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Recombinant Interleukin-1α Augments Granuloma Formation and Cytokine Production but Not Parasite Clearance in Mice Infected with *Leishmania donovani*

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In vivo administration of various doses of recombinant interleukin-1α to B10.D2/n mice chronically infected with *Leishmania donovani* resulted in enhanced formation of granulomas and in vitro production of gamma interferon. By direct microscopical enumeration, reduction in gross parasite burden in the viscera was not observed, however. These data highlight an important discordance between granuloma formation per se and parasite elimination and suggest that interleukin-1 deficiency alone cannot account for the chronicity of this disease.

Evidence is now accumulating to suggest that resolution from infection with the intracellular parasite *Leishmania donovani* is dependent on the coordinated interactions between the components of the cell-mediated immune system. This includes the activation of helper and effector T-cell populations into appropriate cytokine production, the priming/activation of macrophages, and the continued maintenance of these interactions. These events culminate histologically in the granulomatous tissue reaction (17, 24–26, 34, 41).

The cytokine interleukin-1 (IL-1) plays a pivotal role in host defense against microbial infection. It functions to provide important costimulatory signals during T-cell activation and may enhance class II expression on some antigen-presenting cells (11, 15, 20, 23, 29, 35, 40). These events lead to enhanced T-cell production of cytokines such as IL-2 and gamma interferon (IFN-γ). In addition, IL-1 is an important mediator of inflammation (10, 11, 21) and of the cellular influx involved in granuloma formation (1, 6, 11, 22). It is not surprising, therefore, that administration of IL-1 has been shown to confer resistance to a variety of microbes including *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Toxoplasma gondii*, and *Schistosoma mansoni* (3–5, 7–9, 28, 36, 37).

Chronic infection with *L. donovani* is associated with T-cell nonresponsiveness towards both antigen and mitogens (19, 25, 27, 31). In addition, our recent investigations have shown that splenic antigen-presenting cells isolated from infected animals have reduced capacity to support costimulator-dependent T-cell responses, e.g., to anti-CD3 (5a). Given the critical role of IL-1 in cell-mediated immunity, and that decreased IL-1 production has been associated with *L. donovani* infection of murine peritoneal and human peripheral blood monocyte populations in vitro (30, 32), we have administered recombinant human IL-1α (specific activity, 3×10^8 D10 units/mg; gift of P. Lomedico, Hoffman La Roche) in two different schedules to B10.D2/n mice chronically infected with *L. donovani*.

Mice were infected via the lateral tail vein with *L. donovani* amastigotes as previously described (19). Groups of mice were monitored over time to ensure that a stable level of chronic infection had been achieved. Two IL-1 administration schedules were adopted on the basis of previously published protocols. In the first, mice at 36 weeks of

TABLE 1. Administration of recombinant IL-1α does not alter parasite burden in spleens and livers of *L. donovani*-infected B10.D2/n mice[a]

Treatment	Mean log LDU[b] ± SD for organ indicated at week:					
	37		38		39	
	Liver	Spleen	Liver	Spleen	Liver	Spleen
Saline	2.8 ± 0.3	2.4 ± 0.4	2.5 ± 0.1	1.6 ± 0.4	2.6 ± 0.1	1.6 ± 0.2
1× IL-1	2.8 ± 0.1	1.9 ± 0.4	2.4 ± 0.6	1.5 ± 0.2	2.9 ± 0.3	1.8 ± 0.4
2× IL-1	ND[c]	ND	2.4 ± 0.3	1.9 ± 0.2	ND	ND
3× IL-1	ND	ND	ND	ND	2.9 ± 0.5	2.0 ± 0.2

[a] Mice infected with *L. donovani* 36 weeks previously were administered IL-1α (low-dose schedule; see text) or saline, and parasite burdens were determined at the times indicated. Values were not significantly different from those for saline controls at each time.
[b] Log LDU, LDU = (number of parasites/1,000 host cell nuclei) × organ weight.
[c] ND, not determined.

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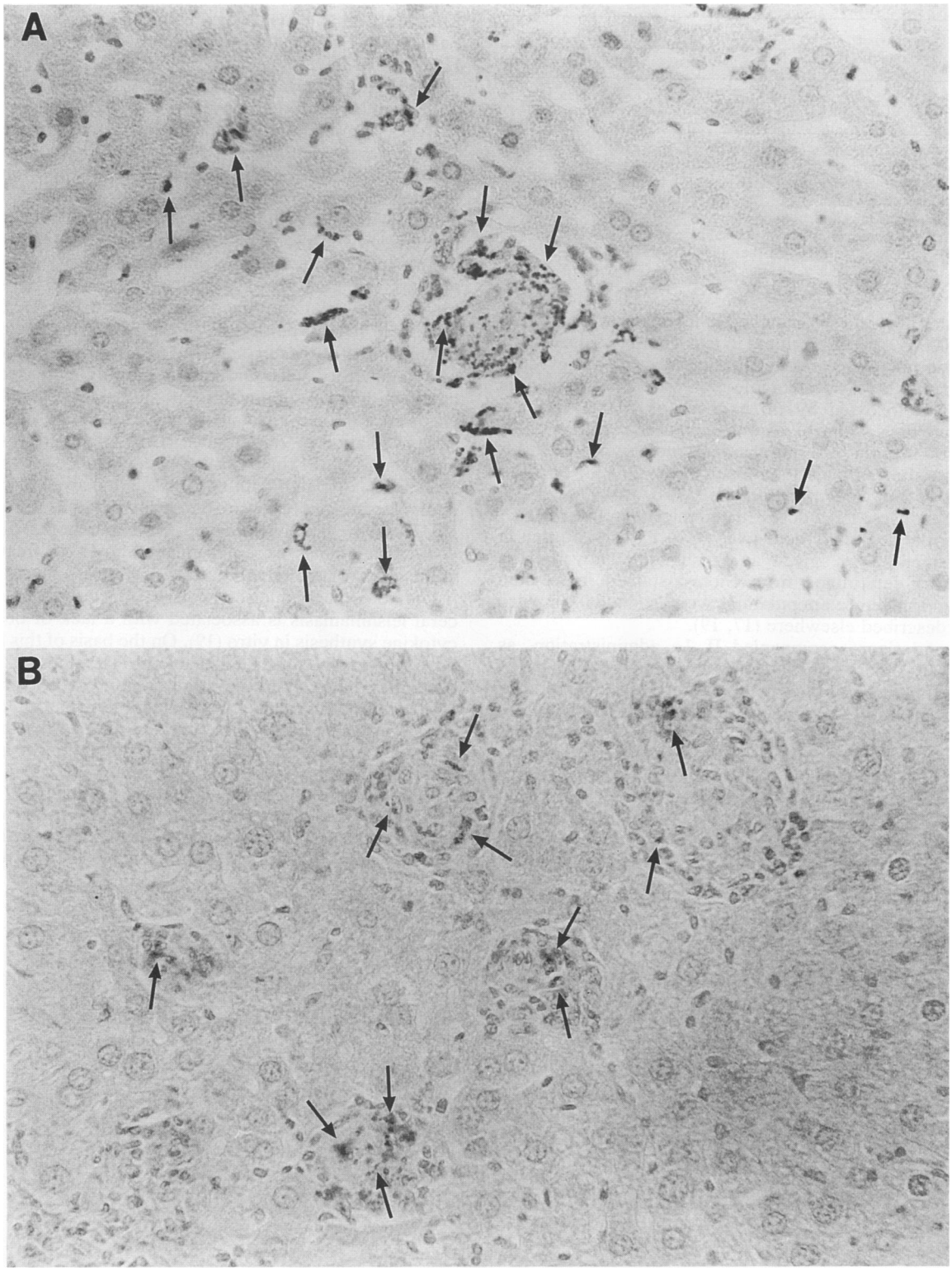

FIG. 1. Histological response to *L. donovani* in normal and IL-1α-treated mice. The localization of amastigotes in paraffin sections of livers from untreated (A) or high-dose IL-1α-treated (B) mice was determined by immunoperoxidase staining by using polyclonal anti-*Leishmania* antisera. Note that in untreated infected livers, infected macrophages (arrows) are observed outside the boundaries of the granuloma. In IL-1α-treated mice, most infected macrophages are detected within the granulomas.

TABLE 2. Parasite distribution and granuloma number in infected B10.D2/n mice treated with IL-1α[a]

Treatment	No. of granulomas[b]	Parasite distribution	
		Within granulomas	Outside granulomas
Saline	25.6 ± 4.3	148 ± 24	174 ± 34
IL-1	49.5 ± 6.4[c]	259 ± 49[d]	89 ± 28[d]

[a] Liver sections from saline or high-dose IL-1α-treated *L. donovani*-infected mice were processed for immunohistological detection of parasites and granulomas as described in detail elsewhere (17). Results are expressed as the mean ± standard deviation.
[b] Per 30 fields (magnification, ×400).
[c] $P < 0.0005$ compared with that for the saline control.
[d] $P < 0.005$ compared with that for the saline control.

infection, or age- and sex-matched uninfected controls, were given 10,000 U of IL-1α (diluted in pyrogen-free saline) once weekly for up to 3 weeks. Alternatively, in a high-dose schedule, mice at 32 weeks of infection were given two doses of 50,000 U followed by three doses of 10,000 U for 5 consecutive days. Control mice in each case received saline alone. Groups of three or more animals were sacrificed 7, 14, or 21 days after a single low dose (weeks 37, 38, or 39 of infection) or 7 days after two (week 38) or three (week 39) weekly low doses. High-dose-treated mice were sacrificed 7 days after the final daily dose (week 34). Parasite burden in the spleen and liver, given as Leishman Donovan units (LDU), was determined from Giemsa-stained impression smears, and spleen cell populations were isolated for in vitro assay as described elsewhere (17, 19).

Fever and rigor accompanied IL-1α administration, as described by others (10, 11). This subsided by 2 to 4 h in uninfected mice and by 24 h in infected mice. Uninfected mice showed both splenomegaly and hepatomegaly of approximately 30 to 40% at sacrifice, whereas those for infected mice were a further 15 to 20% above the enlargements already induced by infection (data not shown). This increase in organ weight probably reflects the capacity of this cytokine to induce cellular influx (11, 13). Analysis of parasite burden in the spleen and liver in low-dose (Table 1)-treated mice revealed that IL-1α did not have any significant effect on disease course. This was also the case for animals given a high-dose regimen (log LDU of 3.33 ± 0.2 and 2.05 ± 0.2 for livers and spleens, respectively, of untreated mice compared with 3.32 ± 0.3 and 2.36 ± 0.2, respectively, for IL-1-treated mice).

Resolution from *L. donovani* infection has been associated with granuloma production (17, 24, 26, 42). Paraffin sections of livers from high-dose-treated mice were therefore stained for the presence of *Leishmania* infection by using polyclonal antisera (raised in rabbits against *L. donovani* promastigote membranes) and immunoperoxidase and then counterstained with hematoxylin to reveal both parasite number and distribution and tissue responses (17). The number of granulomas per 30 microscope fields (magnification, ×400) as well as the number of parasites within and outside the granulomas was determined (Table 2 and Fig. 1). Granulomas were readily detectable in the livers of infected mice, though some of these comprised less mature accumulations of inflammatory cells. Granuloma number increased significantly with IL-1α administration, and, as importantly, there was also a dramatic change in the distribution of the parasites in relation to granulomas. IL-1α treatment resulted in more than 74% of the parasites being localized within these structures compared with only 46% in the absence of IL-1α. A similar augmentation of the granulomatous response by IL-1 was previously observed for experimental listeriosis (7–9). Granulomas were not induced by IL-1α treatment of uninfected control mice.

Unlike cutaneous leishmaniasis (14), chronic murine visceral leishmaniasis is associated with a lack of observable cytokine synthesis in vitro (19). On the basis of this data, we have previously suggested that both Th1 and Th2 populations may have entered a state of nonresponsiveness (18, 19). Analysis of spleen cell populations from infected and IL-1α-treated infected mice revealed that neither the proliferative nonresponsiveness nor the production of IL-2/IL-4 and IL-3 was restored by IL-1α treatment. (In all cases, responses were compared with those of mice at 7 days of infection, which show positive proliferative and cytokine responses; data not shown). The low-dose IL-1α schedule did, however, enhance antigen-dependent IFN-γ production

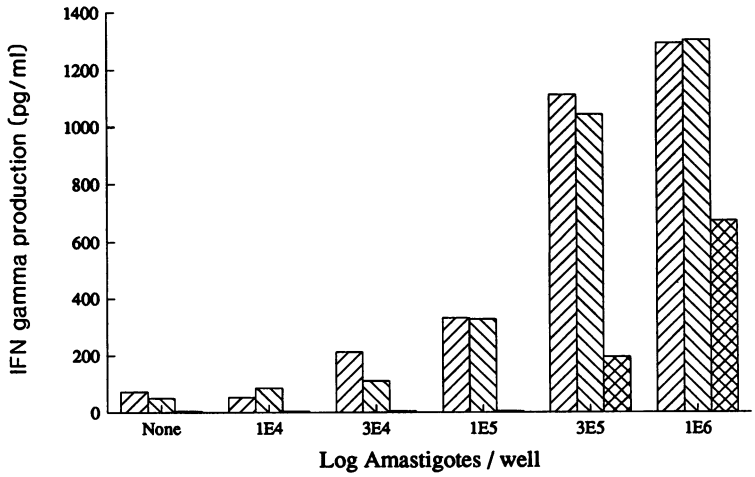

FIG. 2. IFN-γ production by spleen cells from IL-1α-treated *L. donovani*-infected mice. Spleen cells were harvested from mice infected with *L. donovani* for 39 weeks which had received either saline alone (▩) or one (▧) or three (▨) low doses of IL-1α. After incubation with the indicated concentrations of antigen, supernatants were assayed for the presence of IFN-γ by enzyme-linked immunosorbent assay (19). The standard deviation for triplicate cultures was less than 15% of the mean.

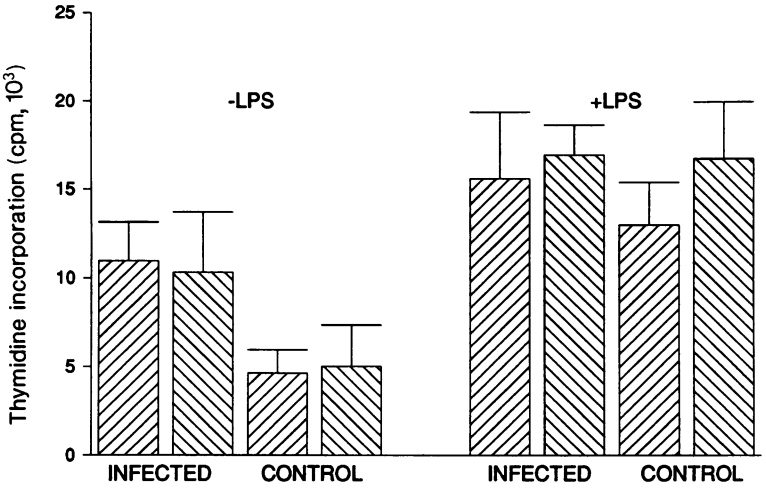

FIG. 3. IL-1 production by spleen cells from infected and IL-1α-treated mice. Spleen cells from infected and control animals which had been given either saline (▧) or IL-1α (▨) were cultured in medium alone or with LPS for 48 h, and supernatant IL-1 levels were determined by bioassay using the D10.G41 cell line (16). Values represent the mean counts per minute ± standard deviation for triplicate cultures.

in vitro (Fig. 2). The level of IFN-γ produced in these animals was still only approximately 20% of that routinely observed in major histocompatibility complex congenic strains of mice cured of infection (see reference 19 for comparisons). In contrast, high-dose IL-1α treatment led to the spontaneous production of IFN-γ (>2 ng/ml) and IL-5 (1.5 to 2 ng/ml) in all groups of animals, including uninfected controls. Addition of antigen did not enhance this production further (data not shown). This finding confirms previous data that under appropriate mitogenic stimuli both Th1 and Th2 cytokines can be produced by spleen cell populations from both naive and these chronically infected animals (19).

We also examined whether IL-1α administration in vivo altered the capacity of spleen cells to produce IL-1 in vitro because of autocrine regulation (12, 39). Spleen cells were cultured in medium alone or in the presence of 10 μg of lipopolysaccharide (LPS, lot L4391; Sigma, Poole, United Kingdom) for 48 h, and the concentration of IL-1 was determined by using the D10.G4.1 cell line (16). Our results (Fig. 3) demonstrate that IL-1α in vivo had no effect on the subsequent capacity to produce IL-1 in vitro in response to LPS. Significantly, however, infected mice had a much higher spontaneous level of IL-1 production in vitro than their uninfected controls, a result which may indicate that some IL-1 could also be produced in vivo in these animals.

In summary, therefore, we have demonstrated that although IL-1α administration in vivo enhances both the ability to form mature granulomas and IFN-γ production in response to antigen in vitro, these combined effects do not lead to detectable enhancement of amastigote killing. Although this may reflect a quantitative deficiency in IFN-γ, recent investigations by others (2, 33, 41) have suggested that IFN-γ alone may be insufficient for inducing leishmanicidal activity, and the strategy we employed here may have failed to significantly augment these additional facets of the response. Dissecting these interactions within the granuloma remains an important future goal. Finally, the studies reported here for a murine model of visceral leishmaniasis demonstrate that IL-1α treatment had little effect on the course of chronic disease. However, as there is only limited information on the immunological regulation of chronic human visceral leishmaniasis, the possibility remains that this cytokine may have an immunomodulatory function in humans.

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