

## Visceral *Leishmania donovani* Infection in Interleukin-13<sup>-/-</sup> Mice

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***Leishmania donovani*-infected interleukin-13<sup>-/-</sup> BALB/c mice showed impaired initial gamma interferon secretion and incomplete granuloma assembly at parasitized liver foci. Nonetheless, control of early parasite replication, resolution of liver infection, and responsiveness to antileishmanial chemotherapy were intact. By itself, interleukin-13 does not appear to materially influence acquired resistance in this intracellular infection.**

In experimental visceral leishmaniasis, host defense against intracellular *Leishmania donovani* is T cell dependent and involves a range of T-cell- and macrophage-activating cytokines (15, 16, 25). In *L. donovani* infection in the liver, interleukin-12 (IL-12) and gamma interferon (IFN- $\gamma$ ) figure prominently in granuloma assembly, macrophage activation, and parasite killing, driving acquired resistance and eventual near resolution of infection (4, 12, 15, 16, 23, 26). In addition, the same inflammatory response supports the efficacy of conventional antileishmanial chemotherapy, pentavalent antimony (Sb) (15).

*L. donovani* infection also provokes expression of Th2-cell-type cytokines (IL-4, IL-10, and IL-13) and transforming growth factor  $\beta$  (18), ordinarily considered counterbalancing, suppressive factors. These cytokines probably limit tissue injury but to various degrees also derail Th1-cell responses, deactivate macrophages, and promote infection (3, 9, 11, 17, 19, 27). In *L. donovani*-infected, susceptible, wild-type (WT) BALB/c mice, neutralization or receptor blockade has demonstrated a striking deactivating effect for IL-10, modest suppressive roles for transforming growth factor  $\beta$  and IL-13, and no role for IL-4 (19). Nevertheless, studies with *L. donovani*-infected IL-4<sup>-/-</sup> BALB/c mice indicate a less well appreciated effect for IL-4 in supporting Th1-type responses and limiting initial liver parasite replication (1, 22, 25). In addition, susceptibility to *L. donovani* is further enhanced in IL-4 receptor  $\alpha$ -deficient (IL-4R $\alpha$ <sup>-/-</sup>) BALB/c mice, in which higher liver parasite burdens correlated with deficient IFN- $\gamma$  secretion and impaired granuloma maturation. Since IL-13 also signals via IL-4 receptor  $\alpha$ , a separate, similarly early-acting antileishmanial role was inferred for IL-13 (25).

To formally test the role of IL-13 alone, IL-13 gene-disrupted mice on a BALB/c background (5) were infected intravenously with  $1.5 \times 10^7$  hamster spleen-derived *L. donovani* amastigotes (1 Sudan strain) (19). However, the course of infection in the liver, measured microscopically with tissue imprints and expressed as Leishman Donovan units (LDU) (19), proved nearly indistinguishable from the course of liver infection for WT BALB/c animals (Fig. 1). In particular, the level of infection was similar at week 4, the point at which liver

parasite burdens in IL-4R $\alpha$ <sup>-/-</sup> mice exceeded those in WT controls, before both groups resolved infection at week 8 (25).

Nevertheless, IL-13<sup>-/-</sup> mice showed defects in two linked expressions of Th1-cell-associated defense against *L. donovani*, IFN- $\gamma$  secretion and granuloma assembly (15, 16). IFN- $\gamma$  was measured in serum by enzyme-linked immunosorbent assay, and a value of 0 was arbitrarily assigned to results showing  $<31$  pg/ml, the limit of detectability (19). IFN- $\gamma$  was not detected in uninfected mice, and in two experiments, levels were lower at week 2 in IL-13<sup>-/-</sup> ( $15 \pm 16$  pg/ml) than in WT ( $56 \pm 21$  pg/ml) animals (mean  $\pm$  standard error of the mean [SEM],  $n = 6$  to 7 mice per group;  $P > 0.05$ ). However, week 4 levels increased in IL-13<sup>-/-</sup> mice ( $140 \pm 57$  pg/ml), suggesting that reduction in IFN- $\gamma$  secretion was transient. While measuring IFN- $\gamma$  in serum is a useful marker of Th1-cell-type responses,

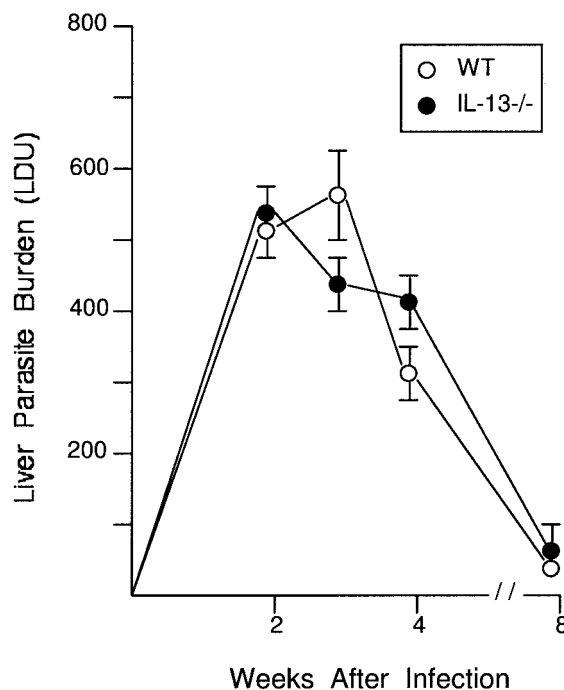


FIG. 1. Course of *L. donovani* liver infection in IL-13<sup>-/-</sup> versus WT BALB/c mice. Results are from two to three experiments and indicate means  $\pm$  SEMs for 6 to 11 mice per group per time point.  $P$  is  $>0.05$  at all time points.

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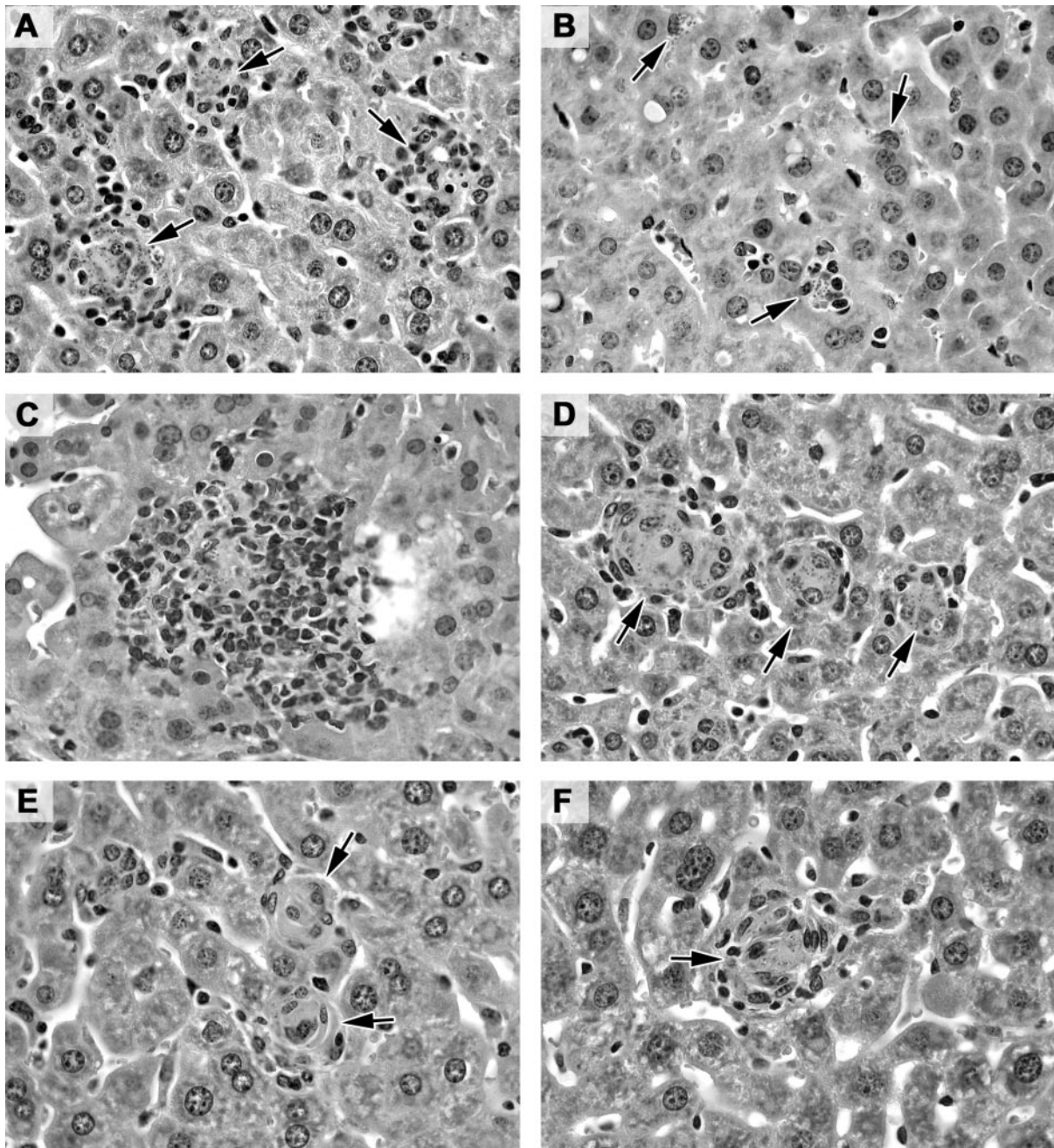


FIG. 2. Liver histologic reactions to *L. donovani* in hematoxylin- and eosin-stained sections from WT (A, C, and E) and IL-13<sup>-/-</sup> (B, D, and F) mice. Arrows indicate infected tissue foci. (A and B) Two weeks after infection, parasitized foci from WT mice (A) show developing granulomas; this is in contrast to little or no cellular response at infected Kupffer cells from IL-13<sup>-/-</sup> mice (B). (C and D) At week 4, granulomas are present in both WT (C) and IL-13<sup>-/-</sup> (D) mice but appear mature only in WT animals (C). (E and F) At week 8, granulomas have involuted in both WT (E) and IL-13<sup>-/-</sup> (F) mice. Magnification,  $\times 400$ .

the physiologic implication of activity in serum (versus in situ IFN- $\gamma$  expression at the infected tissue focus) is unknown.

IL-13<sup>-/-</sup> mice also failed to generate the anticipated early cellular reaction at parasitized Kupffer cell foci at week 2 (Fig. 2B) (16). While granulomas formed by week 4,  $\sim 85\%$  were small and scored as developing (immature) (16), having attracted relatively few recruited mononuclear cells (Fig. 2D). Thus, at week 4, there was little evidence of transition to the

mature-appearing, mononuclear-cell-rich structures (Fig. 2C) associated with control over *L. donovani* in WT mice (16, 25). Subsequent observations indicated no further progression towards granuloma maturation, as IL-13<sup>-/-</sup> mice showed involution of most granulomatous foci at week 8, with residual epithelioid changes similar to those in WT mice (Fig. 2E and F).

Since both IFN- $\gamma$  and mononuclear cell recruitment regulate the leishmanicidal response to Sb chemotherapy within



TABLE 1. Response to treatment<sup>a</sup>

Antimony treatment (mg/kg)	% Parasite killing (mean ± SEM) at day +21	
	WT mice	IL-13 <sup>-/-</sup> mice
500	98 ± 1	96 ± 2
50	12 ± 7	22 ± 6

<sup>a</sup> Two weeks after infection, liver parasite burdens were determined for WT (605 ± 49 LDU) and IL-13<sup>-/-</sup> (559 ± 54 LDU) mice, and single-dose optimal (500 mg/kg) or suboptimal (50 mg/kg) Sb was injected once intraperitoneally 14 days after infection (day +14). Liver burdens were measured 7 days later (day +21) and compared to those at day +14 to determine the percentage of parasite killing (19). Results are from two experiments with seven to eight mice per group.

parasitized tissue (13, 14), we anticipated a deficient response to treatment for IL-13<sup>-/-</sup> mice. However, the efficacy of both optimal- and suboptimal-dose Sb (sodium stibogluconate [Pentostam]) (19) was preserved (Table 1). Suboptimal-dose Sb was used to test for more-subtle effects potentially induced by the absence of IL-13.

Together, our results suggest that IL-13 promotes initial IFN- $\gamma$  production and show that IL-13 influences tissue granuloma assembly and maturation. However, these experiments have not addressed the mechanism(s) by which IL-13 regulates these two expressions of the antileishmanial Th1 response. Possibilities include that IL-13's effects, as reported for IL-4 (1, 24), support Th1-cell development (contributing to initial IFN- $\gamma$  secretion) and shape subsequent responses, including granuloma maturation. Alternatively, although ordinarily considered a suppressive-type cytokine, IL-13 might also act to downregulate other deactivating mechanisms (e.g., IL-10 [7]) which limit initial IFN- $\gamma$  secretion and restrain granuloma development.

Nevertheless, the absence of the two aforementioned effects in IL-13-deficient mice did not materially influence acquired resistance or eventual resolution of *L. donovani* infection in the liver. Similar results for IFN- $\gamma$  and granulomatous responses have been demonstrated well for IL-4<sup>-/-</sup> and in a more pronounced fashion with IL-4R $\alpha$ <sup>-/-</sup> BALB/c mice (25). However, and in contrast to our findings (Fig. 1), these same two effects correlated with increased initial susceptibility to *L. donovani* in both IL-4<sup>-/-</sup> and IL-4R $\alpha$ <sup>-/-</sup> mice (25), possibly indicating a more primary role for IL-4 in supporting initial acquired resistance. Since IFN- $\gamma$ -induced macrophage activation and granuloma formation are clear-cut expressions of the Th1-cell-type response which controls liver infection in this model (16, 17, 19), our findings with IL-13<sup>-/-</sup> mice suggest an early-acting, compensatory, or IFN- $\gamma$ -independent mechanism. Such mechanisms may involve IL-12 and tumor necrosis factor (26) or IL-18 (20). Similarly, *L. donovani*-induced granuloma assembly can also proceed in an IFN- $\gamma$ -independent fashion (18, 26). Further, albeit under separate conditions (10), *L. donovani* infection can also be controlled in the absence of a granulomatous response.

These same IL-13<sup>-/-</sup> BALB/c mice as well as their C57BL/6 counterparts have also been tested with cutaneous *L. major* (5) and *L. mexicana* (2, 24) infection. These studies produced variable results for the role of IL-13, ranging from no role (24) to a protective (2) or a suppressive (2, 5) role. In IL-4R $\alpha$ <sup>-/-</sup> mice, IL-13's inferred role in cutaneous infection has been similarly variable, depending upon the *Leishmania* strain tested (2, 8,

21). In our experiments, in which we used an *L. donovani* strain different from that tested with IL-4R $\alpha$ <sup>-/-</sup> mice (25), the absence of IL-13 did not accelerate control of liver infection, enhance granuloma assembly, heighten IFN- $\gamma$  secretion, or increase responsiveness to Sb chemotherapy-linked expressions of raised Th1-cell reactivity well expressed, for example, in IL-10<sup>-/-</sup> mice (17). Thus, IL-13 by itself does not appear to exert suppressive Th2-type cytokine effects in this model. Conversely, infection in IL-13<sup>-/-</sup> mice was not exacerbated, as would be anticipated (17, 19) if IL-13 played a meaningful prohost defense role. It is also worth pointing out that our observations are limited to the liver; thus, it is possible that different results might have been seen with the spleen, for example.

Nevertheless, endogenous IL-13 does have transient or limited effects in *L. donovani* infection but under quite different conditions: in conjunction with deficient IL-4 (e.g., in IL-4R $\alpha$ <sup>-/-</sup> mice [26]), for intact WT mice injected with soluble IL-13 receptor  $\alpha$ 2 (IL-13R $\alpha$ 2) immunoglobulin G Fc (19), and for IL-13R $\alpha$ 2<sup>-/-</sup> mice (19). The effect of IL-13 again varies, however, depending upon the setting. BALB/c IL-4R $\alpha$ <sup>-/-</sup> mice are initially more susceptible to *L. donovani* at week 4, suggesting an initially protective, albeit dispensable action for IL-13, since infection resolves normally by week 8 (26). Inhibition of IL-13 by IL-13R $\alpha$ 2 immunoglobulin G Fc treatment enhances inhibition of *L. donovani* replication in WT BALB/c mice but does not permit parasite killing (19). Finally, for IL-13R $\alpha$ 2<sup>-/-</sup> mice, which phenotypically resemble IL-13 transgenic animals (6), excess IL-13 suppresses IFN- $\gamma$  secretion and granuloma assembly and promotes *L. donovani* infection; however, for IL-13R $\alpha$ 2<sup>-/-</sup> mice, the latter effect of IL-13 is also transient (19).

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