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Suppression of Interleukin-2 Production by Macrophages in Genetically Susceptible Mice Infected with *Leishmania major*

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Spleen cells from BALB/c mice infected with 2×10^7 *L. major* promastigotes and developing progressive disease produced significantly lower levels of interleukin-2 (IL-2) in response to concanavalin A stimulation than did spleen cells from uninfected mice. In contrast, spleen cells from sublethally irradiated and infected mice, which were able to contain lesion development, produced significantly higher levels of IL-2. The increase in IL-2 production closely paralleled lesion regression. Mice protectively immunized by four intravenous injections with lethally irradiated promastigotes also produced enhanced levels of IL-2, which were sustained after challenge infection. In contrast, spleen cells from BALB/c mice given four s.c. injections of irradiated promastigotes produced high levels of IL-2 before but not after infection. These mice eventually produced levels of IL-2 indistinguishable from those of unimmunized mice with progressive disease. There is thus an inverse relation between disease progression and the ability of spleen cells to produce IL-2. Spleen cells from mice with uncontrolled disease not only produced lower levels of IL-2 but also impaired IL-2 production by normal spleen cells. The ability to inhibit IL-2 was abrogated by (i) passing the cells through a Sephadex G-10 column, (ii) removal of plastic adherent cells, and (iii) removal of carbonyl iron-ingesting cells. Furthermore, Sephadex G-10 column-treated and plastic adherent, nonspecific esterase-positive spleen cells from mice with progressive disease were able to suppress IL-2 production by normal splenic T cells. The suppressive activity of the adherent cells was not affected by treatment with anti-Thy-1.2 antibody and complement. In contrast, adherent spleen cells from uninfected mice were devoid of such suppressor activity. The depressed IL-2 production by spleen cells from progressively infected mice could be restored to that of normal spleen cells by the addition of indomethacin to the culture. There was however, no correlation between IL-2 production and IL-1 activity in infected or immunized BALB/c mice. Thus, it appears that the suppression of IL-2 production is mediated by prostaglandins elaborated by macrophages from chronically infected mice.

Interleukin-2 (IL-2) is produced by T lymphocytes in response to activation. It is essential for T-cell proliferation and differentiation in vitro and has been implicated as an important regulator of immune responses (38). Conclusive evidence for a modifying role for IL-2 in vivo is lacking. However, analysis of IL-2 production has been found relevant for several diseases in which a dysregulation of lymphocyte funciton is apparent. Impairment of IL-2 production has been detected in murine models of acute trypanosomiasis (11), lethal malaria infection (21), and histoplasmosis (42) and in patients with lepromatous leprosy (32). Spleen cells from mice with acute *Leishmania donovani* infection have also been found to produce lower levels of IL-2 in vitro than were produced by normal spleen cells (36). However, it remains unclear whehther suppressed IL-2 production plays a causal role in disease development or is merely a consequence of disease.

The spectrum of murine models reflects the range of clinical leishmaniasis and is amenable to genetic and immunological analysis (24, 29, 30). BALB/c mice are extremely susceptible to *Leishmania major* in that the initial cutaneous lesion develops inexorably, leading to uniformly fatal disseminating and visceralizing disease, even with a minimal infective dose. It is now generally believed that the vulnerability of mice of BALB background to *L. major* infection is largely due to a non-*H-2*-linked autosomal gene, tentatively named *Scl* (J. M. Blackwell, J. G. Howard, F. Y. Liew, and C. Hale, Mouse Newsl., **70**:86, 1984), which is expressed primarily in macrophages. The inability of macrophages to control initial replication of the parasite appears to lead to the preferential induction of specific suppressor T cells which prevent the generation of otherwise curative cell-mediated immunity (9, 12). The suppressor T cell precursors can be inhibited and the effector T cells can be strongly induced by subjecting the mice to sublethal (550 rads) irradiation shortly before infection. Such treatment precludes progresive lesion development, and the majority of mice achieve complete healing and are immune to further *L. major* infection (13). Protective immunity has also been induced by prophylactic immunization with irradiated, killed, or disintegrated *L. major* promastigotes via intravenous (i.v.) or intraperitoneal routes but not by subcutaneous (s.c.) or intramuscular injections (14, 31); s.c. or intramuscular injections of the same promastigote antigens strongly impair the induction and expression of protective immunity resulting from i.v. immunization (23, 26). Suppressive reactivity, convalescent immunity, acquired prophylactic immunity, and the inhibitory effect of s.c. injection can all be adoptively transferred by T cells bearing the Lyt-1^+2^- phenotype (22, 25, 26). Thus, irradiation, infection, and prophylactic immunizations (against *L. major*) of susceptible BALB/c mice offered a unique model for the present study of regulation of the possible role of IL-2 in infectious disease in vivo.

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MATERIALS AND METHODS

Mice. Female BALB/c mice 10 to 12 weeks old were obtained from a colony at the Wellcome Research Laboratories, Beckenham, United Kingdom.

***Leishmania* parasites.** The strain of *L. major* (PLV39) used in this study was kindly provided by R. A. Neal, London School of Hygiene and Tropical Medicine, London, United Kingdom. Promastigotes were cultivated by passaging a piece of nonulcerated lesion into conventional Novy, MacNeal, and Nicolle (NNN) medium blood agar slopes overlaid with 1% glucose–phosphate-buffered saline (pH 7.2). After 4 days at 26°C, the parasites were expanded by passaging into NNN or Schneider *Drosophila* medium (GIBCO Laboratories, Grand Island, N.Y.) containing 30% fetal calf serum (FCS). After centrifugation, the number of organisms was estimated microscopically with a hemacytometer after dilution in a 10% solution of Lugol iodine. Mice were infected s.c. in their shaved rumps with a single dose of 2×10^7 promastigotes in 0.1 ml of 1% glucose–phosphate-buffered saline. The lesions that developed were measured with a calliper gauge. Age-matched, uninfected mice were used as controls.

Antigens. Formaldehyde-fixed (ff) promastigotes were prepared by incubating 2×10^8 parasites per ml in 0.5% Formalin for 5 min at room temperature. They were then washed four times in saline, suspended to 2×10^8 parasites per ml in saline, and stored at 4°C until used.

γ-Irradiation. Mice were irradiated by a cesium (^{137}Cs) source at a rate of 50 rads/min (550 rads per dose) 4 h before infection. The promastigotes used for immunization were exposed to 150 kilorads of γ-irradiation from the same source at 143.7 rads/min. After irradiation, the parasite suspension was stored at 4°C and used within 1 week.

Immunization. Mice were immunized i.v. or s.c. with four weekly doses of 2×10^7 irradiated promastigotes in 0.1 ml of phosphate-buffered saline.

Tissue culture medium. The tissue culture medium consisted of RPMI 1640 (Flow Laboratories, Herts, United Kingdom) supplemented with glutamine (2 mM), penicillin, streptomycin, 2-mercaptoethanol (50 μM), and 5 or 10% heat-inactivated FCS.

In vitro production of IL-1. Peritoneal cells (PC) were isolated by injecting 5.0 ml of cold culture medium into the peritoneal cavity and collecting the cells under sterile conditions. They were washed three times with cold medium and cultured in standing tissue culture flasks (Becton Dickinson Labware, Oxnard, Calif.) at 10^6 cells per ml in culture medium with or without 10 μg of lipopolysaccharide (LPS; Difco Laboratories, Detroit, Mich.) per ml for 4 days at 37°C and 5% CO_2. The supernatants were then filtered through 0.22 μm Millex filters (Millipore SA, Molshiem, France) and stored at −70°C until use. The laboratory standard IL-1 control was prepared similarly from LPS-activated P388 D1 cells (16).

Assay for IL-1 activity. The thymocyte proliferation assay (43) was used for IL-1 titration. Thymocytes from BALB/c mice were adjusted to 10^7 cells per ml in culture medium, and a suboptimal dose of phytohemagglutinin (2.5 μg/ml) was added; 100 μl of this cell suspension was distributed into microtiter plates with 100 μl of individual samples and incubated at 37°C in 5% CO_2 for 72 h. At 16 to 20 h before harvesting, the cultured cells were pulsed with 1 μCi of [^{3}H]thymidine (24 Ci/mmol; Radiochemical Centre, Amersham, United Kingdom) per well. The cells were harvested with an automated cell harvester (Titertek), and radioactivity was measured with a beta-counter (LKB 1215; LKB, Sweden). The results from quadruplicate cultures were expressed as mean counts per minute ± standard error of the mean after subtracting the background counts per minute of the cultures in the absence of LPS.

In vitro stimulation of IL-2 production. Spleen cells from normal, immunized, or infected mice were collected, adjusted to 10^7 cells per ml in medium, and incubated for 24 h with or without 5 μg of concanavalin A (ConA; Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.). The concentration of ConA used was chosen after a series of titrations as optimal for IL-2 production. The supernatants of the cultures were collected, filtered through 0.22-μm Millex filters, and stored at −70°C until tested. In some experiments, the effect of a prostaglandin production inhibitor, indomethacin (Sigma Chemical Co., St. Louis, Mo.), on IL-2 production was tested. Indomethacin dissolved in Tris buffer (pH 8.4) was added at a final concentration of 1, 5, or 10 μg/ml at the beginning of the culture.

Assay for IL-2 activity. IL-2 activity was determined by its capacity to maintain the in vitro proliferation of ConA-induced blasts (18) or to support the growth of a murine IL-2-dependent cytotoxic T cell line (CTL) (7). ConA blasts were obtained by stimulating normal spleen cells (10^7 cells per ml) with 3 μg of ConA per ml in tissue culture medium for 48 h at 37°C in 5% CO_2. The blasts were washed twice in serum-free medium containing 20 mg of α-methyl-D-mannoside (Sigma) per ml and twice in plain RPMI 1640 and suspended at 2×10^5 cells per ml in culture medium supplemented with 10% FCS. The cells were distributed in round-bottom microtiter plates (2×10^4 cells per well in 0.1 ml) and incubated with 100 μl of test samples for 48 h at 37°C in 5% CO_2. At 16 to 20 h before harvesting, the cells were pulsed with 1 μCi of [^{3}H]thymidine; they were processed as described for the IL-1 assay. CTL cells were maintained by continuous passage in culture medium supplemented with 5% FCS and an optimal concentration of IL-2 (ConA-stimulated rat spleen cell culture supernatant). The CTL cells were suspended to 10^5 cells per ml, and 100 μl was cultured with 100 μl of test sample at a final concentration of 5% FCS for 24 h at 37°C in 5% CO_2. Six hours before harvesting, the cultured cells were pulsed with 1 μCi of [^{3}H]thymidine. The cultures were harvested, and IL-2 activity was determined and expressed as described for IL-1.

Lymphocyte proliferation assay. Spleen cells from donor mice were harvested, washed, and suspended to 10^7 or 10^6 cells per ml in culture medium, and 100 μl was incubated with 100 μl of ff promastigotes (10^5) or ConA (1 μg/ml) for 72 h at 37°C in 5% CO_2. The cultures were pulsed and harvested and the uptake of radioactivity was measured as described for the IL-1 assay.

Cell fractionation. Spleen cells from normal or infected BALB/c mice were fractionated by the following procedures.

(i) Antiimmunoglobulin column. The use of the antiimmunoglobulin column was described previously (12). It reduces immunoglobulin-positive cells from 40 to 50% to less than 0.5% as assessed by an indirect immunofluorescence assay.

(ii) Sephadex G-10 column. The Sephadex G-10 column was prepared as described by Ly and Mishell (27).

(iii) Plastic adherence. Unfractionated or immunoglobulin-negative spleen cells (2×10^7) were incubated in 5 ml of medium plus 20% FCS for 4 h in 100-mm plastic petri dishes (Falcon) at 37°C and 5% CO_2. The dishes were precoated with FCS (2 h at 37°C). Nonadherent cells were removed by

vigorous shaking with three changes of warm medium. The adherent cells were detached by incubating the dishes with cold phosphate-buffered saline containing 0.2% EDTA and 5% FCS for 40 min at 4°C followed by vigorous pipetting.

(iv) Carbonyl iron. Sterilized carbonyl iron (100 mg) was added to 10^8 unfractionated or nonadherent spleen cells in 5 ml of medium. The mixture was incubated on a rocking platform at 37°C for 45 min. At the end of the incubation, the iron-containing cells were retained with a strong magnet.

(v) Anti-Thy-1.2 antibody plus complement. In some experiments, adherent cells were treated with anti-Thy-1.2 antibody (F7D5, monoclonal immunoglobulin M; Olac 1976, Ltd., Oxon, United Kingdom) and complement as described previously (26).

Each of the cell fractions was stained with Giemsa solution and esterase (Sigma) by the method described by Tucker et al. (41), with α-naphthyl acetate as the substrate.

Statistical analysis. All experiments were performed two to four times. Statistical significance ($P < 0.05$) was determined by Student's t test.

RESULTS

Production of IL-2 and IL-1 by cells from mice at various times after *L. major* infection. Groups of BALB/c mice were given four weekly i.v. or s.c. injections of 2×10^7 irradiated (150 kilorads) *L. major* promastigotes and then infected s.c. in the shaved rump with 2×10^7 viable promastigotes 7 days after the last injection. Another group was exposed to 550-rad whole-body irradiation and 4 h later, together with nonirradiated mice, infected similarly with 2×10^7 promastigotes. Normal BALB/c mice infected with 2×10^7 promastigotes developed progressive lesions leading to uniformly fatal disease, as demonstrated previously (12, 23). Lesion development was arrested, with the majority achieving complete healing, when the mice were subjected to prior sublethal irradiation (Fig. 1). Substantial resistance to infection was achieved with four i.v. immunizations, whereas 4 s.c. injections not only failed to induce protection but exacerbated lesion development.

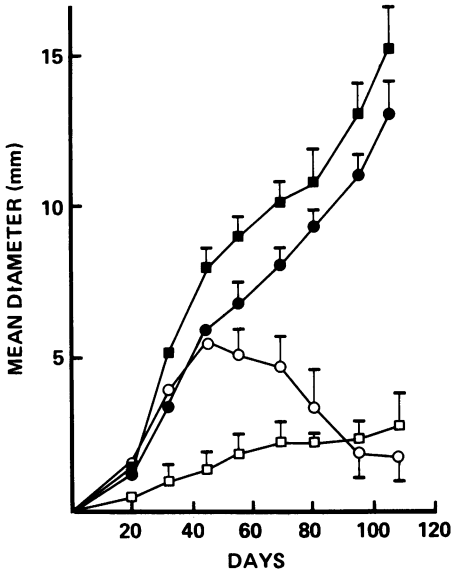

FIG. 1. Lesion size in mice after s.c. infection with *L. major* promastigotes. Groups of mice were given four weekly i.v. (□) or s.c. (■) immunizations with 2×10^7 irradiated (150 kilorads) promastigotes and, together with the controls (●), were infected with 2×10^7 live promastigotes 7 days after the last injection. Another group of mice was given 550-rad whole-body irradiation (○) 4 h before infection. The vertical bars represent one standard error of the mean; $n = 5$ to 7 mice.

At various intervals, groups of three mice were killed and tested for (i) the production of IL-2 by spleen cells in response to ConA (5 μg/ml) stimulation and (ii) the release of IL-1 by PC when activated by LPS (10 μg/ml).

(i) IL-2 production. Just before infection, spleen cells from mice given four s.c. injections produced significantly higher levels of IL-2 than did those from normal mice (Fig. 2). However, this elevated IL-2 production was soon lost, as lesions progressed, and none was detectable after 100 days. Spleen cells from mice immunized four times i.v. produced IL-2 at a level similar to that of normal spleen cells. This increased from 60 days onward and was sustained at a level higher than that of normal spleen cells. Spleen cells from sublethally irradiated mice produced a significantly lower level of IL-2 22 days after irradiation and infection, but the level increased steadily as the lesion regressed and by day 80 became substantially higher than that of normal spleen cells. In contrast, spleen cells from normal mice infected with 2×10^7 promastigotes produced significantly lower levels of IL-2 at all the time points tested.

(ii) IL-1 production. During the early stages of infection, PC from all mice produced higher levels of IL-1 in response to LPS than did cells from the normal control animals (Fig. 3). From day 60 onward, however, PC from mice immunized four times s.c. or i.v. produced IL-1 at levels similar to that of normal PC. PC from sublethally irradiated mice, on the other hand, maintained a significantly higher level of IL-1 production than the controls did throughout the experiment. In contrast, PC from untreated mice produced high levels of IL-1 between 60 and 80 days after infection, and the level returned to that of the normal controls at day 100.

Titration of IL-2 activity in culture supernatants of ConA-stimulated spleen cells. Spleen cells from groups of mice 60 days after infection were stimulated with 5 μg of ConA per

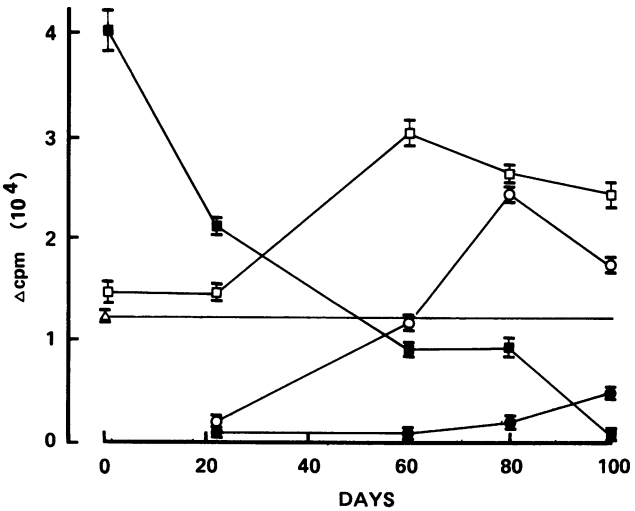

FIG. 2. Production of IL-2 by spleen cells from mice during *L. major* infection. The symbols are defined in the legend to Fig. 1. The horizontal line represents the IL-2 activity produced by uninfected spleen cells (controls). The vertical bars represent one standard error of the mean. Similar results were obtained when the IL-2 activity was assayed with ConA-induced spleen blasts.

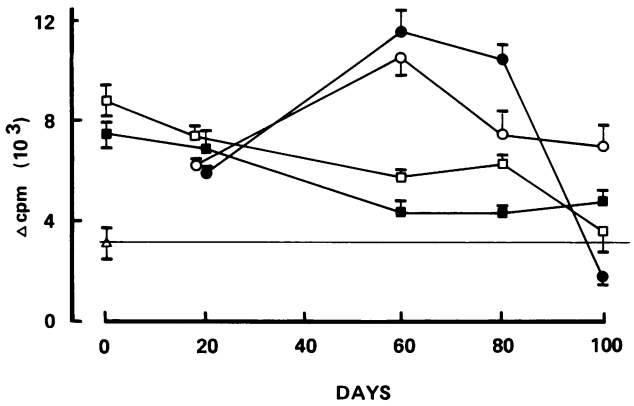

FIG. 3. Production of IL-1 by PC from mice during *L. major* infection. The PC were harvested from the same mice used for the experiment shown in Fig. 2; the symbols are defined in the legend to Fig. 1. The horizontal line represents the IL-1 activity produced by uninfected PC (controls). The vertical bars represent one standard error of the mean; $n = 3$.

ml, and the supernatants were tested for IL-2 activity by using CTL cells. The culture supernatants of cells from mice immunized i.v. or s.c. or given prophylactic sublethal irradiation contained significant levels of IL-2, whereas little or no IL-2 activity was detected in the culture supernatant of cells from mice untreated before infection (Fig. 4). Uninfected spleen cells produced intermediate levels of IL-2.

Proliferative responses of spleen cells to ConA and specific antigens. Since spleen cells from mice with progressive disease produced substantially reduced levels of IL-2, they were also tested for responsiveness to ConA and specific antigens. Spleen cells from mice immunized four times s.c. or infected with 2×10^7 promastigotes 115 days earlier and which had developed progressive lesions responded poorly to ConA stimulation compared with normal cells or cells from mice healing from the infection after sublethal irradiation (Table 1). In contrast, cells which were hyporesponsive

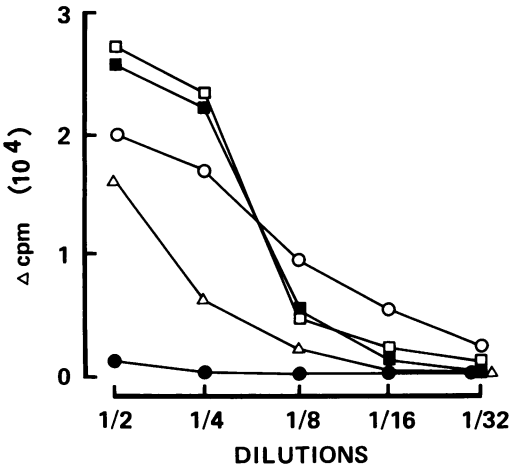

FIG. 4. Titration of IL-2 activity in spleen cell culture supernatants. Spleen cells were obtained from the following groups of mice 62 days after infection with 2×10^7 promastigotes: mice immunized four times i.v. (□) or s.c. (■), 550-rad irradiated mice (○), and normal mice (●). Spleen cells from uninfected mice (△) were included for comparison. There were three mice per group.

TABLE 1. Proliferative responses of spleen cells from normal or treated mice to ConA or ff promastigotes[a]

Mouse group	Uptake of [³H]thymidine (10^3 cpm [mean ± SEM]) after treatment:			
	ConA		ffp	
	+	−	+	−
Normal	21.8 ± 1.1	2.9 ± 0.2	2.0 ± 0.3	1.6 ± 0.2
Progressive infection	8.9 ± 0.5	5.0 ± 0.1	9.6 ± 0.6	3.1 ± 0.3
Cured infection	33.2 ± 1.4	11 ± 0.7	24.6 ± 1.4	3.4 ± 0.2
Immunized i.v.	23.0 ± 1.0	10.3 ± 0.2	31.9 ± 1.2	4.6 ± 0.2
Immunized s.c.	12.4 ± 0.2	2.9 ± 0.2	12.3 ± 0.4	3.4 ± 0.2

[a] Spleen cells were harvested from the following groups of mice as indicated: mice with progressive infection, 100 days after infection with 2×10^7 promastigotes; mice with cured infection, 75 days after 550-rad irradiation and infection with 2×10^7 parasites; mice immunized i.v., 7 days after four weekly injections of 2×10^7 irradiated (150 kilorads) promastigotes; mice immunized s.c., 7 days after four weekly injections of 2×10^7 irradiated parasites. For the mitogenic response, 10^6 cells were stimulated with 1 μg of ConA per ml, and for the specific response, 10^5 cells were cultured with 10^5 ff promastigotes. There were three mice per group.

to ConA underwent significant proliferation in response to ff promastigotes, although at a lower level than did spleen cells from healed mice. As expected, normal spleen cells did not respond to ff promastigotes.

Response of spleen cells to exogenous IL-2. Since spleen cells from mice with progressive disease responded poorly to ConA activation, they were tested in the presence of exogenous IL-2. They were incubated for 48 h with 5 μg of ConA per ml, washed, and recultured with a constant amount of added IL-2. The ConA-activated spleen cells (ConA blasts) responded vigorously to exogenous IL-2, except those from mice infected 100 days earlier with *L. major* and suffering from progressive disease (Table 2). It should be noted that the ConA blasts from mice 23 days after infection were able to respond to added IL-2 and that the unresponsiveness did not occur until a later stage of infection.

Suppressive effect of spleen cells from mice with progressive disease. A possible suppressive effect of cells from mice with progressive disease on IL-2 production by cells from normal, cured, or immunized mice was examined. Spleen cells from mice infected 112 days earlier were cocultured with equal numbers of spleen cells from healed or immunized donors, and the supernatants, harvested 24 h later, were tested for

TABLE 2. Proliferative response of ConA-stimulated spleen cells to exogenous IL-2[a]

Mouse group	Uptake of [³H]thymidine (10^3 cpm [mean ± SEM[b]]) after treatment:	
	No exogenous IL-2	Exogenous IL-2
Normal	2.1 ± 0.4	41.2 ± 0.3
Progressive infection (23 days)	1.0 ± 0.1	24.5 ± 1.4
Progressive infection (100 days)	1.0 ± 0.5	5.4 ± 0.3
Cured infection	2.6 ± 0.2	50.0 ± 1.3
Immunized i.v.	2.8 ± 1.9	41.4 ± 1.0
Immunized s.c.	1.5 ± 0.5	15.9 ± 0.3

[a] Spleen cells from indicated groups of mice (see Table 1, footnote *a*, for details) were cultured for 48 h with 2 μg of ConA per ml to produce ConA blasts. After being washed extensively, 2×10^4 blasts in 100 μl of medium were incubated for another 48 h with 100 μl of an IL-2 preparation (ConA-free T-cell growth factor) or control medium (RPMI plus 10% FCS). Each spleen cell preparation was made up of pooled cells from three mice.

[b] Of quadruplicate cultures.

TABLE 3. Effect of spleen cells from mice with progressive disease on IL-2 production by spleen cells from normal, cured, or immunized mice

Cells[a] ($5 \times 10^7 + 5 \times 10^7$/10 ml)	Uptake of [^{3}H] thymidine (Δcpm [10^3] [mean ± SEM])[b]	% Inhibition[c]
$T_n + T_n$	88.6 ± 8.9	
$T_n + T_s$	8.8 ± 1.2	97.4
$T_r + T_r$	68.4 ± 1.1	
$T_r + T_s$	26.4 ± 4.5	68.0
$T_i + T_i$	54.6 ± 1.5	
$T_i + T_s$	11.8 ± 0.2	89.3
$T_{s.c.} + T_{s.c.}$	21.9 ± 1.3	
$T_{s.c.} + T_s$	8.4 ± 0.1	88.8
Medium	6.7 ± 1.0	

[a] The following unfractionated spleen cells were used: T_n, cells from normal mice; T_s, cells from mice 112 days after infection with 2×10^7 promastigotes; T_r, cells from mice recovered from infection given 53 days earlier 4 h after 550-rad irradiation; T_i, cells from mice given four i.v. immunizations; $T_{s.c.}$, cells from mice given four s.c. immunizations. Each population of cells (5×10^7) was cocultured in 10 ml of medium for 24 h with 5 μg of ConA per ml, and the supernatant was assayed for IL-2 activity.

[b] Values after subtracting the counts per minute of cultures without ConA (Δcpm). Each culture was assayed in triplicate; there were three mice per group.

[c] % Inhibition = {[(control − medium) − (experimental − medium)]/(control − medium)} × 100, where control represents coculture with the same cells and experimental represents coculture with T_s cells.

IL-2 activity. Cells from mice with progressive lesions consistently suppressed IL-2 production by other spleen cell populations (Table 3).

Cell-type analysis of IL-2 suppressive effect of spleen cells. (i) Depletion of adherent cells. Spleen cells from normal or infected mice were depleted of macrophages by plastic adherence, Sephadex G-10 column, and carbonyl iron treatments. The ability of residual nonadherent cells to produce IL-2 was tested in the standard assay. Unfractionated spleen cells from infected mice with progressive disease again produced significantly lower levels of IL-2 than did normal unfractionated spleen cells (Table 4). The suppression was significantly reduced, and in some cases IL-2 production was restored, after depletion of the adherent cells. This was particularly pronounced when the depletion was performed with a Sephadex G-10 column followed by carbonyl iron treatment. The plastic adherence and carbonyl iron procedure reduced the nonspecific esterase-positive cells in the spleen cell populations from 10 to 25% to 5 to 8%, and treatment with Sephadex G-10 and carbonyl iron reduced the level to 1 to 2%. It should be noted that at no time did the removal of adherent cells significantly alter the level of IL-2 produced by uninfected normal spleen cells.

(ii) Effect of adherent cells on IL-2 production by nonadherent cells. Having shown that the nonadherent spleen cells from infected mice could produce normal levels of IL-2 in the absence of adherent macrophages, we sought direct evidence for an IL-2 suppressive effect of the adherent cells. Spleen cells from normal and infected mice were fractionated into adherent and nonadherent cells. Control nonadherent cells from a normal spleen (NA-C) were cocultured in various ratios with adherent cells from uninfected (A-C) or infected (A-I) mice. IL-2 production by NA-C was significantly reduced in the presence of A-I compared with that in the presence of A-C (Table 5). In all cases, the suppressive effect of A-I decreased as the NA-C/A-I ratio increased. Similar results were obtained with different cell-fractionating procedures and with A-I harvested 40 days after infection.

Effect of indomethacin on IL-2 production by spleen cells. It is well documented that activated macrophages release, among other materials, prostaglandins which may be responsible for the interference with IL-2 production by T cells.

TABLE 4. Effect of depletion of adherent cells[a]

Source of spleen cells (time postinfection [days])	Treatment of cells	Uptake of [^{3}H]thymidine (Δcpm [10^3] [mean ± SEM])	% Inhibition[b]	% Change after depletion[c]
Normal	Unfractionated	8.9 ± 1.1		29.2
	Plastic + iron	11.5 ± 2.2		
Infected (63)	Unfractionated	2.0 ± 0.2	77.5[d]	250.0
	Plastic + iron	7.0 ± 0.5	39.1[e]	
Normal	Unfractionated	26.4 ± 0.4		−20.4
	G-10 + iron	21.0 ± 1.3		
Infected (77)	Unfractionated	9.4 ± 0.9	64.4[d]	110.6
	G-10 + iron	19.8 ± 2.1	5.7	
Normal	Unfractionated	16.0 ± 0.1		6.2
	Plastic + iron	15.0 ± 1.6		
Infected (105)	Unfractionated	5.0 ± 0.5	68.7[d]	84.0
	Plastic + iron	9.2 ± 0.3	38.6[e]	

[a] Pooled spleen cells from four to eight normal or infected mice (63 to 105 days postinfection) were left unfractionated or were depleted of adherent cells by plastic adherence followed by carbonyl iron treatment or by passage through a Sephadex G-10 column followed by carbonyl iron treatment. Both the fractionated and unfractionated cells were cultured at 10^7 cells per ml with 5 μg of ConA per ml for 24 h, and the supernatants were assayed for IL-2 activity in triplicate with CTL cells.

[b] % Inhibition = [(Δcpm normal − Δcpm infected)/Δcpm normal] × 100.

[c] % Change = [(Δcpm fractionated − Δcpm unfractionated)/Δcpm unfractionated] × 100.

[d] $P < 0.001$ compared with corresponding control (e.g., line 1 versus line 3).

[e] $P < 0.01$ compared with corresponding control (e.g., line 2 versus line 4).

TABLE 5. Suppressive effect of adherent spleen cells from mice with progressive disease on IL-2 production by normal nonadherent cells

Cell fractionation procedure(s)	Cells (time postinfection [days]) and cell ratio[a]	Uptake of [^{3}H]thymidine (Δcpm [10^3] [mean ± SEM])	% Inhibition[b]
Plastic adherence	NA-C + A-C		
	2:1	24.4 ± 0.9	
	4:1	22.9 ± 0.2	
	8:1	21.2 ± 0.4	
	NA-C + A-I (49)		
	2:1	13.5 ± 1.0	44.6
	4:1	15.8 ± 1.0	31.0
	8:1	16.5 ± 1.2	22.1
Anti-immunoglobulin column + plastic adherence[c]	NA-C + A-C		
	1:1	103.3 ± 4.4	
	2:1	114.9 ± 6.6	
	4:1	108.1 ± 4.4	
	8:1	101.0 ± 6.6	
	NA-C + A-I (56)		
	1:1	12.3 ± 0.8	88.1
	2:1	16.4 ± 0.6	85.7
	4:1	33.8 ± 1.8	68.7
	8:1	60.0 ± 2.3	40.6
G-10 column + anti-Thy-1.2 + complement[d]	NA-C + A-C		
	1:1	26.0 ± 2.2	
	4:1	26.6 ± 1.2	
	8:1	27.0 ± 2.4	
	NA-C + A-I (77)		
	1:1	10.5 ± 2.1	59.6
	4:1	12.0 ± 1.9	54.8
	8:1	14.0 ± 3.3	48.1
Plastic adherence + anti-Thy-1.2 + complement[d]	NA-C + A-C		
	2:1	19.2 ± 0.3	
	4:1	18.8 ± 0.3	
	8:1	18.7 ± 0.9	
	NA-C + A-I (105)		
	2:1	7.8 ± 0.3	59.3
	4:1	9.0 ± 1.9	52.1
	8:1	9.3 ± 2.1	50.2

[a] Each cell population was pooled from four to eight mice. The ratio is of nonadherent to adherent cells. The total number of cells in the culture was constant at 10^7 cells per ml (±5 μg of ConA per ml).

[b] % Inhibition = {[Δcpm control (NA-C + A-C) − Δcpm experimental (NA-C + A-I)]/[Δcpm control (NA-C + A-C)]} × 100. All figures are significantly different from those for the corresponding controls ($P < 0.05$) (e.g., line 1 versus line 4 and line 2 versus line 5).

[c] Spleen cells were first depleted of immunoglobulin-positive cells by passage through an antiimmunoglobulin column. The immunoglobulin-negative cells were then further fractionated by plastic adherence.

[d] Spleen cells were fractionated by plastic adherence or a G-10 column, and the adherent cells, after detaching from the column, were treated with anti-Thy-1.2 antibody and complement to eliminate residual T cells.

Experiments were performed to test the production of IL-2 by spleen cells in the presence of various concentrations of indomethacin. Whereas indomethacin (1 to 10 μg/ml) had no effect on IL-2 production by normal spleen cells, it significantly increased the production of IL-2 by unfractionated spleen cells from mice with progressive disease (Fig. 5).

DISCUSSION

It is generally accepted that cell-mediated immunity plays a causal role in recovery from leishmanial infection (29, 30) and that the failure of highly susceptible mouse strains of BALB background to contain cutaneous leishmaniasis is attributable to suppression of such curative immunity (13, 24). Two distinct forms of immune suppression have been identified: (i) antigen-specific T cell-mediated suppression (13) and (ii) adherent-cell (macrophage)-mediated nonspecific suppression of the mitogen-induced or antigen-activated proliferative response (37). The mechanism of suppression is unclear in both cases. It has also been postulated that the suppression might be due to an excess of L3T4$^+$ Lyt-2$^-$

helper T cells (40) or colony-stimulating factors (29a). We present evidence in this report that progressive cutaneous leishmaniasis in BALB/c mice infected with *L. major* is accompanied by a depressed level of IL-2 production by spleen cells in response to ConA. These results are in agreement with and extend the findings of an earlier study (36) that abnormal phytohemagglutinin-induced spleen cell activation in BALB/c mice with *L. donovani* infection is associated with impaired production of IL-2. In addition, our data support an in vivo functional role for IL-2 in cutaneous leishmaniasis. Disease progression or regression was correlated with the IL-2-producing capacity of spleen cells from mice with cured infection or which had protective i.v. immunization or the deleterious s.c. injection with irradiated promastigotes. It seems unlikely, however, that IL-2 alone is capable of containing disease progression since four s.c. injections exacerbated the disease despite an initial capability of producing a relatively high level of IL-2.

A major finding of the present study was that depressed IL-2 production was not attributable to a primary defect in T cells but rather to the presence of a population of adherent suppressor cells whose suppressive activity could be reversed by a prostaglandin inhibitor, indomethacin. Adherent suppressor cells have been demonstrated in numerous systems (1, 3, 5, 15, 17, 33). Several lines of the present evidence imply that the suppressor cells regulating IL-2 production are macrophages: (i) the suppressive activity was removed by treatment with carbonyl iron and passage through a Sephadex G-10 column, (ii) it was retained in the plastic- and Sephadex G-10-adherent fractions, and (iii) it was not modified by treatment with anti-Thy-1.2 antibody and complement. Nevertheless, the possibility was not formally excluded that the suppression observed was mediated by an adherent T cell population with a low density of Thy-1 antigen on its surface. It is unlikely, however, that depressed IL-2 production in spleen cell populations is due to a numerical reduction of IL-2-producing T cells. Although there is a significant increase in the percentage of macrophages (from 10 to 20%) among spleen cells after *L. major* infection (19), there is little corresponding decrease in the percentage of $L3T4^+$ T cells (our unpublished data), which are believed to be mainly responsible for IL-2 production (34). It is also unlikely that the suppresion is due to leishmanial antigens carried over by infected macrophages since the deliberate addition of live promastigotes or amastigotes into the NA-C + A-C culture did not lead to significant reduction of IL-2 production (data not shown).

Suppression of IL-2 production has been reported for several infectious disease systems. These include *Trypanosoma cruzi* (11), *Plasmodium berghei* (20, 21), and *Histoplasma capsulatum* (42) infection in mice and *Mycobacterium leprae* (32) infection in humans. It has been reported that an IL-2 suppressive factor is released by nonspecific T acceptor cells in the T suppressor circuit of the contact sensitivity system (28). The present results show that IL-2 production by mitogen-stimulated T cells can also be suppressed by macrophages from mice infected with *L. major*. However, attempts to obtain evidence for the presence of a similar suppressor factor (10) were unsuccessful (data not shown).

The secretion of IL-2 by T cells can be influenced by IL-1 (39). However, it is unlikely that the suppression of IL-2 production was caused by a deficiency of IL-1 in the present system since the PC of all mice tested produced normal or elevated levels of IL-1 in response to LPS. Furthermore, no correlation between IL-1 production and disease development was observed. Although we used peritoneal macrophages instead of spleen cells for IL-1 production, such production is consistently higher by spleen cells than by peritoneal exudate cells (21, 43). Suppression of IL-2 production in murine trypanosomiasis (11), histoplasmosis (42), malaria (21), and clinical leprosy (32) has been shown to be IL-1 independent.

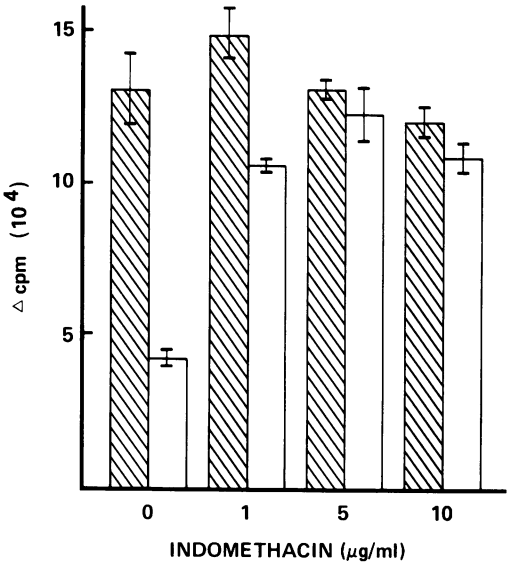

FIG. 5. Effect of indomethacin on IL-2 production by spleen cells from mice infected 150 days earlier with 2×10^7 promastigotes. The cells were cultured for 24 h with or without 5 μg of ConA per ml in the presence of various concentrations of indomethacin, and the IL-2 activity in the supernatant was assayed. The hatched columns show the IL-2 activity produced by uninfected spleen cells (controls) in parallel cultures. The vertical bars represent one standard error of the mean; there were four mice per group.

Unlike acute malarial infection, in which depressed mitogen-induced proliferation can be fully restored by exogenous IL-2, the proliferation of ConA blasts from BALB/c mice with progressive *L. major* infection (100 days postinfection) was restored only minimally by exogenous IL-2 (Table 2). This failure to mount a vigorous response to added IL-2 was apparently not due to the absence of an IL-2 receptor (as is the case for lepromatous leprosy) since the splenic T cells, in the absence of adherent cells, produced normal elvels of IL-2 (Table 4) and had an IL-2 receptor density similar to that of normal splenic T cells (our unpublished data). Adherent suppressor cells seem likely to affect the ability of T cells to respond to or release IL-2.

The action most commonly attributed to indomethacin is inhibition of prostaglandin synthetase; the importance of prostaglandins in regulation of the immune response has been well documented (8). Prostaglandins are produced by activated macrophages (6), and prostaglandin E_2 (PGE_2) can inhibit IL-2 production by human T cells (2, 35). The increase in the production of PGE_2 by macrophages at the height of infection is thought to be responsible for the generalized immunosuppression associated with African trypanosomiasis (4) and leishmaniasis (37). In the present system, the suppressed IL-2 production by spleen cells from mice with progressive leishmaniasis was readily reversed by the addition of indomethacin. Thus, our results suggest that in fatal cutaneous leishmaniasis macrophages may secrete PGE_2, which would in turn inhibit the production of IL-2,

which is essential for the induction of protective immunity. This immunosuppression could represent an additional pathway, distinct from antigen-specific T-cell-mediated modulation, contributing to the failure of genetically susceptible BALB/c mice to contain *L. major* infection.

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