Interleukin-10 (IL-10) in Experimental Visceral Leishmaniasis and IL-10 Receptor Blockade as Immunotherapy

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Interleukin-10 (IL-10) is thought to promote intracellular infection, including human visceral leishmaniasis, by disabling Th1 cell-type responses and/or deactivating parasitized tissue macrophages. To develop a rationale for IL-10 inhibition as treatment in visceral infection, Th1 cytokine-driven responses were characterized in Leishmania donovani-infected BALB/c mice in which IL-10 was absent or overexpressed or its receptor (IL-10R) was blocked. IL-10 knockout and normal mice treated prophylactically with anti-IL-10R demonstrated accelerated granuloma assembly and rapid parasite killing without untoward tissue inflammation; IL-12 and gamma interferon mRNA expression, inducible nitric oxide synthase reactivity, and responsiveness to antimony chemotherapy were also enhanced in knockout mice. In IL-10 transgenic mice, parasite replication was unrestrained, and except for antimony responsiveness, measured Th1 cell-dependent events were all initially impaired. Despite subsequent granuloma assembly, high-level infection persisted, and antimony-treated transgenic mice also relapsed. In normal mice with established infection, anti-IL-10R treatment was remarkably active, inducing near-cure by itself and synergy with antimony. IL-10's deactivating effects regulate outcome in experimental visceral leishmaniasis, and IL-10R blockade represents a potential immunomodulatory strategy in this infection.

Translating the host T-cell-mediated immune response into a feasible form of treatment has been a sought-after therapeutic objective in leishmaniasis, an intracellular protozoal infection which targets tissue macrophages. In disseminated (visceral) leishmaniasis, this aim has been pursued experimentally and/or clinically using three interrelated approaches: (i) identifying specific components of macrophage-activating, T-cell-dependent pathways which can act alone as immunotherapeutics, (ii) understanding how this same Th1 cell-associated mechanism regulates in vivo responsiveness to chemotherapy, and (iii) blending immunotherapeutic and chemotherapy to optimize intracellular Leishmania killing in the tissues (32, 33).

Effective defense towards visceralizing strains, including Leishmania donovani, depends strictly upon T (Th1) cells, and acquired resistance is governed by T-cell- and macrophage-activating cytokines (5, 16, 17, 27, 32, 33, 59). Among the latter (32, 33, 34, 35, 58), interleukin-12 (IL-12) and gamma interferon (IFN-γ) play particularly prominent experimental roles. These cytokines initiate and/or drive the basic antileishmanial Th1 cell response and direct the assembly of tissue granulomas, structures within which intracellular parasites are killed by IFN-γ-activated mononuclear phagocytes (17, 32, 33, 36–38, 51, 57, 58). In addition, along with host T cells, endogenous IL-12 and IFN-γ are also required for expression of the leishmanicidal effect of conventional chemotherapy, pentavalent antimony (36, 39–41). Not surprisingly, then, employing pro-host defense cytokines in exogenous form, either alone or with antimony, represents the primary immunotherapeutic approach thus far tested experimentally and/or clinically in visceral infection (3, 4, 13, 29, 32–36, 42, 54, 55, 58).

Downregulating mechanisms have also been identified in both experimental and human visceral leishmaniasis (kala-azar), including Th2 cell-associated responses (2, 5, 11, 18, 19, 22, 27–29, 33, 50, 56). This information, coupled with immunopathogenetic lessons learned from models of cutaneous Leishmania major infection (7, 12, 20, 21, 43, 47, 60), has pointed in a separate immunotherapeutic direction — targeting (inhibiting) the effects of simultaneously induced, suppressive cytokines. While transforming growth factor beta (60) and perhaps Th2 cell-derived IL-4 and/or IL-13 may exert such effects in visceral infection (2, 43, 49, 50), considerable evidence supports a central, deactivating role for endogenous IL-10.

IL-10 is induced in both experimental and human infection, and its broad actions compromise antigen-presenting cell and T-cell costimulatory mechanisms, T-cell proliferation, and secretion of and macrophage responsiveness to activating Th1 cell-associated cytokines (5, 7–9, 11, 18, 19, 22, 28–30, 33, 43, 56, 60). Although not devoid of proinflammatory effects (24), IL-10 clearly has the potential to disable host antileishmanial defense and foster visceral infection and, in parallel, impair responsiveness to chemotherapy (antimony).

To characterize IL-10’s role, we studied the behavior of L. donovani in modified mice which lack or overexpress endogenous IL-10 and then tested IL-10 receptor (IL-10R) blockade,
used alone or with antimony, as immunotherapy in established infection.

**MATERIALS AND METHODS**

**Animals.** BALB/c IL-10−/− (knockout) mice were produced by backcrossing the IL-10−/− gene mutation (24) eight generations onto the BALB/c background (1). BALB/c IL-10 (human IL-10) transgenic mice were produced as previously described previously (20); transgene-negative littermates, designated here as wild-type mice, served as controls. These three strains were bred at the DNAX Research Institute (Palo Alto, Calif.) (1, 20). BALB/c mice from Jackson Laboratory (Bar Harbor, Maine), designated here as normal BALB/c mice, were used as controls for knockout mice. Mice were 8 to 16 weeks old when challenged with *L. donovani*. Knockout, transgenic, and wild-type mice were male and female; purchased BALB/c mice were female. Some normal BALB/c mice were injected subcutaneously once weekly for 4 weeks with 0.2 ml of saline containing 1.5 × 10⁷ heat-killed *L. major* promastigotes. This procedure induces a cross-reacting Th2 cell-associated response upon subsequent challenge with viable *L. donovani* (43).

**Visceral infection and tissue response.** Groups of three to five mice were injected via the tail vein with 1.5 × 10⁷ hamster spleen-derived *L. donovani* amastigotes (1 Sudan strain) (43). Visceral infection was followed microscopically using Giemsa-stained liver imprints in which liver parasite burdens were measured by blinded counting of the number of amastigotes per 500 cell nuclei × liver weight in milligrams (Leishman-Donovan units [LDU]) (43). The histologic response to infection was assessed microscopically in liver sections stained with hematoxylin and eosin. The number of granulomas (infected Kupffer cells which had attracted ≥5 mononuclear cells) was counted in 100 consecutive 40× fields, and at 100 parasitized foci, the reaction was scored as (i) none (infected Kupffer cell with no mononuclear cell infiltrate) or (ii) developing or mature granuloma (37, 38). The latter were defined as a core of fused infected Kupffer cells surrounded or well infiltrated by numerous mononuclear cells. Granulomas which contained no visible amastigotes were designated parasite-free.

**Cytokine mRNA expression.** Total cellular RNA was extracted from liver homogenates from three to four mice per group, and qualitative reverse transcript-PCR using primers for IL-12 p40, IFN-γ, and β-actin was carried out as described previously elsewhere (6, 25). Densitometry of amplified bands was performed with a Phosphorimagere (Molecular Dynamics, Sunnyvale, Calif.), and results were normalized to the density of β-actin.

**Detection of immunoreactive inducible nitric oxide synthase.** Formalin-fixed, paraffin-embedded liver sections from three to four mice per group were processed for immunocytochemistry as described previously elsewhere (6), using an automated immunostainer (Ventana, Tucson, Ariz.) and polyclonal rabbit anti-mouse inducible nitric oxide synthase (1:500) (Calbiochem, La Jolla, Calif.).

**Treatment with antimony and/or anti-IL-10R monoclonal antibody.** Two weeks after *L. donovani* challenge (day +14), liver parasite burdens were determined, and mice then received no treatment or a single intraperitoneal injection of antimony (42). Antimony (sodium stibogluconate; Pentostam; Wellcome Foundation Ltd., London, United Kingdom) was given at optimal (500 mg/kg) or suboptimal (25, 50, or 100 mg/kg) doses (42). Seven days later (day +21), mice were sacrificed, and liver parasite burdens were measured. Percent parasite killing was determined as (day ×14 LDU – day ×21 LDU in treated mice)/(day ×14 LDU) × 100. Since IL-10 knockout mice rapidly controlled infection, single-dose antimony was given 1 week earlier on day +7, and treatment effects were determined on day +14. Differences in liver parasite burdens were analyzed using a two-tailed Student’s *t* test.

To test IL-10R blockade, normal BALB/c mice were injected intraperitoneally with 0.5 ml of saline containing 1 mg of anti-IL-10R monoclonal antibody (1B1.3A) (1, 10) or 1 mg of an isotype control monoclonal antibody (GL117.41, anti-β-galactosidase) (DNAX Research Institute). To determine prophylactic effects, monoclonal antibody injections were started 1 day after *L. donovani* challenge (day +1) and given once weekly; to test therapeutic effects, monoclonal antibody was given once on day +14 with or without a second injection on day +21. To test chemotherapy in the presence of IL-10R blockade, mice were injected once with monoclonal antibody on day +12 and then once with suboptimal antimony 2 days later. This 2-day interval was selected from a preliminary experiment (not shown) in which the efficacy of combined treatment was approximately doubled by giving anti-IL-10R 48 h before versus simultaneously with or 48 h after antimony.

**RESULTS**

**IL-10 regulates the kinetics of visceral infection.** Normal BALB/c and wild-type mice initially supported progressive *L. donovani* replication in the liver; after week 3, infection was controlled and parasite burdens declined (Fig. 1A). Although mRNA for both activating Th1 cell- (IL-12, IL-2, and IFN-γ) and suppressive Th2 cell-associated cytokines (IL-4, IL-10) is induced early on in parasitized liver and spleen in normal BALB/c mice (16, 17, 27, 29, 57), their self-curing phenotype suggested little overall impact for either IL-4 or IL-10 in this model (29).

Nevertheless, the kinetics of visceral infection were strikingly different in IL-10 knockout mice (Fig. 1A). Antileishmanial effects were apparent by week 2 and then clearly expressed by enhanced parasite killing and accelerated resolution of infection. These results mirror those recently reported in IL-10 knockout mice also challenged with *L. donovani* (31) as well as *L. major* (7, 21). Cytokine gene-deficient mice may respond to *L. donovani* with compensatory mechanisms not ordinarily developed in intact animals (40). Therefore, starting 1 day after infection, normal BALB/c mice were treated once weekly with anti-IL-10R monoclonal antibody to induce a functional IL-10-deficient state. Anti-IL-10R treatment permitted normal mice to control and rapidly resolve visceral infection (Fig. 1B).

In direct contrast, initial *L. donovani* replication in transgenic mice was unremarked (Fig. 1A). While liver parasite levels then peaked and declined somewhat, there was no further progress towards resolution of infection. In one experiment carried out to week 12, liver burdens in transgenic mice remained persistently high (4,329 ± 462 LDU, *n* = 3 mice).

**Effects on antileishmanial mechanisms.** To characterize IL-10’s actions in directing outcome of infection, liver tissue was assayed for granuloma assembly and expression of IL-12 and IFN-γ mRNAs and inducible nitric oxide synthase reactivity. In this model, granulomas enclose parasitized tissue macro-
phages with influxing T cells and blood monocytes (37). IL-12 likely initiates and maintains the basic Th1 cell response (17, 36, 51), and IFN-γ and inducible nitric oxide synthase act together at the level of the macrophage to regulate intracellular L. donovani killing (44, 57).

(i) Granuloma assembly. Livers of both control and IL-10 knockout mice demonstrated little histologic response 1 week after challenge (not shown). By week 2, however, events accelerated markedly in knockout mice; granuloma number was increased, structural maturation with epithelioid changes was advanced, and functional activity (parasite-free granulomas) was well established (Fig. 2 and 3). Similar kinetic (data not shown) and nearly identically enhanced histologic effects were induced in normal BALB/c mice by once-weekly prophylactic treatment with anti-IL-10R (Fig. 4B).

As judged by cell morphology, granuloma composition was also altered. In both types of IL-10-deficient animals, these structures were largely populated by mononuclear phagocytes, likely influxing blood monocytes (26, 37), and appeared to contain few surrounding lymphocytes (Fig. 3D, 3E, and 4B). In contrast, the latter microscopically distinct cells (previously identified as T cells [26]) were well represented at developing infected foci in both normal BALB/c and control monoclonal antibody-injected mice (Fig. 3C and 4A). In knockout and anti-IL-10R-treated mice, the initially exuberant inflammatory reaction appropriately subsided once parasites had been largely eliminated. By week 4, >50% of previously established granulomas had involuted; most remaining collections matured, became parasite-free, and then disassembled by week 8 (Fig. 2 and 3F).

In IL-10 transgenic mice, early granuloma assembly was inhibited (Fig. 2 and 5). At week 2, for example, 31% ± 3% of parasitized foci showed no mononuclear cell recruitment versus 7% ± 2% in wild-type mice. However, inhibition was transient, since granulomas subsequently developed and the majority evolved to a morphologically mature appearance. Nevertheless, the suppressive effect of IL-10 on macrophage function was clear: <10% of week 4 or week 8 granulomas in transgenic mice were parasite-free, and most remained heavily infected (Fig. 2 and 5F).

(ii) Detection of Th1 cytokines and inducible nitric oxide synthase. In normal, knockout, and wild-type BALB/c mice, the trend of results for IL-12 p40 mRNA expression (Fig. 6A), taken to reflect activity of the Th1 cell-associated response, suggested a correlation between control over visceral L. donovani and IL-12 p40 upregulation. In infected IL-10 transgenic mice, the IL-12 p40 response appeared to be deficient. Trends for IFN-γ mRNA expression (Fig. 6B) and results for inducible nitric oxide synthase immunoreactivity (Fig. 7), reflecting activity of the effector arm of the antileishmanial Th1 mechanism, were consistent with the preceding correlation and also suggested impaired responses in the presence of excess IL-10.

Inducible nitric oxide synthase reaction product was readily visualized at most parasitized liver foci 2 weeks after infection in normal BALB/c and wild-type mice, was particularly prominent in the numerous granulomas of knockout animals, and was reduced at most infected areas in transgenic mice (Fig. 7). This apparent differential expression of inducible nitric oxide synthase was also maintained at week 4 (not shown), at which time immunoreactivity was detected at ∼100% of parasitized foci in normal, knockout, and wild-type mice but at only one-third of granulomas in infected transgenic animals.

Interaction of IL-10 and chemotherapy. In vivo expression of antimony’s leishmanicidal activity is also Th1 cell dependent and specifically requires and is enhanced by IL-12 and IFN-γ (36, 39). Since IL-10 targets the same pathway, we treated IL-10 knockout and transgenic mice, anticipating that antimony’s efficacy would be upregulated by the absence and downregulated by the overexpression of IL-10.

(i) Effect on initial response to antimony. The results in Fig. 8A confirmed the first part of the preceding hypothesis—suboptimal doses of antimony which had no (25 to 50 mg/kg) or partial static (100 mg/kg) effects in normal animals induced killing in knockout mice. However, initial responses to optimal (500 mg/kg) (Fig. 8B) as well as suboptimal (100 mg/kg) (not shown) antimony were intact in transgenic mice.

(ii) Effect of IL-10 in combination with IL-4. Since the sustained presence of IL-10 sufficiently deactivated the Th1 cell-associated response to promote high-level infection, it was surprising that antimony’s initial efficacy was not similarly disabled in transgenic mice (36, 39). The observation suggested that a cofactor might be required for even more thorough suppression of the effects of IL-12 and/or IFN-γ before the response to antimony is impaired. IL-4 is not a determinant of outcome in mouse models of visceral infection (16, 29, 43, 52, 60); however, IL-4 acts synergistically with IL-10 to better suppress in vivo antileishmanial mechanisms (43, 48), and its

FIG. 2. Liver granuloma assembly (A), maturation (B), and antileishmanial function (C) after L. donovani challenge in IL-10 knockout (solid circles) and IL-10 transgenic (solid squares) mice. Results for normal BALB/c and wild-type controls were similar and were pooled (open circles). Data are from two experiments and indicate mean ± standard error of the mean values for four to five mice per group at each time point.
secretion is not upregulated in these IL-10 transgenic mice (20).

Therefore, antimony was tested in BALB/c mice immunized with heat-killed *L. major* promastigotes. This procedure conditions normal mice to cross react to *L. donovani* with a Th2 cell-type response, dependent upon both IL-10 and IL-4, which induces the noncure phenotype (43). Two-week-infected heat-killed *L. major* promastigote-stimulated mice, however, responded normally to single-dose antimony treatment (86% *L. donovani* killing induced by 500 mg/kg, three experiments, *n* = 10 mice), indicating that in the presence of increased IL-4, excess endogenous IL-10 also did not impair antimony’s visceral efficacy.

(iii) Recurrence of infection posttreatment. Although antimony was active in transgenic mice at the time drug was given, we also tested whether persistent IL-10-associated deactivation could undermine the durability of the treatment response. Wild-type and transgenic mice were injected with optimal-dose antimony and left undisturbed for an additional 10 weeks. In wild-type controls, the parasite load declined further after treatment to low levels (Fig. 8B). In contrast, liver burdens increased fivefold in transgenic mice, suggesting that once the drug effect waned, sustained IL-10 can foster progression of residual infection.

**Treatment of established infection with anti-IL-10R.** Two of the prior findings appeared to have therapeutic application:
prophylactic anti-IL-10R treatment induced rapid control over L. donovani (Fig. 1B), and antimony’s activity was enhanced in the absence of IL-10 (Fig. 8A). To translate these observations into a treatment approach, we completed this study by using normal BALB/c animals with established visceral infection to test two related hypotheses — that blocking IL-10’s effect would (i) induce parasite killing, and at the same time, (ii) also augment host responsiveness to chemotherapy.

(i) Effect of anti-IL-10R alone. Fourteen days after infection, mice were given a single injection of anti-IL-10R. This treatment enhanced granuloma formation (Fig. 5D) and proved surprisingly active in inducing appreciable parasite killing (65%) within 7 days (Fig. 9A). With no further treatment, this antileishmanial effect persisted for an additional week (e.g., to day +28). Injecting anti-IL-10R twice, on days +14 and +21, led to near resolution of infection by day +28, at which time parasite burdens were 11-fold lower than in mice treated twice with control monoclonal antibody.

(ii) Effect of anti-IL-10R combined with antimony. To test combination therapy, mice were injected on day +12 with monoclonal antibody and on day +14 with suboptimal antimony (50 mg/kg); liver parasite burdens were determined on day +21. Monoclonal antibody was given before antimony to allow for waning of apparent residual IL-10-induced effects (see Materials and Methods). As judged by comparing day +21 to day +14 liver burdens (Fig. 9B), antimony treatment failed to reduce parasite loads (e.g., did not induce L. donovani killing) in either control (group 1) or control monoclonal antibody-injected mice (group 2). In contrast, parasite killing reached 79% on day +21 in anti-IL-10R-injected mice (group 3) treated with antimony. This high-level leishmanicidal effect was similar to the 89% killing induced in control mice treated on day +14 with 10-fold more antimony (500 mg/kg). While again indicating the activity of treatment with anti-IL-10R alone (group 3), the results in Fig. 9B also show that low-dose antimony reduced the day +21 liver burden in anti-IL-10R-treated mice by an additional 63%. In control and control monoclonal antibody-injected mice, corresponding reductions induced by the same antimony treatment were 15 and 19%, respectively. Thus, in the presence of IL-10R blockade, there was a threefold increase in the efficacy of chemotherapy.

**DISCUSSION**

Since IL-10 has been implicated as an immunodeactivating factor in human visceral leishmaniasis (5, 11, 15, 18, 19, 22, 55, 56), inhibiting its action represents a logical therapeutic approach. Our results, in animals modified to express both ends of the spectrum of IL-10’s effects, firmly identify the cytokine as appropriate to inhibit and provide a rationale for targeting
IL-10 as an antileishmanial strategy in visceral infection. One method of implementing this strategy, monoclonal antibody-induced blockade of IL-10R signaling, clearly proved effective in established infection. The demonstration that anti-IL-10R was active by itself and with antimony indicates two parallel directions for future testing as immuno- or immunochemotherapy.

The findings described here, those recently reported in L. donovani- and L. major-infected IL-10 knockout mice (7, 21, 31) and L. major-infected IL-10 transgenic mice (20), and previous results with other diverse pathogens (30) together reemphasize the breadth of IL-10's regulatory effects in experimental infections. IL-10's actions appear particularly relevant in promoting intracellular infections in which host defense is governed by Th1 cells, mediated by cytokines including IL-12 and IFN-γ, and requires activated macrophages. In such infections, reducing IL-10 activity almost invariably increases resistance (30), even in the host with an established, apparently satisfactory Th1 cell response.

This effect, demonstrated in our normal BALB/c mice treated therapeutically with anti-IL-10R monoclonal antibody, has also been well illustrated in C57BL/6 mice chronically infected with L. major (7). While these animals, considered resistant to L. major, healed their primary skin lesions, persistent, low-level cutaneous infection was not eliminated until the mice were injected with anti-IL-10R (7). Thus, the benefits of

**FIG. 5.** Histologic effects of sustained IL-10 in infected livers. (A, C, and E) Control wild-type mice show initial granuloma assembly at week 2 (A), full maturation at week 4 (C), and granuloma involution with resolution of infection at week 8 (E). (B, D, and F) IL-10 transgenic mice show poor cell recruitment, suppressed granuloma assembly, and heavily parasitized Kupffer cells at week 2 (B), emerging granulomas at week 4 (D), and mature granulomas at week 8 (F). Despite intact structure, week 8 granulomas are filled with amastigotes. Magnification: (A, B, and F) 500×; (C to E) 315×.
inhibiting IL-10’s effects also extend beyond the acute stage of infection.

Primary Th1 cell-mediated antileishmanial events — restraint of initial *L. donovani* replication, granuloma assembly, inducible nitric oxide synthase expression, rapid resolution of infection (acquired resistance), and responsiveness to chemotherapy (29, 33, 36, 37, 39, 44, 57) — were all more prominent and/or more efficiently induced in animals devoid of IL-10. Each of the preceding responses requires IFN-γ, largely induced by IL-12 (33, 36, 51). Murphy and colleagues (31) have reported that restimulated spleen cells from the same infected knockout mice show increased IFN-γ secretion in vitro, which in turn correlated with accelerated tissue expression of inducible nitric oxide synthase and similarly rapid *L. donovani* killing.

While leishmanicidal activity in IL-10 knockout animals clearly requires both endogenous IL-12 and IFN-γ, treatment of knockout mice with neutralizing anti-IL-12 or anti-IFN-γ monoclonal antibody did not fully restore parasite replication (31). This finding may reflect incompletely neutralized, residual IL-12 or IFN-γ acting on sensitized or more efficiently activated effector cells no longer handcuffed by IL-10. Alternatively, the observation also suggests that the generalized immunoenhanced state in knockout animals may be supported by other antileishmanial mediators ordinarily limited by IL-10, for example, tumor necrosis factor (9, 58).

Responses in *L. donovani*-infected IL-10 transgenic mice appeared to be more complex than simply the result of persis-
tent, global suppression of Th1 cell mechanisms. Induction of Th1 cell-dependent events was indeed initially suppressed (except for responsiveness to antimony), entirely consistent with IL-10’s recognized effects (30), and associated with a rapid intracellular parasite replication. With time (e.g., by week 4), however, granuloma assembly emerged in transgenic mice accompanied by some inducible nitric oxide synthase expression. Thus, in this second phase, in which infection was converted from unrestrained to high-level chronic, IL-10’s direct macrophage-deactivating effects (9, 30) may have assumed more prominence. The presence of heavily parasitized macrophages within structurally mature granulomas 8 weeks after infection likely reflected this state of deactivation. That IL-10 can act primarily at the level of the macrophage and inhibit the effector function of the Th1 cell response without necessarily impairing IL-12 or IFN-γ secretion has been reported in IL-10 transgenic mice (20).

We would have predicted that if IL-10 overexpression inhibited one Th1 cell-dependent antileishmanial mechanism, it would likely inhibit all such responses. However, the intact initial response to antimony in transgenic mice and their granuloma assembly (albeit delayed) suggested compensatory mechanisms unrelated to Th1 cell cytokines or impervious to IL-10. For example, expression of ICAM-1, required for both granuloma formation and optimal antimony efficacy (45), is preserved in these transgenic mice (20). However, these observations also introduce the notion of differential in vivo sensitivity to IL-10, as previously documented in vitro for macrophage mechanisms ranging from tumor necrosis factor release to secretion of microbicidal reactive oxygen and nitrogen intermediates (9).

Thus, despite a common denominator (strict requirement for T cells, IFN-γ, and IL-12), the basic Th1 cell- and cytokine-mediated responses in this model showed hierarchical inhibition by sustained IL-10: (i) acquired resistance, dependent upon macrophage activation and induction of inducible nitric oxide synthase (44), appears most susceptible to deactivation, (ii) granuloma-initiating events show transient susceptibility, and (iii) the mechanism underlying expression of antimony’s in vivo leishmanicidal effect appears resistant.

Since these mechanisms in L. donovani-infected mice are complex, differential in vivo sensitivity to IL-10 may well reflect the influence of more than one factor. Two possibilities include effects on endogenous tumor necrosis factor which variably contributes to each of the preceding three antileishmanial responses (40, 58) and is readily suppressed by IL-10 (9), or different tissue thresholds (or ratios of IFN-γ to IL-10 [46]) which need to be reached for satisfactory expression of cytokine-induced antileishmanial events. Thus, even in the presence of excess IL-10, some Th1 cell reactivity induced in infected transgenic mice might be sufficient to exceed the threshold required for antimony responsiveness and granuloma assembly but not for induction of acquired resistance.

The preceding speculation about thresholds extends to using anti-IL-10R-induced immunostimulation might well prove critical in other infections associated with inflammation, but was not necessary in this model or in C57BL/6 mice chronically infected with L. major (7).

While anti-IL-10R treatment clearly accelerated granuloma assembly in infected livers, repeated injections did not release uncontrolled inflammation. This finding, together with the self-limited histologic response in knockout mice, indicated that IL-10 is not required to terminate the inflammatory response initiated by L. donovani. In some models of infection caused by other pathogens, however, IL-10 knockout mice have indeed developed runaway, sometimes fatal hyperinflammation — a pathological state thought to be largely induced by exaggerated and/or unopposed secretion of tumor necrosis factor, IL-12, and/or IFN-γ (30). In our anti-IL-10R-treated and IL-10 knockout animals, multiple factors may have limited such potential tissue damage, including other downregulating cytokines induced by L. donovani infection (transforming growth factor beta, IL-13, and possibly IL-4 [2, 33, 50, 60]), the apparent paucity of infiltrating T cells at parasitized foci, and the absence of a compensatory neutrophil response (14, 53).

Our findings in transgenic mice implicated sustained IL-10 secretion in relapse after chemotherapy, correlating with clinical observations (18, 56). Nonetheless, IL-10 overexpression neither by itself nor accompanied by increased IL-4 impaired the initial response to antimony. In BALB/c mice with a polarized, IL-4-driven Th2 cell response induced by cutaneous L. major infection, responsiveness to antimony is also intact during the time drug is administered (47). However, as in our treated IL-10 transgenic mice, L. major infection also recurred.

FIG. 8. Response to antimony treatment in IL-10 knockout (A) and transgenic (B) mice. (A) One week after infection (day +7), liver parasite burdens in normal BALB/c (655 ± 58 LDU, n = 6) and knockout mice (620 ± 43 LDU, n = 8) were similar and are indicated by intersection of the arrow-headed line with the vertical axis. Normal (open circles) and IL-10 knockout (solid circles) mice were injected once intraperitoneally on day +7 with the indicated suboptimal doses of antimony, and LDUs were measured on day +14. Day +14 results (two experiments, mean ± standard error of the mean values for seven to nine mice per group) above the horizontal line indicate parasite replication and below the line indicate parasite killing. (B) Wild-type (open squares) and IL-10 transgenic (solid squares) mice were injected once with optimal-dose antimony (500 mg/kg) on day +14 and then received no further treatment. Results indicate mean ± standard error of the mean values from two experiments for 6 to 10 mice at each time point. In A, P < 0.05 for treated knockout versus normal mice at all antimony doses. In B, P < 0.05 for transgenic versus wild-type controls at week 12.
once the drug was discontinued, suggesting that sustained IL-10- or IL-4-induced deactivation likely promoted progression of residual visceral or cutaneous infection posttreatment.

In the L. major model, cotreatment with anti-IL-4 monoclonal antibody plus antimony prevented recurrence of local infection and led to eventual cure (47). However, multiple studies (33), including those in IL-4 knockout, IL-4 transgenic, and anti-IL-4-treated normal BALB/c mice (29, 43, 52), have shown that IL-4 plays little or no role in experimental L. donovani infection and is therefore not a candidate for neutralization. IL-4 also is not consistently expressed in human kala-azar (2, 19, 22, 23, 56).

Our results in established visceral infection in normal mice support two potential applications for anti-IL-10R monoclonal antibody: first, as monotherapy, since injection of monoclonal antibody by itself induced rapid leishmanicidal activity; and second, in combination with chemotherapy to stimulate a synergistic effect. We do not yet know how blockade of IL-10R signaling is actually translated into L. donovani killing in vivo. Results reported by Belkaid and colleagues in anti-IL-10R-treated mice with chronic L. major infection do not point to IFN-γ upregulation or a more prominent immune response (7). Rather, the results suggest that macrophage responsiveness to the existing level of Th1 cell reactivity is enhanced by removing the limiting effects of IL-10 (7).

This conclusion conforms to the notion that even physiologic levels of IL-10, produced normally in response to inflammatory microbial stimuli, are capable of restraining the efficacy of the Th1 cell mechanism (30). Thus, as our results in established infection in normal mice suggest, the potential therapeutic application of anti-IL-10R monoclonal antibody extends beyond states in which IL-10 is pathologically elevated, provided the infected host has or is capable of expressing a satisfactory level of Th1 cell reactivity.

The BALB/c mice used in our anti-IL-10R treatment experiments fulfilled this requirement (29, 33, 38); studies in human visceral infection also indicate simultaneous expression of a Th1 cell cytokine response, albeit reduced or deactivated (15, 19, 22, 23, 33, 56). IL-10R blockade also appears to represent a viable alternative to administering activating Th1 cytokines in exogenous form (33, 38), the prior strategy employed to induce immunoenhancement in this L. donovani model.

Used in conjunction with antimony, conventional chemotherapy for visceral leishmaniasis in most regions (32, 33), anti-IL-10R treatment also allowed a substantial reduction (∼10-fold) in the dose of antimony required to kill the majority of liver amastigotes. Such an effect is similar to that achieved in the initial experimental approach to immunotherapy in which exogenous IFN-γ or IL-12 was combined with antimony (36, 42). In human visceral infection, antimony is administered daily for 28 days (33). We suspect that anti-IL-10R injection could also be used to reduce treatment duration, and experiments to test this effect are under way. Since antimony toxicity may relate to drug accumulation (32), reduction in either dose or duration of therapy permitted by coadministration of anti-IL-10R might also diminish toxicity while making conventional treatment more effective and less arduous.

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