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Visceral Leishmaniasis in Congenic Mice of Susceptible and Resistant Phenotypes: T-Lymphocyte-Mediated Immunosuppression

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This paper continues a comparative study (A. D. Nickol and P. F. Bonventre, Infect. Immun. 50:160–168, 1985) describing immune responses exhibited by congenic, Lsh^s mouse strains C57B1/10 (cure) and B10.D2 (noncure) during the course of disseminated leishmaniasis. We report that sublethal whole-body irradiation of B10.D2 mice before challenge with *Leishmania donovani* converted the noncuring mice to a curing phenotype. Splenic lymphocytes from *L. donovani*-infected B10.D2 mice failed to proliferate in response to parasite antigen stimulation in vitro. Splenic lymphocytes from irradiated, cured B10.D2 mice regained the capacity to respond to the parasite antigen stimulus. Transfer of T cells but not B cells from *L. donovani*-infected B10.D2 mice prevented the acquisition of immunity and recovery from infection in X-irradiated mice. In addition, a splenic T-cell population from *L. donovani*-infected B10.D2 mice suppressed the proliferation in vitro of parasite antigen-stimulated lymphocytes of irradiation-cured B10.D2 mice. Suppressor T cells were not demonstrable in the spleens of spontaneously cured C57B1/10 mice. Splenic lymphocytes from infected B10.D2 mice were deficient in the production of macrophage-activating factor (MAF) upon stimulation by *L. donovani* antigens in vitro. Deficient MAF production was specific for parasite antigen stimulation, because MAF production subsequent to concanavalin A stimulation of splenic lymphocytes from infected B10.D2 mice was not suppressed. The data suggest that a genetically based immunological defect in B10.D2 mice prevents the acquisition of effective cell-mediated immunity and subsequent elimination of *L. donovani* from tissue macrophages. The immunological deficit, not apparent in the curing C57B1/10, appears to be caused by the development of parasite antigen-specific suppressor T cells during the course of the disseminated leishmaniasis.

It is generally accepted that cellular immunity mediated by macrophages and sensitized T cells plays a primary role in recovery and acquisition of immunity to *Leishmania donovani* infection (18, 28). During the course of human visceral leishmaniasis, a significant proportion of infected individuals exhibit evidence of immune suppression (4, 5, 13, 16). Infection of certain inbred strains of mice with *L. donovani* constitutes a useful experimental model for the study of immune suppression in visceral leishmaniasis. In cure strains of mice, parasite burdens fall dramatically after 30 to 60 days, whereas noncure strains maintain elevated parasite burdens chronically (2). The immune status of mouse strains which demonstrate either the development of acquired immunity or, alternatively, suffer a chronic infection, have not been compared previously. Recent studies show that the noncure response is associated with a state of immune suppression of cell-mediated immunity (3, 19, 20, 26).

Elsewhere, we described a nonspecific, adherent-cell-mediated immunosuppression in *L. donovani*-infected (Lsh^s) congenic mice (20). In this paper we show that, in addition, B10.D2 noncure mice develop a suppressor T-cell population which inhibits parasite antigen-induced lymphocyte proliferation in vitro and also prevents the development of acquired immunity in vivo.

MATERIALS AND METHODS

Animals. Inbred C3Heb/Fej (C3H), BALB/cJ (BALB/c), and congenic C57B1/10SnJ (C57B1/10) and B10.D2/nSnJ (B10.D2) mice were purchased from Jackson Laboratory, Bar Harbor, Maine. Syrian golden hamsters were purchased from Engle Laboratory Animals, Farmersburg, Ind.

Leishmania amastigotes and promastigotes. L. donovani amastigotes and promastigotes were propagated as previously described (20).

Determination of parasite burden in infected animals. Infection was initiated by intravenous (i.v.) inoculation of amastigotes, and the parasite burden in the liver and spleen was estimated as described previously (20).

Lymphocyte proliferative responses. Lymphocytes were obtained from mouse spleens as described previously (20). The cells were suspended to 5.0×10^6 cells per ml in RPMI 1640 medium plus 20% heat-inactivated fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.), and 0.1-ml volumes were distributed into round-bottomed multiwell tissue culture plates (Costar, Cambridge, Mass.). For the experiments containing distinct cell populations in coculture, 5.0×10^5 cells of each population were used unless otherwise noted. Promastigote antigens were added in 0.1 ml of RPMI 1640 medium without serum at a concentration of 10^7 *L. donovani* promastigote equivalents per ml. After lymphocyte cultures were incubated for 42 or 66 h, [^{3}H]thymidine (specific activity, 6.7 Ci/mmol; ICN Pharmaceuticals Inc., Irvine, Calif.) incorporation was determined as described previously (20). Data are expressed as corrected counts per minute, i.e., the arithmetic mean of counts per minute from stimulated cultures minus the mean of unstimulated cultures. All samples were cultured and processed in quadruplicate.

T-cell enrichment. Spleen cell populations were enriched for T cells and depleted of macrophages and B cells by passage through nylon-wool columns (20) and resuspended in the appropriate medium. Yields of T-cell-enriched lym-

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phocytes were approximately 25% of total spleen cells from uninfected mice and 16% of spleen cells from infected mice.

T-cell depletion. Thy 1.2 antigen-bearing T cells were eliminated by lysis with monoclonal anti-Thy 1.2 antiserum (CL8600-A; Accurate Chemical and Scientific Corp., Westbury, N.Y.) and complement. Spleen cells were suspended at 10^7 cells per ml in RPMI 1640 plus 3% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) and anti-Thy 1.2 antiserum at a final dilution of 1:500. The cells were incubated for 1 h at 4°C. Cells were then centrifuged, and the pellet was suspended to the original volume in RPMI 1640 medium plus 3% bovine serum albumin and guinea pig complement (Accurate Chemical and Scientific) diluted 1:10. The cell suspension was incubated at 37°C for 1 h to effect lysis, and the remaining cells were washed twice and suspended in the appropriate medium.

Production of MAFs. Spleen cell supernatants containing macrophage-activating factor (MAF) were produced by a modification of the procedure described by Hockmeyer et al. (14). Spleen cells from normal or infected mice were suspended to 5.0×10^6 cells per ml in minimal essential medium plus 5% heat-inactivated fetal calf serum. Samples (20 ml) or the cell suspension with 5 μg of concanavalin A (ConA; purified ConA provided by D. Behnke, Cincinnati, Ohio) or 5.0×10^6 promastigote equivalents per ml were incubated in tissue culture flasks (75 cm^2) in an upright position for 48 h at 37°C under 5% CO_2. ConA or parasite antigen was added to control cultures after 48 h at 37°C. Spleen cells were removed by centrifugation, and Sephadex G-10 (Pharmacia Fine Chemicals, Piscataway, N.J.) (10 mg/ml) was added to ConA-generated supernatants and mixed for 10 min to bind residual ConA (14). The lymphokine-containing and control supernatants were stored at −20°C after filter sterilization.

Assay of MAFs. MAF activity was measured by the capacity of spleen cell supernatants to induce leishmanicidal activity in cultured peritoneal macrophages (10). Macrophages were obtained from female C57B1/10 or B10.D2 mice 3 to 4 days after the injection of 2.0 ml of 3% Brewer's thioglycolate medium (BBL Microbiology Systems, Cockeysville, Md.) intraperitoneally. Peritoneal exudate cells were cultured as monolayers on cover glasses for 24 h and infected with *L. donovani* amastigotes (10). Infected macrophage monolayers were incubated in 2 ml of minimal essential medium plus 10% heat-inactivated fetal calf serum containing either the ConA- or parasite antigen-stimulated spleen cell supernatant at different concentrations. Control monolayers were cultured in minimal essential medium plus 10% heat-inactivated fetal calf serum plus 15% (vol/vol) supernatant of normal spleen cell cultures. Media were exchanged daily for 3 days, after which infected monolayers were rinsed in minimal essential medium and stained with Diff-Quick (American Scientific Product, Obetz, Ohio). Estimates of the antileishmanial activities of the MAF preparations were based on the physical elimination of ingested parasites (10). Values are expressed as the percentage of amastigotes eliminated by MAF treatment with untreated infected monolayers as the 100% baseline.

Irradiation of mice and spleen cell transfer. Mice were sublethally irradiated with 415 rad of whole-body X-irradiation in a Westinghouse Quadrocondex therapy unit. Mice were irradiated 24 h before i.v. challenge with 5.0×10^6 *L. donovani*. One hour after irradiation, groups of B10.D2 (noncure) mice received a specific spleen cell population isolated from chronically infected B10.D2 donor animals. The spleen cells were obtained and T-cell enrichment was accomplished as described previously (20). T-cell-depleted spleen cells were obtained by lysis of Thy 1.2-bearing cells with monoclonal anti-Thy 1.2 antibody and complement. Spleen cell populations were suspended in Hanks balanced salt solution containing no Ca^{2+}, Mg^{2+}, and phenol red, and 10^7 cells were injected i.v. into irradiated recipient mice as indicated.

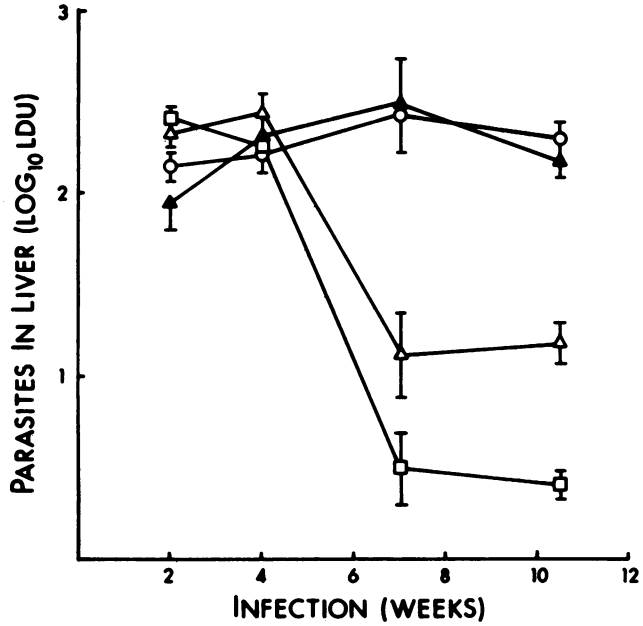

FIG. 1. Protective effect of sublethal whole-body irradiation of B10.D2 mice infected with *L. donovani*. Mice were X-irradiated with 415 rad 24 h before challenge with 5.0×10^6 amastigotes. One hour after irradiation, recipients received ca. 10^7 T-cell-enriched or B-cell-enriched spleen cells from B10.D2 donor mice infected 60 to 90 days previously. The course of infection in four groups (five mice per group) was charted over a 12-week period by microscopic evaluation of liver imprints. Symbols: ▲, untreated B10.D2 mice; □, irradiated B10.D2 mice; ○, irradiated recipients receiving 10^7 T-cell-enriched spleen cells from infected donors; △, irradiated recipients receiving 10^7 B-cell-enriched spleen cells from infected donors.

RESULTS

Evidence for T-cell suppression of acquired immunity to *L. donovani*. The results (Fig. 1) show that irradiated B10.D2 mice exhibited a curing pattern of infection similar to that of the C57B1/10 (cure) mouse. The data also show that T cells from infected (suppressed) donors, but not B cells, abrogated the protective effects of irradiation. This experiment confirmed the recent observations of Blackwell and Ulczak (3).

Two cell types suppress lymphocyte proliferation to parasite antigens in noncuring mice. Splenic lymphocytes from C57B1/10 (cure) mice, which have eliminated parasite burdens by development of naturally acquired immunity, responded briskly when stimulated with parasite antigens (Table 1, line 1). Similarly, splenic lymphocytes from 7- to 10-week-infected B10.D2 mice converted to a cure phenotype by irradiation also proliferated in response to parasite antigens (Table 2, line 2). However, the parasite antigen responses of spleen cells from infected C57B1/10 mice before curing (Table 1, line 2) and nonirradiated infected B10.D2 mice (Table 2, line 2) were markedly inhibited. Splenic lymphocytes from 6-week-infected C57B1/10 mice (Table 1, line 5) or 9- to 12-week-infected B10.D2 mice

TABLE 1. Restoration of parasite antigen-induced proliferation of spleen cells infected C57B1/10 mice after removal of nylon-wool-adherent cells[a]

Spleen cell population[b]	Proliferation	
	Corrected cpm ± SEM $\times 10^{-3}$[c]	% of Control
1. Cured	8.7 ± 0.9	100
2. Infected	3.1 ± 1.1	36
3. NWNA infected	0.2 ± 0.2	2
4. Cured + cured	11.3 ± 1.6	100
5. Cured + infected	2.8 ± 0.3	25
6. Cured + NWNA infected	9.8 ± 0.9	87

[a] C57B1/10 mice were infected i.v. with 10^7 viable *L. donovani* and used 6 weeks (infected) or 20 weeks (cured) after infection.

[b] Microtiter wells containing 5.0×10^5 cells of each cell population were incubated for 90 h with 5.0×10^6 promastigote equivalents per ml. [^{3}H]thymidine (1.0 μCi per well) was added for the last 18 h, and the incorporation of radiolabel was determined. NWNA, Nylon wool nonadherent.

[c] The corrected counts per minute was calculated as the difference between counts per minute of cultures containing antigen and counts per minute of cultures without antigen.

(Table 2, line 4) cocultivated with spleen cells from cured mice inhibited lymphocyte proliferative responses to parasite antigens. The T-cell-enriched spleen cells from infected C57B1/10 mice (Table 1, line 5), unlike total spleen cells (Table 1), failed to suppress the parasite antigen-induced proliferation of spleen cells from cured C57B1/10 mice. Thus, it appears that the suppression of parasite antigen responses by total spleen cells of infected C57B1/10 mice observed in this case was due to adherent suppressor cells as previously described (20). The ability of T-cell-enriched spleen cells of infected C57B1/10 mice to respond to mitogens (20) but not to parasite antigens (Table 1, line 3) may be explained by the more stringent requirement for macrophages in specific antigen-stimulated proliferation of sensitized lymphocytes (27).

In contrast to the solely adherent cell suppression manifested in curing C57B1/10 mice, T-cell-enriched spleen cells from infected B10.D2 mice suppressed parasite antigen-stimulated lymphoproliferative responses. The elimination of T cells with anti-Thy-1.2 and complement failed to abolish suppression of the parasite antigen-specific response (Table 2, line 5). Thus, the suppressive activity demonstrated by both the T-cell-enriched and T-cell-depleted populations appeared to be caused by two distinct suppressor cell types present in the spleens of chronically infected B10.D2 mice. The evidence indicates the presence of adherent suppressor cells (Table 2, line 5), as was demonstrated in C57B1/10 mice and, in addition, suppressor T cells unique to B10.D2 (noncure) mice (Table 2, line 6). To test this possibility further, nonadherent spleen cells were treated with anti-Thy 1.2 and complement. The 28% of the nonadherent cells which survived lysis by anti-Thy 1.2 and complement consisted of B cells, macrophages, and null cells which did not adhere to nylon wool. These residual nonadherent cell populations when added to cultures at ca. 1.4×10^5 cells per well (i.e., 28% of 5×10^5) did not inhibit the parasite antigen responses of spleen cells from irradiation-cured B10.D2 mice (Table 2, line 7). As already noted, T-cell-enriched spleen cells of infected B10.D2 mice were suppressive at a concentration of ca. 10^5 (Table 2), and the data show, therefore, that the infected noncuring mice developed parasite antigen-specific suppressive T lymphocytes as well as adherent suppressor cells (20) during infection.

Production of macrophage-activating lymphokines by lymphocytes of infected B10.D2 and C57B1/10 mice. Sustained activation of *L. donovani*-infected macrophages by lymphokines results in parasite elimination (10, 19). Murray et al. (19) reported that ConA- or parasite antigen-stimulated spleen cells from *L. donovani*-infected BALB/c mice (Lsh^s) were transiently deficient in the production of macrophage-activating lymphokine and presented suggestive evidence that adherent spleen cells were responsible. In efforts to corroborate these observations, we tested the C57B1/10 (cure) and B10.D2 (noncure) congenic mice during the course of their infections. Macrophage-activating activity was determined by incubating infected macrophages with diminishing concentrations of spleen cell supernatants elicited by parasite antigens. The lymphocyte culture supernatants prepared from spleen cells of 6- and 20-week-infected C57B1/10 cure mice completely eliminated amastigotes at dilutions as low as 5% (vol/vol) (Fig. 2). However, supernatants prepared from spleen cells of 6- or 20-week-infected B10.D2 (noncure) mice failed to completely eliminate amastigotes when used at a concentration of 15% and were inactive at a concentration of 5%. Thus, spleen cells from the infected noncure mice produced considerably less MAF upon parasite antigen stimulation than did spleen cells from cure mice.

To determine whether the defect in MAF production by *L. donovani*-infected B10.D2 mice was restricted to responses to parasite antigens, MAF-containing supernatants were also prepared by ConA stimulation of spleen cells from uninfected and infected C57B1/10 and B10.D2 mice. *L. donovani*-infected macrophage monolayers were exposed to media containing 15, 10, and 5% (vol/vol) of the ConA-

TABLE 2. Dual suppression of parasite antigen-induced proliferation of spleen cells from irradiation-cured B10.D2 mice by macrophages and T cells from infected B10.D2 mice[a]

Spleen cell populations[b]	Proliferation [corrected cpm ± SEM $\times 10^{-3}$][c]	
	Expt 1	Expt 2
1. Cured (irradiated)	2.4 ± 13 (100)	5.4 ± 0.9 (100)
2. Infected (unirradiated)	0.9 ± 0.5 (38)	1.1 ± 0.9 (20)
3. Cured + cured	3.4 ± 0.6 (100)	6.7 ± 0.5 (100)
4. Cured + infected	0.5 ± 0.7 (15)	1.3 ± 0.3 (19)
5. Cured + T cell depleted, infected[d]	0.5 ± 0.4 (15)	1.7 ± 0.4 (25)
6. Cured + T cell enriched, infected[e]	ND	2.3 ± 0.4 (34)
7. Cured + adherent cell depleted, T cell depleted, infected[f]	ND	5.7 ± 1.3 (85)

[a] Mice were infected i.v. with 10^7 viable *L. donovani*. Cured B10.D2 mice received 415 rad of whole-body irradiation 24 h before infection which caused parasite burdens to be eliminated by 8 weeks after infection.

[b] Spleen cells were isolated from animals 9 and 12 weeks after infection for experiment 1 and 2, respectively. Microtiter wells containing 5.0×10^5 cells of each cell population were incubated for 66 or 90 h in experiment 1 and 2, respectively, with 5.0×10^6 disrupted promastigotes per ml. [^{3}H]thymidine (1.0 μCi per well) was added for the last 18 h, and the incorporation of radiolabel was determined.

[c] The corrected counts per minute was calculated as the difference between counts per minute of cultures containing antigen and counts per minute of nonstimulated cultures. Numbers in parentheses are percentages of cured control values. ND, Not done.

[d] Total spleen cells were treated with anti-Thy 1.2 and complement.

[e] Each well contained 1.4×10^5 NWNA (nylon-wool-nonadherent) spleen cells from infected B10.D2 mice.

[f] B10.D2 spleen cells were passaged through nylon wool (NWNA), treated with anti-Thy 1.2 and complement, and readjusted to 1.4×10^5 cells per well.

elicited supernatants derived from the curing and noncuring strains. There was no measurable difference in the macrophage-activating activity of ConA supernatants at any of the concentrations tested. Parasites were eliminated from macrophages at a concentration of 5% (Fig. 3) and 10 or 15% (data not shown) of lymphokine. Spleen cell supernatants from uninfected, 7-week-infected, and 20-week-infected mice of both the curing and noncuring phenotypes caused 95 to 100% elimination of amastigotes. Thus, although spleen cells from 6- to 8-week-infected C57B1/10 and B10.D2 mice fail to proliferate in response to ConA stimulation (20), no defect in ConA-induced MAF production was noted. Lymphokine preparations at less than 5% (vol/vol) were not tested, and thus it is possible that a defect in ConA-induced MAF might be demonstrable at a lesser concentration. We do not consider this likely, however, because in our experience ConA supernatants from any source failed to eliminate *L. donovani* amastigotes from infected macrophages at a concentration less than 5% (vol/vol).

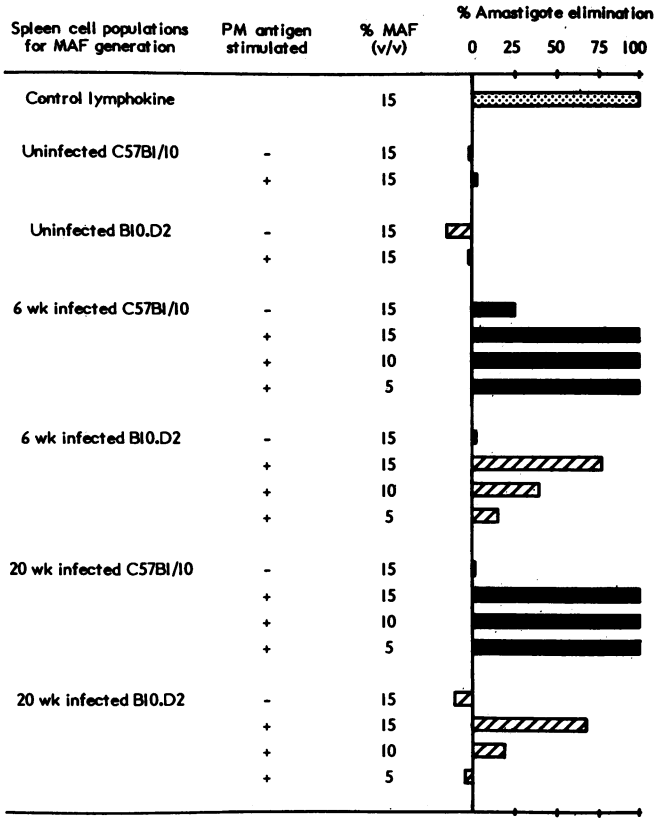

FIG. 2. Capacity of lymphokines generated by promastigote antigen stimulation of spleen cells from *L. donovani*-infected C57B1/10 and B10.D2 mice to eliminate amastigotes from infected peritoneal macrophage monolayers. Spleen cells were isolated from uninfected mice or mice injected i.v. with 10^7 viable *L. donovani* amastigotes 6 or 20 weeks previously. Lymphokine-containing supernatants were prepared by incubating 10^8 spleen cells for 48 h in 20 ml of culture medium with 10^8 freeze-thaw-disrupted promastigotes. Adherent, thioglycolate-induced macrophages from normal C57B1/10 or B10.D2 mice were infected with viable *L. donovani* amastigotes. Lymphokine activity was assayed by incubating infected macrophage monolayers with media containing lymphocyte supernatants at different concentrations for 72 h. Media were changed daily. The number of residual intact amastigotes per 100 macrophages after 72 h was enumerated. Results are presented as percent amastigote elimination when compared with the infected macrophage monolayers incubated in medium without lymphokines. Control infected monolayers contained ca. 500 amastigotes per 100 macrophages. A ConA-induced control lymphokine generated by C3H spleen cells is included for comparative purposes.

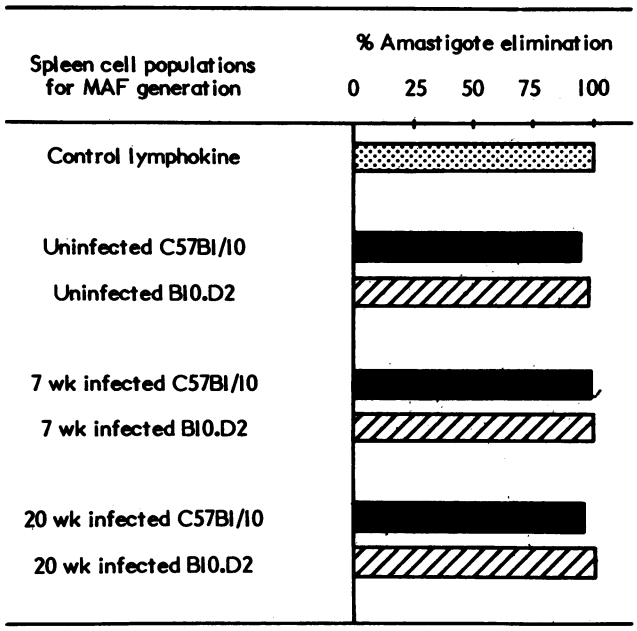

FIG. 3. Capacity of lymphokines generated by ConA stimulation of spleen cells from *L. donovani*-infected C57B1/10 and B10.D2 mice to eliminate amastigotes from infected peritoneal macrophage monolayers. Leishmanicide-generating activity in supernatants of normal spleen cells stimulated with ConA (5 μg/ml) for 48 h was assayed as with the promastigote antigen-generated supernatants (see the legend to Fig. 2). A 5% (vol/vol) concentration of ConA lymphokine was used in all cases. A control lymphokine generated by ConA stimulation of C3H spleen cells is included for comparison.

DISCUSSION

Sublethal, whole-body irradiation applied 1 day before infection with *L. donovani* amastigotes converted the noncure B10.D2 phenotype to a cure phenotype similar to that seen in spontaneously curing C57B1/10 mice. Transfer of enriched T-cell populations from chronically infected B10.D2 mice suppressed the expression of the cure phenotype in irradiated B10.D2 mice (Fig. 1). The data suggest that noncure B10.D2 mice fail to eliminate *L. donovani* owing to the appearance of radiation-sensitive T-suppressor lymphocytes or their precursors during disseminated leishmaniasis. These results confirm the recent observations of Blackwell and Ulczak (3) who also characterized the suppressor cell as having a Thy-1^+ Lyt-1^+2^- surface phenotype (3). Howard et al. (15) also have demonstrated suppressor T cells in BALB/c mice during the course of visceralized *Leishmania tropica* infection. As shown by Blackwell and Ulczak (3) and by us here with *L. donovani*-infected congenic mice, X-irradiation also permits the development of curative immunity during *L. tropica* infection of BALB/c mice (15). The suppressor T cell in *L. tropica*-infected BALB/c mice has also been identified as of Thy-1^+ Lyt-1^+2^- surface phenotype (17). Thus, there appears to be considerable similarity in the nature of the immunosuppression evoked in both murine models of disseminated leishmaniasis.

The results of our studies and those of Ulczak and Blackwell (3, 26) and Howard et al. (15) may be compared with experimental models investigating immune suppression during the growth of malignant tumors. Both situations involve chronic antigen stimulation of increasing intensity as parasite and tumor burden progress. Whole-body irradiation can retard tumor growth (25), inhibit the establishment of tumors (24), or cause tumor regression (12). These phenomena have been attributed to the elimination of radiation-sensitive suppressor T cells or their precursors (12, 24, 25). North and his colleagues (1, 6) observed that transfer of T cells from tumor-immune to nonimmune recipient mice prevented the growth of recently implanted homologous tumors. Additionally, they found that suppressor T cells neutralized the protective function of immune T cells when both T-cell populations were transferred to tumor-bearing recipients. Using a similar mouse tumor model, Fujimoto et al. (7) demonstrated that a sequential activation of two suppressor T-cell subsets and the production of soluble suppressor factors occurred during tumor development (7, 9, 22).

We have identified a T-cell population in *L. donovani*-infected B10.D2 mice which suppresses in vitro splenic lymphocyte responses to leishmanial antigens. Concomitant with the acquisition of immunity by irradiated B10.D2 mice, splenic lymphocytes from the cured mice regained the capacity to proliferate in response to stimulation by parasite antigens in vitro. A Thy 1.2^+ suppressive lymphocyte population from chronically infected B10.D2 mice inhibited the parasite antigen-induced proliferation of sensitized lymphocytes from irradiation-cured B10.D2 mice (Table 2). Thus, we have demonstrated an in vitro indicator of parasite antigen-specific suppressor T cells in *L. donovani*-infected noncure mice. Suppression of specific antigen responses by suppressor T cells, however, was demonstrable only after the removal of suppressor macrophages also present in the total spleen cell populations. Removal of either the suppressor T cells or the adherent cells individually from total spleen cells failed to restore normal proliferative responses. Thus, the data indicate that parasite antigen-stimulated proliferation of sensitized lymphocytes from infected B10.D2 mice is suppressed by an adherent cell-mediated mechanism (20) and also by suppressor T cells. The import of the T-cell suppression was established clearly by irradiation experiments in which the B10.D2 mice were restored to immunological competence during the course of infection. C57B1/10 mice which eliminated their parasite burden spontaneously after several months of infection did not develop significant T-cell suppression. Removal of nylon-wool-adherent cells from spleen cells of infected C57B1/10 mice fully restored *L. donovani* antigen-stimulated lymphocyte proliferation in vitro. This would indicate that in the curing C57B1/10 mice only the adherent cell-mediated suppression is expressed (20).

We also showed that splenic lymphocytes from *L. donovani*-infected noncure B10.D2 mice, when stimulated in vitro with parasite antigens, were deficient in the production of MAF required for the elimination of intracellular amastigotes. The deficiency in lymphokine production was specific for parasite antigen stimulation, because ConA-stimulated lymphocytes from uninfected and infected B10.D2 mice released equivalent levels of macrophage-activating lymphokine (Fig. 3). In contrast, splenic lymphocytes from *L. donovani*-infected C57B1/10 mice produced optimal levels of MAF when stimulated with either ConA or parasite antigens. Generation of MAF occurred during a period when the in vitro lymphocyte proliferative responses of C57B1/10 mice to either ConA or parasite antigens were markedly suppressed. This would suggest that the production of MAF in vitro by lymphocytes does not necessitate lymphocyte proliferation. Similar results have been obtained by Nogueria et al. (21); spleen cells from *Trypanosoma cruzi*-infected, resistant mice produced more MAF upon *T. cruzi* antigen stimulation than did spleen cells from infected, susceptible mice. These investigators also showed that the MAF-producing lymphocytes of *T. cruzi*-infected mice failed to proliferate in the presence of *T. cruzi* antigen or ConA. Others have demonstrated that lymphokine production by stimulated lymphocytes may occur in the absence of cellular proliferation. Hecht et al. (11) showed that gamma-interferon was released by irradiated T cells responding to antigen. In addition, migration inhibitory factor was produced by irradiated ConA-stimulated lymphocytes (8) and by antigen-stimulated lymphocytes treated with 5-bromodeoxyuridine (23). This same phenomenon may explain our data showing an inhibition of proliferation by spleen cells of infected C57B1/10 mice with no apparent defect in production of MAF (Fig. 3). Our data are at odds with those reported by Murray et al. (19) who found reduced MAF production by ConA-stimulated lymphocytes from *L. donovani*-infected BALB/c mice. The reason for this discrepency is uncertain, but it may be related to differences in the responses of BALB/c and the congenic mice used in our studies. Our data, however, appear to be unequivocal.

Because ConA is theoretically capable of stimulating all T lymphocytes, we suggest that sufficient numbers or T cells in the cultures of spleen cells from infected C57B1/10 and B10.D2 mice respond by releasing significant quantities of MAF in the absence of cellular proliferation. This situation differs with that of parasite antigen-stimulated spleen cells of infected C57B1/10 and B10.D2 mice. The quantity of MAF produced in this case would be proportional to the number of antigen-reactive T cells in these spleen cell populations. Curing C57B1/10 mice may develop a significantly larger population of antigen-reactive T cells capable of releasing MAF than do the noncuring B10.D2 mice. When extrapolated to the in vivo situation, a more abundant production of MAF may account for the greater activation of systemic macrophages during disseminated leishmaniasis in C57B1/10 than in B10.D2 mice (20). This augmented macrophage activation in C57B1/10 mice may lead subsequently to parasite elimination. In contrast, B10.D2 mice, which produce smaller quantities of MAF, fail to activate systemic macrophages sufficiently to cause parasite elimination. A partial activation of tissue macrophages in B10.D2 mice may account for the maintenance of the stable, chronic infection seen in noncuring mice, rather than the development of a fatal infection. The failure of B10.D2 mice to develop a significant population of antigen-reactive T lymphocytes in vivo may be the result of the suppressor T-cell population appearing during *L. donovani* infection. Suppressor T cells may prevent expansion of antigen-specific T-cell clones and thus restrict lymphokine-producing T cells required to provide adequate cell-mediated immunity and elimination of the parasite burden.

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