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RECIPROCAL EXPRESSION OF INTERFERON γ OR INTERLEUKIN 4 DURING THE RESOLUTION OR PROGRESSION OF MURINE LEISHMANIASIS

Evidence for Expansion of Distinct Helper T Cell Subsets

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Leishmania species cause a spectrum of illness that ranges from asymptomatic seroconversion to progressive and ultimately fatal infection. Although many manifestations of this disease are due to differences among the Leishmania species themselves, host factors are clearly important in determining whether infection is controlled or allowed to progress inexorably (1).

Murine infection with *Leishmania major* mimics human infection due to *L. donovani*. Both self-limited and progressive visceral infections occur; the latter is accompanied by hepatosplenomegaly, anemia, and hypergammaglobulinemia. Among inbred mouse strains, non-MHC II-linked genetic susceptibility to dissemination has been well characterized. Although both C57BL/6 and BALB/c mice are susceptible to infection with *L. major*, C57BL/6 mice resolve infection with the establishment of lasting immunity, whereas BALB/c mice fail to control local replication of the parasite and suffer fatal progressive disease. In C57BL/6 mice, the ability to control infection has been linked directly to parasite-specific Th by various cell transfer and depletion experiments (2, 3); neither Lyt-2$^+$ T cells (4) nor NK cells (5) are critical for resolution of infection. C57BL/6 mice heal concomitant with the appearance of mRNA for IFN-γ in the lymphoid organs of infected animals (6), together with the ability of lymphocytes to generate IFN-γ after stimulation with Leishmania antigens in vitro (7). Neither occurs among nonhealer BALB/c mice with progressive disease. Experiments examining IFN-γ production in vitro among infected or healing human patients have reported a similar association (8, 9). Evidence to date suggests that IFN-γ is a critical macrophage-activating factor mediating cellular defense against the obligate intracellular amastigote (10, 11).

The ability of BALB/c lymphocytes to respond to infection by generating IFN-γ is not due to a failure of L3T4$^+$ cells to proliferate; in fact, these cells expand more rapidly in infected BALB/c mice than in healer strains (12–14). Recent studies have documented two subsets of helper L3T4$^+$ T cells among murine T cell clones (15).

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The presence of these subsets has been established in vivo (16, 17). To date, no surface antigens have been identified that distinguish these two subsets, and their distinction depends upon the unique lymphokine profiles generated by these cells in response to antigens and mitogens. IFN-γ is uniquely secreted by one subset, designated Th1, that has been demonstrated to mediate delayed-type hypersensitivity (18). The other subset, designated Th2, uniquely releases several B cell growth and stimulatory factors, including IL-4 and IL-5 (19). Our finding that resolution of murine leishmaniasis was associated with the production of IFN-γ prompted us to examine the hypothesis that progression of disease concomitant with $L3T4^+$ cell expansion was consistant with Th2 cell proliferation and would be associated with the production of IL-4 in the same lymphoid tissues. We demonstrate here that IFN-γ and IL-4 are produced reciprocally in resolving or progressive infection in murine leishmaniasis, consistent with the hypothesis that distinct Th subsets moderate the spectrum of this infectious disease. Further, passive administration of a neutralizing anti-IL-4 mAb attenuated the progression of Leishmania infection in susceptible mice, suggesting that activities mediated by this lymphokine favor the progression of infection.

Materials and Methods

Special Reagents. Rat mAbs were partially purified by ammonium sulfate precipitation from ascites produced by the hybridomas GK1.5 (anti-L3T4, IgG2b; American Type Culture Collection, Rockville, MD) (20), 11B11 (antimurine IL-4; IgG1; kindly provided by Dr. T. R. Mosmann, DNAX Research Institute, Palo Alto, CA) (21), and R4-6A2 (antimurine IFN-γ, IgG1; American Type Culture Collection) (22). Both the 11B11 and R4-6A2 hybridomas produce neutralizing antibodies. Plasmids containing specific cDNA were gifts from the following individuals: murine IFN-γ (550-bp Eco RI-Eco RV fragment), Dr. P. W. Gray, Genentech, Inc., South San Francisco, CA (23); IL-2 (330-bp Pst I-Hind III) and IL-4 (370 bp PstI), Dr. T. Yokota, DNAX Research Institute, Palo Alto, CA (24, 25), and β-Actin (2,000-bp Pst I), Dr. D. W. Cleveland, Princeton University, Princeton, NJ (26). Plasmid preparation, restriction digests, and fragment isolation from low-melting point agarose gel were performed as described (27). Dr. R. Devos (Biogent, Ghent, Belgium) provided murine IL-4 cDNA subcloned into pGEM-I (Promega Corp., Madison, WI) that was used for the preparation of the IL-4 RNA probe. An oligonucleotide probe complementary to the DNA sequence encoding residues 207-220 (28) of murine IL-1β (TGT TGA AGA CAA ATC GCT TTT CCA TCT TCT TCT TTG GGT A) was obtained from the Biomolecular Resource Center at UCSF Medical Center.

Parasites. *L. major* promastigotes (World Health Organization strain WHOM/IR/-/173) were propagated in M199 medium (Gibco Laboratories, Grand Island, NY) containing 30% FCS (Sterile Systems, Logan, UT) penicillin G (100 U/ml), streptomycin (100 mg/ml), and amphotericin B (40 mg/ml), as described (7). Promastigotes were harvested from stationary-phase culture by centrifugation, washed, and enumerated microscopically after immobilization of an aliquot in 30% ethanol/20% glycerol in PBS, pH 7.2, before use.

Mice. Female 8-10-wk-old BALB/c and C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in the animal care facility at UCSF Medical Center. Mice were inoculated in each hind footpad with 5×10^6 promastigotes in HBSS. In selected experiments, BALB/c mice were pretreated with 1 mg of GK1.5 anti-L3T4-mAb i.p. 18 h before infection as described (6). Designated mice were treated with 0.5 mg of either 11B11 anti-IL-4 or R4-6A2 anti-IFN-γ mAb i.p. at the time of infection and weekly thereafter.

Sample Tissues and Cells. Uninfected and infected animals (3, 4, 6, and 8 wk of infection) were euthanized, the excised spleens and draining lymph nodes were minced against a No. 100 steel mesh screen, and the suspended cells were washed in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA). Erythrocytes were lysed using tris-buffered ammonium chloride

and the cells were washed twice in 0.15 M sodium chloride, 15 mM sodium citrate buffer, pH 7.2 (SSC), that had been pretreated with diethylcarbonate (DEPC, Sigma Chemical Co., St. Louis, MO). Blood was collected from the tail veins of infected and uninfected BALB/c and C57BL/6 mice, and the prepared serum was stored at −20°C before use.

Quantitation of Tissue Parasites. At designated periods after infection, the footpad thickness was measured using calipers to assess the progression of the local infection (14). The footpads were removed, soaked in alcohol, allowed to dry, crushed against a steel screen using a plastic syringe barrel, and strained through a No. 100 mesh screen to remove large debris. The draining popliteal lymph nodes were excised, minced, and strained into separate single cell suspensions in HBSS through a steel mesh screen. Cell suspensions were washed, treated with tris-buffered ammonium chloride, pH 7.5, to lyse erythrocytes, rewashed, and brought to 10 ml (footpad suspensions) or 1 ml (lymph node suspensions) volumes in HBSS. The numbers of viable tissue parasites were established by culture of dilutions of these cell suspensions in vitro for 3 d at 27°C to allow conversion of amastigotes to the motile promastigote forms under conditions that do not support the viability of host cells. Detection of viable organisms was accomplished both by direct microscopic observation and by a modification of the tetrazolium reduction assay used for mammalian cells as previously described (29). In brief, 100-μl aliquots of the tissue suspensions were distributed to 900 μl of M199 containing antibiotics and 30% heat-inactivated FCS and subsequently subjected to six serial 10-fold dilutions. The final dilutions of the footpad tissue ranged from 10^3 to 10^8, and of the lymph nodes, 10^2–10^7. Aliquots of 100 μl from each dilution tube were transferred in quadruplicate samples to 96-well microtiter trays and incubated at 27°C in 5% CO_2 air. After 72 h the numbers of surviving and/or proliferating promastigotes were quantitated by colorimetric reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma Chemical Co.) as described (29). Briefly, 50 μg MTT was added to each well for 4 h, 80 μl of 0.05 M HCl in isopropanol was added to dissolve the blue formazan crystals, and the color was quantitated at 560 nm using a Microelisa plate scanner (Bio-Tek Instruments, Inc.; Burlington, VT) against a reference wavelength of 690 nm.

Isolation of RNA. Poly-A^+ mRNA was purified from cell preparations using guanidine HCl lysis (30) and oligo-dT-affinity chromotography (31), subjected to electrophoresis in formaldehyde-denatured, 1% agarose gel (5 or 10 μg/lane), stained with ethidium bromide, photographed, and transferred to nylon membranes (Amersham Corp., Arlington Heights, IL) using capillary blotting. Ethidium bromide staining was used to confirm that comparable quantities of RNA were present in each lane.

Preparation of Labeled Probes. Cytokine-specific cDNA fragments were labeled to a specific activity of 1–2 × 10^8 cpm/μg using a nick-translation kit (Bethesda Research Laboratories, Gaithersburg, MD) and ^{32}P-α-dCTP (sp act, 800 Ci/mmole, New England Nuclear, Boston, MA) (27). Antisense RNA probes were produced using an SP6 RNA polymerase kit (Promega Biotec, Madison, WI) and ^{32}P-α-CTP (3,000 Ci/mmol, New England Nuclear). Oligonucleotide probes were end-labeled to a sp act of 2–5 × 10^9 cpm/μg using T4 polynucleotide kinase (International Biotechnology Inc., New Haven, CT) and ^{32}P-γ-ATP (3,000 mCi/mol, New England Nuclear) (27).

Northern Hybridizations. For use with cDNA probes, membranes were preincubated overnight at 42°C in prehybridization solution (50% formamide, 0.1% Ficoll, 0.1% polyvinyl-pyrrolidone, 0.1% BSA, 1.5 M NaCl, 25 mM phosphate buffer, pH 7.4, 1 mg/ml yeast RNA, and 200 mg/ml denatured salmon sperm DNA) and then further incubated with 5 × 10^6 cpm of nick-translated probe at 42°C for 18 h in prehybridization solution containing 10% dextran. The blots were washed using high-stringency conditions (0.2 × SSC, 0.1% SDS at 55°C) and exposed to x-ray film (XAR-5, Eastman Kodak Co., Chester, NY) with intensification screens at −70°C. Membranes hybridized with oligonucleotide probes were preincubated in 0.2% polyvinyl-pyrrolidone, 0.2% Ficoll, 0.2% BSA, 150 μg/ml salmon sperm DNA, 1.7 mg/ml yeast RNA, 2× SSC overnight at room temperature, and then further incubated with 5 × 10^6 cpm of probe in the same buffer at 57°C. The membranes were then washed in 2× SSC for 1 h at 50°C. In indicated experiments, ^{32}P-labeled antisense RNA probe was used to detect IL-4 mRNA using hybridization techniques similar those used for nick-translated probes, but with incubations and washes performed at 65°C and a final incu-

bation with 20 μg/ml of RNase A (Sigma Chemical Co.) to remove nonspecifically bound probe (27). The intensity of hybridization was analyzed by laser densitometry (LKB; Bromma, Sweden). After autoradiography, the membranes were washed in 20 mM Tris/0.1% SDS/10 mM EDTA at 85°C for 90 min to remove hybridized probe.

Serum IgE Determinations. Serum was assayed for IgE using a solid-phase ELISA technique. A double-sandwich assay using rabbit antimurine IgE antiserum, and a rat mAb specific for mouse IgE (EM95) was used as described (32).

Results

Groups of healer C57BL/6 and nonhealer BALB/c mice were infected with *L. major* in the footpads, and the draining lymph nodes and spleen were harvested at 3, 4, 6, and 8 wk after inoculation. Previous studies have established that infection under these conditions causes comparable local infection for the first 3 wk, but thereafter, infection in the C57BL/6 mice is eliminated whereas infection progresses to footpad ulceration and visceral dissemination to the spleen and liver in the BALB/c by 8 wk of infection (7). The lymphoid cells were immediately disrupted, the mRNA was extracted, and the poly(A)$^+$ RNA was purified and subjected to electrophoresis before sequential hybridizations with selected cDNA probes (Fig. 1). In healer C57BL/6 mice, mRNA for IFN-γ was present in spleen by 3 wk of infection, and was continuously expressed over the subsequent 8 wk. Message for IFN-γ was present in the draining lymph nodes at all times (not shown) and continued to be heavily expressed at 8 wk. In contrast, in the BALB/c mice, mRNA for IFN-γ was not present in spleen until 4 wk, peaked at 6 wk, and subsequently disappeared; IFN-γ mRNA was not present in the lymph node cell populations. Message for IFN-γ was not

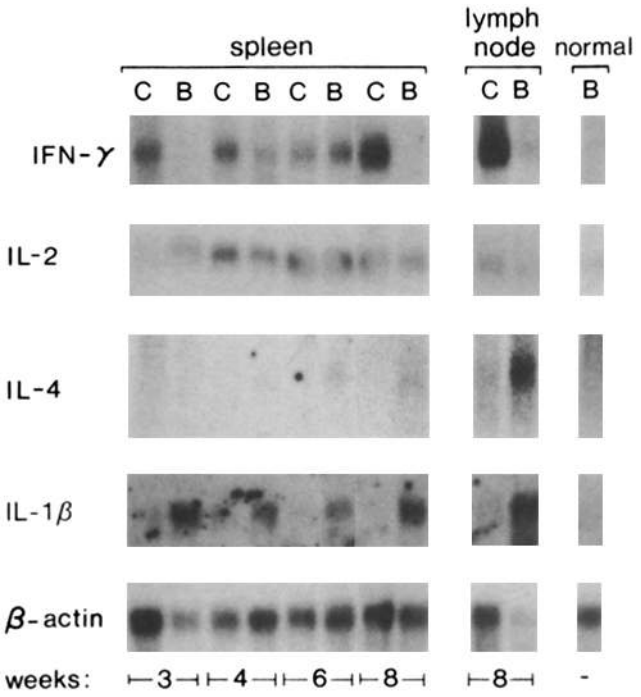

FIGURE 1. Cytokine mRNA during leishmaniasis. Poly(A)$^+$ RNA (5 μg) was isolated from the spleens and lymph nodes of BALB/c (B) and C57BL/6 (C) mice at designated times after infection with *L. major*. Northern hybridizations were performed using the indicated cytokine-specific probes (see Materials and Methods). Control RNA is from uninfected BALB/c spleen.

present in normal C57BL/6 or BALB/c splenic tissue. The presence of mRNA correlates with previous measurements of IFN-γ protein produced after stimulation of these lymphocyte populations with leishmania antigens in vitro (6, 7).

Rehybridization of the same blots with an IL-2 cDNA probe revealed that, at most periods after infection, comparable levels of message for this lymphokine were produced. Although resting C57BL/6 spleen had relatively low levels of IL-2 message (not shown), resting BALB/c splenic tissue contained detectable IL-2 mRNA.

Rehybridization using an IL-4 cDNA revealed significant differences as compared with IFN-γ. Thus, IL-4 expression was present at 4, 6, and 8 wk in BALB/c spleen, and at 8 wk in BALB/c lymph node cells, but was not present in C57BL/6 cell preparations. IL-4 message was not present in spleen from either mouse strain under resting conditions.

Recent reports have suggested that the T cell growth factor activity of IL-4 requires IL-1 as an accessory growth factor for Th2 cells (33). When the Northern blots were rehybridized using a labeled cDNA for murine IL-1, IL-1 message was substantially more expressed in tissues from infected BALB/c as compared with C57BL/6 mice (Fig. 1). Although expressed at low levels in normal BALB/c spleen, mRNA for IL-1 was increased 10–20-fold in lymphoid tissues from BALB/c mice at all times during the course of Leishmania infection.

Finally, the blots were assayed using ethidium bromide fluorescence at the beginning of the assay (not shown) and β-actin hybridization at the end of the sequential hybridizations to confirm that comparable poly(A)$^+$ RNA had been analyzed in each lane (Fig. 1).

BALB/c mice that have been pretreated with mAb GK1.5 to deplete L3T4$^+$ cells at the time of infection heal Leishmania infection (6, 34). To ascertain that species differences did not account for the previous discrepant findings, sequential cytokine hybridizations were repeated using tissues harvested from infected BALB/c mice and from infected BALB/c mice that had been pretreated with GK1.5 antibody. Tissues were harvested at 8 wk at a time when L3T4$^+$ T cells have repopulated the lymphoid tissues of GK1.5-treated mice (6). A group of C57BL/6 mice were coinfected and assayed concurrently. The final hybridization utilized an IL-4 antisense RNA probe followed by RNase digestion to improve the sensitivity of IL-4 detection. As previously demonstrated, infected C57BL/6 mice demonstrated message for IFN-γ and IL-2 in spleen but no message for IL-4 (Fig. 2 *A*). Conversely, BALB/c demonstrated message for IL-2 and IL-4, but little message (20-fold less) for IFN-γ. Finally, BALB/c that had been pretreated with GK1.5 demonstrated tissue expression of IFN-γ and IL-2, but not IL-4. The draining lymph node tissues from the BALB/c, but not from the GK1.5-pretreated BALB/c, expressed readily detectable IL-4 message (Fig. 2 *B*). Message for IL-4 (and IFN-γ) was consistently more heavily expressed in lymph node than in splenic tissues (Figs. 1 and 2), presumably reflecting the ascending nature of the infection from the footpads, to the draining lymph nodes, and thence to the spleen (6).

To assess IL-4 biologic activity in vivo, serum IgE levels were quantitated in C57BL/6, BALB/c, and GK1.5-pretreated BALB/c mice before and after 8 wk of infection (Table I). Serum levels in infected BALB/c mice, but not in control, C57BL/6-, or GK1.5-pretreated mice, were significantly elevated, and were comparable with levels reported after nematode infection in BALB/c mice (35).

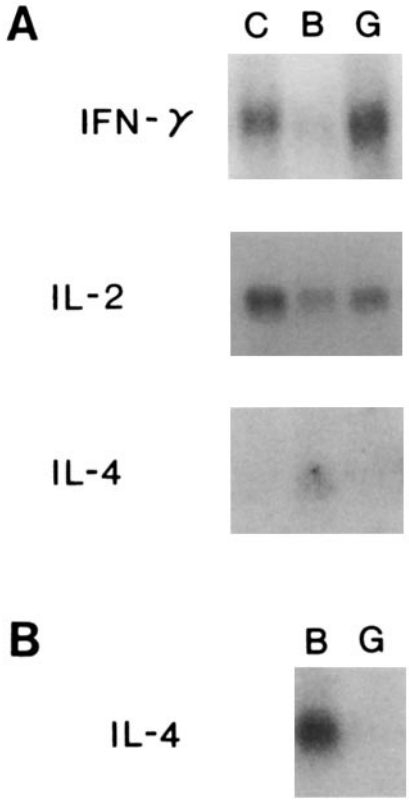

FIGURE 2. Lymphokine mRNA generation after transient in vivo depletion of L3T4$^+$ cells. (*A*) Poly(A)$^+$ RNA (10 μg/lane) was isolated from the spleens of C57BL/6 (C), BALB/c (B) and GK1.5-pretreated BALB/c mice (G) after 8 wk of infection and hybridized with the indicated cDNA probes. To increase sensitivity, IL-4 mRNA was detected using ^{32}P-labeled antisense RNA and was followed with RNase digestion to remove unbound probe. (*B*) Poly(A)$^+$ RNA (10 μg) was obtained from the draining nodes of infected BALB/c (B) and GK1.5-pretreated BALB/c mice (G) after 8 wk of infection and hybridized with IL-4-specific antisense RNA.

The presence of IL-4 mRNA in lymphoid tissues during progression of infection, together with the prior demonstration of L3T4$^+$ cell expansion during the course of disease (12–14), suggested that these L3T4$^+$ cells might represent Th2 cells that have previously been defined at the clonal level (15). In an attempt to interfere with

TABLE I

Effect of L. major Infection on Serum IgE Levels in Normal and GK1.5-pretreated Mice

Mouse strain	GK1.5 Pretreatment	Duration of infection	Serum IgE level
		wk	*μg/ml ± SD*
BALB/c	–	0	0.22 ± 0.11
BALB/c	–	6	28.55 ± 7.48
BALB/c	+	0	0.10 ± 0.02
BALB/c	+	6	1.24 ± 1.68
C57BL/6	–	0	<0.10 ± ND
C57BL/6	–	6	<0.10 ± ND

Groups of three BALB/c, mAb GK1.5-pretreated BALB/c, or C57BL/6 mice were infected with *L. major*, and the sera were collected both immediately (0 wk) and after 6 wk of infection before being assayed for IgE (see Materials and Methods).

IL-4-mediated functions in vivo, we administered neutralizing anti-IL-4 mAb to susceptible BALB/c mice and monitored the course of subsequent Leishmania infection. As assessed by thickness of the primary lesion at 5 and 8 wk (Fig. 3) and by direct quantitation of parasites in the footpads and lymph nodes (Table II), anti-IL-4 significantly attenuated the progression of leishmaniasis in susceptible BALB/c mice. Protection against tissue swelling and ulceration was most significant at 5 wk, and was associated with a 5-log reduction in the numbers of recoverable tissue parasites from the primary (footpad) lesions. Serum IgE concentrations were reduced from 8.8 ± 2.1 μg/ml in the infected BALB/c to 0.4 ± 0.2 μg/ml in infected BALB/c mice that had received anti-IL-4 antibody. The anti-IL-4-treated mice eventually developed progressive infection, although the course of disease continued to be delayed even at 8 wk. Treatment with an mAb against IFN-γ of the same isotype had a minimal effect on the course of infection (slight exacerbation), consistent with the limited amounts of IFN-γ produced by these mice. Comparable results were obtained in three separate experiments investigating the effects of anti-IL-4 administration on the course of Leishmania infection.

Discussion

Previous studies have documented that L3T4$^+$ lymphocytes taken from BALB/c mice during progressive infection or after subcutaneous immunization with *L. major* can adversely influence the course of disease when transferred into recipient animals (36, 37). Conversely, L3T4$^+$ lymphocytes taken from BALB/c mice that have recovered from Leishmania infection after prior immunologic manipulations, such as thymectomy or sublethal irradiation, confer immunity on recipient mice (3, 37). We and others have previously shown that the numbers of L3T4$^+$ cells within the

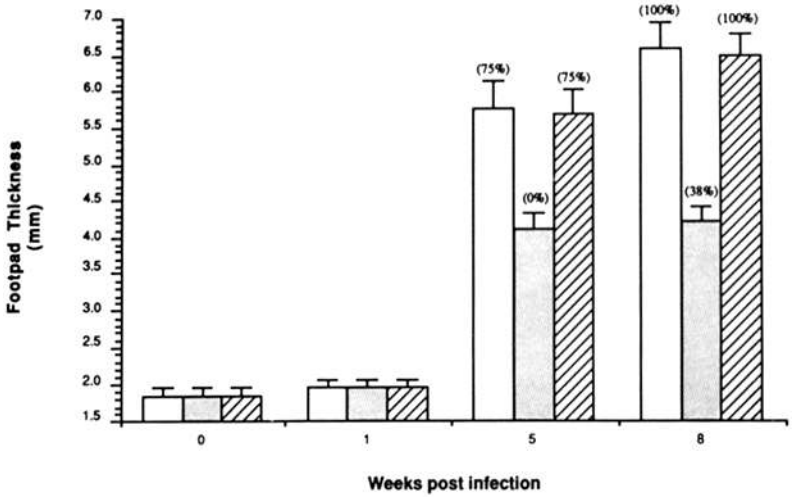

FIGURE 3. Effect of anti-IL-4 on the course of leishmaniasis. Groups of four BALB/c mice were infected with *L. major* in the hind footpads and the size of the lesions measured at 0, 1, 5, and 8 wk after no therapy (□) or weekly intraperitoneal injections of anti-IL-4 (▨) or anti-IFN-γ (▨) mAb. Mean and SD of the measurements are shown. Numbers in parentheses at the top of the bars indicates the percentage of lesions that were ulcerated in each group.

TABLE II

Tissue Amastigotes in BALB/c Mice Treated with Anti-IL-4 or Anti-IFN-γ During Leishmania Infection

	Dilution of tissue yielding viable leishmania	
Tissue	5 wk	8 wk
Footpads		
Control	10^7	$>10^8$
Anti-IL-4	10^2	10^6
Anti-IFN-γ	10^6	$>10^8$
Lymph nodes		
Control	10^4	10^6
Anti-IL-4	10^3	10^5
Anti-IFN-γ	10^5	$>10^7$

Groups of three BALB/c mice were either untreated (control) or received weekly intraperitoneal injections of anti-IL-4 or anti-IFN-γ mAbs after infection with *L. major*. At 5 and 8 wk the footpads and popliteal lymph nodes draining the lesions were harvested, minced, and diluted before in vitro culture for 3 d. Numbers indicate final dilutions of tissue yielding viable parasites.

lymph nodes and spleens of infected BALB/c mice are greater than or comparable with those in healer strains of mice by 6–8 wk of infection (12–14). As shown here, however, lymphoid tissues from healer C57BL/6 mice or from BALB/c mice pretreated with anti-L3T4 antibody contain message for IFN-γ in vivo, whereas lymphoid tissues from BALB/c mice with progressive infection contain message for IL-4, but little for IFN-γ. These results are most consistent with the hypothesis that the expansion of $L3T4^+$ cells during the resolution of leishmaniasis reflects preferential activation of a different subset of $L3T4^+$ cells than those activated during progressive infection. The disparate expression of IFN-γ and IL-4 suggests that these subsets are the in vivo correlates of the recently described subsets of murine $L3T4^+$ T cell clones (15).

The description of two subsets of murine Th clones, designated Th1 and Th2 based on their profiles of secreted lymphokines (15, 19), was followed by reports documenting the presence and activation of both subsets after antigenic stimulation in vivo (16, 17). Th1 cells preferentially secrete IFN-γ and IL-2 (15, 19), and have been shown to mediate delayed-type hypersensitivity (18). Th2 cells preferentially secrete IL-4 and IL-5 in response to mitogenic or antigenic stimulation (19). Although IL-2 will also support growth of Th2 cells, IL-4 acts as an autocrine growth factor in the presence of IL-1 (33, 38). By analogy with studies using cloned T cells (33, 38), the production of both IL-4 and IL-1 message in the lymphoid organs of mice with progressive Leishmania infection, as shown here, would presumably favor expansion of Th2 cells. Although the presence of IL-4 mRNA provides only indirect evidence for Th2 cell activation, data from Th2 clones suggests that IL-4 is transcriptionally regulated in response to activation stimuli (19).

Besides its activity as a T cell growth factor for Th2 cells, IL-4 has major B cell growth and maturational activities (39, 40), and has been identified as an Ig switch factor for IgG1 and IgE production (41). Marked hypergammaglobulinemia is characteristic of visceral leishmaniasis, and would be consistent with the sustained production of B cell stimulatory factors during the course of disease. Importantly, the pro-

nounced elevations of serum IgE that were measured in BALB/c mice with progressive leishmaniasis provide further evidence for the functional activation of Th2 cells. The IgE levels were compatable with those accompanying infection with the intestinal nematode *Nippostrongylus brasiliensis* (35). As in the studies reported here in leishmaniasis, elevated IgE levels in Nippostrongylus infection could be completely suppressed with anti-IL-4 antibody (35).

The ability of prior anti-L3T4 antibody treatment to confer a healer phenotype on susceptible BALB/c mice was associated both with the disappearance of mRNA for IL-4, marked attenuation of the IgE response, and the appearance of mRNA for IFN-γ. These findings are consistent with activation of Th1, as opposed to Th2, cells, and suggest that immunologic manipulations such as anti-L3T4 treatment or sublethal irradiation must preferentially affect Th2 cell populations, perhaps due to differences in the repopulation kinetics of these two $L3T4^+$ T cell subsets after their transient depletion. Despite the association of IL-2 secretion with Th1 clones (15), there was little relationship between the levels of IL-2 mRNA expression and the outcome of Leishmania infection. At late time periods (8 wk), IL-2 mRNA was expressed at approximately twofold greater levels in healing mice (Fig. 2). Our preliminary experiments utilizing cells enriched after complement-mediated antibody lysis indicate that significant amounts of the IL-2 mRNA in nonhealing mice are associated with $Lyt\text{-}2^+$ cells (unpublished observations). Evidence from $Lyt\text{-}2^+$ depleted mice (42) and nude mice selectively reconstituted with either $L3T4^+$ or $Lyt\text{-}2^+$ cells (4) suggests that $Lyt\text{-}2^+$ cells play at best a minor role in control of Leishmania.

The association of IFN-γ expression with healing has been shown in both murine (7) and human (8, 9) leishmaniasis. Leishmania exist as obligate intramacrophage amastigotes in the mammalian host. Activation of macrophages is required for the elimination of the organism in vitro, and IFN-γ has been identified as the major macrophage-activating factor mediating defense against Leishmania (10, 11). In the kinetic experiments described here, susceptible BALB/c mice did show evidence for IFN-γ production in the spleen during early infection, although message for this lymphokine disappeared by the time that visceral dissemination had occurred. IFN-γ mRNA was not present in the cells taken from the draining lymph nodes. The disappearance of message from the spleen during the progression of disease is consistent with the appearance of a suppressor influence during infection. Data presented here suggest that some of this influence may be mediated by IL-4.

Because of the pleiotropic effects of IL-4 on the immune system, we administered neutralizing anti-IL-4 antibody to infected BALB/c mice to establish whether activities mediated by this lymphokine might be causally related to the progression of infection. As demonstrated, anti-IL-4 therapy significantly attenuated the progression of leishmaniasis as assessed both by the size of the local lesion and the numbers of tissue parasites. The treated mice did eventually develop progressive disease as evidenced by the increase in tissue parasites at 8 wk. This may have been related to the development of antibodies to the rat mAb 11B11 as documented using a capture-sandwich ELISA to detect the presence of anti-rat antibodies (data not shown).

The mechanisms by which IL-4 might exacerbate Leishmania infection are unknown. We presume that neutralization of IL-4 selectively interferes with Th2 cell expansion and subsequent Th2-mediated suppression, although this is difficult to

prove in the absence of an understanding of the mechanisms for the latter process. Because B cell depletion by administration of anti-μ antibody before infection also ameliorates subsequent disease (43), it is possible that anti-IL-4 interrupts critical B cell circuits important for disease progression. Since passive transfer of antibody has no effect on the course of disease (44), this putative B cell effect would be independent of antibody. Additionally, IL-4 might directly interfere with macrophage activation by IFN-γ. Although IL-4 has some macrophage-activating capacities of its own, including stimulation of class II antigen expression (45) and up-regulation of CR3 (46), a macrophage receptor for Leishmania (47, 48), it cannot alone activate macrophages to resist parasitization by Leishmania (49). Previous studies have documented that administration of IFN-γ can ameliorate the course of Leishmania infection in mice (50). Although IFN-γ can directly activate macrophages to kill the organism (7, 10, 11), it is possible that some of the beneficial effects are due to the capacity of IFN-γ to inhibit directly many of the effects of IL-4 (39, 51, 52), including clonal expansion of Th2 cells (53). A combination of IFN-γ and anti-IL-4 might have major immunomodulatory effects on the course of Leishmania infection.

The data presented here suggest that progression of murine leishmaniasis occurs with preferential activation of Th2 L3T4$^+$ lymphocytes as demonstrated by tissue expression of IL-4 and profound elevations of serum IgE. In contrast, resolution of infection occurs with preferential activation of Th1 L3T4$^+$ lymphocytes as demonstrated by tissue expression of IFN-γ, a lymphokine known to stimulate eradication of intracellular Leishmania by infected macrophages, and by the acquisition of delayed-type hypersensitivity responses to Leishmania antigens. These findings suggest that the host response to infection with Leishmania, as assessed by the immunologic bias towards Th1 or Th2 L3T4$^+$ T cell activation, determines whether infection is contained or allowed to progress. These data would predict that Leishmania antigens that preferentially stimulate Th1 cells would serve as effective vaccine candidates, whereas those that stimulate Th2 cells might not. Whether bias among Th subsets occurs in other spectral infectious diseases, such as leprosy or coccidiodomycosis, will be important areas for further investigation.

Summary

We purified poly(A)$^+$ mRNA from the spleen and lymph nodes at designated times after infection with *Leishmania major* in genetically susceptible BALB/c and resistant C57BL/6 mice. The steady-state levels of IL-2, IFN-γ, IL-4, and IL-1β mRNA were determined using Northern hybridizations. IL-2 mRNA levels in the infected organs of BALB/c and C57BL/6 mice were comparable after infection, but IFN-γ and IL-4 mRNA levels were reciprocally expressed. Levels of IFN-γ mRNA in C57BL/6 draining nodes and spleen were significantly greater than in BALB/c mice except at 4 and 6 wk of infection, when splenic IFN-γ mRNA levels were transiently comparable. In contrast, IL-4 mRNA was apparent only in BALB/c and not in C57BL/6 nodes and spleen. Tissue levels of IL-1β mRNA were 10–20-fold greater in BALB/c mice. BALB/c mice were pretreated with GK1.5 mAb, a manipulation that promotes healing of subsequent infection by transiently depleting L3T4$^+$ cells. At 8 wk of infection, by which time lymphoid organs were repopulated with L3T4$^+$ cells, GK1.5-pretreated BALB/c mice produced IFN-γ, but not IL-4 message. Serum levels of IgE were markedly elevated in infected BALB/c, but not in infected C57BL/6

or GK1.5-pretreated BALB/c mice, consistent with in vivo biologic activity of IL-4 in nonhealing mice. Treatment of infected BALB/c mice with neutralizing anti-IL-4 antibody abolished the elevation of serum IgE and significantly attenuated the progression of disease as assessed by size and ulceration of the lesion, and by reduction in the number of tissue parasites.

Both protective and deleterious responses to Leishmania infection have previously been shown to be L3T4$^+$ cell dependent. Our findings are consistent with the differential expansion of protective, IFN-γ-producing Th1 cells in healing mice, and the expansion of deleterious, IL-4-producing Th2 cells in nonhealing mice. The inverse relationship of IFN-γ and IL-4 gene expression during leishmaniasis may underlie the divergence of cellular and humoral immunity that occurs during chronic infection with Leishmania and possibly other intracellular parasites.

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