CUTANEOUS LEISHMANIASIS

The Defect in T Cell Influx in BALB/c Mice

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The Leishmania are intracellular pathogens replicating in the parasitophorous vacuoles of visceral and cutaneous macrophages. Depending upon subtle strain differences they demonstrate tropisms for either the skin or central parenchymatous organs such as liver and spleen in humans. In some individuals the cutaneous lesions are self-healing, whereas in others the disease leads to deformities of skin and mucous membranes (1, 2). A somewhat similar course of disease can be observed in susceptible and resistant mouse strains (3, 4). As with other intracellular pathogens, host resistance depends upon the expression of cell-mediated immunity (5). It is generally considered that the macrophage is the important effector cell, its antimicrobial potential being regulated by lymphokines derived from Th cells (6). In particular, IFN-γ plays a central role (7).

Detailed studies by Liew et al. (8, 9) have focused on the T cell phenotypes that are associated with either host resistance or susceptibility. Adoptive transfer studies have indicated the role of Ts cells of the Lyt-1^+2^- phenotype in decreasing the resistance of mice to cutaneous disease. Ts cells have also been shown to play a central role in antitumor immunity in the mouse (10). In neither instance, however, has there been an in-depth analysis of the nature of the cells that accumulate in the local lesion. For this reason we have performed a longitudinal analysis of the cell types that are present in the expanding lesions of the susceptible BALB/c mouse and the regressing lesions of the C57BL/6 mouse. Among other findings, we associate the susceptibility of the BALB/c animal with its inability to mount a T cell response into the cutaneous lesion.

Materials and Methods

Mice. BALB/c mice were obtained from The Rockefeller University Laboratory Animal Research Center breeding colony. C57BL/6 mice were purchased from Charles River Breeding Laboratories, Wilmington, MA. All mice were male and weighed 18–20 g when infected.

Leishmania Parasites. The *Leishmania mexicana amazonensis* strain was kindly provided by Dr. J. S. Keithly, Cornell Medical Center, New York. The protozoa were cultivated from aspirates of infected mouse lesions in Schneider's *Drosophila* medium (Gibco Laboratories, Grand Island, NY) with 15% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin in the dark at 26°C. When stationary growth phase was reached, the promastigotes were washed three times in sterile PBS, pH 7.4, and adjusted to 10^8

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TABLE I
Rat mAbs for Mouse Leukocytes

Clone (ATCC number)	Immunoglobulin subclass	Specificity	Reference
M1/70 (TIB 128)	IgG2b	Macrophages, polys[1] (C3bi receptor)	13
F4/80	IgG2b	Macrophages	14
B21.2	IgG2b	$I\text{-}A^{b,d}$	15
M5/114.14.2 (TIB 120)	IgG2b	$I\text{-}A^{b,d,q}$, $I\text{-}E^{d,k}$	16
53-7.313 (TIB 104)	IgG2a	Lyt-1	17
53-6.72 (TIB 105)	IgG2a	Lyt-2	17
GK1.5, L3T4 (TIB 207)	IgG2b	Mouse T4	18
RA3-3A1/6.1 (TIB 146)	IgM	220 kD Glycoprotein on B cells	19
B5.3	IgG2b	Thy-1.2	20

organisms per milliliter in PBS. 1 d before infection, the hair over the mouse rumps was removed with depilatory cream. The rumps were infected intradermally with a 0.1-ml suspension of 10^7 promastigotes. Lesions were measured in two perpendicular diameters with a dial caliper (Mitutoyo, Tokyo, Japan) at weekly intervals.

Tissue Processing. Mice were killed by CO_2 inhalation. Skin lesions, livers, spleens, and lymph nodes were removed and cut into small portions for histologic, immunocytochemical, and electron microscopic studies. For routine H and E staining, biopsies were fixed in formalin and embedded in paraffin blocks. For ultrastructural studies, biopsies were fixed in 2% glutaraldehyde in 0.1 M cacodylate-buffered 0.1 M sucrose solution, pH 7.4, postfixed in 2% osmium tetroxide, stained with 0.25% uranyl acetate, dehydrated in alcohol, and embedded in epon blocks (11). Sections were examined and photographed using a JEOL USA (Cranford, NJ) JEM 100cx transmission electron microscope.

Immunocytochemistry. Biopsies were fixed 3–4 h in periodate-lysine-paraformaldehyde fixative (12), and suspended in graded sucrose solutions up to 25% sucrose with 5% glycerol. Frozen sections were cut onto 1% gelatin-coated multiwell slides (Carlson Scientific Inc., Peotone, IL), dried overnight, and rehydrated in PBS. Sections were incubated with culture supernatants from growing hydridomas containing rat anti-mouse mAbs, specificities of which are denoted in Table I. Biotinylated rabbit anti-rat IgG, mouse-adsorbed (Vector Laboratories, Inc., Burlingame, CA), was used as secondary antibody, followed by avidin-biotin-peroxidase complex (Vector Laboratories). The label was subsequently developed with 0.01% hydrogen peroxide in 0.02 M Tris buffer, pH 7.6, 0.4 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (Polysciences, Inc., Warrington, PA). Sections were gently washed with PBS between each reactant. Nonspecific staining was monitored at each step by substituting PBS with 0.2% BSA. Positive controls were stained simultaneously with each study for comparison. Tissues were counterstained with Gill's hematoxylin (Formulation No. 1, Fisher Scientific Co., Fairlawn, NJ). A Nikon Microphot FX light microscope was used for examination of immunoperoxidase staining and photography.

Quantitation of Peripheral Blood Leukocytes. Mice were injected intraperitoneally with 100 U of heparin in 0.1 ml volume. Blood was collected following decapitation into tubes containing heparin. A drop of blood was smeared onto coverslips and stained with Wright's-Giemsa stain; differential counts per 100 leukocytes were taken. After washing with PBS to remove serum, packed blood cells were diluted 1:2 with PBS, layered over Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) (21), and centrifuged at 2,100 rpm for 20 min at room temperature. Mononuclear cells at the interface were removed, treated with 0.83% ammonium chloride to remove contaminating erythrocytes, and

[1] *Abbreviations used in this paper:* HEV, high endothelial venules; polys, polymorphonuclear leukocytes.

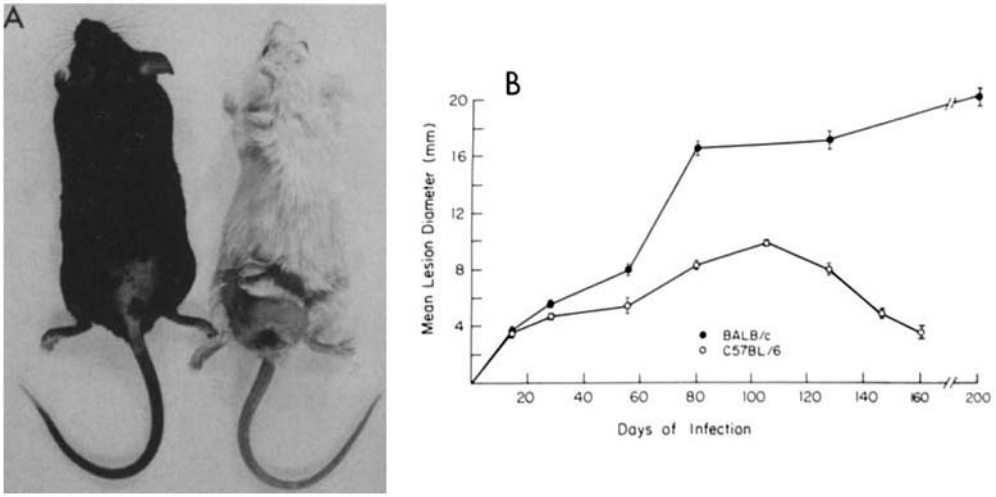

FIGURE 1. Comparison of cutaneous *L. mexicana amazonensis* infection in C57BL/6 and BALB/c mice. (*A*) The C57BL/6 (*left*) lesion consists of a healing flattened ulcer at 18 wk of infection. The BALB/c (*right*) lesion at 18 wk is elevated and expanding with central ulceration. (*B*) Progression of lesion size over 21 wk of infection is shown in the two mouse strains. Measurements do not include elevation.

washed three times with PBS. Viability by trypan blue exclusion was >95%. Cells were attached to poly-L-lysine–coated multiwell slides and reacted with mAb supernatants (Table I), biotinylated rabbit anti–rat IgG, and fluorescein avidin D (Vector Laboratories) at 4°C. After establishing negative controls, percent positive fluorescent cells were counted using a Nikon Optiphot microscope.

Results

The Structural Modifications in the Skin of BALB/c and C57BL/6 Mice. Palpable skin lesions were detected in both C57BL/6 and BALB/c mice after 2 wk of infection. Central ulceration occurred in most animals by 4 wk and lesions progressively enlarged over 12 wk. Fig. 1 illustrates the cutaneous lesion development in the two mouse strains. After 12 wk of infection no further increase in lesion size was noted in C57BL/6 mice, and the lesions slowly healed over the next 8–10 wk. BALB/c mice failed to heal their primary lesions and by 25 wk these measured over 2 cm in diameter. Nonulcerating cutaneous dissemination over the face, trunk, and limbs of BALB/c mice, but not C57BL/6 mice, developed by 20 wk of infection.

Examination of formalin-fixed, paraffin-embedded, H and E–stained cutaneous lesions by light microscopy revealed close similarities in histopathological changes over the first 10 wk of infection in BALB/c and C57BL/6 mice. The epidermis thickened from 1–2 to 6–10 cell layers by 12 wk of infection, but no extracellular or intracellular parasites were seen in any of these layers. The dermis contained numerous leishmania amastigotes within large parasitophorous vacuoles of macrophages (Fig. 2). Parasitized macrophages and eosinophils infiltrated subcutaneous fat and muscle and in some areas completely replaced normal tissues. Although amastigotes appeared predominantly in macrophages, transmission electron microscopy revealed partially degraded parasites within eosin-

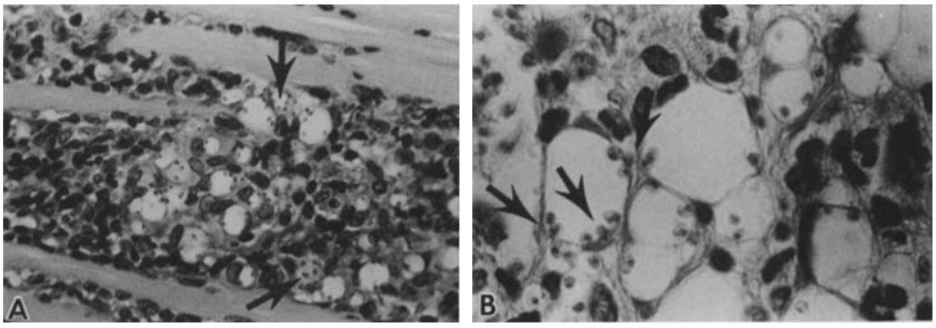

FIGURE 2. Photomicrographs of H and E-stained dermal lesions at 6 wk of infection. (*A*) Parasitized macrophages and eosinophils have infiltrated and partially replaced muscle and fat. × 225. (*B*) Higher magnification. × 562.

ophils (Fig. 3). By 8–12 wk areas of hemorrhagic necrosis were scattered through the dermis. The number of infected macrophages began to fall in the C57BL/6 mice, and by 21 wk very few organisms were seen in the healing skin lesions (Table II). In contrast, BALB/c skin lesions continued to demonstrate massive numbers of leishmania organisms with surrounding eosinophils between 12 and 21 wk of infection.

Immunocytochemical studies of skin lesions consistently showed membrane staining of infected macrophages for the macrophage surface antigens F4/80 and MAC-1. The anti-class II MHC Ia mAb stained Langerhans' cells in the epidermis as well as the majority of infected macrophages in the dermis. Control sections incubated without mAbs demonstrated large numbers of cells with endogenous peroxidase-positive granules (Fig. 4). These granulocytes contained reaction product throughout the cytoplasm and were easily distinguishable from staining with specific monoclonal reagents. The majority of the granulocytes proved to be eosinophils by electron microscopic examination, although occasional polymorphonuclear leukocytes were also seen.

The Influx of B and T Lymphocytes into the Cutaneous Lesions. Both BALB/c and C57BL/6 mouse lesions up to 8 wk of infection showed a paucity of small round lymphoid cells on H and E-stained sections. This finding was confirmed by the near absence of both T and B lymphocytes by immunocytochemical studies using anti-L3T4, anti-Lyt-1, anti-Lyt-2, and anti-B cell mAbs. However, T lymphocyte migration was apparent in C57BL/6 mice by 12–15 wk of infection. This was exemplified by small round cells in histologic sections and staining with mAbs specific for T cell subsets. ~60% of the total T cells were L3T4$^+$, Lyt-1$^+$, representing the Th cells, and 40% were Lyt-2$^+$ representing the T cytotoxic/suppressor cells (Table III). Further increase in T lymphocyte influx was demonstrated in the C57BL/6 by 21 wk. ~50% stained with L3T4 and 50% stained with Lyt-2. The influx of both helper and cytotoxic/suppressor T cells was associated with a marked reduction in parasitized macrophages in the C57BL/6 mice.

In contrast to the C57BL/6 lesions, BALB/c mice failed to show a similar lymphoid cell infiltrate and only scant numbers of T lymphocytes could be demonstrated with L3T4, Lyt-1, or Lyt-2 subset markers by immunoperoxidase

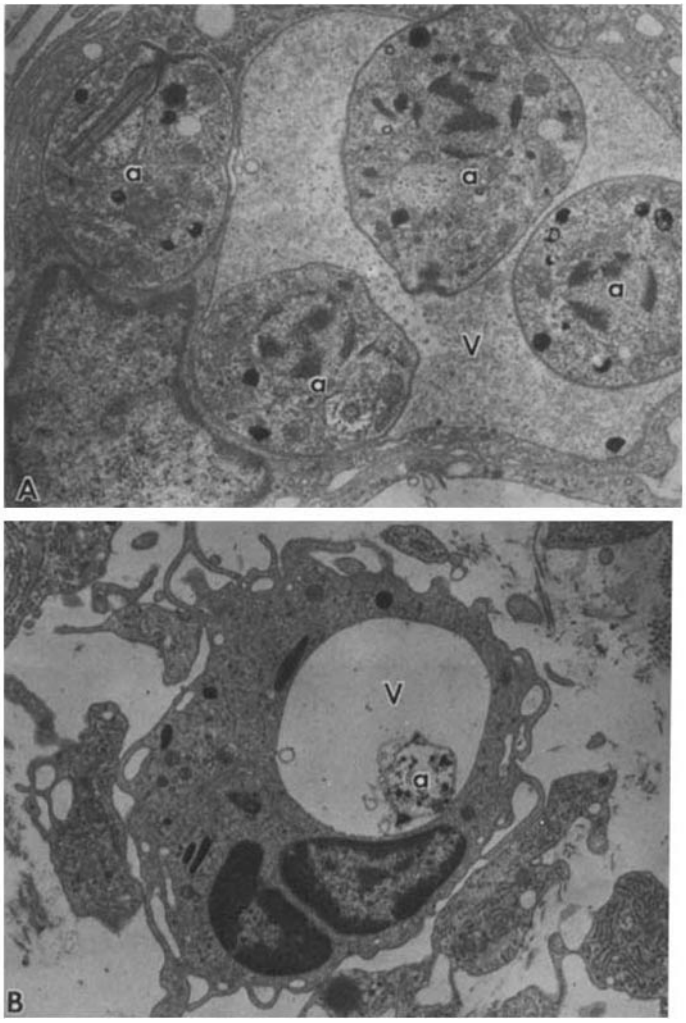

FIGURE 3. Transmission electron micrographs of a BALB/c cutaneous infiltrate at 5 wk of infection. (*A*) The large parasitophorous vacuole (*v*) of an infected macrophage contains several amastigotes (*a*). × 8,300. (*B*) Numerous eosinophils surround the infected macrophages. The eosinophil seen here has a partially degraded amastigote (*a*) within a large vacuole. × 6,600.

staining up to 23 wk of infection (Fig. 5). The lack of lymphocyte migration and an organized granulomatous response in the progressive skin lesions of BALB/c mice was associated with the massive accumulation of infected macrophages in the dermis and subcutaneous tissue.

Epidermal Alterations in the Lesions of BALB/c and C57BL/6 Mice. The influx of T lymphocytes after 12 wk of infection in C57BL/6 mice was associated with a diminution in lesion size and healing of the cutaneous ulceration. These changes were also associated with intense staining of epidermal keratinocytes for class II MHC Ia markers, B21.2 and TIB 120 (Fig. 6). Ia^+ keratinocytes persisted in the epidermis throughout 15 wk, but by 21 wk this staining pattern had disappeared. Comparative studies in BALB/c mouse lesions failed to show a similar Ia^+

TABLE II
Phagocyte Infiltration in Cutaneous Leishmania mexicana amazonensis Lesions

Mouse strain	Length of infection	Infected macrophages		Number of eosinophils counted per 20 fields
		Number counted per 20 fields*	Percent of total cells counted	
	wk		%	
BALB/c	3	55	14	141
BALB/c	12	132	52	23
BALB/c	21	117	68	20
C57BL/6	3	71	17	98
C57BL/6	12	38	12	32
C57BL/6	21	3	<1	34

* Cells located in a 12.5-μm^2 grid were counted in 20 random fields. The dermal lesions were stained with H and E and examined under oil immersion at × 100 magnification.

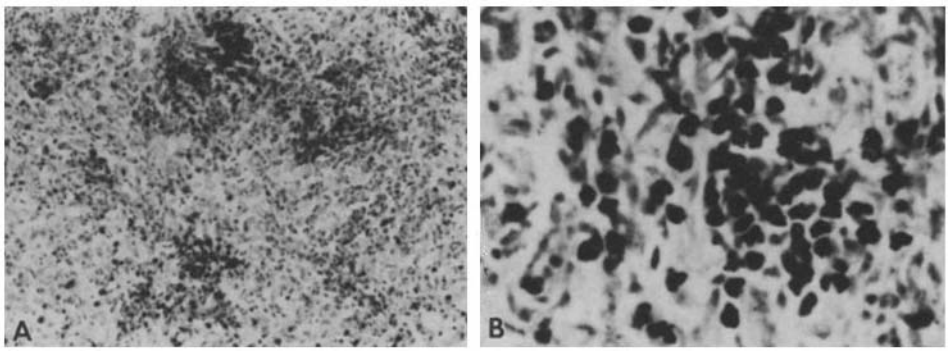

FIGURE 4. Photomicrograph of BALB/c cutaneous lesion showing influx of peroxidase-positive eosinophils. Dense accumulations as shown here are typical only in areas of necrosis. Sections were stained with diaminobenzidine tetrahydrochloride. The primary mAb secondary biotinylated rabbit anti-rat antibody and the avidin-biotin–horseradish peroxidase complex were omitted. (*A*) × 80. (*B*) × 160.

keratinocyte response. In the BALB/c lesion, Langerhans' cells were the only epidermal cells staining for the class II MHC markers (Fig. 6).

The Quantitation of Leukocytes in the Peripheral Blood of BALB/c and C57BL/6 Mice. The defective migration of lymphocytes in the cutaneous lesions of BALB/c mice raised the possibility that a paucity of such cells might exist in the circulation. We therefore analyzed the circulating leukocytes, including T cell subsets in the resistant and susceptible strains at various times after infection. Both C57BL/6 and BALB/c mice displayed decreasing numbers of circulating lymphocytes with a concomitant rise in circulating polymorphonuclear leukocytes as shown in Table IV. Blood monocyte levels in infected mice were no different from uninfected mice. Despite substantial accumulations of eosinophils in the infected skin lesions, a peripheral eosinophilia was not evident. Circulating blood mononuclear cells were further characterized by a biotin-avidin immunofluorescence technique using mAbs and results are shown in Table V. Th and T

TABLE III

Lymphocyte Migration into Cutaneous Leishmania mexicana amazonensis Lesions

Mouse strain	Length of infection	Number of Cells Per 20 Fields*		
		L3T4$^+$ T helper	Lyt-2$^+$ cytotoxic/suppressor	Total T cells‡
	wk			
BALB/c	3	0	0	0
BALB/c	12	5	6	11
BALB/c	21	7	9	16
C57BL/6	3	0	0	0
C57BL/6	12	46	32	78
C57BL/6	21	67	61	128

* Cells located in a 12.5-μm^2 grid were counted in 20 random fields. The dermal lesions were stained by the immunoperoxidase technique and examined under oil immersion at × 100 magnification.

‡ Numbers obtained by adding the number L3T4$^+$ and Lyt-2$^+$ cells.

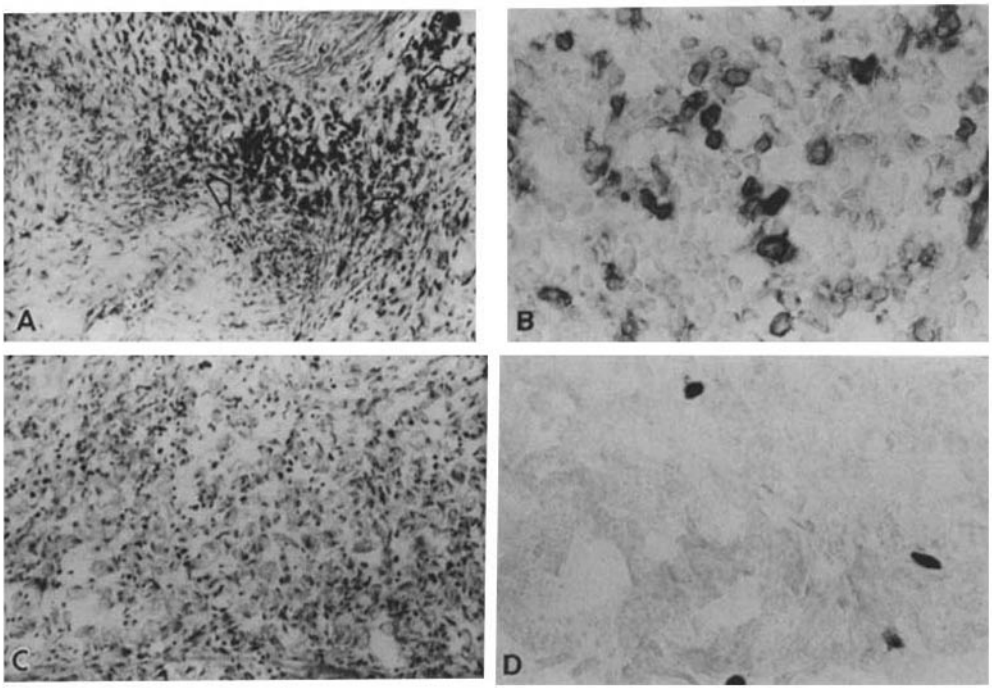

FIGURE 5. Photomicrographs of immunoperoxidase staining with mAb L3T4, specific for Th cells. (*A*) C57BL/6 dermal lesion at 15 wk showing influx of Th cells (*arrow*). Only a few scattered infected macrophages were found. × 36. (*B*) C57BL/6 dermal lesion without hematoxylin counterstain, demonstrating cellular membrane staining with L3T4. × 354. (*C*) BALB/c dermal lesion at 15 wk. × 36. Note the marked absence of Th cells and persistence of heavily infected macrophages. All peroxidase-positive cells are eosinophils. × 20. (*D*) BALB/c dermal lesion without hematoxylin counterstain, demonstrating absence of L3T4 staining and a few eosinophils. × 354.

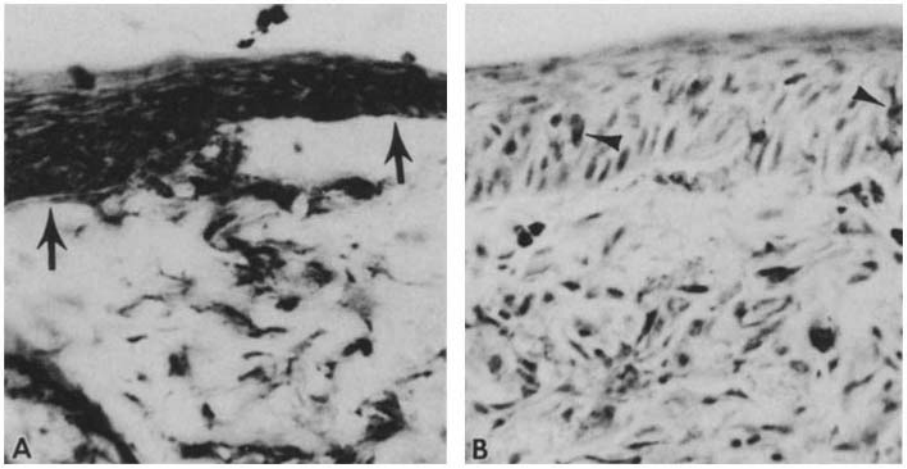

FIGURE 6. Photomicrographs of immunoperoxidase staining for class II MHC antigen, Ia, using mAb B21.2. × 160. (*A*) C57BL/6 mouse skin lesion at 12 wk. Note the Ia^+ keratinocytes in the thin and thickened layers of the epidermis (*arrows*). (*B*) BALB/c mouse skin lesion at 12 wk, showing Ia^+ Langerhans' cells (*arrowheads*) in the epidermis but no Ia^+ keratinocytes.

TABLE IV

Differential Counts of Peripheral Blood Leukocytes from Leishmania mexicana amazonensis-infected and Uninfected Mice

Mouse strain	Length of infection	Lympho-cytes	Polys	Monocytes	Eosinophils
	wk			%	
BALB/c	0	67	29	4	0
BALB/c	3	51	44	5	0
BALB/c	10	64	31	4	1
BALB/c	24	46	47	7	0
C57BL/6	0	66	30	4	0
C57BL/6	3	64	31	5	0
C57BL/6	10	52	44	4	0
C57BL/6	24	32	65	2	0

TABLE V

Quantitation of Mononuclear Cells from Peripheral Blood by Immunofluorescence

Mouse strain	Length of infection	Positive staining			
		T cells		B cells	Monocytes
		L3T4	Lyt-2	B220	C3biR
	wk			%	
BALB/c	0	81	10	4	5
BALB/c	3	70	5	12	13
BALB/c	10	60	4	18	18
C57BL/6	0	44	28	17	11
C57BL/6	3	55	8	18	19
C57BL/6	10	57	16	18	9

cytotoxic/suppressor cell levels were no different in infected BALB/c than in uninfected BALB/c mice, while levels of circulating B lymphocytes appeared to increase with progressive infection. A rise in MAC-1$^+$ cells in BALB/c infection likely represents both monocytes and contaminating polymorphonuclear leukocytes that may not have separated below the Ficoll-Paque gradient. B and T lymphocyte levels in infected and uninfected C57BL/6 were similar, with overall higher levels of T cytotoxic/suppressor cells than in BALB/c mice.

T Cell Distribution in the Liver and Spleen of Infected Animals. Visceral dissemination of leishmania amastigotes over the course of infection varied between the two mouse strains. Microscopic examination of liver sections after 18 wk of infection revealed the formation of small granulomas in the C57BL/6 mice. Intracellular amastigotes were not visualized within the granulomas or in isolated Kupffer cells along the sinusoids. Clusters of uninfected MAC-1$^+$ macrophages made up the central core of the C57BL/6 hepatic granulomas. Both L3T4$^+$ and Lyt-2$^+$ T cells were scattered along the granuloma rim (Fig. 7).

In contrast, widespread leishmania infection was found in BALB/c mice by 18 wk of infection, with infected macrophages replacing most of the normal liver architecture. Isolated Kupffer cells along sinusoids and large interstitial granulomas were heavily infected with leishmania amastigotes. Infected macrophages in both sites failed to stain with the MAC-1 mAb. An occasional L3T4$^+$ or Lyt-2$^+$ T cell was found along the outer edge of the infected areas, but most infected areas lacked T cells identifiable with anti-L3T4, anti-Lyt-1, and anti-Lyt-2 mAb (Fig. 7). The absence of T cells and the presence of high levels of organisms in the hepatic lesions mimicked the results at the cutaneous site.

Spleen size increased progressively over 8–21 wk of infection in both mouse strains. BALB/c mice had massive splenomegaly before death. T lymphocytes identified by mAb staining were appropriately located in the white pulp. Both L3T4$^+$ Th and Lyt-2$^+$ T cytotoxic/suppressor cells increased in amounts proportional to the overall splenic enlargement in each strain. In BALB/c spleens the red pulp was filled with leishmania-infected macrophages.

Discussion

We have performed a longitudinal study of the cellular phenotypes accumulating in the cutaneous leishmania lesions of susceptible BALB/c and resistant C57BL/6 mouse strains. Immunocytochemical analyses indicate that there is a profound deficiency in T cells in the expanding lesions of BALB/c mice (Table III, Fig. 5). This finding is associated with a progressive increase in the number of leishmania amastigotes in the lesions as well as metastatic spread to other skin sites and viscera. The failure of T cells to migrate into the cutaneous lesions cannot be attributed to a central absence of T cell subsets because appropriate numbers were found both in peripheral blood and in the spleens of BALB/c mice with cutaneous lesions (Tables IV and V). This lack of T cell response also extended to the BALB/c liver where in late infection, despite numerous heavily infected macrophages, very few T cells were detected (Fig. 7). In comparison, as the cutaneous lesions in C57BL/6 mice regressed and parasite load diminished, both Th and T cytotoxic/suppressor subsets were plentiful and present in approximately equal amounts (Fig. III, Fig. 5). Parallel studies in C57BL/6 livers

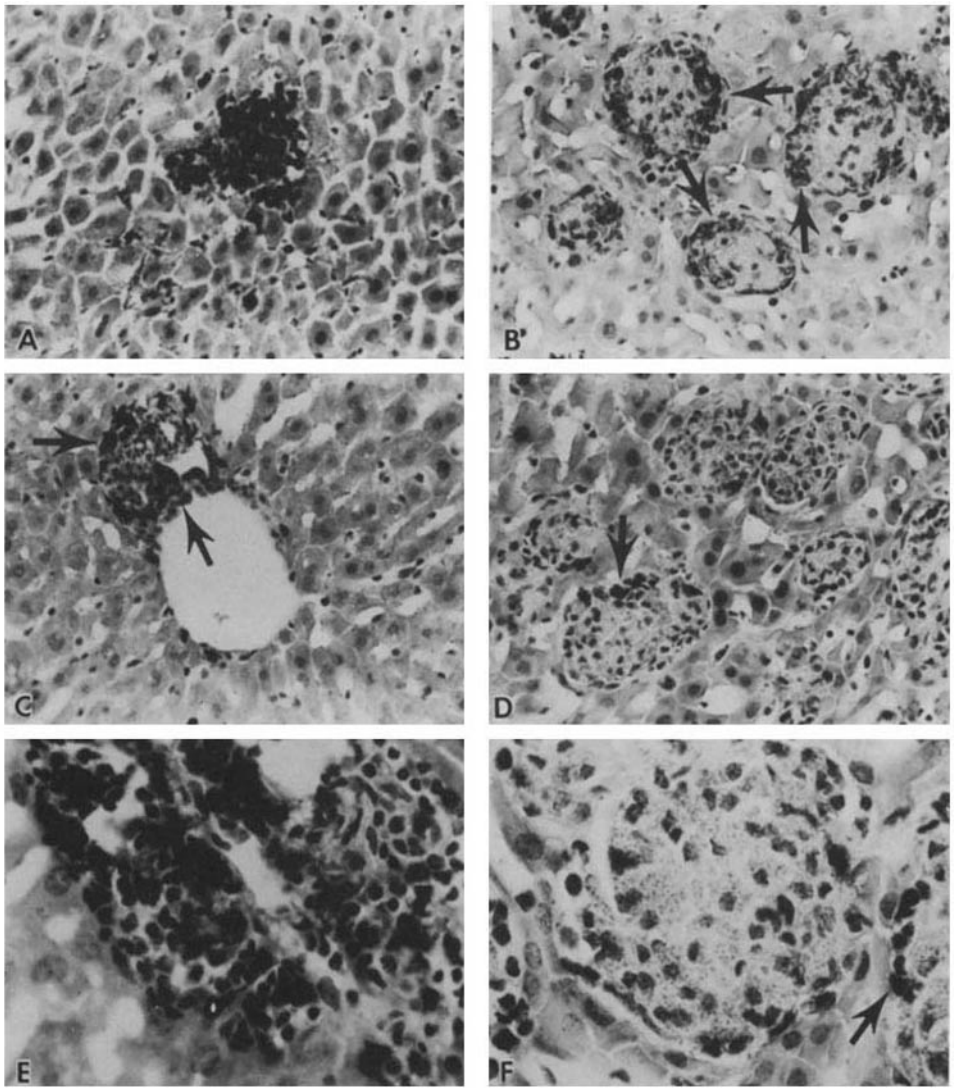

FIGURE 7. Photomicrographs of mouse livers at 18 wk of cutaneous leishmania infection. (*A*) C57BL/6 liver stained with MAC-1 mAb shows clusters of uninfected mononuclear phagocytes within granulomas. Most of the normal liver architecture remains intact. × 160. (*B*) BALB/c liver stained with MAC-1. Positive cells (*arrows*) surround the heavily infected multinucleated giant cells. Much of the liver parenchyma has been replaced with infected macrophages. × 160. (*C*) C57BL/6 liver stained with L3T4 shows Th cells within and along the edge of the granuloma (*arrows*). × 160. (*D*) BALB/c liver with an occasional L3T4$^+$ Th cell along the periphery of infected areas (*arrows*). × 160. (*E*) Higher magnification (× 344) of *C*. (*F*) Higher magnification (× 344) of *D*.

demonstrated small granulomas that were almost devoid of parasites but replete with T cells (Fig. 7).

The mechanism whereby susceptible BALB/c mice are unable to mount a migratory T cell response is not known. The presence of postcapillary high endothelial venules (HEV) is believed to influence T cell migration into lymphoid

tissue through lymphocyte homing receptors (22), but we found no electron microscopic evidence for HEV at the site of infection. Second, BALB/c mice may be unable to sensitize T cells to certain leishmania epitopes and thus may fail to promote a migratory response. Previous studies (3, 23–25) examining T cell response to leishmania antigen in BALB/c mice have produced varying results depending upon whether in vivo or in vitro tests of responsiveness were used. Infections with different Leishmania strains and the use of different Leishmania antigen preparations have further complicated the issue. Unlike other intracellular pathogens such as *Mycobacteria bovis* and *Listeria monocytogenes*, which cause acute illness and an accelerated healing response, our results indicate that *L. mexicana amazonensis* infection, even in a self-healing mouse strain (C57BL/6), is associated with a chronic disease course and overall delayed T cell response in the cutaneous lesion for up to 12 wk of infection. The ability of leishmania amastigotes to survive and multiply intracellularly for several weeks in self-healing mice suggests that the parasite has means to prevent or evade a cell-mediated immune response. Liberation of leishmania products during excessive proliferation may block antigen-induced T cell responsiveness. Alternatively, leishmania-specific products may inhibit T cell chemotaxis directly or indirectly through altered adherence and passage through skin endothelial cells at the inflammatory focus.

The migratory failure appears specific for T cells. Both C57BL/6 and BALB/c cutaneous lesions contain large numbers of newly emigrated monocytes as well as older resident macrophages as defined by immunocytochemical staining. The latter harbor and support the multiplication of leishmania amastigotes. Polymorphonuclear leukocyte and eosinophil influx appears unperturbed.

Resistance to leishmania infection associated with local T cell migration into healing C57BL/6 lesions may occur through at least two mechanisms. Lymphokines, particularly IFN-γ, are released from sensitized T cells and activate surrounding infected macrophages to undergo an oxidative response (7). Oxygen intermediates released, particularly hydrogen peroxide, are toxic to leishmania amastigotes (26). IFN-γ release in C57BL/6 lesions is suggested by the reduction in the parasite load after T cell influx. Keratinocyte Ia staining in C57BL/6 cutaneous lesions after 12 wk of infection may also be associated with local production of IFN-γ. In vitro treatment of human keratinocytes with rIFN-γ (27) as well as intradermal injection of rIFN-γ into the lesions of lepromatous leprosy patients (28) have been shown to induce the expression of Ia antigen on the surface of keratinocytes. An association between the generation of delayed reactions and keratinocyte Ia expression has also been observed in delayed responses to intradermal PPD and in the chronic granulomas of tuberculoid leprosy and cutaneous leishmaniasis (29). Alternatively, T cells may kill parasitized macrophages. Viable amastigotes may be liberated into the extracellular environment and become subject to destruction by humoral factors (e.g., eosinophil peroxidase coupled with hydrogen peroxide or pore-forming protein released from cytotoxic T cells). Coating of amastigotes with leishmania-specific host antibody may be necessary for extracellular destruction.

A number of in vivo systems have been described in which purported Ts cells inhibit the ability of small rodents to reject tumor allografts or inhibit the

replication of intracellular bacteria or parasites (30). Suppressor cells have been reported to be operative in the response to cutaneous leishmaniasis (8), and in this and similar models Ts are sensitive to ionizing radiation or cytotoxic drugs (31, 32). Although a histological description of the course of *L. mexicana* infection in C57BL/6 and BALB/c mice has been reported (33), to our knowledge no detailed analysis has been made of the local lesion and its composition of effector cells. It may be, as described in this report, that suppressor networks have as their distal expression the inhibition of T cell migration into the infective foci.

A major defect in T cell migration in the BALB/c mouse has been documented in both cutaneous and visceral infection. Efforts to detect sensitized T cells and stimulate T cell migration in BALB/c are in progress. Reduction of antigen load by treatment with sodium stibogluconate, immunization with leishmania antigen to presensitize T cells before infection, and sublethal irradiation of mice to remove potential Ts cells that may inhibit migration, are several approaches being pursued. Understanding why T cells fail to localize in BALB/c lesions should elucidate more general mechanisms involved in cell-mediated immune depression in disseminated cutaneous infection.

Summary

Local cellular responses to cutaneous infection with *Leishmania mexicana amazonensis* were examined in susceptible (BALB/c) and resistant (C57BL/6) mouse strains by immunocytochemical and electron microscopic studies. Infection during the first 8 wk in both animal strains was characterized by progressively enlarging lesions, epidermal thickening and ulceration, and accumulation of eosinophils and Ia^+ infected macrophages. Healing of C57BL/6 mouse lesions began after 12 wk of infection and was associated with local influx of both Th ($L3T4^+$) and T cytotoxic/suppressor ($Lyt\text{-}2^+$) cells into the dermis, and Ia antigen expression on epidermal keratinocytes. T lymphocyte infiltration was marked and intracellular parasites were scarce by 21 wk of C57BL/6 infection. Similarly, granulomas in C57BL/6 livers contained $L3T4^+$ and $Lyt\text{-}2^+$ T lymphocytes and no visible intracellular parasites by 21 wk of infection.

In contrast, BALB/c mouse lesions continued to enlarge and never healed. Throughout the entire course of infection, T lymphocyte influx into the heavily infected dermis was minimal. Keratinocyte Ia expression was absent in BALB/c lesions. BALB/c livers were heavily infected by 18 wk of cutaneous infection, with few demonstrable T lymphocytes. A systemic absence of T cells could not be demonstrated in BALB/c mice. Both $L3T4^+$ and $Lyt\text{-}2^+$ T cells were found in the peripheral blood in normal numbers in both mouse strains.

Our results support the role of T cells as important local effector cells in the healing response of murine cutaneous leishmaniasis. We suggest that local T lymphocyte infiltration may provide lymphokines, particularly IFN-γ, that can activate infected macrophages to destroy the intracellular parasites. Alternatively, T cells may play a cytotoxic role, killing infected macrophages and allowing local humoral factors to destroy released extracellular parasites.

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