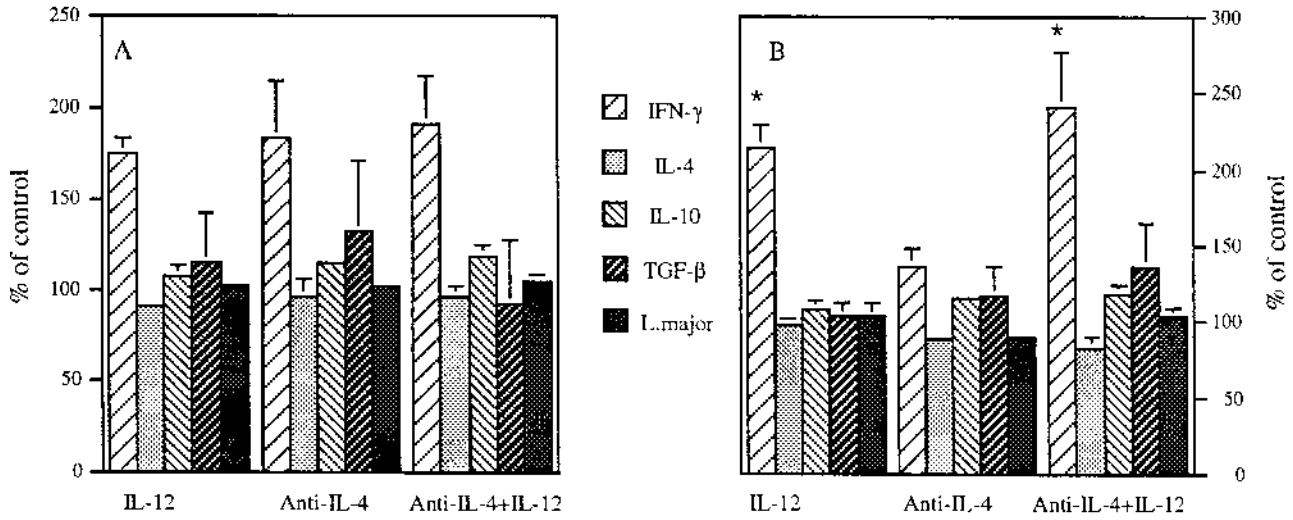

FIG. 4. Percent change in mRNA levels within footpad lesions (A) and draining lymph nodes (B) from infected BALB/c mice. Mice were treated with IL-12, anti-IL-4 MAb, or both IL-12 and anti-IL-4 MAb at 4 weeks of infection as described in the legend to Fig. 2. Gene expression was determined on day 4 following treatment. Values represent percent change in mRNA levels relative to mRNA levels in untreated control mice, which were designated as 100%. *, $P < 0.05$ compared to untreated control mice.

lymph node tissues of groups of mice treated with IL-12 or anti-IL-4 MAb, but combined treatment did not increase transcript levels above those noted in the individual treatment groups (Fig. 4).

Effects of IL-12 and/or anti-IL-4 antibody treatment on the course of infection in CB6F1 mice. In order to assess the long-term effects of treatment on the immune response and ultimate course of disease, groups of mice were injected with IL-12 and/or 11B11 MAb at 4 weeks of infection as described above and lesion size was monitored for the next 6 weeks. As can be seen in Fig. 5, anti-IL-4 treatment, alone, had no significant effect on the course of infection since lesion development progressed at a rate similar to that observed in control mice. IL-12 treatment, in contrast, appeared to slow the course of lesion development. However, the effects were only temporary since by 5 weeks posttreatment, lesion size in the IL-12-treated group was not significantly different from that in controls. Combined treatment with IL-12 and anti-IL-4 antibody had a much more dramatic effect on lesion progression, as evidenced by the fact that lesion size did not increase in this group beyond 2 weeks posttreatment and lesions remained significantly smaller than control lesions throughout the study period. The protective effect of IL-12 plus anti-IL-4 MAb treatment is further supported by the fact that parasite numbers in the lesions of mice receiving the combined treatment were significantly reduced compared to those in the individual treatment groups and the nontreated control group (Fig. 6).

Although neither IL-12 nor anti-IL-4 treatment, alone, significantly altered the course of an *L. major* infection, each treatment did modify the resulting immune response. Serum IgE levels were significantly reduced in IL-12-treated mice and reduced even further in mice receiving anti-IL-4 antibody (Fig. 7), consistent with the IL-4 dependence of IgE production (6). Neither IL-12 nor anti-IL-4 treatment, alone, had a measurable effect of the production of parasite-specific IgG2a, a Th1-associated antibody isotype (Fig. 7), but mice receiving either of these treatments had lower levels of anti-parasite IgG1, an isotype generally associated with Th2-type responses (data not shown). Mice treated with both IL-12 and anti-IL-4 antibody had the lowest serum IgE levels and highest titers of parasite-specific IgG2a, consistent with the development of a Th1-type response (Fig. 7). With respect to cytokine-producing cells, mice treated with anti-IL-4 antibody and sacrificed at 6 weeks posttreatment had similar frequencies of IL-4- and IFN-γ-producing cells, suggesting that anti-IL-4 treatment, alone, did not promote a Th1-dominant response (Fig. 8). Mice treated with IL-12, alone, had a higher frequency of IFN-γ-producing

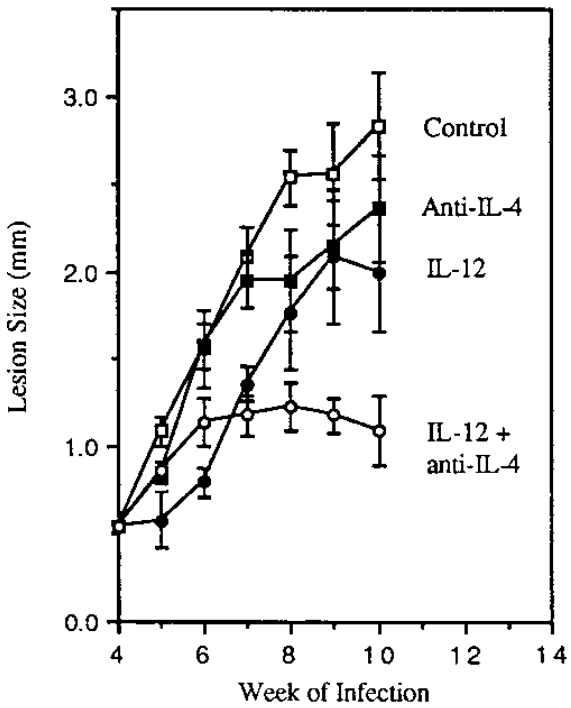

FIG. 5. Course of infection in CB6F1 mice following treatment with IL-12, anti-IL-4 MAb, or both IL-12 and anti-IL-4 MAb at 4 weeks of infection as described in the legend to Fig. 2. Values represent the mean ± SE of at least four mice per group.

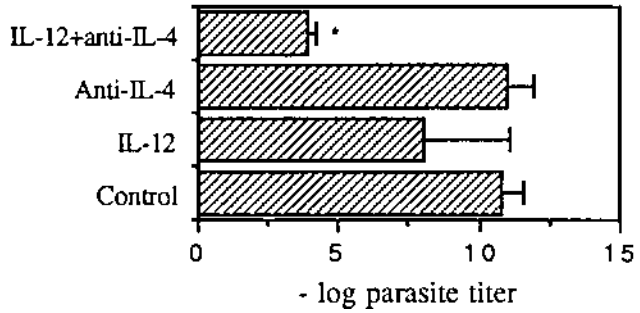

FIG. 6. Lesion parasite burdens at 6 weeks posttreatment in the mice described in the legend to Fig. 5. Footpads were homogenized in a fixed volume, and then the homogenates were serially diluted and examined after 1 week for the presence of parasites. The data represent the mean ± SE of four mice per group. *, $P < 0.05$ compared to untreated control mice.

cells but exhibited no reduction in the numbers of IL-4-producing cells compared to control mice. Only mice which received combined IL-12 and anti-IL-4 treatment developed Th1-dominant responses characterized by increased numbers of IFN-γ-producing cells and a decrease in IL-4-producing cells (Fig. 8). The development of a Th1-dominant response in the combined treatment group reflects the fact that this was the only group of mice which progressed towards healing.

DISCUSSION

We have utilized CB6F1 mice chronically infected with *L. major* to test the effects of IL-12 and/or anti-IL-4 MAb treatment on both cytokine production and disease progression. Infection in the CB6F1 strain elicits an immunological response with characteristics of that seen in both the resistant C57BL/6 and susceptible BALB/c parental strains (18). Most notably, cells from footpad-infected CB6F1 mice produce significant levels of both Th1- and Th2-type cytokines for an extended period of time following parasite inoculation (18). The prolonged production of IL-4 and IFN-γ by cells from CB6F1 mice contrasts with the rapid evolution of Th1- or Th2-dominant responses observed in the resistant and susceptible parental strains, respectively, and probably accounts for the fact that lesion sizes are larger and healing is considerably

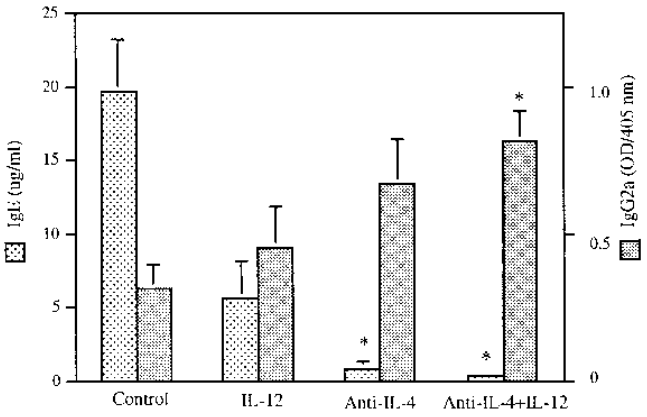

FIG. 7. Total IgE and parasite-specific IgG2a levels in the serum of CB6F1 mice. Mice were treated with IL-12, anti-IL-4 MAb, or both IL-12 and anti-IL-4 MAb at 4 weeks of infection. IgE and IgG2a levels were determined at 6 weeks posttreatment as described in Materials and Methods. Values represent the mean ± SE of 4 mice. *, $P < 0.05$ compared to untreated control mice.

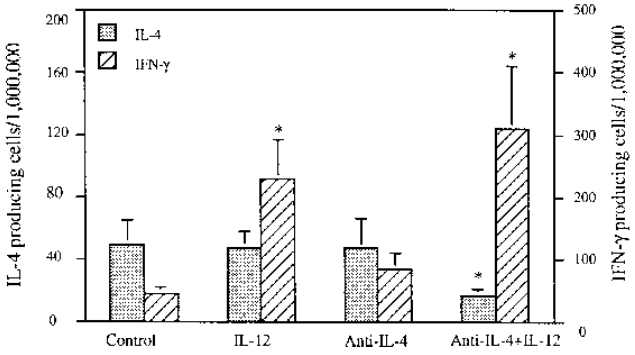

FIG. 8. Number of IFN-γ- and IL-4-producing cells per 10^6 draining lymph node cells of CB6F1 mice. Mice were treated with a single injection of IL-12, anti-IL-4 MAb, or both IL-12 and anti-IL-4 MAb at 4 weeks of infection. Lymph node cells were harvested 6 weeks after treatment, and ELISPOT assays were performed to determine numbers of cytokine-producing cells. Each value represents the mean ± SE for four mice per group. *, $P < 0.05$ compared to untreated control mice.

delayed in CB6F1 mice compared to C57BL/6 mice. When treated at 4 weeks of infection with a single inoculation of either IL-12 or anti-IL-4 MAb, CB6F1 mice exhibited rapid changes in cytokine production within infected lesions and draining lymph nodes as assessed by analysis of mRNA levels. IL-4 transcript levels were reduced within lesions of both IL-12- and anti-IL-4-treated mice by 4 days posttreatment, while transcripts for TGF-β, and to a lesser degree IL-10, were reduced within lesions of the anti-IL-4-treated group. Given the capacity of IL-12 to promote IFN-γ production and for IL-4 to suppress Th1-type responses, we were surprised to observe no changes in the levels of IFN-γ transcripts within lesions of either treated group. The failure of IL-12 to enhance IFN-γ transcript levels within infected lesions following intraperitoneal inoculation was not due to a failure in cytokine delivery to the local environment since injection of IL-12 directly into lesions also failed to promote a significant increase in lesion IFN-γ transcripts (data not shown). In contrast to the effects noted within lesions, IL-12 did induce a twofold increase in IFN-γ transcripts in draining lymph nodes, suggesting that differences in the local cell composition in lymph nodes and lesions may influence the response to this cytokine. The response to treatment in CB6F1 mice was dramatically enhanced when both IL-12 and anti-IL-4 MAb were administered together. Most notable were a marked suppression of IL-4 and TGF-β transcript levels within infected lesions and a marked increase in IFN-γ transcripts within draining lymph node tissues. Lesion transcripts for an *L. major* small ribosomal subunit were also decreased by about 70% in mice treated with both IL-12 and anti-IL-4 MAb, suggesting that the changes noted in cytokine production were accompanied by a rapid decrease in parasite numbers. Parasite rRNA levels were also reduced within lesions of mice treated with anti-IL-4 antibody, but not to the degree noted in the combined treatment group. The response of CB6F1 mice to exogenous IL-12 and anti-IL-4 antibody was clearly different from that observed in highly susceptible BALB/c mice. Although treatment of BALB/c mice elicited a modest increase in IFN-γ production within lesions, it had little effect on the production of IL-4 or TGF-β transcripts. The failure of IL-12 and anti-IL-4 treatment to dramatically modify cytokine production in BALB/c mice was further evidenced by the fact that these treatments also failed to alter the subsequent course of infection in these animals (data not shown).

It has been previously noted that IL-12 or anti-IL-4 antibody treatment of *L. major*-infected BALB/c mice initiated after the first 1 to 2 weeks of infection, as opposed to treatment at the time of infection, generally fails to promote healing (4, 21, 26, 28). These results are consistent with the concept that various immunotherapies promote the differentiation of naive $CD4^+$ cells to Th1-type cells but fail to influence the function of established Th2-type effector cells. Indeed, a prior study has shown that IL-12 administered to BALB/c mice at day 14 of infection not only fails to suppress Th2-type cytokine levels, but may actually promote IL-4 and IL-10 production, as well as the production of the Th1 cytokine, IFN-γ (28). We did not note any significant increase or decrease in the levels of IL-4 or IL-10 transcripts following IL-12 therapy, although the experimental protocols used in this and the prior study differed (we gave a single injection of IL-12 to mice at 4 weeks of infection, as opposed to four daily injections of IL-12 to 2-week-infected BALB/c mice). Given the moderate effect on IFN-γ production and the lack of effect on IL-4 production, it is not surprising that treatment with IL-12 and/or anti-IL-4 did not influence parasite numbers within BALB/c lesions in a manner similar to that observed in lesions of CB6F1 mice.

BALB/c and CB6F1 differ in their immunological response to *L. major*, and differences in the levels of cytokine production were evident in these strains of mice prior to IL-12 and/or anti-IL-4 treatment. For example, IFN-γ transcripts in parasitized lesions of CB6F1 mice were 2-fold higher than in BALB/c mice, while IL-4 transcripts were 3.5-fold higher in BALB/c than in CB6F1 lesions (data not shown). Whether simple differences in the levels of Th1- and Th2-type cytokines and/or other genetically predetermined factors influence a response to treatment are still unclear. Prior studies using T-cell receptor (TCR) transgenic mice have shown that under neutral culture conditions, naive BALB/c T cells differentiate into Th2-type cells following antigenic stimulation while B10.D2 cells differentiate into a Th1-type population (13). In addition, the population of BALB/c cells generated in response to antigen lost responsiveness to IL-12, while responsiveness to IL-12 was maintained in populations of B10.D2 cells, suggesting that the genetic background of the host may influence not only patterns of initial T-cell differentiation but also subsequent responses to cytokines such as IL-12 (8). The degree of Th subset polarization may also influence the response to immunotherapy (17). Recent studies have shown that T-cell populations which appear to be strongly polarized may, in fact, be composed of individual cells which are highly heterogeneous with respect to cytokine production and stage of differentiation (19). These cell populations may be more amenable to manipulation with cytokines or anti-cytokine antibodies than are fully differentiated Th1 and Th2 effector cell populations. Although cells from chronically infected BALB/c mice do retain some capacity to respond to IL-12 since we have previously shown that treatment of mice with IL-12, in combination with an antileishmanial drug, will promote healing and the development of a Th1-type response (16), it is clear that the BALB/c cells are more polarized toward a Th2-type population than are cells from CB6F1 mice.

Combined treatment of CB6F1 mice with IL-12 and anti-IL-4 antibody not only altered patterns of cytokine production but also enhanced resistance to infection and the more rapid development of a Th1-dominant response. By week 10 of infection, the combined treatment group also had the lowest levels of serum IgE and highest levels of parasite-specific IgG2a compared to the control and individual treatment groups. Lymph nodes from these mice also had a higher frequency of IFN-γ-producing cells and a lower frequency of IL-4-producing cells characteristic of a Th1-dominant response. It is interesting that the immediate effects of IL-12 and/or anti-IL-4 treatment, as assessed by measurements of lesion and lymph node cytokine transcripts on day 4 after treatment, appear to predict the long-term effects of therapy on the course of disease.

One of the more interesting effects of combined IL-12 and anti-IL-4 noted in this study was suppression of TGF-β mRNA levels within parasitized lesions. Although the production of bioactive TGF-β is regulated in a complex and tissue-specific manner (2) and TGF-β mRNA levels do not necessarily correlate with the production of active TGF-β protein (1), it is probable that the decrease in TGF-β transcripts observed in treated mice reflects a decrease in local TGF-β production. Treatment of mice with an anti-TGF-β antibody has been shown to increase resistance to *Leishmania amazonensis* (3) and antigen-stimulated cells from *L. major*-infected mice produce elevated levels of IFN-γ in vitro in the presence of anti-TGF-β antibody (22). In addition, TGF-β has been shown to suppress the IL-12-mediated induction of IFN-γ production by normal spleen cells (14). Together, these results suggest that high levels of TGF-β may suppress IFN-γ production and the development of resistance to an *L. major* infection. Multiple cell types including T cells and macrophages are known to produce this cytokine, and TGF-β protein can be easily detected within parasitized lesions (25). Since parasite numbers are reduced within lesions of treated mice and parasitized macrophages are known to produce TGF-β (15), it is possible that reduced levels of TGF-β transcripts simply reflect a decrease in numbers of parasitized macrophages in treated mice rather than a specific cytokine signal to decrease TGF-β production. It remains to be determined whether a decrease in TGF-β production is integral to the development of resistance and a Th1-dominant response in these chronically infected mice.

In summary, our results show that a single injection of IL-12 plus anti-IL-4 antibody is sufficient to promote more rapid healing and a Th1-type response in mice with chronic *L. major* infections, provided that the existing response prior to treatment is not highly polarized towards a Th2-type response. Therapeutic effects could be noted within a few days of treatment, including marked suppression in transcript levels for IL-4 and TGF-β within infected lesions. Given the capacity of these cytokines to promote Th2-type responses, it is tempting to speculate that their rapid suppression is important to the eventual development of an IFN-γ-dominant Th1 response.

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