INFECTION AND IMMUNITY, Mar. 1994, p. 1058–1063
0019-9567/94/$04.00+0

Vol. 62, No. 3

Th1 and Th2 Cell-Associated Cytokines in Experimental Visceral Leishmaniasis

G. DIEGO MIRALLES,[1] MARK Y. STOECKLE,[1] DAVID F. McDERMOTT,[1] FRED D. FINKELMAN,[2] AND HENRY W. MURRAY[1]*

Division of Infectious Diseases, Cornell University Medical College, New York, New York,[1] and Department of Medicine, Uniformed Services University of Health Sciences, Bethesda, Maryland[2]

Received 8 October 1993/Returned for modification 16 November 1993/Accepted 7 December 1993

In experimental *Leishmania donovani* infection in BALB/c mice, initial susceptibility gives way to T-cell-dependent acquired resistance and eventual control over visceral infection. Since various cytokines appear to underlie the host response to *Leishmania* infection, we examined infected liver tissue for gene expression of cytokines associated with Th1 (gamma interferon [IFN-γ] and interleukin-2 [IL-2]) and Th2 cells (IL-4 and IL-10). By Northern (RNA) blot analysis, only IFN-γ mRNA expression was detected in livers of infected euthymic mice. To determine whether activation of Th1 cells develops selectively in this model, qualitative PCR analysis was used. These results indicated that mRNAs for IFN-γ, IL-2, IL-4, and IL-10 were all induced by *L. donovani* infection. The potentially negative Th2 cell-associated response did not appear to play a functional role, however, since resistance was acquired, anti-IL-4 monoclonal antibody treatment did not accelerate control over visceral infection, and serum immunoglobulin E levels remained low. As judged by PCR analysis, IL-4 and IL-10 mRNAs were also expressed under three other conditions without apparent effect: in naive euthymic mice treated with IL-2, which induces leishmanicidal activity; in rechallenged immune mice, which resist reinfection; and in nude mice, which fail to control *L. donovani*. These results suggest that, like other *Leishmania* species, *L. donovani* infection may trigger a potentially suppressive Th2 cell-associated cytokine response. However, in T-cell-intact mice able to control *L. donovani*, this response either is insufficient to influence outcome or more likely is overshadowed by the Th1 cell response.

Although initially susceptible to experimental infection caused by the intracellular protozoan *Leishmania donovani*, BALB/c mice proceed to develop a T-cell-dependent immune response which results in control over visceral parasite replication (43). With time, visceral parasite burdens are reduced by >85%, and thereafter these chronically infected animals are resistant to rechallenge (42). This spontaneous acquisition of resistance to *L. donovani* appears to reflect the effects of lymphokine-secreting T cells, activated macrophages, and a well-established tissue granulomatous reaction (43). Endogenously-generated gamma interferon (IFN-γ) (50) and interleukin-2 (IL-2) (40), lymphokines primarily produced by the Th1 subset of $CD4^+$ T cells (12, 37), have already been implicated in this response.

Akin to the preceding results in experimental infection, recent epidemiologic studies of human visceral leishmaniasis suggest that up to 85% of infected individuals may also spontaneously control infection (2, 10, 16, 24, 34). These individuals either remain asymptomatic or develop oligosymptomatic infection which eventually resolves without treatment (2, 10, 16, 24, 34). This response is associated with skin test reactivity and appears to correlate with intact antigen-specific production of IFN-γ (10, 24, 34, 45), a lymphokine which induces macrophages to express leishmanicidal activity (39, 41). In contrast, in patients who proceed to develop or have clinically active visceral infection (kala-azar), in vitro secretion of antigen-stimulated IFN-γ and IL-2 is suppressed (9, 10, 15, 24, 45), enhanced induction of IL-10 and/or IL-4 mRNA can be demonstrated in the tissues (19, 26), and IL-4 can sometimes be detected in the circulation (24, 51). The presence of IL-4 and IL-10, Th2 cell-associated cytokines (38), appears important pathogenetically since both can suppress the secretion and/or host defense effects of activating cytokines, including IFN-γ and IL-2 (25, 31, 38, 49).

This same preferential Th2 cell response also appears to largely (11, 22, 23, 30, 46), but perhaps not exclusively (4, 6–8, 36, 47), underlie the failure of BALB/c mice to control cutaneous infection with *Leishmania major*. Th2 cell activation may also partially exert a negative effect in experimental *Leishmania amazonensis* cutaneous infection (1). In view of these observations about noncuring experimental cutaneous disease and the recent data from patients with visceral infection (2, 10, 16, 19, 24, 26, 34), one would predict that, in BALB/c mice capable of controlling *L. donovani*, a Th2 cell response either is not triggered in vivo or is overshadowed by a Th1 cell-associated response. The present results support the latter conclusion.

MATERIALS AND METHODS

Visceral infection. Congenitally athymic nude (*nu*/nu) 20- to 30-g female mice bred on a BALB/c background and their euthymic (*nu*/+) littermates were purchased from Life Sciences (St. Petersburg, Fla.) and maintained under similar conventional conditions. To establish visceral infection, mice were injected by tail vein with infected hamster spleen homogenate containing 10^7 *L. donovani* 1S amastigotes (43). Animals were sacrificed at various intervals for up to 8 weeks, and liver parasite burdens, expressed as Leishman-Donovan units (43), were determined microscopically in Giemsa-stained tissue imprints. Immune BALB/c mice were rechallenged with 10^7 amastigotes and sacrificed at various intervals for up to 2 weeks (42). These animals, which solidly resist reinfection (42), were originally infected 7 to 12 months before use.

* Corresponding author. Mailing address: 1300 York Ave., New York, NY 10021.

IL-2 treatment. *Escherichia coli*-derived recombinant human IL-2 (7×10^6 U/mg; Amgen, Thousand Oaks, Calif.) was administered in saline continuously for 7 days by a subcutaneously implanted osmotic pump (Alzet model 2002; Alza, Palo Alto, Calif.) which delivered 6.4×10^4 U/day (40). Controls received pumps filled with saline alone.

RNA extraction. Sections of liver were pressed through a wire mesh to obtain single-cell suspensions, and RNA was isolated by the guanidium thiocyanate–phenol-chloroform method (RNAzol B; BIOTECX, Houston, Tex.) (14). Each sample yielded 300 to 500 μg of total RNA.

Northern blot analysis. For Northern (RNA) blot analysis, 20 μg of total RNA from each sample was separated by electrophoresis on a 1.2% agarose–formaldehyde gel, transferred to a nylon filter by capillary blotting, and hybridized with ^{32}P-labeled cDNA (42). Probes were prepared by a random primer method with 0.5- to 0.8-kb fragments of murine IFN-γ, IL-2, IL-4, and IL-10 cDNAs and human glyceraldehyde-3-phosphate dehydrogenase (GAP) cDNA.

Qualitative RT-PCR. Reverse transcription PCR (RT-PCR) was performed as follows. To synthesize cDNA, 1 μg of each RNA sample was reacted with murine leukemia virus reverse transcriptase and oligo(dT)$_{18}$. Reagents were incubated at 37°C for 1 h and heated to 65°C for 10 min to denature the murine leukemia virus-reverse transcriptase, and samples were diluted to 100 μl. Ten microliters of each cDNA solution was subjected to PCR in a volume of 50 μl containing 2 mM (each) deoxynucleoside triphosphate, 20 μM (each) specific primer, buffer, and 1.25 U of *Taq* polymerase (Perkin-Elmer Cetus). The primer sequences were as follows: IFN-γ, sense TCA GGA AGC GGA AAA GGA GTC, antisense TCA AGT CAC TTG AGA CAC TGC; IL-2, sense CAG CTC GCA TCC TGT GTC ACA, antisense GAT GAT GCT TTG ACA GAA GGC; IL-4, sense GTA CCA GGA GCC ATA TCC ACG, antisense GAG TCT CTG CAG CTC CAT GAG; IL-10, sense CTG GAA GAC CAA GGT GTC TAC, antisense GAG CTG CTG CAG GAA TGA TGA; and GAP, sense GAT GAC ATC AAG GTG GT, antisense TCT TGC TCA GTG TCC TTG CTG. Each primer pair amplified across an intron. Each cycle consisted of 94°C for 30 s, 55°C for 1 min, and 72°C for 1.5 min. Amplification was performed with 40 cycles for IFN-γ, IL-2, IL-4, and IL-10 and 22 cycles for GAP. IL-2 amplification yielded multiple bands. To improve specificity, 2 μl of each IL-2 amplification reaction sample was reamplified for 15 cycles with internal primers: sense CAT CTT CAG TGC CTA GAA GAT (312 to 333), antisense GAA GGC TAT CCA CTC CCT CAG (524 to 503). After amplification, 10 μl of each sample was applied to a 2% agarose gel, and the products were visualized by ethidium bromide staining (40). The specificity of the amplified bands was confirmed by size, restriction enzyme digest, and Southern blot. GAP was used as a control for the amount of cDNA used in each sample.

Treatment with IL-4 in vitro and anti-IL-4 MAb in vivo and serum IgE determination. To establish cultures of human monocyte-derived macrophages (41), 1.5×10^6 peripheral blood mononuclear cells were added to 13-mm-diameter round glass coverslips and then processed and maintained in standard medium for ≥7 days as reported previously (41). These monocyte-derived macrophages were then pretreated for 3 days with medium alone or medium containing 1,000 U of recombinant human IFN-γ per ml (10^7 U/mg; Amgen), 10 to 100 U of recombinant human IL-4 per ml (2×10^7 U/mg; Amgen), or both IFN-γ and IL-4. Cultures were challenged for 1 h with 5×10^6 *L. donovani* amastigotes, washed to remove uningested parasites (time zero), and then reincubated for 72 h in medium alone (41). With fixed coverslips, the number of amastigotes per 100 macrophages at time zero was compared with the number present at 72 h to determine parasite killing or the fold increase in intracellular replication (41).

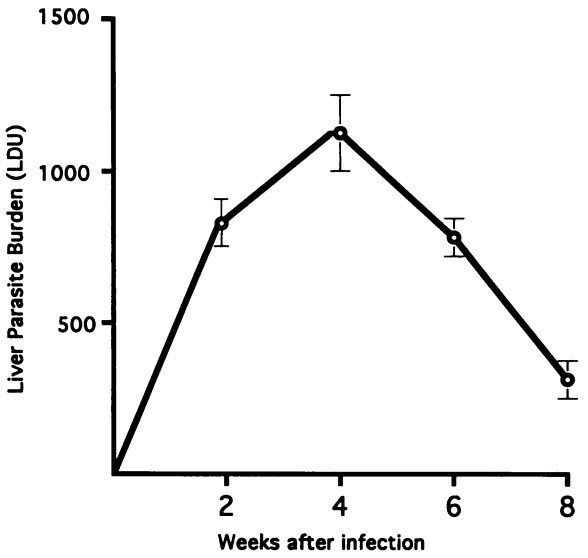

FIG. 1. Course of hepatic infection in euthymic BALB/c mice following challenge with *L. donovani* amastigotes. Results are the means ± standard errors for 8 to 10 mice at each time point from four to five experiments. LDU, Leishman-Donovan units.

Starting 1 day after infection and once weekly thereafter, groups of three mice were injected intraperitoneally with 1 mg of anti-murine IL-4 monoclonal antibody (MAb) (11B.11, lot 3-287-880217) generously provided by C. Reynolds (Biological Response Modifiers Program, National Cancer Institute, Frederick, Md.). Liver parasite burdens were determined 2 and/or 4 weeks after infection. Serum immunoglobulin E (IgE) levels were measured by enzyme-linked immunosorbent assay as described elsewhere (17).

RESULTS AND DISCUSSION

Induction of cytokine mRNA by *L. donovani* infection. After challenge with *L. donovani*, liver parasite burdens in naive euthymic BALB/c mice progressively increase, peak at 4 weeks as resistance is acquired, and then decline to low levels (Fig. 1) (43). Cytokine mRNA analysis of liver tissue from infected mice was initially performed by Northern blot. By this method, IFN-γ mRNA was induced 2 weeks after infection and was more fully expressed at week 4 (Fig. 2). Thereafter, IFN-γ mRNA expression declined and was not detected 8 weeks after infection. Using the same liver samples, we were unable to detect expression of IL-2, IL-4, or IL-10 mRNA at any time point (Fig. 2).

Qualitative PCR analysis. We next reexamined the same liver samples for the induction of cytokine mRNA by using RT-PCR and focused on tissue from mice infected for 4 weeks, the time at which acquired resistance was first expressed. Four weeks after infection, mRNAs for IFN-γ and IL-2 as well as for IL-4 and IL-10 were induced (Fig. 3). At our limits of detection, mRNAs for these four cytokines were not expressed in liver tissue from either uninfected controls (Fig. 3) or controls injected with spleen homogenates from uninfected hamsters (not shown). mRNA for each of the four cytokines was first detected by RT-PCR 10 days after infection; mRNAs for IL-2, IL-4, and IL-10 were also detected at weeks 6 and 8

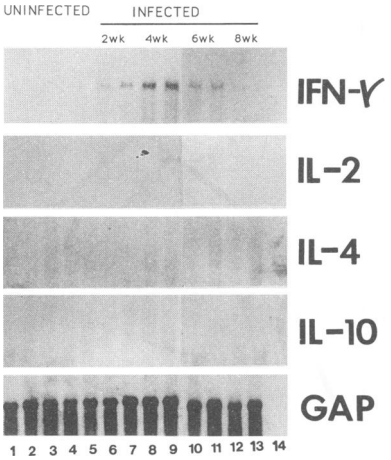

FIG. 2. Northern blot analysis of cytokine mRNA in livers of BALB/c mice infected with *L. donovani*. Each lane represents a single mouse. Lanes: 1 to 5, uninfected age-matched littermates; 6 to 13, mice at the indicated postinfection time points. Autoradiographs of 10- to 14-day exposures for IFN-γ, IL-2, IL-4, and IL-10 are shown. Only IFN-γ mRNA was detected. GAP is a control for the amount of RNA in each sample (exposure of 1 day). Lane 14 has no RNA.

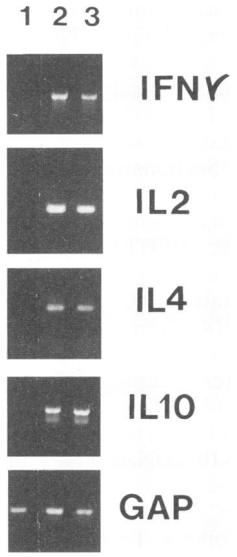

FIG. 3. RT-PCR analysis of cytokine mRNA in livers of euthymic BALB/c mice 4 weeks after *L. donovani* infection. RNA preparations for each RT-PCR sample are the same as those used in Fig. 2. Lanes: 1, uninfected control; 2 and 3, infected mice.

(not shown). In contrast, IFN-γ mRNA expression (Fig. 4) was not detected by PCR analysis 8 weeks after infection, paralleling the Northern blot results shown in Fig. 2.

These PCR data suggested two conclusions. First, *L. donovani* infection in the liver induces mRNA expression of both Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-10) cell-associated cytokines. Other tissues infected by *L. donovani* (spleen, bone marrow) were not examined in this study. Since the PCR assay employed was qualitative, no conclusion can be drawn about the relative amounts of the various cytokine mRNAs detected. Nevertheless, it seems reasonable to infer from the Northern blot results (only IFN-γ mRNA detected) that *L. donovani* infection in the liver preferentially induced a Th1 cell response. It is also possible that infection activated a separate subset of $CD4^+$ cells (e.g., Th0 cells [18, 35, 38, 44]) capable of secreting multiple cytokines. Second, in addition to correlating with the course of visceral infection, the kinetics of IFN-γ mRNA expression in both the Northern blot and PCR assays also appeared to support prior observations derived from treating infected mice with anti-IFN-γ MAb (50). In those experiments, the inhibition of antileishmanial defense induced by anti-IFN-γ MAb was most pronounced early on (at week 4) when acquired resistance was first expressed (50). In contrast, despite 8 weeks of continued anti-IFN-γ MAb treatment and measurable anti-IFN-γ activity, hepatic infection nevertheless came under control (50). This finding led us to postulate that the eventual resolution of acute infection with conversion to a chronic low-level state (43) may involve a separate IFN-γ-independent mechanism (50). The apparent absence of IFN-γ mRNA expression at week 8 (Fig. 2 and 3) supports this conclusion. Other more recent studies suggest that tumor necrosis factor alpha may mediate this late-acting mechanism (50a).

Role of IL-4. In addition to the basic capacity of BALB/c mice to control infection, other observations have also implied that the primary response to *L. donovani* is apparently Th1 cell dependent: in vitro-stimulated spleen cells from infected mice secrete IFN-γ and IL-2 (28, 43) and not IL-4 (28), and treatment with MAb directed at endogenous IFN-γ and IL-2 impairs initial acquired resistance (40, 50). Nevertheless, as judged by PCR analysis, IL-4 and IL-10 mRNAs were also expressed by week 2, raising the possibility that a Th2 cell response might contribute to the logarithmic increase in parasite replication during the first weeks after challenge (Fig. 1) (43).

To examine the potential consequences of Th2 cell activation, we focused on IL-4 and used three approaches. First, IFN-γ-activated macrophages infected with *L. donovani* were treated in vitro with IL-4. Second, mice were injected with anti-IL-4 MAb. Third, serum was assayed for IgE, a marker for the presence of IL-4 (17, 21, 22, 46). In three experiments, human macrophages (41) pretreated for 3 days with IFN-γ alone (1,000 U/ml) killed 41 ± 2% of ingested amastigotes 72 h after infection, while untreated cells supported a 2.2- ± 0.4-fold increase in intracellular replication. The addition of 10 U of IL-4 per ml during the IFN-γ pretreatment period abolished killing and permitted a 1.7- ± 0.4-fold increase in intracellular replication. Pretreating macrophages with up to

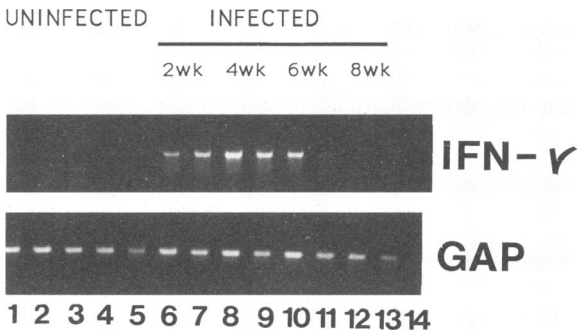

FIG. 4. RT-PCR analysis of the kinetics of IFN-γ mRNA expression in livers of euthymic BALB/c mice 2 to 8 weeks after infection with *L. donovani* (lanes 6 to 13, two mice per time point). Lanes: 1 to 5, uninfected mice; 14, negative control for PCR.

100 U of IL-4 per ml alone did not influence amastigote intracellular replication. These data, indicating the capacity of IL-4 to inhibit macrophage activation, confirm results from a previous study (31).

In two experiments, anti-IL-4 MAb treatment had no effect on visceral infection in vivo. Liver parasite burdens in naive mice treated once weekly with 1 mg of anti-IL-4 starting the day after challenge were not different from those of untreated animals at week 2 (not shown) and were somewhat higher rather than lower at week 4 in anti-IL-4-treated mice (578 ± 68 versus 408 ± 89 Leishman-Donovan units, mean ± standard error, $n = 6$ mice per group). In a single experiment, initiating once-weekly anti-IL-4 treatment 1 day prior to *L. donovani* challenge also had no effect at either week 2 or week 4 (not shown). These results contrast directly with the salutary effect of anti-IL-4 MAb in nonhealing BALB/c mice infected with *L. major* (46).

In addition, and as previously reported by Kaye and colleagues (28) for both self-curing and noncuring *L. donovani*-infected mice, we also found that serum IgE concentrations were low at levels 10- to 30-fold below those reported in *L. major*-infected BALB/c mice (21, 22). In two experiments, the serum IgE level (mean ± standard error) for uninfected mice was 0.9 ± 0.2 μg/ml ($n = 5$ mice). Levels in 2-, 4-, and 8-week-infected BALB/c mice were 1.4 ± 0.4, 3.1 ± 0.5, and 3.1 ± 0.8 μg/ml, respectively ($n = 6$ to 8 mice per group). Although there was a modest increase in IgE level at week 4, this increase coincided with the onset of acquired resistance and control of infection. Separate results suggested that the anti-IL-4 MAb was active in infected mice after four once-weekly injections; IgE levels were reduced from 2.4 ± 0.2 μg/ml to 0.6 ± 0.1 μg/ml, a concentration similar to the 0.8 ± 0.2 μg/ml measured in uninfected mice ($n = 3$ mice per group).

Responses in immune and T-cell-deficient mice. In prior studies, we also determined the response to *L. donovani* in two other populations of BALB/c mice: previously-infected euthymic mice, which become solidly resistant (immune) to rechallenge, and T-cell-deficient athymic (nude) animals, which permit progressive visceral infection (42, 43). Thus, we also extended this analysis to these two other quite distinct hosts.

In immune mice rechallenged with *L. donovani*, qualitative PCR analysis indicated that mRNAs for IFN-γ, IL-4, and IL-10 were expressed within 1 day (Fig. 5A), 10 times faster than in naive euthymic mice. The rapid induction of IL-4 mRNA appeared to be of little consequence, however, since (i) immune mice resisted rechallenge as judged by minimal increases in parasite burdens 1 and 2 weeks after reinfection (not shown) (42), and (ii) anti-IL-4 MAb treatment (1 mg/week starting the day before rechallenge) did not alter liver burdens 2 weeks after reinfection (two experiments, data not shown).

Two weeks after infection in nude mice, IFN-γ and IL-10 mRNAs (Fig. 5B) and IL-4 mRNA (not shown) were also detected in liver samples by qualitative PCR. However, mRNAs for these cytokines were not detected in the same samples by Northern blot analysis (not shown) suggesting a difference between nude and euthymic animals for IFN-γ mRNA expression (Fig. 2). In view of reports of T-cell-independent mechanisms of IFN-γ production (3, 20, 27, 48), we suspect that natural killer cells (or perhaps γ/δ cells [5]) are the most likely sources of the IFN-γ mRNA detected by PCR in nude mice (3, 20, 27, 48). However, nude mice fail to control *L. donovani* visceral infection (43). Thus, it appears that, in the absence of a full complement of T cells, either IFN-γ is not sufficient by itself to induce antileishmanial activity or, alternatively, the IFN-γ produced by other cells may not be sufficient to lead to effective resistance. Indeed, twice-weekly

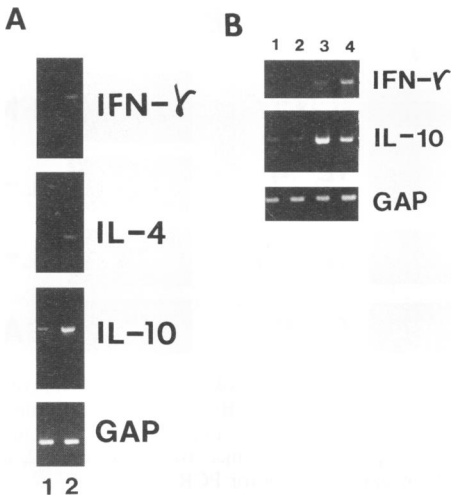

FIG. 5. RT-PCR analysis of cytokine mRNA in livers of immune (A) and nude (B) BALB/c mice after challenge with *L. donovani*. (A) Lane 1, unrechallenged immune mouse; lane 2, 1 day after rechallenge. (B) Lanes 1 and 2, uninfected nude mice; lanes 3 and 4, nude mice 2 weeks after infection.

treatment for 4 weeks with anti-IFN-γ MAb, using a preparation (R4-6A2 [42, 50]) and injection schedule which impairs acquired resistance in naive euthymic animals (42, 50), did not exacerbate already progressive visceral infection in nude mice (two experiments, data not shown). In addition, as judged by undetectable levels of IgE in serum (<0.07 μg/ml) in 4-week-infected nude mice and the finding that once-weekly injections of 1 mg of anti-IL-4 did not affect liver burdens (two experiments, data not shown), endogenous IL-4 also did not appear to exert an effect in nude mice. While these observations suggest that mRNAs for IFN-γ, IL-4, and IL-10 may all be expressed in non-T cells in this model, it is worth pointing out that nude mice may have residual T cells as well (29).

Effect of treatment with IL-2. In both the exogenous and endogenous forms, IL-2 appears to exert contrasting effects in BALB/c mice infected with *L. major* (negative or no effect [21, 32, 33]) compared with those infected with *L. donovani* (positive effects [40]). Therefore, we completed these experiments by using this Th1 cell-associated cytokine as a treatment. Since *L. donovani* infection by itself induced cytokine mRNA in the PCR assay, uninfected mice were examined. As shown in Fig. 6, 7 days of continuous IL-2 treatment resulted in the expression of IFN-γ, IL-4, and IL-10 mRNAs in the livers of both euthymic and nude mice. Cytokine mRNA was not detected in livers from mice treated with pump-delivered saline alone (not shown). In nude mice, the Th1 and Th2 cell responses associated with IL-2 treatment appeared to be of little functional significance, since in experiments performed in parallel (40), this same IL-2 regimen induced neither a positive nor a negative effect on visceral infection (40). In contrast, in euthymic mice, continuous IL-2 treatment achieved leishmanicidal activity (40), suggesting that the IL-2-induced appearance of IL-4 and IL-10 mRNAs also yielded little effect in these animals. However, the observed Th1 cell response to IL-2 (IFN-γ mRNA induction) did appear relevant, since the antileishmanial activity of exogenous IL-2 is reversed by anti-IFN-γ MAb treatment (40).

Together, and as judged by qualitative PCR-detectable induction of IL-4 and IL-10 mRNAs, these results suggest that

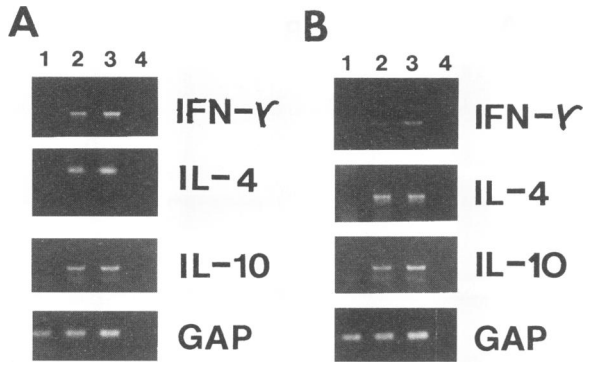

FIG. 6. RT-PCR analysis of cytokine mRNA in livers of uninfected euthymic (A) and nude (B) BALB/c mice treated continuously with IL-2 for 7 days. For both panels A and B, lane 1 represents untreated mice, lanes 2 and 3 represent mice treated with IL-2, and lane 4 represents the negative control for PCR.

L. donovani (as well as the Th1 cell-derived stimulus IL-2) may trigger a Th2 cell-associated response in a variety of immunologically distinct hosts. However, this reaction, particularly as it relates to IL-4, does not appear to be sufficient to influence primary Th1 cell responses and therefore the course of visceral infection. In a separate model of *L. donovani* infection in B10.D2/n mice (28), the noncuring phenotype is associated with an extinguished Th1 cell response. However, the suppression of this response and disease progression did not appear to be related to activation of Th2-like cells, since there was no increase in serum IgE, and spleen cells did not produce IL-4 or IL-5 in vitro (28). While our findings suggest that *L. donovani* stimulates a Th2 cell-associated response, this response does not appear to develop fully and is inhibited (13, 38), or at least is overshadowed, by the opposing effects of Th1 cells in mice capable of expressing antileishmanial resistance.

ACKNOWLEDGMENTS

We are particularly grateful to Rachel Teitelbaum and June Hariprashad for technical assistance and Homa Yeganegi (Amgen) for providing IL-2, IL-4, and IFN-γ.

This work was supported by NIH research grants AI 16963 and R29GM 46890 and the Aaron Diamond Foundation.

REFERENCES

1. **Afonso, L. C. C., and P. Scott.** 1993. Immune responses associated with susceptibility of C57BL/10 mice to *Leishmania amazonensis*. Infect. Immun. **61:**2952–2959.
2. **Badaro, R., T. C. Jones, and E. M. Carvalho.** 1986. New perspectives on a subclinical form of visceral leishmaniasis. J. Infect. Dis. **154:**1003–1111.
3. **Bancroft, C. J., R. D. Schreiber, G. C. Bosma, J. J. Bosma, and E. R. Unanue.** 1987. A T cell-independent mechanism of macrophage activation by interferon-γ. J. Immunol. **139:**1104–1109.
4. **Belosevic, M., C. Davis, M. Meltzer, and C. Nacy.** 1988. Regulation of activated macrophage antimicrobial activities. J. Immunol. **141:**890–896.
5. **Bluestone, J., and L. Matis.** 1989. TCR γ/δ cells—minor redundant T cell subset or specialized immune system component. J. Immunol. **142:**1785–1788.
6. **Bogdan, C., S. Stenger, M. Rollinghoff, and W. Solbach.** 1991. Cytokine interactions in experimental cutaneous leishmaniasis. Interleukin-4 synergizes with interferon-γ to activate murine macrophages for killing of *Leishmania major* amastigotes. Eur. J. Immunol. **21:**327–333.
7. **Bretscher, P., G. Wei, J. Menon, and H. Bickfeldt-Ohman.** 1992. Establishment of stable cell-mediated immunity that makes susceptible mice resistant to *Leishmania major*. Science **257:**539–542.
8. **Carter, K., G. Gallagher, A. Ballic, and J. Alexander.** 1989. The induction of protective immunity to *Leishmania major* in the BALB/c mouse by interleukin-4 treatment. Eur. J. Immunol. **19:**779–782.
9. **Carvalho, E. M., R. Badaro, S. G. Reed, T. C. Jones, and W. D. Johnson, Jr.** 1985. Absence of gamma interferon and interleukin-2 production during active visceral leishmaniasis. J. Clin. Invest. **76:**2066–2069.
10. **Carvalho, E. M., A. Barral, D. Pedral-Sampario, M. Barral-Netto, R. Badaro, H. Rocha, and W. Johnson.** 1992. Immunologic markers of clinical evolution in children recently infected with *Leishmania donovani chagasi*. J. Infect. Dis. **165:**535–540.
11. **Chatelain, R., K. Varkila, and R. L. Coffman.** 1992. IL-4 induces a Th2 response in *Leishmania major*-infected mice. J. Immunol. **148:**1182–1187.
12. **Cherwinski, H. M., J. H. Schumacher, K. D. Brown, and T. R. Mosmann.** 1987. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. J. Exp. Med. **166:**1229–1239.
13. **Chomarat, P., M. Rissoan, J. Banchereau, and P. Miossee.** 1993. Interferon-γ inhibits interleukin-10 production by monocytes. J. Exp. Med. **177:**523–527.
14. **Chomczynski, P., and N. Sacchi.** 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-choroform extraction. Anal. Biochem. **162:**156–162.
15. **Cillari, E., F. Y. Liew, P. L. Campo, S. Milano, S. Mansueto, and A. Salerno.** 1988. Suppression of IL-2 production by cryopreserved peripheral blood mononuclear cells from patients with visceral leishmaniasis in Sicily. J. Immunol. **140:**2721–2726.
16. **Evans, T. G., M. J. Teixeira, I. T. McAuliffe, A. W. Vasconcelos, A. Q. Sousa, J. W. Lima, and R. D. Pearson.** 1992. Epidemiology of visceral leishmaniasis in northeast Brazil. J. Infect. Dis. **166:**1124–1132.
17. **Finkelman, F. D., I. M. Katona, J. F. Urban, Jr., C. M. Snapper, J. Ohara, W. E. Paul.** 1986. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. Proc. Natl. Acad. Sci. USA **83:**9675–9678.
18. **Firestein, G. S., W. D. Roeder, J. A. Laxer, K. S. Townsend, C. T. Weaver, J. T. Hom, J. Linton, B. E. Torbett, and A. L. Glasebrook.** 1989. A new murine CD4+ T cell subset with an unrestricted cytokine profile. J. Immunol. **143:**518–525.
19. **Ghalib, H., M. Piuvezam, Y. Skeily, M. Siddig, F. Hashim, D. Russo, and S. Reed.** 1993. Interleukin-10 production correlates with pathology in human *Leishmania donovani* infections. J. Clin. Invest. **92:**324–329.
20. **Hanada, K., R. Suzuki, H. Matsui, Y. Shimizu, and K. Kumagai.** 1983. Natural killer (NK) cells as responders to interleukin 2 (IL-2). II. IL-2-induced interferon-γ production. J. Immunol. **130:**988–994.
21. **Heinzel, F., R. Perko, F. Hatam, and R. Locksley.** 1993. IL-2 is necessary for the progression of leishmaniasis in susceptible murine hosts. J. Immunol. **150:**3924–3931.
22. **Heinzel, F. P., M. D. Sadick, B. J. Holaday, R. L. Coffman, and R. M. Locksley.** 1989. Reciprocal expression of interferon-γ or interleukin-4 during the resolution of progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. J. Exp. Med. **169:**59–72.
23. **Heinzel, F. P., M. D. Sadick, S. S. Mutha, and R. M. Locksley.** 1991. Production of interferon-γ, interleukin-2, interleukin-4, and interleukin-10 by CD4+ lymphocytes in vivo during healing and progressive murine leishmaniasis. Proc. Natl. Acad. Sci. USA **88:**7011–7015.
24. **Holaday, B. J., M. Pompeu, T. Evans, D. Braga, M. Texeira, A. Sousa, M. Sadick, J. Abrams, R. Pearson, and R. Locksley.** 1993. Correlates of *Leishmania*-specific immunity in the clinical spectrum of infection with *Leishmania chagasi*. J. Infect. Dis. **167:**411–417.
25. **Howard, M., and A. O'Garra.** 1992. Biological properties of interleukin-10. Immunol. Today **13:**198–200.
26. **Karp, C., T. Wynn, M. Satti, A. Kordofani, F. Hashim, F. Neva, T.**

Nutman, and D. Sacks. 1993. In vivo cytokine profiles in patients with kala-azar. Marked elevation of both interleukin-10 and interferon-gamma. J. Clin. Invest. **91:**1644–1648.

27. **Karupiah, G., R. V. Blanden, and I. A. Ramshaw.** 1990. Interferon-γ is involved in the recovery of athymic nude mice from recombinant vaccinia virus/interleukin-2 infection. J. Exp. Med. **172:**1495–1503.
28. **Kaye, P. M., A. J. Curry, and J. M. Blackwell.** 1991. Differential production of Th1- and Th2-derived cytokines does not determine the genetically controlled or vaccine-induced rate of cure in murine visceral leishmaniasis. J. Immunol. **146:**2763–2770.
29. **Kung, J., and C. Thomas.** 1988. Athymic nude CD4+8− T cells produce IL-2 but fail to proliferate in response to mitogenic stimuli. J. Immunol. **141:**3691–3696.
30. **Leal, L., D. Moss, R. Kuhn, W. Muller, and F. Liew.** 1993. Interleukin-4 transgenic mice of resistant background are susceptible to *Leishmania major* infection. Eur. J. Immunol. **23:**566–569.
31. **Lehn, M., W. Y. Weiser, S. Engelhorn, S. Gillis, and H. G. Remold.** 1989. IL-4 inhibits H_2O_2 production and antileishmanial capacity of human cultured monocytes mediated by IFN-γ. J. Immunol. **143:**3020–3024.
32. **Lexama-Davila, C., D. Williams, G. Gallagher, and J. Alexander.** 1992. Cytokine control of *Leishmania* infection in BALB/c mice: enhancement and inhibition of parasite growth by local administration of IL-2 and IL-4 is species and time dependent. Parasite Immunol. **14:**37–48.
33. **Mazingue, C., J. Louis, M. Kweider, C. Avriautt, and A. Capron.** 1989. In vitro and in vivo effects of interleukin 2 on the protozoan parasite *Leishmania*. Eur. J. Immunol. **19:**487–491.
34. **Meller-Melloul, C., C. Farnarier, S. Dunan, B. Faugere, J. Franck, C. Mary, P. Bongrand, M. Quilici, and S. Kaplanski.** 1991. Evidence of subjects sensitized to *Leishmania infantum* on the French Mediterranean coast: differences in gamma interferon production between this population and visceral leishmaniasis patients. Parasite Immunol. **13:**531–536.
35. **Moll, H., and M. Rollinghoff.** 1990. Resistance to murine cutaneous leishmaniasis is mediated by Th1 cells but disease-promoting CD4+ cells are different from Th2 cells. Eur. J. Immunol. **20:**2067–2074.
36. **Morris, L., A. B. Troutt, K. S. McLeod, A. Kelso, E. Handman, and T. Aebischer.** 1993. Interleukin-4 but not gamma interferon production correlates with severity of murine cutaneous leishmaniasis. Infect. Immun. **61:**3459–3465.
37. **Mosmann, T. R., H. Cherwinski, M. W. Bond, A. Giedlin, and R. L. Coffman.** 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. **136:**2348–2354.
38. **Mosmann, T. R., and R. L. Coffman.** 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. **7:**145–163.
39. **Murray, H. W.** 1990. Effect of continuous administration of interferon-γ in experimental visceral leishmaniasis. J. Infect. Dis. **161:**992–994.
40. **Murray, H. W., G. D. Miralles, M. Y. Stoeckle, and D. F. McDermott.** 1993. Role and effect of interleukin-2 in experimental visceral leishmaniasis. J. Immunol. **151:**929–938.
41. **Murray, H. W., B. Y. Rubin, and C. D. Rothermel.** 1983. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon-γ is the activating lymphokine. J. Clin. Invest. **72:**1506–1510.
42. **Murray, H. W., K. E. Squires, C. D. Miralles, M. Y. Stoeckle, A. M. Granger, A. Grangelli-Piperno, and C. Bodgan.** 1992. Acquired resistance and granuloma formation in experimental visceral leishmaniasis. Differential T cell and lymphokine roles in initial versus established immunity. J. Immunol. **148:**1858–1863.
43. **Murray, H. W., J. J. Stern, K. Welte, B. Y. Rubin, S. M. Carriero, and C. F. Nathan.** 1987. Experimental visceral leishmaniasis: production of interleukin-2 and interferon-γ, tissue immune reaction, and response to treatment with interleukin-2 and interferon-γ. J. Immunol. **138:**2290–2297.
44. **Pallard, X., R. de Waal, Malefijt, D. Blanchard, I. Chretien, J. Abrams, J. de Vries, and H. Spits.** 1988. Simultaneous production of IL-2, IL-4, and interferon-gamma by activated human CD4+ and CD8+ T cell clones. J. Immunol. **141:**849–854.
45. **Sacks, D., S. Lal, S. Shrivastava, J. Blackwell, and F. Neva.** 1987. An analysis of T cell responsiveness in Indian kala-azar. J. Immunol. **138:**908–912.
46. **Sadick, M. D., F. P. Heinzel, B. J. Holaday, R. T. Pu, R. S. Dawkins, and R. M. Locksley.** 1990. Cure of murine leishmaniasis with anti-interleukin-4 monoclonal antibody. Evidence for a T cell-dependent, interferon-γ-independent mechanism. J. Exp. Med. **171:**115–127.
47. **Sadick, M. D., N. Street, T. R. Mosmann, and R. M. Locksley.** 1991. Cytokine regulation of murine leishmaniasis: interleukin-4 is not sufficient to mediate progressive disease in resistant C57BL/6 mice. Infect. Immun. **59:**4710–4714.
48. **Salkowski, C. A., and E. Balish.** 1991. A monoclonal antibody to gamma interferon blocks augmentation of natural killer cell activity during systemic cryptococcosis. Infect. Immun. **59:**486–493.
49. **Silva, J. S., P. H. Morrissey, K. H. Grabstein, K. M. Mohler, D. Anderson, and S. G. Reed.** 1992. Interleukin-10 and interferon-γ regulation of experimental *Trypanosoma cruzi* infection. J. Exp. Med. **175:**169–174.
50. **Squires, K. E., R. D. Schreiber, M. J. McElrath, B. Y. Rubin, S. L. Anderson, and H. W. Murray.** 1989. Experimental visceral leishmaniasis: role of endogenous IFN-γ in host defense and tissue granulomatous response. J. Immunol. **143:**4244–4249.

50a. **Tumang, M., C. Keogh, L. L. Moldawer, R. Teitelbaum, J. Horiprashad, and H. W. Murray.** Submitted for publication.

51. **Zwingenberg, K., G. Harms, C. Pedrosa, B. Sandkamp, and S. Neifer.** 1990. Determinants of the immune response in visceral leishmaniasis: evidence for predominance of endogenous interleukin-4 over interferon-γ production. Clin. Immunol. Immunopathol. **57:**242–249.