9.36

The Langerhans Cell. Ed. J. Thivolet, D. Schmitt. Colloque INSERM/John Libbey Eurotext Ltd. © 1988. Vol. 172, pp. 479-490.

Immunocytochemical analysis of Langerhans cells in murine cutaneous leishmaniasis

F.J. Tapia[1], E. Rojas[1], G. Kraal[2], W. Mosca[1], J. Convit[1]

[1]Instituto de Biomedicina, Apartado 4043, Caracas 1010A, Venezuela. [2]Dept. Histology, Medical Faculty, Free University, Amsterdam, The Netherlands

ABSTRACT

Las Células de Langerhans (CL) son células inmunocompetentes de la piel que funcionan como accesorias y presentadoras de antígeno. La importancia de estas células en la leishmaniasis cutánea no ha sido aún enclarecida. El presente estudio realiza una caracterización y cuantificación de las CL a diferentes estadios de la leishmaniasis murina experimental, y muestra nuestra experiencia en pacientes con leishmaniasis cutánea americana. Ratones consanguíneos C57BL/6 fueron infectados con Leishmania mexicana amazonensis (Lma) cepa M1132. Algunos de estos animales fueron tratados con éter monobenzil de hidroquinona (MBEH), un agente que causa incremento en la densidad de CL. Los resultados en pacientes con leishmaniasis cutánea americana demuestran un aumento importante de las CL en las distintas formas clínicas de la enfermedad. Este aumento fue significativamente mayor en pacientes con leishmaniasis cutánea localizada. Los ratones C57BL/6 (n=60) fueron inoculados subcutáneamente en la almohadilla plantar con 10^3 amastigotes de Lma. Los animales bajo tratamiento (n=30) recibieron en forma tópica una solución al 20% de MBEH por 7 días antes de la infección. El análisis fue llevado a cabo sobre cortes congelados y epidermis separada. La caracterización inmunocitoquímica se realizó utilizando la técnica avidina-biotina inmunoperoxidasa y el anticuerpo monoclonal NLDC-145, un nuevo marcador de células dendríticas. Los animales infectados con Lma mostraron un aumento significativo de CL/mm² de epidermis. Estos valores fueron el doble de los obtenidos después del tratamiento con MBEH y cuatro veces los observados en animales sanos. Un análisis similar se realizó en ratones BALB/c infectados con Lma, mostrando un incremento de las CL durante el curso de la infección. En estos animales se observó que la almohadilla no inoculada con Lma también mostraba un incremento en CL, pero un una escala mayor que la observada en la almohadilla inoculada. Esto último enfatiza el concepto de la piel como órgano integral. Los resultados expuestos demuestran una asociación entre la densidad de las CL y la infección leishmánica.

KEYWORDS: avidin-biotin immunoperoxidase, cell immunity, dendritic cells, immunocytochemistry, Langerhans cells, leishmaniasis.

INTRODUCTION

The clinical pathological spectrum of American cutaneous leishmaniasis (ACL) in its diverse forms is the expression of the immunological response of the human host to infection by the Leishmania parasite (Convit & Pinardi, 1974). The two forms at either end of that spectrum are diffuse cutaneous leishmaniasis (DCL), in which patients are non-reactor to Leishmania antigens in tests of cell-mediated immunity, and localized cutaneous leishmaniasis (LCL), in which patients have a cell-mediated immune response to the protozoan (Convit et al., 1972; Castés et al., 1983). Another clinical form of the disease is mucocutaneous leishmaniasis (MCL), characterized by lesions that contain very few parasites and chronic mucous membrane ulcers, appearing usually after a healed primary skin lesion.
In murine models, depending on the Leishmania strain and the number of inoculated parasites, it has been possible to reproduce the distinct clinical forms observed in humans. Thus, BALB/c mice reproduce lesions similar to DCL, C57BL mice are resistant reproducing LCL-like lesions, and DBA mice can show intermediate forms of the disease (Preston et al., 1972; Howard et al., 1981; Pérez, 1982).

The use of immunocytochemical techniques and monoclonal antibodies for the localization of specific antigens allows the precise characterization of the different cell subsets involved in the immune response of various skin disorders (Modlin et al., 1985; Tapia et al., 1986; Gross et al., 1987). The type of information obtained from the application of these techniques is not only a descriptive one but it could also give quantitative information contributing to a better understanding of the dynamics of granuloma formation or epidermal involvement.

American cutaneous leishmaniasis is an important public health problem in Latin America. In Venezuela alone there are about 4000 new cases a year. The disease mainly affects people living in rural areas; recently due to urban expansion, the population is penetrating the forest where the phlebotomi live causing an increase in new cases.

In this study, we will present our experience in the immunocytochemical characterization of Langerhans cells (LC) in human and experimental models of ACL. In addition, in order to understand LC involvement in the pathogenesis of leishmaniasis, we have used in the murine models of the disease, monobenzyl ether of hydroquinone (MBEH), a known LC promoting agent (Rheins & Nordlund, 1986).

MATERIAL AND METHODS

Patients

Patients with LCL (n=20), DCL (n=2) and MCL (n=1) were studied at the Instituto de Biomedicina. The diagnosis was made by established clinical, histological, microbiological and immunological criteria (Convit, 1974). All the LCL lesions were ulcerated. Smears, hamster foot incoculation and Montenegro tests were positive in the LCL patients.

Animals and infection
C57BL/6 female mice (n=60) aged 6-8 weeks old were inoculated subcutaneously in the left footpad with 10^3 amastigotes of L. mexicana amazonensis (Lma) (strain M1132, Lainson, Belem, Brazil). The right footpad was left intact as a control. At various times after infection, groups of 2-4 mice were killed by cervical dislocation, and both footpads removed. A small group (n=30) BALB/c female mice aged 8-10 weeks old were also inoculated with Lma. A distinct group of C57BL/6 mice were treated with a solution of MBEH (Aldrich Chemical Co., U.S.A.) prepared in 95% ethanol. Half of these animals were infected with Lma as described above. The rest were left as controls to evaluate the effect of MBEH.

Tissue preparation
Scalpel skin biopsy specimens were embedded in OCT medium (Miles Labs, U.S.A.) and rapidly frozen in liquid Nitrogen. The tissues were then stored at -20° C until sectioning. In the animal models LC characterization was also carried out on EDTA-separated epidermis. Briefly, scalpel skin biopsies (2-3 mm^2) were soaked in PBS for 5 min, and then in a solution of 0.76 % EDTA in PBS for 2.5 hrs at 37° C. After washing with PBS, the epidermis was separated by micromanipulation under a stereo microscope.

ATPase staining
The APTase staining for murine LC cells was carried out according to Baker et al. (1983). Briefly, EDTA-separated epidermis was fixed in 2% formaldehyde in cacodylate buffer pH 7.3 for 20 min at 4° C. After 3 washings (10 min each) with saline, the epidermis was incubated at 37° C for 15 min in the substrate (10 µg adenosine 5 triphosphate in 5ml 5% $MgSO_4$, 3ml 2% $Pb(NO_3)_2$, and 42ml TRIS-maleate, pH 7.2 containing 8.55% glucose). After washing in saline and development with ammonium sulphide at room temperature, the epidermis was mounted on glycerin-gelatin.

Monoclonal antibodies
A murine monoclonal antibody directed to the CD1 molecule on human LC (Leu-6; 1:100) and purchased from Becton Dickenson, Inc, U.S.A. was used in the cases of ACL. A rat monoclonal antibody NLDC-145, a newly developed dendritic cell (including epidermal LC) marker (Kraal et al., 1986), was used 1:10 (culture supernatant) for the detection of epidermal LC on the experimental cutaneous leishmaniasis. Dilutions and immunostaining were performed using a modified phosphate-buffered saline (PBS) pH 7.2 (Hofman et al., 1982).

Immunoperoxidase staining procedure
Frozen sections (5µm) were cut with a cryostat and air dried overnight before the immunostaining. Similarly, EDTA-separated epidermis was mounted on coverslips and air dried for a few hours before the immunostaining. Some material was also stained with hematoxylin and eosin (H&E), and Giemsa. Immunostaining was performed using the avidin-biotin immunoperoxidase technique (Hsu et al., 1981), the sections being treated as follows: fixation in fresh acetone, 10 min; PBS, 5 min; primary monoclonal antibody (optimal dilution) 30-90 min; PBS, 5 min; incubation for 30 min in biotinylated horse-anti mouse IgG 1:100 (for Leu-6) or rabbit-anti-rat IgG 1:100 (for NLDC-145), both purshased from Amersham, U.K.; PBS, 5 min; strepavidin-peroxidase 1:60, Amersham, U.K.; PBS, 5 min; developing for 10 min with 90 µM H_2O_2 and 3-amino-9-ethyl-

carbazole (final concentration 0.88 µM) which was dissolved in 50 mM N,N-dimethylformamide in 0.1 M acetate buffer, pH 5.2; rinse in water; counterstaining with Mayer's hematoxylin and mounting in glycerin-gelatin.

LC quantification
Cells were counted using a light microscope with millimetered scale calibrated to determine the number of cells/mm² of epidermis or granuloma. Only cells showing red-brownish immunostaining and a visible nucleus were counted as positive.

Statistical analysis
Cell counts were expressed as mean ± SD per mm² of epidermis or granuloma. Statistical test t-Student and Mann-Whitney were applied for independent samples, and t-Student and Wilcoxon for paired samples.

RESULTS

Epidermal LC (CD1-positive) were increased, as compared to normal values, in all the clinical forms of ACL. However, the numbers were higher in the epidemis of LCL patients (Fig. 1). These cells were also observed in the dermal granulomas.

LC counting in the murine models of leishmaniasis was carried out on separated epidermis and frozen sections using immunocytochemistry for NLDC-145 and ATPase staining. The results using ATPase showed a significant drop in LC density after the fourth week, most certainly due to an increase in background staining which makes the identification of the cells difficult. For this reason this cytochemical technique was put aside for further analysis.

The numbers of LC in the Lma-infected mice were different between the BALB/cJ and C57BL/6 mice. Normal density values for BALB/c mice are 1400 LC/mm², whereas C57BL/6 have about 500 LC/mm², values which are similar to those found in normal human epidermis.

The immunocytochemical analysis using antibody NLDC-145 showed an exponential increase of LC during the first twelve weeks of infection in C57BL/6 (Fig. 2). After this period the LC values varied, reaching a peak of 1865 LC/mm² epidermis during the fifteenth week, and a 40% decrease during the sixteenth week (Table 1). Morphologically, the cells from the fifteenth week were smaller than those observed at the begining of the infection. The chages in LC density observed during the course of infection (Table 1) were statistically different ($P<0.05$) from those observed in the non-inoculated footpad. These values were also significantly different from those observed in normal C57Bl/6 mice.

Lma-infected BALB/c J mice also showed an increase in the numbers of epidermal LC, starting with values similar to those found in normal mice (1300 LC/mm²) (Table 2; Fig. 3). These animals showed a decrease in LC density on the first week (day 7) (903 LC/mm²) and a peak value (2054 LC/mm²) on the fourth week (day 25). In all these experiments, the non-inoculated footpads of the same animals were used as controls. An important finding was a similar cell kinetic curve between the infected and the non-infected footpads. However,

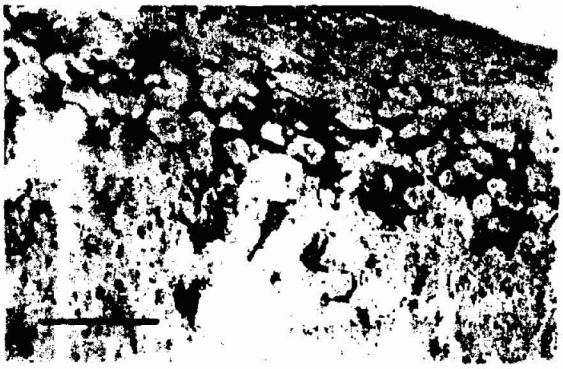

Fig. 1
Numerous epidermal Langerhans cells in a lesion of localized cutaneous leishmaniasis. Avidin-biotin immunoperoxidase using anti-CD1. Bar = 20µm.

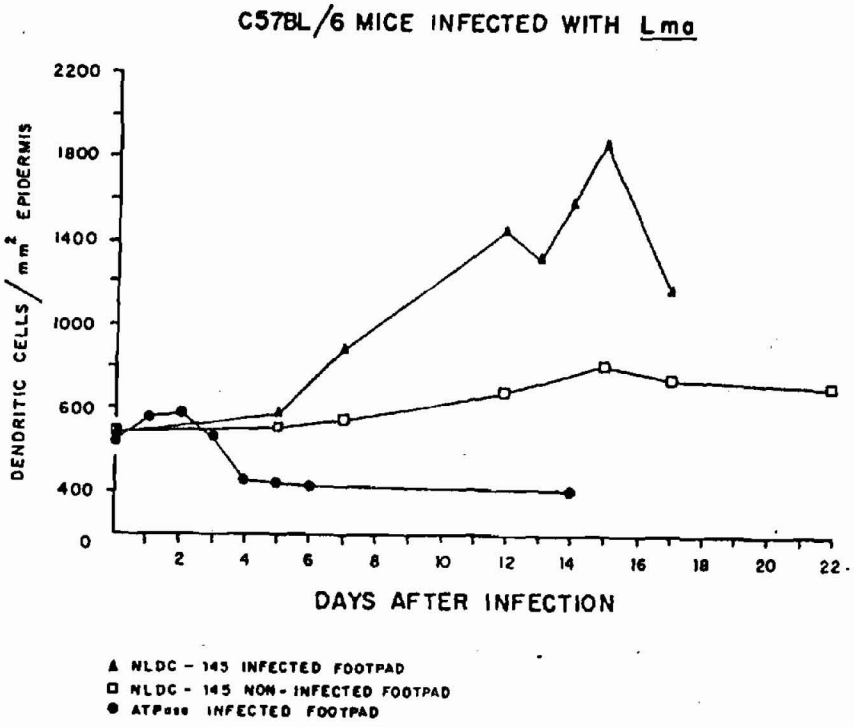

Fig. 2
Changes in Langerhans cells density in Lma-infected C57BL/6 mice. Note that the LC density is higher in the non-inoculated footpad.

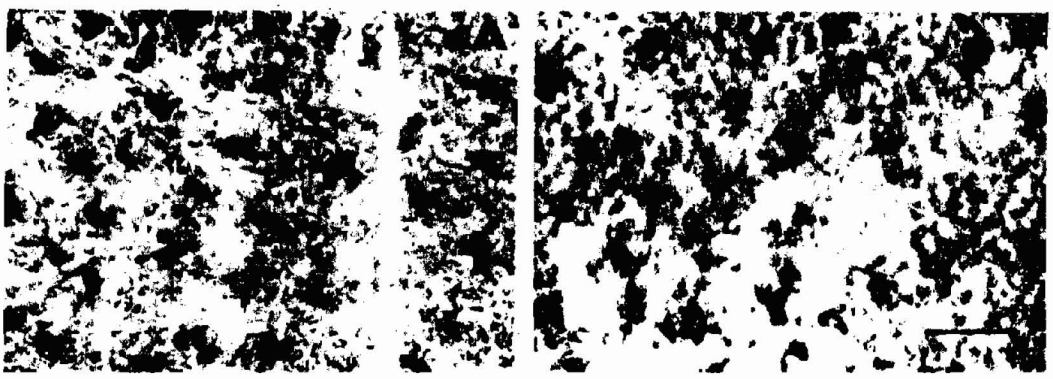

Fig. 3
Langerhans cell characterization in separated epidermis of BALB/c mice. A) normal footpad. B) Lma-inoculated footpad. Avidin-biotin immunoperoxidase using anti-NLCD-145. Bar = 10μm.

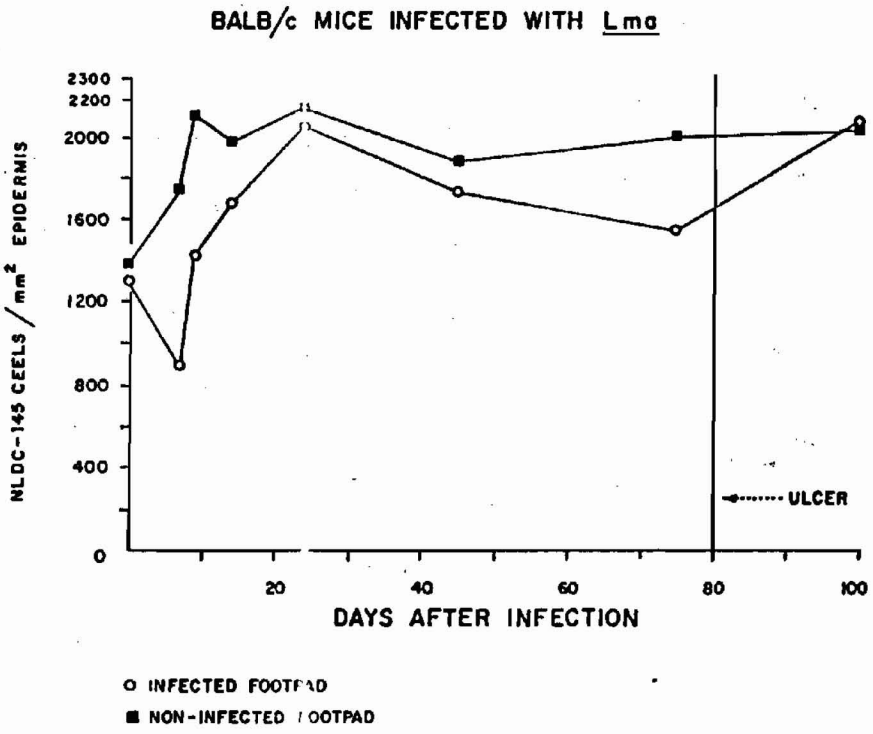

Fig. 4
Langerhans cells kinetics in Lma-infected BALB/c mice. Note that the LC density is higher in the non-inoculated footpad.

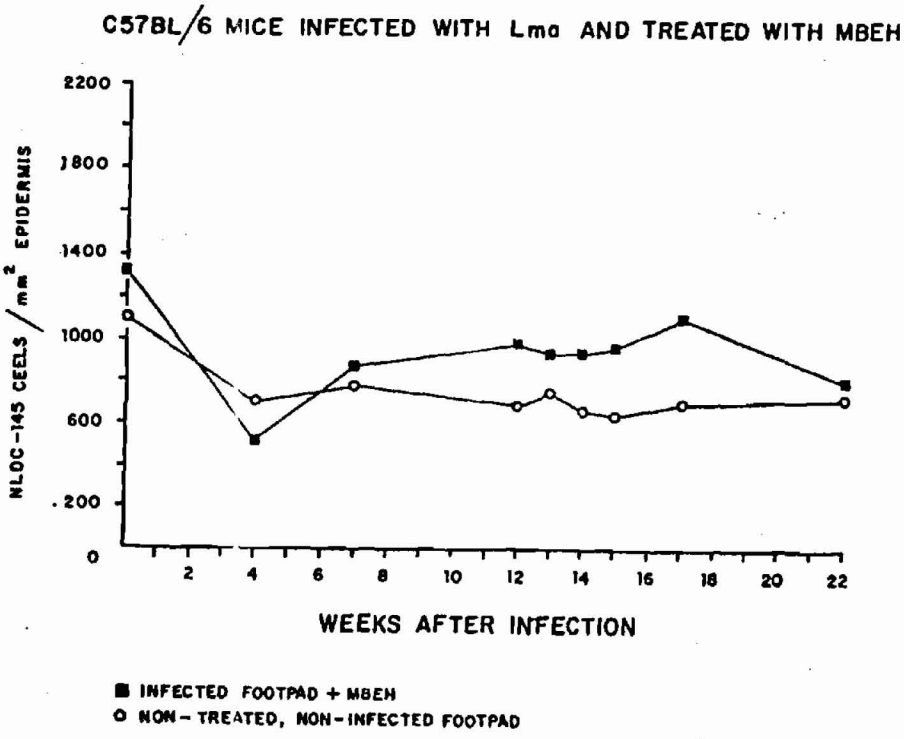

Fig. 5
Changes in Langerhans cells density in Lma-infected C57BL/6 mice treated with MBEH. Note the decrease in LC numbers during the fourth week.

TABLE 1

EPIDERMAL LANGERHANS CELLS DENSITY IN C57BL/6 MICE INFECTED WITH LEISHMANIA MEXICANA AMAZONENSIS.
(NLDC-145+ CELLS/mm² EPIDERMIS)

WEEK	INFECTED FOOTPAD (A)	NON-INFECTED FOOTPAD (B)	STATISTICAL SIGNIFICANCE*	DIFFERENCES AMONG GROUPS**
0	472 ± 38	486 ± 55	NS	
4	ND	ND	ND	
5	567 ± 66	520 ± 41	NS	A-X: P<0,05
7	881 ± 77	547 ± 64	P<0,05	B-X --> NS
12	1447 ± 194	693 ± 67	P<0,01	
13	1305 ± 74	ND	ND	
14	1580 ± 136	ND	ND	
15	1065 ± 141	809 ± 58	P<0,01	
17	1163 ± 124	738 ± 74	P<0,05	
22	ND	ND	ND	

* T-STUDENT AND WILCOXON FOR PAIRED SAMPLES
** T-STUDENT AND MANN-WHITNEY FOR INDEPENDENT SAMPLES
VALUES EXPRESSED IN MEAN ± STANDARD DEVIATION
NS: NOT STATISTICALLY SIGNIFICANT
ND: NOT DETERMINED
X: NORMAL C57BL/6 MICE

TABLE 2

EPIDERMAL LANGERHANS CELL DENSITY IN BALB/c MICE.
(MLDC-145+ CELLS/mm^2).

Days	(A) Healthy Mice	(B) Infected Mice Infected Footpad	(C) Infected Mice Non-infected Footpad	Statistical Significance*	Differences Among Groups**
0	1417 ± 124	1315 ± 84	1390 ± 67	NS	
7	ND	903 ± 53	1745 ± 115	p<0.01	A-B:p<0.05
9	1266 ± 93	1429 ± 165	2112 ± 152	p<0.01	A-C:p<0.01
14	ND	1670 ± 195	1976 ± 171	p<0.05	
24	1385 ± 135	2054 ± 151	2148 ± 120	NS	
45	1310 ± 40	1726 ± 202	1873 ± 123	NS	corr$^{\theta}$ B-C:
75	1484 ± 82	1537 ± 192	1984 ± 240	p<0,05	V:0.57
100	1459 ± 74	2078 ± 136	2027 ± 52	NS	p<0.05

* T-STUDENT FOR PAIRED SAMPLES
** T-STUDENT FOR INDEPENDENT SAMPLES
VALUES EXPRESSED IN MEAN ± STANDARD DEVIATION
NS : NOT STATISTICALLY SIGNIFICANT
ND : NOT DETERMINED
θ KENDALL CONCORDANCE COEFFICIENT

TABLE 3

DENDRITIC CELLS MLDC-145+ IN GRANULOMAS OF LBA-INFECTED C57BL/6 MICE (POSITIVE CELLS/mm^2)

Week	NBEN Treated	Infected	Statistical* Significance
4	19 ± 2	17 ± 3	NS
12	18 ± 2	25 ± 3	NS
17	17 ± 1	24 ± 5	NS

* T-STUDENT AND MANN-WHITNEY FOR INDEPENDENT SAMPLES
VALUES EXPRESSED IN MEAN ± STANDARD DEVIATION
NS : NOT STATISTICALLY SIGNIFICANT

TABLE 4

EPIDERMAL LANGERHANS CELLS DENSITY IN NORMAL C57BL/6 MICE TREATED WITH NBEN (MLDC-145+ CELLS/mm^2).

Days	Treated Footpad (A)	Non-treated Footpad (B)	Statistical Significance*	Different Among Groups**
0	598 ± 74	552 ± 63	NS	
1	660 ± 61	ND	ND	A-X:p<0.05
3	1100 ± 162	671 ± 55	p<0,01	B-X:p<0.05
5	1194 ± 53	917 ± 51	p<0.01	
8	1378 ± 61	1204 ± 90	p<0.01	
15	1563 ± 155	851 ± 77	p<0.001	corr$^{\theta}$. A-B
22	935 ± 52	1024 ± 38	NS	V:1;p<0.05
32	978 ± 79	1019 ± 41	NS	

* T-STUDENT FOR PAIRED SAMPLES
** T-STUDENT AND MANN-WHITNEY FOR INDEPENDENT SAMPLES
VALUES EXPRESSED IN MEAN ± STANDARD DEVIATION
NS : NOT STATISTICALLY SIGNIFICANT
ND : NOT DETERMINED
θ : KENDALL CONCORDANCE COEFFICIENT DURING TREATMENT
X : NORMAL C57BL/6 MICE

TABLE 5

EPIDERMAL LANGERHANS CELLS DENSITY IN LBA-INFECTED C57BL/6 MICE TREATED WITH NBEN (MLDC-145+ CELLS/mm^2).

Week	Infected Footpad (A)	Non-infected Footpad (B)	Statistical Significance*	Difference Among Groups**
0	1318 ± 70	1095 ± 90	p<0,05	
4	518 ± 44	701 ± 85	NS	A-X: p<0.01
5	ND	ND	ND	B-X: p<0.01
7	868 ± 62	767 ± 69	NS	
12	976 ± 33	685 ± 51	p<0,05	
13	926 ± 79	743 ± 64	NS	
14	935 ± 82	658 ± 39	p<0.05	Correl. A-B
15	954 ± 58	632 ± 78	p<0.05	V:0.6
17	1091 ± 62	694 ± 35	p<0.05	p<0.01
22	798 ± 104	710 ± 28	NS	

* T-STUDENT FOR PAIRED SAMPLES
** T-STUDENT AND MANN-WHITNEY FOR INDEPENDENT SAMPLES
VALUES EXPRESSED IN MEAN ± STANDARD DEVIATION
NS : NOT STATISTICALLY SIGNIFICANT
ND : NOT DETERMINED
X : NORMAL C57BL/6 MICE

the non-inoculated footpad had an order of magnitude higher than the infected footpad. These differences were statistically significant ($p < 0.05$) with a positive correlation ($P<0.05$, $W=0.59$) (Fig. 4).

In all infected animals LC were also found in the dermis as single, scattered cells or forming part of the leishmanial granuloma (Table 3). A particular topographical distribution of these cells was not observed in either human or murine lesions.

The use of MBEH induced an increment in the numbers of epidermal LC in C57BL/6 mice with a peak of 1378 LC/mm². This increment persisted one week after the topical application of MBEH with values reaching 1563 LC/mm². From that moment, LC numbers started to decrease until days 20-30, when they reached constant values. The LC kinetics of the non-MBEH treated footpad were very similar to the treated footpad but with much lower numbers (Table 4).

The Lma-infected C57BL/6 mice when treated with MBEH showed a marked decrease of epidermal LC during the fourth week (518 LC/mm²) and a smooth increase with a peak of 1091 LC/mm². However, these values were lower than those observed at the beginning of the inoculation (1318 LC/mm²) (Fig. 5, Table 5). The non-infected footpad showed a small and progresive decrease of LC from the moment of infection. The LC kinetics for the infected and non-infected footpads were similar, and statistically different from those observed in normal mice footpads. Morphologically, the LC in the MBEH-treated footpads showed an apparent loss of the dendritic forms in close association with an increase in cellular density. the Kendall coefficient revealed a positive correlation ($W=0.605$; $P<0.05$) between the values observed in the infected MBEH-treated footpad and the non-treated footpad.

DISCUSSION

The present study shows an association between epidermal LC and the leishmanial infection in murine cutaneous leishmaniasis. This association was made evident through changes in LC density observed during the course of infection.

The LC characterization in normal C57BL/6 mice using cytochemical ATPase staining and immunostaining with NLDC-145 showed the same degree of sensitivity. However, when these techniques were applied during the course of the leishmanial infection, the cytochemical procedure was very limited in detecting LC beyond the fourth week. This limitation may be the consequence of LC hyperplasia and increase in background staining. Other investigators have found difficulties in detecting LC by ATPase staining in contact dermatitis (Juhlin & Shelley, 1977). Similarly, it has been demonstrated that the known effect of UV irradiation on LC may be affecting the ATPase production (Aberer et al., 1981; Iacobelli et al., 1983).

The use of Leishmania-resistant C57BL/6 and susceptible BALB/c mice allowed the study of LC kinetics in the epidermis and dermal granulomas of two distinct clinical forms. The hyperplasia of LC occurs during the fourth week in C57BL/c mice. This difference may be associated with the type of immune response mounted. C57BL/6

mice develop localized-type lesions with 3.6 increment in LC density as compared to normal epidermal values. In contrast, in BALB/c mice the increament is only 1.5 times higher than normal values. A similar situation has been observed in tuberculoid leprosy (Modlin et al., 1984) and in localized cutaneous leishmaniasis (Modlin et al., 1985) when compared with the most severe forms of both diseases.

It was interesting to note that BALB/c mice showed higher numbers of LC in the non-inoculated footpad than in the Leishmania-inoculated footpads, whereas in resistant C57BL/c animal the results were similar between the non-inoculated footpads and normal footpads. These results may reflect the differences in the effectivity of both mice strains in eliminating the Leishmania parasite. Furthermore, the apparent excessive number of LC in BALB/c mice may contribute to an exacerbated function of these cells which affects the effectivity to eliminate the parasite.

The findings using MBEH demonstrated that this agent is an effective modifier of LC density, that could be very useful in understanding the involvement of these cells in the pathogenesis of leishmaniasis. The present study did not reveal any histological or immunocytochemical differences between MBEH-treated and non-treated Lma-infected animals. However, we have some evidence (data not shown) that demonstrates an increase in T helper-inducer L3T4+ lymphocytes in the granulomas of Lma-infected mice treated with MBEH. This result seems to indicate that a modification in LC density may alter the granuloma and may promote eventually a change in the immune response. This observation coincides with that of Giannini (1986) which demonstrates that after UV-irradiation of the epidermal inoculated site the granuloma cell component is modified.

Further studis, now in progress, will try to correlate LC density with the parasites load, delayed-type hypersensitivity response and the development of clinical disease in cutaneous leishmaniasis.

ACKNOWLEDGEMENTS

We are grateful to Dr Marian Ulrich for reading and commenting on this manuscript, Miss Sara Egui and Mr Luis Alexander Silva for technical assitance, and Miss Dilia Hernández for her secretarial support.
Part of this work has been financially supported by grant M-10.16.-87 from CDCH-UCV.

REFERENCES

Aberer, W., Schuler, G., Honigsmann, H. & Wolff, K. (1981) Ultraviolet light depletes surface markers of Langerhans cells. J. Invest. Dermatol. 76, 202.

Baker, K.W. & Habowsky, J.E.J. (1983) EDTA Separation and ATPase Langerhans cell staining in the mouse epidermis. J. Invest. Dermatol. 80, 104.

Castés, M., Agnelli, A., Verde, O. & Rondon, A.J. (1983) Characterization of the cellular immune response in American cutaneous leishmaniasis. Clin. Immunol. Immunopathol. 27, 176.

Pérez, H. (1982) Inmunología de la leishmaniasis cutánea experimental. Adel. Microbiol. Enf. Infecc. 1, 103.

Preston, P.M., Carter, R.L., Leuchars, E., Davies, A.J.S. & Dumonde, D.C. (1972) Experimental cutaneous leishmaniasis. III. Effects of thymectomy on the course of infection of CBA mice with Leishmania tropica. Clin. Exp. Immunol. 10, 337.

Rheins, L.A. & Nordlund, J.J. (1986) Modulation of the population density of identifiable epidermal Langerhans cells associated with enhancement or suppression of cutaneous immune reactivity. J. Immunol. 136, 867.

Tapia, F.J., Gross, A., Castés, M., Mosca, W., Modlin, R.L. & Convit, J. (1986) Cellular immune response in the skin lesions of patients with american cutaneous leishmaniasis. VI International Congress for Parasitology, Brisbane, Australia.

Résumé

Les cellules de Langerhans (CL) sont les cellules immunocompétantes de la peau qui agissent comme cellules accessoires présentant les antigènes. L'importance de ces cellules dans la leishmaniose cutanée n'est pas encore parfaitement connue. Nous présentons ici une étude portant sur la caractérisation et la quantification des CL à différents stades de la leishmaniose murine experimentale et humaine.

Des souris consanguines C57BL/6 ont été infectées par Leishmania mexicana amazonensis (Lma) souche M 1132. De plus des animaux ont été traités par le monobenzyl ether d'hydroquinone (MBEH) qui est connu pour agir sur la densité des CL.

Chez les patients présentant une leshmaniose cutanée américaine nous avons observé une agumentation importante des CL dans toutes les formes cliniques de la maladie.

Les souris C57BL/6 (n = 60) furent inoculées en sous-cutané au niveau des coussinets plantaires à raison de 10^3 amastigotes de Lma Un groupe de 30 souris BalbB/C femelles agées de 8 à 10 semaines ont également été inoculées avec Lma. Un autre groupe de souris C57BL/6 a été traité avec une solution alcoolique de MBEH. La moitié de ces animaux a été infectée par Lma. Le reste a constitué le controle pour evaluer f'effet du MBEH.

La recherche des CL a été effectuée sur biopsies congelées de peau par immunoperoxydase en biotine avidine et des anticorps monoclonaux (Leu6 et NLDC-145). La meme recherche et la coloraiton par l'ATPase ont également été effectuées sur épiderme entier séparé du derme par l'EDTA.

Les animaux infectés par Lma montrent une augmentation significative des CL/mm^2 d'épiderme. Les valeurs mesurées atteignent le double de celles observées chez les animaux traités par MBEH et/ou les animaux sains. Une analyse similaire est réalisée chez les souris BalB/C infectées par Lma où là encore une augmentation du nombre des CL est observée.
Chez tous les animaux infectés, des CL ont été observées dans le derme, dans le granulome sans distribution topographique particulière.

Les résultats obtenus montrent une association entre la densité des CL et le développement de l'infection à Leishmania.