Int. J. Exp. Path. (1993) **74**, 481–491

Leishmania (Viannia) panamensis-induced cutaneous leishmaniasis in Balb/c mice: pathology

JOSÉ ISAAC ROJAS*, EDNÉIA TANI†, ANDERS ÖRN‡, CEFERINO SÁNCHEZ* AND HIRO GOTO‡§

**Department of Pharmacology, School of Medicine, University of Panamá, Panamá, Panamá,*
‡Department of Immunology, Karolinska Institute, Stockholm, Sweden, and
§Laboratory of Infectious Diseases' Pathology, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

Received 28 January 1993
Accepted 28 May 1993

Summary. *Leishmania* (*Viannia*) *panamensis* infected Balb/c mice developed a progressive swelling in the injected footpad that grew to a tumour-like lesion from day 80 onwards. We did not observe any typical ulcera, necrosis or metastasis to other parts of the skin. Neither did we observe any histopathological changes in liver or spleen during the experiment.

At the site of injection, we observed progressive changes ranging from a moderate, mixed inflammatory infiltrate with few leishmania amastigotes in the macrophages to an extensive inflammation composed of monomorphic vacuolated macrophages containing large numbers of parasites. A granulomatous pattern with presence of epithelioid cells and a few multinucleated giant cells was observed at the initial phase of the infection. During later stages, focal necrosis with polymorphonuclear neutrophils was seen. Lymph nodes presented granulomatous lesions in the subcapsular area, numerous plasma cells in the medullary cords and macrophages with leishmania organisms in dilated cortical sinuses at the 4th and the 6th months of infection.

This *Leishmania* (*Viannia*) *panamensis* infected Balb/c mice seems to be a good model for continued studies of the pathogenesis of cutaneous leishmaniasis and also for drug trials in the development of new therapeutic tools.

Keywords: cutaneous leishmaniasis, *Leishmania* (*Viannia*) *panamensis*, pathology, mouse model

Leishmaniases are diseases caused by protozoa of the genera *Leishmania*. In the New World, the aetiologic agents of cutaneous and/or mucocutaneous leishmaniasis are a number of *Leishmania* species. In Panamá, where there is a high incidence of cutaneous leishmaniasis, *Leishmania* (*Viannia*) *panamensis* has been identified as the most frequent species (Walton 1987). This species is also known to occur in Western Colombia, and it is probably responsible for most cutaneous leishmaniasis cases in Costa Rica. *L.* (*V.*) *panamensis* seems to be prevalent over almost all Central America. Clinical presentations in patients differ from those caused by other species, having associated lymph node involvement in chain, resembling sporotrichosis, as a common feature (Walton 1987).

Correspondence: Hiro Goto, Faculdade de Medicina da USP, Departmento de Patologia, Av. Dr. Arnaldo, 455, 01246-903-São Paulo, SP, Brazil.

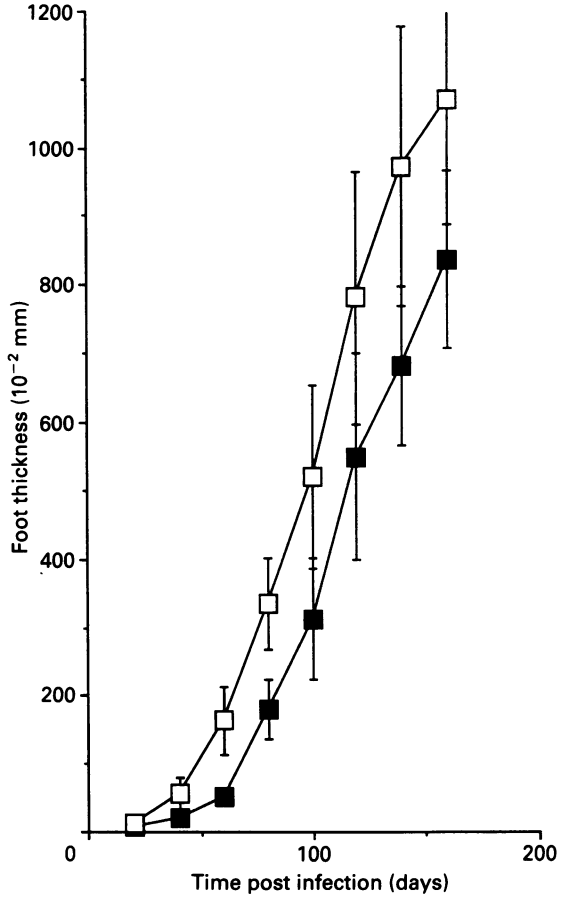

Figure 1. Evolution of footpad swelling (mean of 8–14 mice ± s.d.) in Balb/c mice injected with ■, 10^7 or □, 10^8 *L. (V.) panamensis* promastigotes in 20 to 160 days post-infection period. One of three similar experiments is shown. The maximum measurement obtained in control mice was $(2.0 \pm 3.8) \times 10^{-2}$ mm throughout the experiments. For details see Materials and methods.

The establishment of animal models, particularly in mice, provides powerful tools for a number of studies in different fields such as immunology, pharmacology and pathology. For leishmaniasis, there are well established mouse models with *Leishmania* (*Leishmania*) *major* (Handman *et al.* 1979; Howard *et al.* 1980) and *Leishmania* (*Leishmania*) *amazonensis* (Barral-Neto *et al.* 1987a; McElrath *et al.* 1987).

We know of only one report indicating susceptibility of Balb/c strain to *L. (V.) panamensis* infection (Neal & Hale 1983). Since this report does not describe further details of the model, we previously studied the susceptibility and resistance of three strains of mice to this particular *Leishmania* species (J.I. Rojas, H. Goto, L. Sporrong, C. Sanchez, P. de Carreira & A. Örn. 1992, unpublished). We observed that Balb/c mice are very susceptible in contrast to C57B6 and CBA mouse strains which are resistant. Cell-mediated and antibody responses in susceptibile Balb/c mice were also studied (J.I. Rojas *et al.* 1992, unpublished).

In this paper we present the evolution of histopathological changes in the cutaneous lesions of Balb/c mice. We also describe the histopathology of spleen and liver as indicators of the absence of any obvious visceral involvement.

Table 1. Histological findings at various time points in skin of foot pads injected with 10^7 and 10^8 promastigotes of *L. (V.) panamensis*

Days post infection	Histological findings	Group injected with 10^7 parasites	Group injected with 10^8 parasites
20	parasites	5/6*	4/5
	granuloma	3/6	3/5
40	parasites	6/6	6/6
	granuloma	0/6	1/6
60	parasites	6/6	5/5
	granuloma	1/6	0/5
80	parasites	6/6	5/5
	granuloma	0/6	0/5
	necrosis	4/6	4/5
130	parasites	6/6	5/5
	granuloma	0/6	0/5
	necrosis	6/6	5/5
	ulceration	4/6	3/5
160	parasites	6/6	5/5
	granuloma	0/6	0/5
	necrosis	6/6	5/5
	ulceration	6/6	5/5

* Number of animals with referred findings/number of animals in the group.

Materials and methods

Mice

Inbred Balb/c mice from the colony maintained at the Department of Immunology of Karolinska Institute, Stockholm, Sweden, 6–10 weeks old, of either sex were used throughout the experiment.

Parasite

Leishmania (*Viannia*) *panamensis*, HSJD-1 strain, was isolated from a patient at the San Juan de Dios Hospital, in San José, Costa Rica in 1972. It was characterized as *Leishmania* (*Viannia*) *panamensis* by isoenzyme typing by Professor W. Peters at Liverpool School of Tropical Medicine and by hybridization studies with insert probes of *L. (V.) panamensis* and *L. (L.) amazonensis* by Dr P. de Carreira at the Research Center of Parasitic Disease,

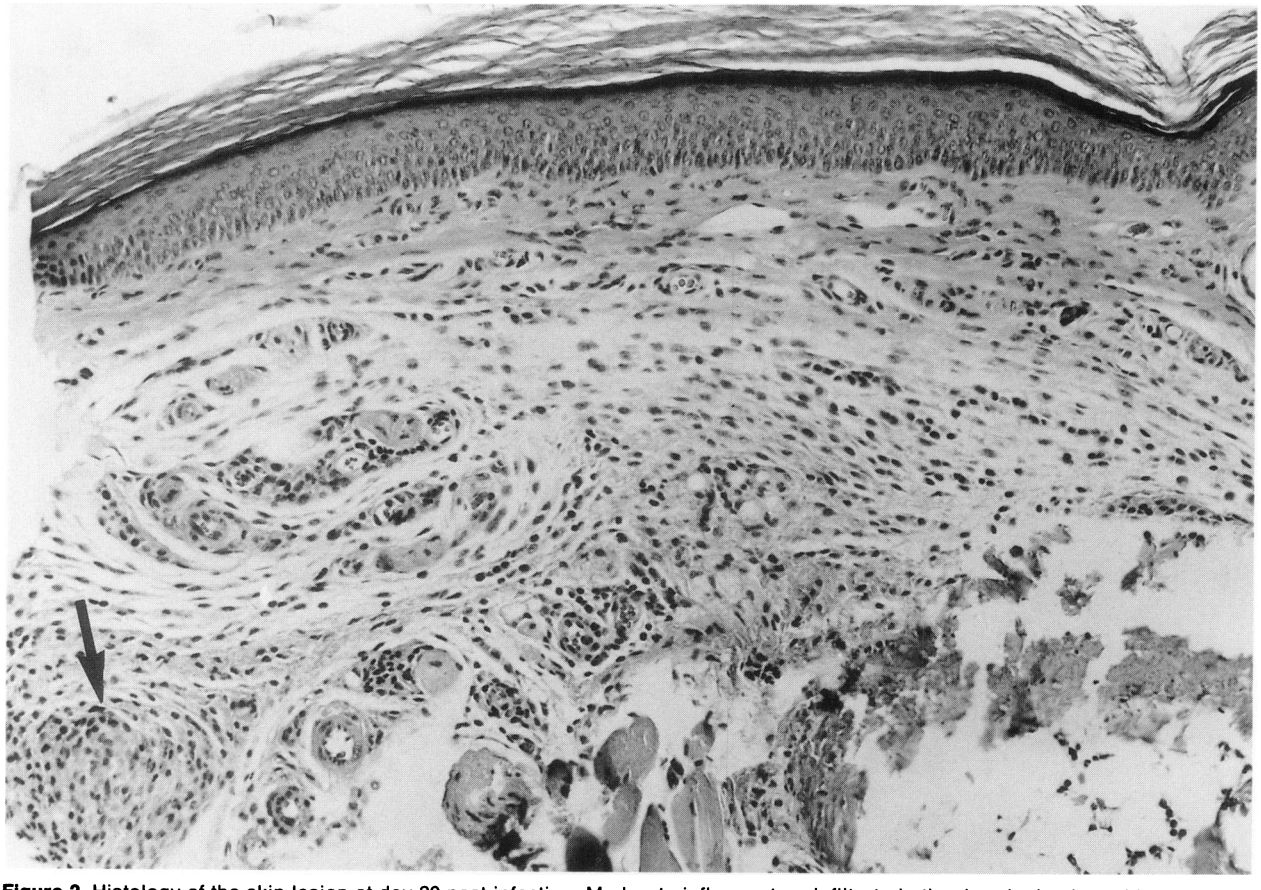

Figure 2. Histology of the skin lesion at day 20 post infection. Moderate inflammatory infiltrate in the dermis dominated by lymphocytes, macrophages and few granulocytes, and with granulomatous formation (arrow). H&E. × 40.

University of Panamá. In our department, parasites were now maintained in NNN medium over-layered by RPMI 1640 medium supplemented with 10% FCS, at 25°C, with periodical in-vivo passage in hind footpad of Balb/c mice. The parasites isolated in NNN medium from footpad lesions of mice were expanded in RPMI 1640 medium supplemented with 10% FCS. Parasites in stationary phase of growth were washed three times in sterile PBS and either 10^7 or 10^8 promastigotes in 50 μl PBS were injected subcutaneously in one hind footpad of mice. The control mice were injected subcutaneously with 50 μl PBS in one hind footpad.

Evaluation of lesion development

Mice were observed every 20 days during 160 days. Size of lesion, ulceration in the injected footpad and appearance of any other skin lesions were recorded. The injected and non-injected footpads were measured every 20 days with a dial caliper (Starrett, Athol, Mass., USA) and the difference between the measurements was taken as the extent of swelling.

Histopathological studies

Six mice injected with 10^7 promastigotes, five with 10^8 promastigotes and four control mice were sacrificed on days 20, 40, 60, 80, 130 and 160 post-infection. Fragments of skin lesion, spleen and liver were taken at each time point and draining popliteal lymph nodes on day 130 and 160. Specimens were fixed in phosphate buffered 4% formaldehyde pH 7.0, embedded in paraffin and stained in haematoxylin–eosin (H&E) for histological studies.

Results

Course of infection in Balb/c mice

Macroscopically, it was possible to detect swelling from day 20 post-injection in the footpad. This swelling increased progressively to an almost tumour-like lesion. Mice injected with 10^7 and 10^8 promastigotes of *Leishmania (Viannia) panamensis* had a similar course of development except that in the initial phase, those that received 10^7 parasites showed significantly smaller

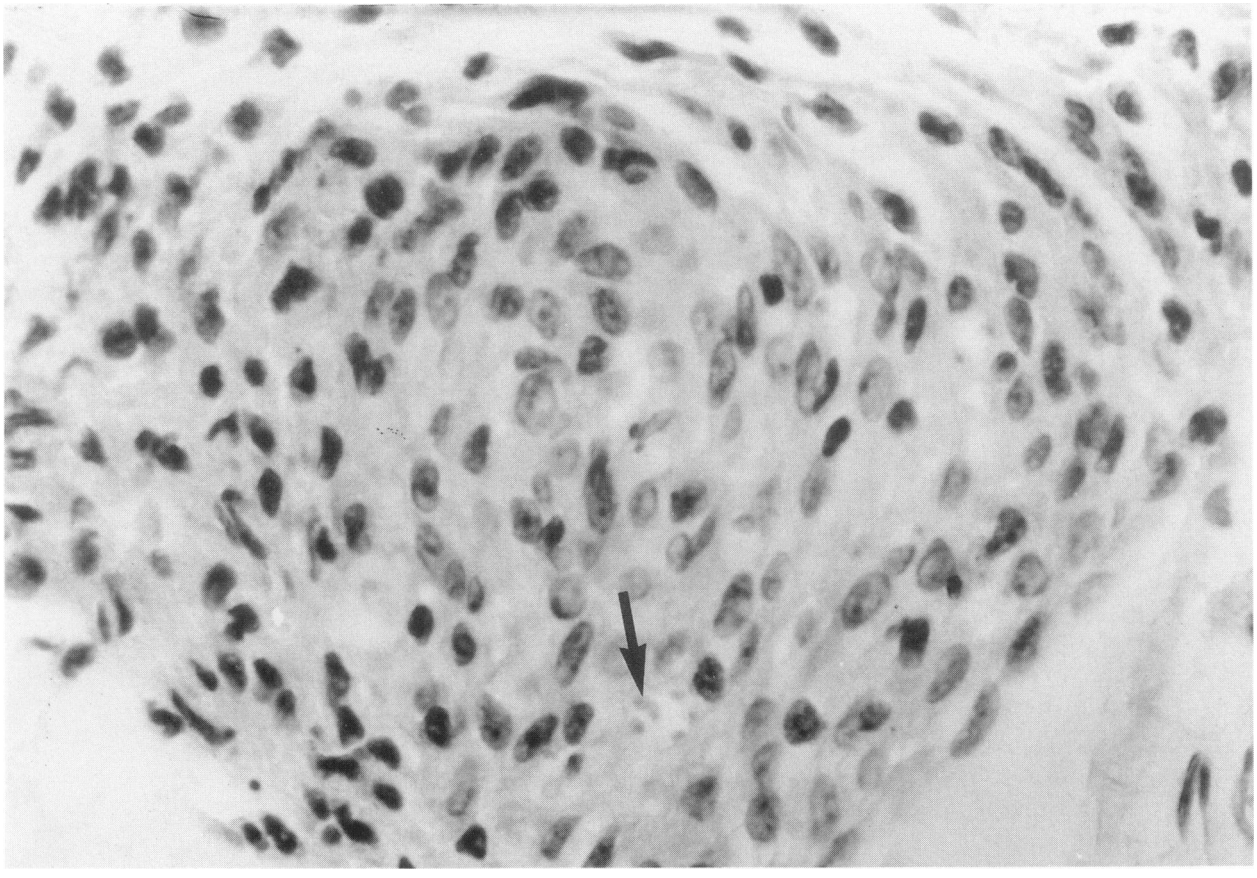

Figure 3. Skin lesion at day 20 post infection. Granuloma with the presence of epithelioid cells and few parasites (arrow) in the cytoplasm of macrophages. H&E. ×200.

lesion size, around half that of the 10^8-injected group (Figure 1). Throughout the experiments, we observed no typical ulceration. From day 100 in the 10^8-injected mice and from day 120 in the 10^7-injected group, we observed localized crust formation on the skin of the footpad which resembled the consequence of a traumatic process.

No other skin lesions were ever detectable in other parts of the body. No death was recorded during the observing period, up till day 160 post-infection.

Histopathology.

Cutaneous lesions. Since the histological findings were very similar in mice injected with 10^7 and 10^8 parasites during the whole study period, we describe the data all together (Table 1).

Twenty days after inoculation there was a moderate inflammatory infiltrate in the dermis dominated by lymphocytes, macrophages and few granulocytes (Figure 2). In 10 of 11 animals, few leishmania amastigotes were identified in the cytoplasm of macrophages. A granulomatous pattern with the presence of epithelioid cells and a few giant multinucleated cells was observed in six of 11 animals (Figure 3). No change was observed in the epidermis.

Forty days after inoculation more intense inflammatory processes were observed in the dermis (Figure 4). The inflammatory infiltrate was dominated by vacuolated macrophages containing higher amounts of parasites (Figure 5). In all animals the leishmanias were identified. A tendency to a granulomatous reaction was observed in one animal, while another animal presented focal necrosis in the inflammatory process.

From the 60th day, the skin lesions increased progressively in size, extending close to the epidermis, always disclosing an extensive inflammatory infiltrate. This infiltrate was composed of monomorphic vacuolated macrophages containing the parasites and showed a pattern similar to adipose tissue. In the periphery of the infiltrate there were few lymphocytes, monocytes and plasma cells. Focal necrosis with polymorphonuclear neutrophils was observed in three of 11 animals (Figure

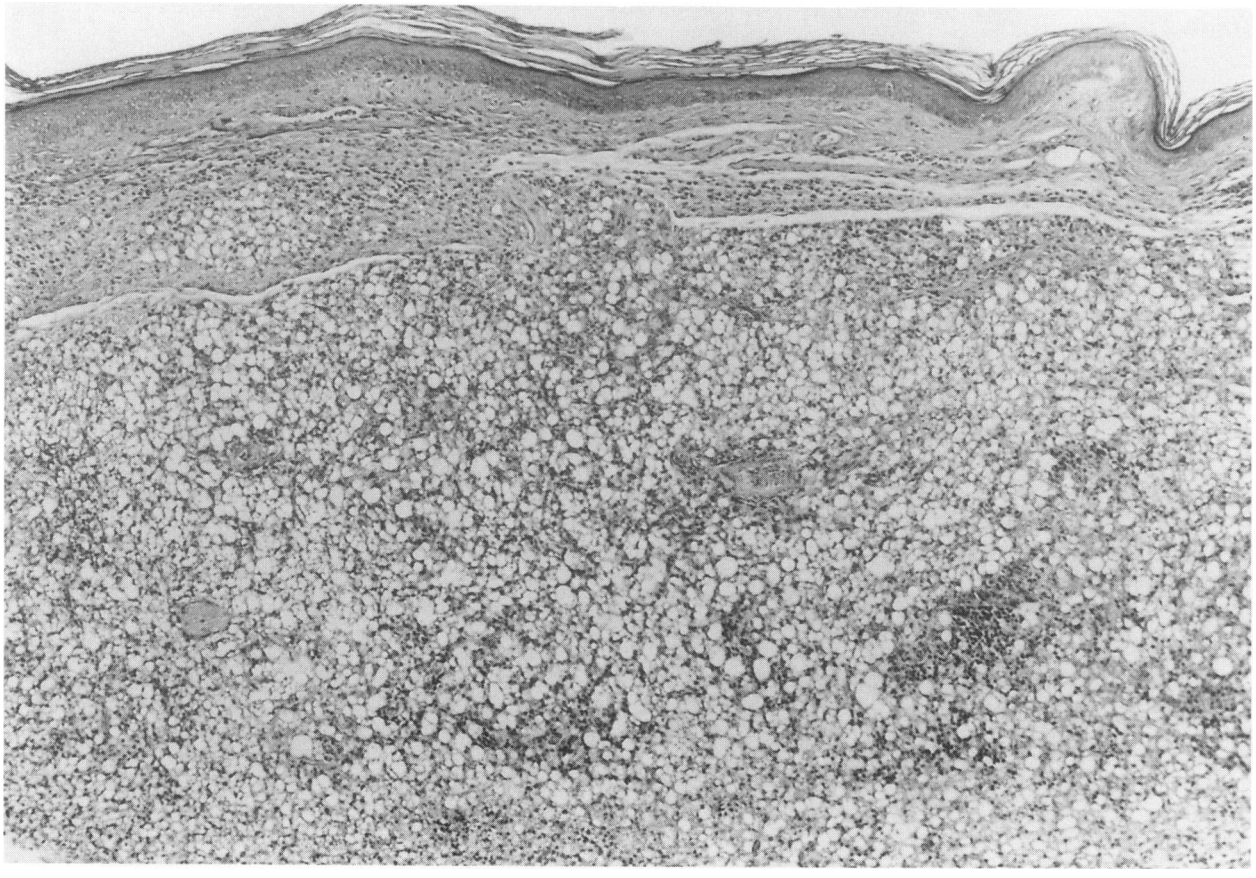

Figure 4. Skin lesion at day 40 post infection. Intense inflammatory infiltrate in the dermis dominated by vacuolated macrophages is shown. The inflammatory process extends close to the epidermis and shows a pattern similar to adipose tissue. H&E. × 20.

6). No tendency to granulomatous formation was observed.

Eighty days after inoculation, the histological picture was very similar to that described above for 60 days post infection. However, the inflammatory infiltrate was more extensive and focal necrosis was seen in eight of 11 animals.

A nodular, tumorous foam cell lesion overtaking all the dermis, including the subepidermic area, was seen in all animals 130 and 160 days after inoculation. Focal necrosis was seen. Due to the tumorous process in the dermis, the epidermis was stretched and ulcerated (Figure 7) in six of 11 and 11 of 11 animals after 130 and 160 days, respectively. Only a few parasites were seen in the cytoplasm of vacuolated cells in the epidermis.

Neither acanthosis nor hyperplasia of the epidermis was observed at any time point. Further, there was no involvement of nerves in the process.

Liver and spleen. No histological changes were observed in liver and spleen. Neither hyperplasia nor hypertrophy of Kupffer cells or of macrophages was observed in liver or spleen. No parasites were seen in our material stained by haematoxylin and eosin.

Lymph nodes. The draining popliteal lymph nodes were examined in mice at day 130 (six animals) and day 160 (nine animals) post inoculation. All animals of both groups presented numerous plasma cells in the medullary cords. The cortical sinuses were dilated and contained macrophages with leishmania organisms in the cytoplasma of five of six and nine of nine animals at 130 and 160 days, respectively (Figure 8). Granulomatous lesion was observed in four of six animals in the subcapsular area after 130 days and in six of nine animals after 160 days of infection. Lesions were composed of epithelioid cells, macrophages with parasites and few multinucleated giant cells (Figure 9).

Discussion

Balb/c mice are susceptible to *Leishmania* (*Viannia*)

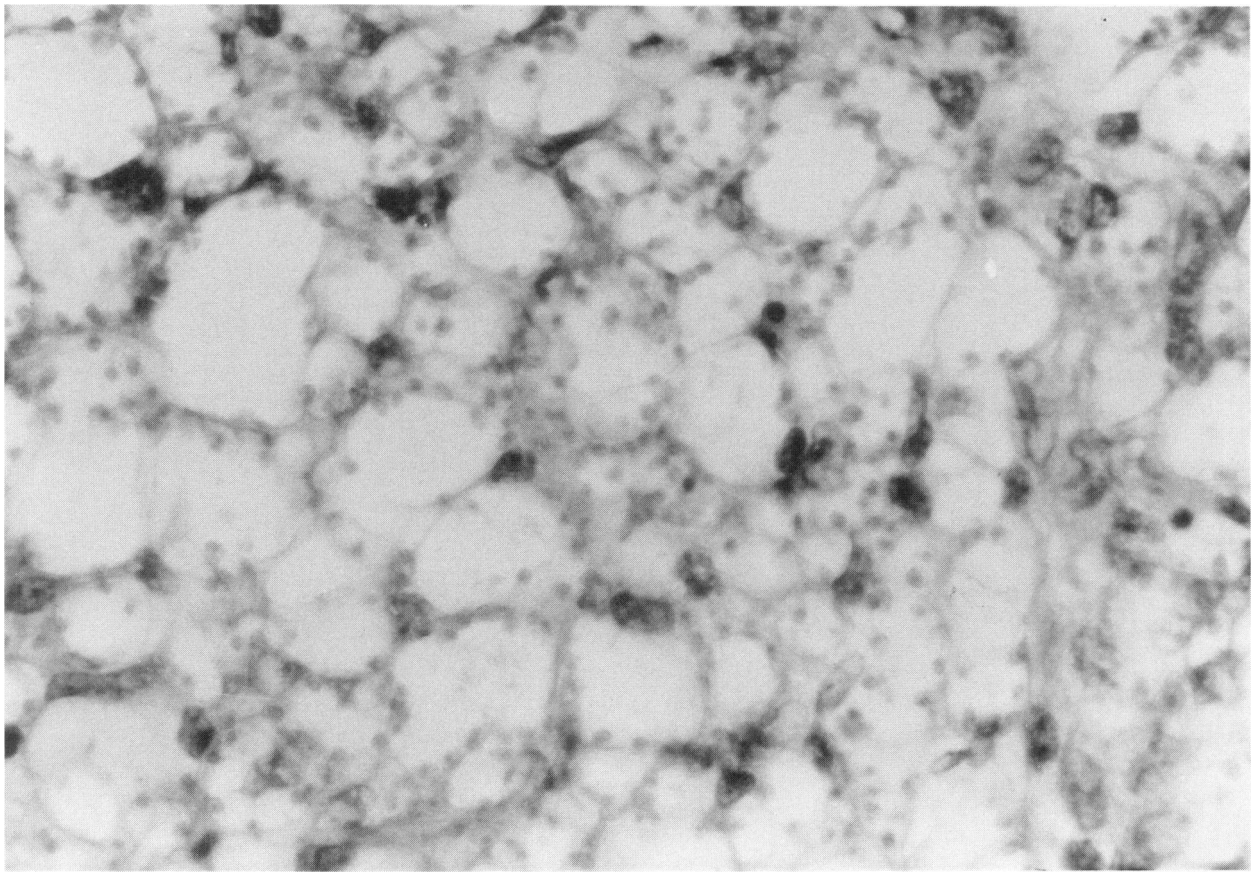

Figure 5. Monomorphic vacuolated macrophages containing numerous parasites at day 40 post infection. H&E. × 200.

panamensis infection and here we analysed the histopathology of the skin, draining lymph node, liver and spleen during the course of the infection.

In Balb/c mice infected with *L.* (*V.*) *panamensis* we observed characteristics that differentiate this model from infections caused by other well known species, i.e. *Leishmania* (*Leishmania*) *major* and *Leishmania* (*Leishmania*) *amazonensis*. With *L.* (*V.*) *panamensis* we recorded tumour-like lesion but without typical ulcera, necrosis, skin metastasis and visceralization leading to death that were described in the above mentioned Balb/c models with other *Leishmania* species (Handman *et al.* 1979; Howard *et al.* 1980; Barral-Neto *et al.* 1987a; McElrath *et al.* 1987). Regarding visceralization, we could isolate parasites from draining popliteal lymph nodes and spleen in samples from the 4th and the 6th months of infection (J. I. Rojas *et al.* 1992, unpublished) but histopathology of spleen and liver showed no evidence of establishment of the infection in these places where amastigotes proliferate in the case of visceral spread. Our data indicate that this species of parasite circulates systemically but it is not able to establish the infection in the viscera, probably because of the physiology of this *Leishmania* species. Similar evidence was provided by Hill (1988) in *L.* (*L.*) *amazonensis* infection where parasites were detected and grown from viscera. However, these parasites did not establish lesions in any internal organs, although they were capable of metastasizing in skin.

Comparing infection of Balb/c mice with these three species of *Leishmania*, we can classify *L.* (*L.*) *major* by its capacity to metastasize to skin and viscera (Handman *et al.* 1979; Howard *et al.* 1980), *L.* (*L.*) *amazonensis* as one that mostly affects skin but less the viscera (Barral-Neto *et al.* 1987a; Hill 1988), and *L.* (*V.*) *panamensis* as without disseminating capacity either to other parts of skin or to internal organs.

The route of dissemination for *Leishmania* parasites in general is unclear but Travi *et al.* (1988) showed that *L.* (*V.*) *panamensis* disseminates through the lymphatic system and not through the blood in the hamster. The histopathology of draining lymph nodes in our material would also suggest that the parasites circulate via lymphatic vessels from the injected site, coming to lymph

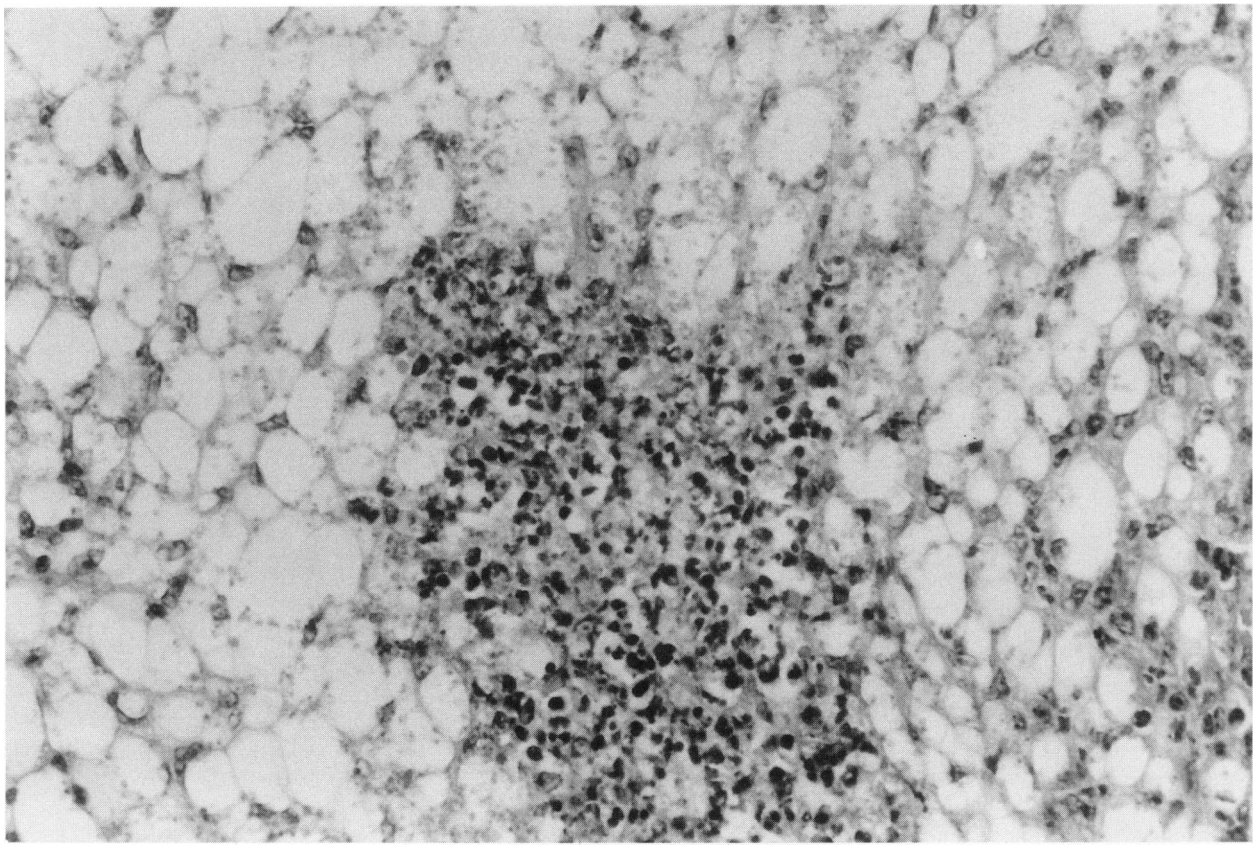

Figure 6. Focal necrosis with polymorphonuclear neutrophils in the inflammatory process constituted by vacuolated macrophages containing parasites at 60 days post infection. H&E. × 100.

nodes through cortical sinuses where they are phagocytosed by macrophages and apparently proliferate. The clinical picture of cutaneous leishmaniasis in Panamá, with involvement of lymph nodes in chain, resembling sporotrichosis (Walton 1987), might suggest preferential dissemination of this species via the lymphatic system.

The granulomas found in the subcapsular area may indicate a reaction of this lymphoid organ controlling the progress and spread of infection. Granuloma has been related to resistance in immunized Balb/c mice infected with *L.* (*L.*) *amazonensis* (Barral-Neto *et al.* 1987b), and also to absence of amastigotes and healing with less total dose of antimonial treatment in leishmaniasis patients (Gutierrez *et al.* 1991). Further, multinucleated giant cells found in granuloma have been related to the effect of interferon γ (Weinberg *et al.* 1985; Murray *et al.* 1987; Belosevic *et al.* 1989) and interferon γ is known to be effective in activating macrophages to kill leishmanias intracellularly (Murray *et al.* 1983). It is noteworthy that these granulomatous reactions were detected in samples from 4 and 6 months post infection when another type of process was going on with apparently uncontrolled multiplication of the parasites in the injected footpad, inside the histiocytes and without any sign of granulomatous reaction.

Footpad swelling was significantly smaller at the beginning of the experiment with 10 times lower dose of the parasite, but in the later periods, lesions became tumour-like and reached similar levels. The histopathological picture showed that the process taking place was very similar in both groups. Our histopathological findings suggest that even in susceptible Balb/c mice, there is a response by the host tending to control the lesion in the beginning. We observed predominant lymphocyte infiltration and granulomatous reactions with multinucleated cells indicative of a potentially effective immune mechanism, as if it was actually inducing interferon γ production, as discussed above in relation to lymph nodes. This also could be correlated to the lymphoproliferative response of lymph node cells to the leishmania antigens detected on day 20 (J. I. Rojas *et al.* 1992, unpublished). However, it is not a truly protective reac-

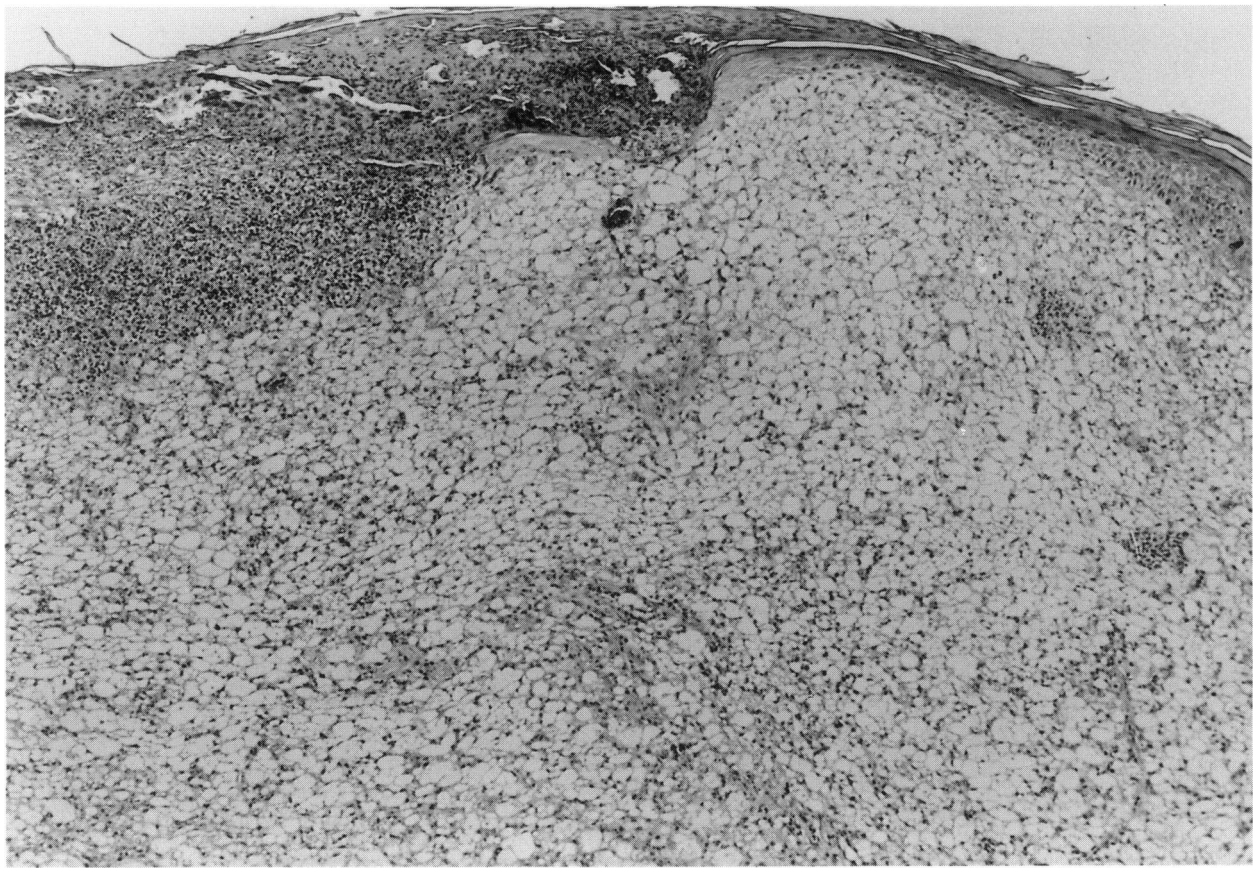

Figure 7. Skin lesion at day 130 post infection. Nodular, tumorous foam cell type of lesion overtaking all the dermis, including the subepidermic area. Focal necrosis and ulceration of the epidermis are seen. H&E. × 20.

tion because it progressed to a lesion with predominance of vacuolated histiocytes containing many amastigotes inside and with few lymphocytes. In this period a depression in cell-mediated immune response to leishmania antigens was recorded (J.I. Rojas *et al.* 1992, unpublished).

Different human cutaneous leishmaniasis lesions have been classified by Ridley (Ridley *et al.* 1980; Ridley 1987) and the initial lesion can be considered as belonging to their group III. Subsequently, the picture changes to the polar anergic form like that described in the disseminated disease, with almost exclusively histiocytes harbouring many amastigotes inside (Bryceson 1969; Ridley 1987).

The foci of necrosis appeared in the sequence, but they are neither extensive nor numerous. Necrosis has been claimed as one of the effective mechanisms eliminating the leishmania (Ridley & Ridley 1986). On the contrary, Gutierrez *et al.* (1991) related necrosis to the presence of amastigotes and to the necessity for higher total dose of antimonial treatment, using extensive human leishmaniasis material. Andrade *et al.* (1984) described very similar skin lesion in Balb/c mice infected with *L.* (*L.*) *amazonensis* with anergic changes dominated by fully parasitized histiocytes, but with more extensive necrosis. They could not directly relate the necrosis to the protection, but comparing alterations found in resistant mice which also presented necrosis, they observed the following differences: in susceptible Balb/c mice, the necrosis was coagulative and in sequence became invaded by polymorphonuclear neutrophils and seemed to be ischaemic; in resistant A/J mice it was fibrinoid and became substituted by fibrous tissue. The necrosis in our material resembled that found in susceptible Balb/c mice described by these authors and, considering the progressive course of infection, it does not seem to contribute to control the infection.

In this period, both specific cell-mediated responses and IgG responses were detectable (J.I. Rojas *et al.* unpublished). Antibodies have never convincingly been related to host protection but there might be some role for antibodies in the immunopathology related to the

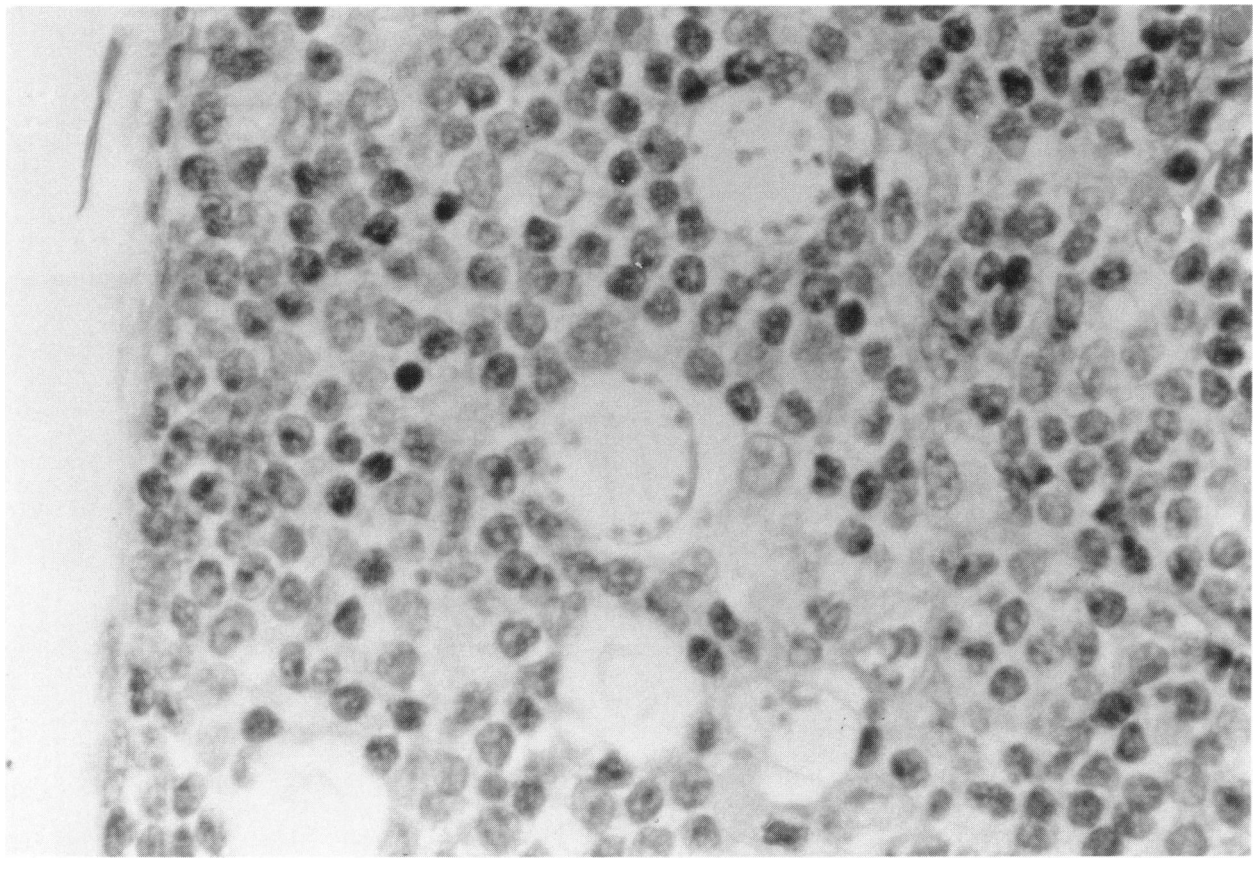

Figure 8. Draining popliteal lymph node showing vacuolated macrophages harbouring parasites in draining popliteal lymph node at day 160 post infection. H&E. × 200.

necrotic process. Serum antibody titres have previously been associated with the presence of amastigotes and necrosis (Gutierrez *et al.* 1991). One could speculate that an induction of lymphokines like IL-4 and IL-10 reported in progressive murine leishmaniasis (Heinzel *et al.* 1991) would favour production of non-protective antibodies at this stage of the infection (Paul 1991). The cell-mediated response in this period might also have some role in the necrotic process, but it does not seem to exert any definitive role for protection.

We saw no significant alteration in the epidermis. The ulceration observed in microscopic examination suggests that it results from the convergence of different processes: an intense inflammatory process in the dermis that reaches the subepidermic area in later periods and a traumatic process because of the large size of the lesion.

To understand the underlying mechanisms of either susceptibility or resistance to the infection with *Leishmania* parasites, histopathological studies of the lesion site are of utmost importance since that is where the interaction of either protective or disease promoting immune elements takes place. In addition, immunological studies, usually of draining lymph node cells and peripheral blood lymphocytes, should be done. Further studies are planned of the histopathology of resistant mouse strains and to identify the invading lymphocyte subpopulation during the course of the skin infection.

The mouse model of cutaneous leishmaniasis is of course useful not only in the study of pathogenesis of *L.* (*V.*) *panamensis*-induced infection but would also be useful in drug trials in the development of new therapeutic tools.

Acknowledgements

Hiro Goto was supported by a scholarship from the Universidade de São Paulo—Banco Interamericano de Desenvolvimento programme. We acknowledge the Karolinska Institute Research Training programme and SAREC for supporting this project.

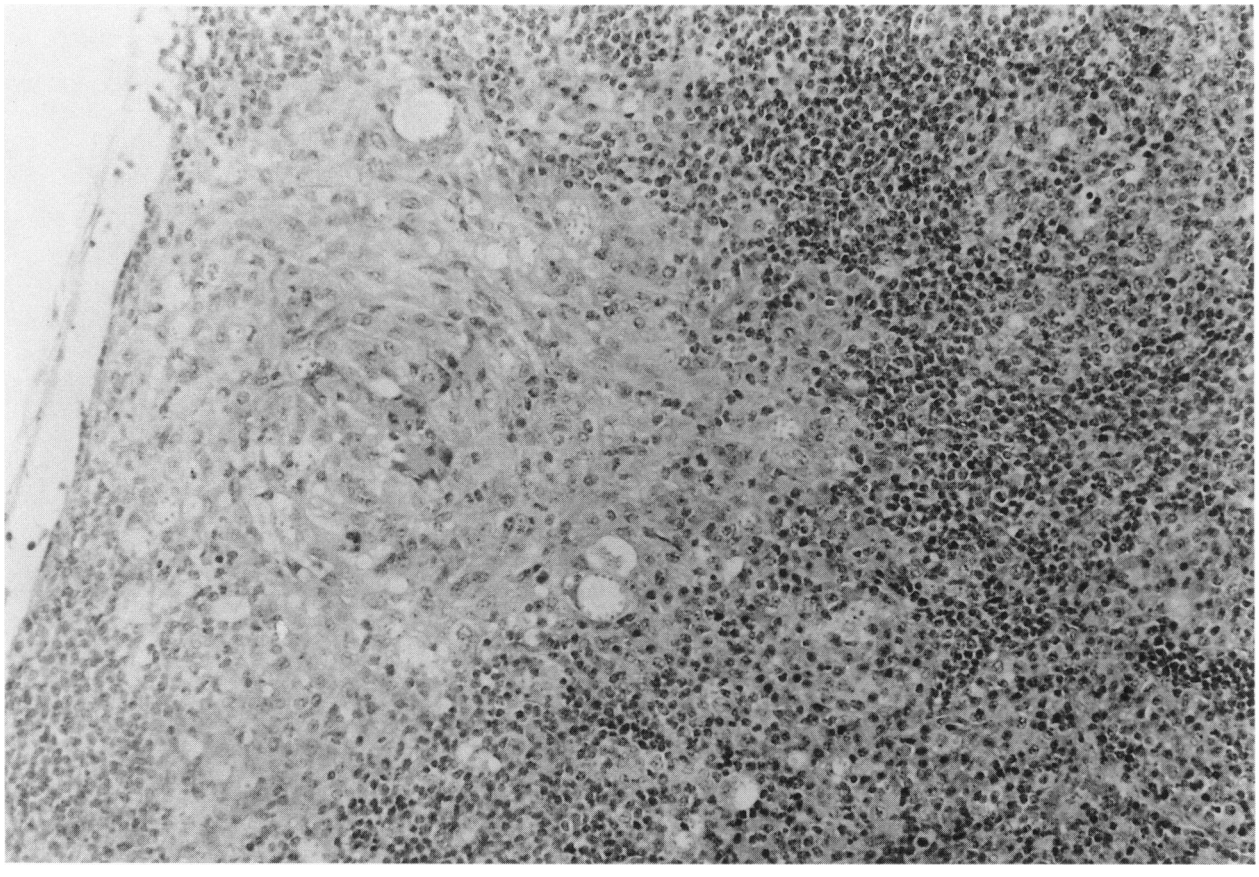

Figure 9. Draining popliteal lymph node at day 160 post infection. Granulomatous lesion in the subcapsular area composed of epithelioid cells, macrophages with parasites and few multinucleated giant cells are shown. H&E. × 50.

References

ANDRADE Z.A., REED S.G., ROTTERS S.B. & SADISGURSKY M. (1984) Immunopathology of experimental cutaneous leishmaniasis. *Am. J. Pathol.* **114,** 137–148.

BARRAL-NETO M., CARDOSO S.A. & BARRAL A. (1987a) Different patterns of disease in two inbred mouse strains infected with a clone of *Leishmania mexicana amazonensis*. *Acta Trop. (Basel)* **44,** 5–11.

BARRAL-NETO M., DE FREITAS L.A.R. & ANDRADE Z.A. (1987b) Histopathologic changes induced by vaccination in experimental cutaneous leishmaniasis of Balb/c mice. *Am. J. Pathol.* **127,** 271–278.

BELOSEVIC M., FINBLOOM D.S., VAN DER MEIDE P.H., SLAYTER M.V. & NACY C.A. (1989) Administration of monoclonal anti-IFN-γ antibodies in vivo abrogates natural resistance of C3H/HeN mice to infection with *Leishmania major*. *J. Immunol.* **143,** 266–274.

BRYCESON A.D.M. (1969) Diffuse cutaneous leishmaniasis in Ethiopia. I. The clinical and histological features of the disease. *Trans. R. Soc. Trop. Med. Hyg.* **63,** 708–737.

GUTIERREZ Y., SALINAS G.H., PALMA G., VALDERRAMA L.B., SANTRICH C.V. & SARAVIA N.G. (1991) Correlation between histopathology, immune response, clinical presentation, and evolution in *Leishmania braziliensis* infection. *Am. J. Trop. Med. Hyg.* **45,** 281–289.

HANDMAN E., CEREDIG R. & MITCHELL G.F. (1979) Murine cutaneous leishmaniasis: disease patterns in intact and nude mice of various genotypes and examination of some differences between normal and infected macrophages. *Aust. J. Exp. Biol. Med. Sci.* **57,** 9–29.

HEINZEL F.P., SADICK M.D., MUTHA S.S. & LOCKSLEY R.M. (1991) Production of interferon γ, interleukin 2, interleukin 4, and interleukin 10 by $CD4^+$ lymphocytes in vivo during healing and progressive murine leishmaniasis. *Proc. Natl Acad. Sci. USA* **88,** 7011–7015.

HILL J.O. (1988) Pathophysiology of experimental leishmaniasis: the role of parasite physiology in the development of metastatic disease. *Am. J. Trop. Med. Hyg.* **39,** 256–260.

HOWARD J.G., HALE C. & CHAN-LIEW F.Y. (1980) Immunological regulation of experimental cutaneous leishmaniasis. I Immunogenetic aspects of susceptibility to *Leishmania tropica* in mice. *Parasite Immunol.* **2,** 303–314.

MCELRATH M.J., KAPLAN G., NUSRAT A. & COHN Z.A. (1987) Cutaneous leishmaniasis. The defect in T cell influx in Balb/c mice. *J. Exp. Med.* **165,** 546–559.

MURRAY H.W., RUBIN B.Y. & ROTHERMEL D. (1983) Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon-γ is the activating lymphokine. *J. Clin. Invest.* **72,** 1506–1510.

MURRAY H.W., STERN J.J., WELTE K., RUBIN B.Y., CARRIERO S.M. & NATHAN C.F. (1987) Experimental viceral leishmaniasis: pro-

duction of interleukin 2 and interferon-γ, tissue immune reaction, and response to treatment with interleukin 2 and interferon-γ. *J. Immunol.* **138,** 2290–2297.

NEAL R.A. & HALE C. (1983) A comparative study of susceptibility of inbred and outbred mouse strains compared with hamsters to infection with New World cutaneous leishmaniasis. *Parasitology* **87,** 7–13.

PAUL W.E. (1991) Interleukin 4: prototypic immunoregulatory lymphokine. *Blood* **77,** 1859–1870.

RIDLEY R.D. (1987) Pathology. In *The Leishmaniasis in Biology and Medicine*. Eds W. Peters & R. Killick-Kendrick. London: Academic Press Inc. pp. 665–701.

RIDLEY D.S., MARSDEN P.D., CUBA C.C. & BARRETO A.C. (1980) A histological classification of mucocutaneous leishmaniasis in Brazil and its clinical evaluation. *Trans. R. Soc. Trop. Med. Hyg.* **74,** 508–514.

RIDLEY M.J. & RIDLEY D.S. (1986) Monocyte recruitment, antigen degradation and localization in cutaneous leishmaniasis. *Br. J. Exp. Path.* **67,** 209–218.

TRAVI B., REY-LADINO J. & SARAVIA N.G. (1988) Behaviour of *Leishmania braziliensis* s.l. in golden hamsters: evolution of the infection under different experimental conditions. *J. Parasitol.* **74,** 1059–1062.

WALTON B.C. (1987) American cutaneous and mucocutaneous leishmaniasis. In *The Leishmaniasis in Biology and Medicine*. Eds W. Peters & R. Killick-Kendrick. London: Academic Press Inc. pp. 637–664.

WEINBERG J.B., HOBBS M.M. & MISUKONIS M.A. (1985) Phenotypic characterization of gamma interferon-induced human monocyte polykaryons. *Blood* **66,** 1241–1246.