Available online at www.sciencedirect.com
SCIENCE DIRECT®
Acta Tropica 87 (2003) 377–384
ACTA TROPICA
www.elsevier.com/locate/actatropica

Lymphocyte subsets, macrophages and Langerhans cells in actinomycetoma and eumycetoma tissue reaction

Claudia Cenci Guimarães [a], Luiz Guilherme M. Castro [b], Mírian N. Sotto [a,b,*]

[a] *Department of Pathology, University of São Paulo Medical School, Av. Dr. Arnaldo, 455 sala 1118, 01246-903 São Paulo, SP, Brazil*
[b] *Department of Dermatology, University of São Paulo Medical School, Av. Dr. Arnaldo, 455 sala 1118, 01246-903 São Paulo, SP, Brazil*

Received 19 August 2002; received in revised form 14 April 2003; accepted 28 April 2003

Abstract

The aim of this work was to demonstrate, quantify and compare cell elements in the inflammatory infiltrate of 23 skin lesions of actinomycetoma (ACM) and 17 of eumycetoma (EUM). Epidermal Langerhans cells (LC) population was also analyzed in 18 ACM, 13 EUM and ten normal skin samples as control group. Tissue response in both groups of mycetoma showed CD4 and CD8 T lymphocytes surrounding the neutrophils aggregates with macrophages, revealed by CD68 antibody, among them. B lymphocytes were not identified. ACM lesions showed a higher number of CD8+ lymphocytes ($P = 0.02$) and macrophages ($P = 0.01$) when compared with EUM lesions. As well as morphologically altered, displaying irregular and short dendritic processes, LC were depleted both in ACM and EUM lesions ($P = 0.0004$) when compared with normal skin but no difference between both types of mycetoma ($P > 0.05$) was found. Results suggest that cellular mediated immunity may play a role in mycetoma pathogenesis. The morphological alterations and marked reduction of LC in mycetoma lesions might reflect a depressed cellular immune response, partially explaining the chronic course and unresponsiveness to treatment of this group of diseases.

Keywords: Mycetoma; Immunopathology; Immunohistochemistry; Lymphocyte subsets; Macrophages; Langerhans cells

1. Introduction

Immunological studies of mycetoma have addressed more frequently humoral aspects. Few reports have addressed the role of cellular immunity in humans (Mahgoub et al., 1977) and experimental mycetoma pathogenesis (Mahgoub, 1978; Salinas-Carmona et al., 1999; Salinas-Carmona, 2000). Studies on the immunopathologic aspects of tissue reaction are much less frequent. Immunecomplex deposits in actinomycotic grains as well as in surrounding inflammatory zones were demonstrated in experimentally induced *Nocardia brasiliensis* actinomycetoma (ACM) (Conde et al., 1983).

Polymorphonuclear leucocytes are the predominant cell type in the inflammatory infiltrate in the

* Corresponding author. Tel./fax: +55-11-3066-7238.
E-mail address: mnsotto@ajato.com.br (M.N. Sotto).

0001-706X/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0001-706X(03)00139-6

skin lesions. In vitro experiments demonstrated that these cells migrate when exposed to ACM and eumycetoma (EUM) antigens (Yousif and Hay, 1987).

Cellular immunity in the skin is related to a specialized lymphoid system described by Streilein (1983), who demonstrated an integrated system of immune surveillance designed uniquely for the skin and named it skin-associated lymphoid tissues (SALT). It provides the skin with an unusual set of immunologic abilities and is comprised by a distinctive population of recirculating T lymphocytes, LC and keratinocytes.

Recently El Hassan et al. (2001) demonstrated T lymphocytes, CD68+ macrophages as well as immunoglobulins G, M and complement in *S. somaliensis* ACM lesions from three patients. They also showed that cytokine profile in the lesions and lymph nodes was of a dominant Th_2 pattern.

In order to verify the cellular elements related to cell mediated immunity in tissue response in mycetoma we demonstrated, quantified and compared populations of macrophages, B cells, epidermal Langerhans cells (LC) and T lymphocytes (and their subsets) in ACM and EUM skin lesions. The epidermal LC population in ACM and EUM skin lesions was compared with normal skin control group.

2. Material and methods

Twenty-three biopsies of ACM and 17 of EUM were retrieved from the files of the Dermatopathology Laboratory of the Dermatology Clinic of the University of São Paulo School of Medicine among the biopsies examined in the period of 1948–2000. Selection criteria included: diagnosis confirmed by culture and/or by presence of well preserved grains in tissue sections and availability of tissue for the technical procedures. Among the ACM group *N. brasiliensis* was isolated in five cases, *Nocardia asteroides* in one, *Spreptomyces* sp. in one case and in the remaining cases the agent was characterized in tissue specimen. *Madurella grisea* was the agent of the infection in four cases of EUM, *Madurella mycetomatis* in one and *Acremonium kiliense* in two. In the remaining EUM cases the grains were composed of hyaline hyphae. Ten normal skin biopsies taken during orthopedic knee joint trauma surgery were used as control group for epidermal LC population.

Age of ACM patients ranged from 21 to 63, while for EUM patients it ranged from 20 to 62. ACM group consisted of 18 males and five females and EUM group of 13 males and four females. 12 biopsies of ACM were taken from foot lesions, six from the lower limb, four from the upper limb and one from the chest. 13 biopsies of the EUM group were from the foot, three from the lower limb and one from the hand.

In order to classify each case according to the morphology and the characteristics of the grains in the tissue sections, hematoxilyn-eosin, Fite-Faraco's, Grocott's and Brown and Brenn's modification of Gram stain were used to stain all mycetoma biopsies.

Five μm histological sections were obtained from each paraffin-embedded specimen, placed on glass slides coated with 3-aminopropyltrietoxy-silane (Sigma Chemical Co, St. Louis, MO, USA).

Immunohistochemistry technique with streptABC complex/HRP (Dako Corporation, Carpinteria, CA, USA) according to Hsu et al. (1981) was used to demonstrate the following antigens (antibody dilutions and sources in parentheses): CD20 (monoclonal 1:200), CD3 (policlonal 1:200), CD4 (monoclonal OPD4 1:200), CD8 (monoclonal 1:100) CD68 (monoclonal 1:200); all these antibodies were purchased from Dako Corporation; CD1a (monoclonal 1:20) was obtained from Immunotech, Marseille, France. The reaction to demonstrate CD1a cells was performed with the catalyzed signal amplification (CSA) method (Dako Corporation; Key and Phillips, 1996). The reactions were revealed with 3,3 Diamino-benzidine tetrahydroxychloride (Sigma Chemical Co) chromogen and counterstained with haematoxylin. Lymph node and Langerhans histiocytosis skin sections represented positive controls. Negative controls were obtained by omitting each primary antibody that was replaced by phosphate buffered saline (pH 7.4, 0.01 M).

Since the number of epidermal LC of soles and palms is smaller than in other skin sites (Ashworth

et al., 1986) plantar skin and biopsies without epidermal representation were excluded, and only 18 biopsies of ACM and 13 of EUM group were submitted for CD1a demonstration.

The quantification of each immune-labeled cell population was obtained using a ×10 eyepiece with a square grid and a ×40 objective. The area of each microscopic field was 0.0625 mm^2. The grid was placed on four orthogonal imaginary lines that cross the grain and/or the center of the inflammatory focus. All labeled cells that were confined to each grid area were counted. At least seven (minimum) and 16 (maximum) grid area/biopsy were counted.

CD1a+ epidermal expression was evaluated using an eyepiece with a grid with 100 hits (points of crosses of two lines) and a ×40 objective. The fraction of area of epidermis positive for CD1a antigen was obtained by counting the number of hits over CD1a+ reaction and the total number of hits over the entire epidermis (excluding the cornified epidermal layer) of each specimen. The ratio of the number of hits over CD1a+ reaction: total number of hits over the epidermis corresponded to the epidermal fraction area occupied by the LC (Bieber et al., 1988).

Statistical analysis of the results was performed using the non-parametric Mann–Whitney and Kruskal–Wallis tests at the 95% level of significance.

3. Results

Tissue reaction pattern was similar in both groups of mycetoma. Inflammatory foci were composed mainly of neutrophils. The innermost neutrophils were closely attached to the surface of the grains. Outside this zone there was granulation tissue containing macrophages, lymphocytes, plasma cells and few neutrophils. Several macrophages had large vacuolated cytoplasm. Concentrically arranged fibrin layers, giving an onionskin appearance, surrounded capillaries and veins. Arterioles showed hypertrophic muscular layer, thickened and edematous intima and narrowed lumen. The dermis displayed variable degree of fibrosis.

Tissue reaction classification according to Fahal et al. (1995) revealed 22 ACM biopsies with type I reaction (i.e. inflammatory foci with predominance of neutrophils) and only one with type II (i.e. macrophages and multinuclear giant cells besides the neutrophils in inflammatory foci). 16 biopsies of EUM showed type I reaction and one biopsy an intermediate reaction between type I and II.

ACM exhibited small grains with a network of thin filaments embedded in hard cement with filamentous border (Fig. 1a). Filaments were Gram+ and in six cases were partially acid fast resistant. EUM showed grains composed by a network of branched and septated hyphae and

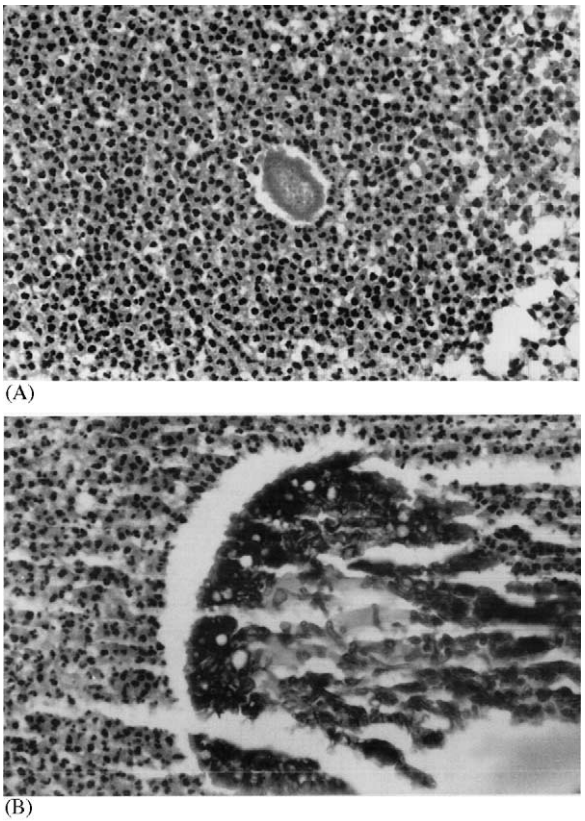


Fig. 1. (a) Actinomycetoma lesion. Neutrophils exsudate around a small irregular grain bordered by short irregular spicules of eosinophilic material; (b) eumycetoma lesion. Grain composed of interwoven hiphae surrounded by neutrophils exsudate. Haematoxilyn and eosin (×400 original magnification).

vesicles, sometimes embedded in a cement-like matrix (Fig. 1b). Five specimens exhibited dematiaceous hyphae.

B lymphocytes were not seen among the inflammatory cells in tissue reaction of either group. T lymphocytes (CD3+) were observed around the neutrophils aggregates as small clusters and also interspersed among other mononuclear cells. CD4+ and CD8+ lymphocytes exhibited the same disposition in the inflammatory process (Fig. 2a, b). Macrophages, with cytoplasm labeled by CD68 antibody, were observed within the inflammatory foci. Giant multinucleated cells seen in type II reaction were strongly labeled by CD68 antibody. The same type of tissue reaction was found in both groups of mycetoma (Fig. 2c, d).

The number of CD3+ and CD4+ cells did not differ between both groups. On the other hand, the number of CD8+ lymphocytes and macrophages was higher in ACM lesions ($P = 0.02$ and 0.01, respectively). Fig. 3 shows the distribution of the immune labeled cells/mm^2 of both mycetoma groups.

Epidermal LC were morphologically altered, displaying irregular and short dendritic processes. When compared with the control group, both ACM and EUM specimens showed a statistically significant ($P = 0.0004$) decrease in number of LC (Fig. 4). Interestingly no difference was found

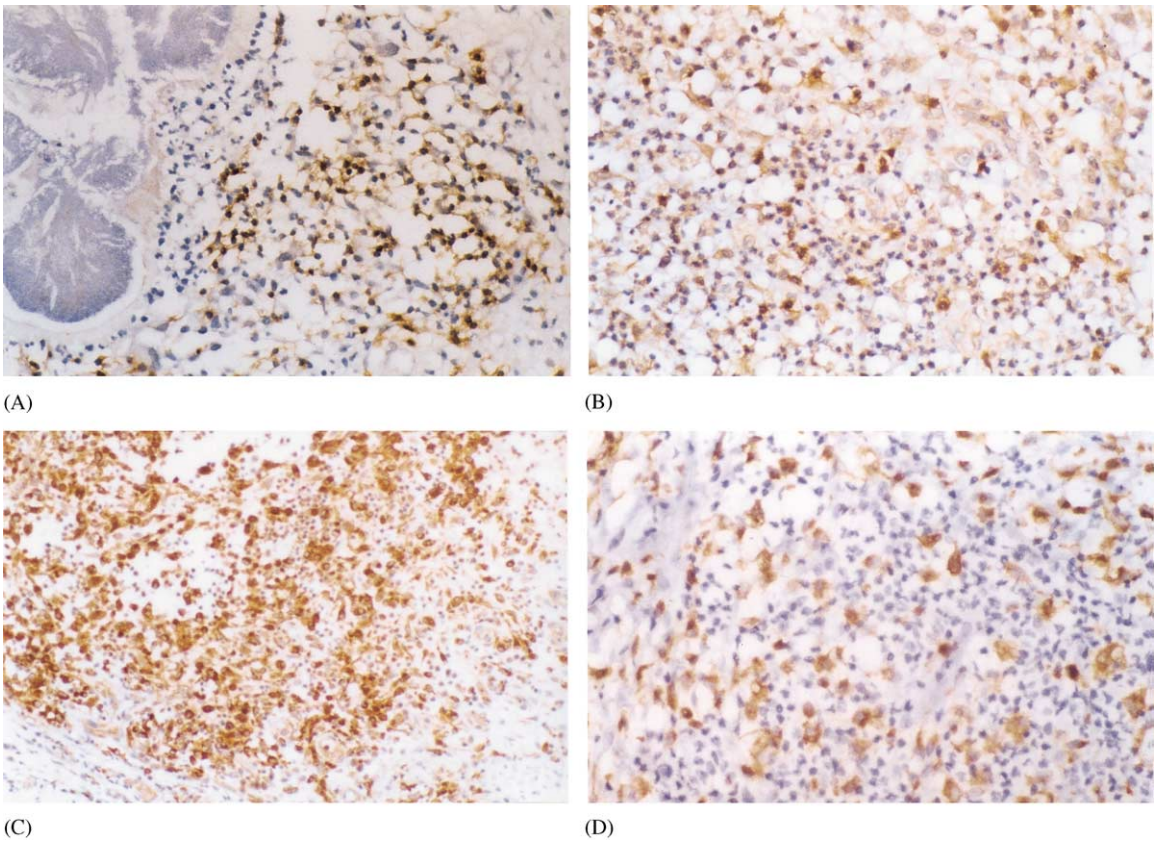


Fig. 2. (a) Actinomycetoma lesion. CD8+ lymphocytes around a grain (× 200 original magnification). (b) Eumycetoma lesion. CD8+ lymphocytes intermingled with neutrophils in an eumycotic inflammatory focus (× 200 original magnification). (c) Actinomycetoma lesion. Numerous CD68+ macrophages in inflammatory focus (× 200 original magnification). (d) Eumycetoma lesion. CD68+ macrophages among neutrophils in the inflammatory focus (× 400 original magnification). Immunohistochemistry technique SABC complex.

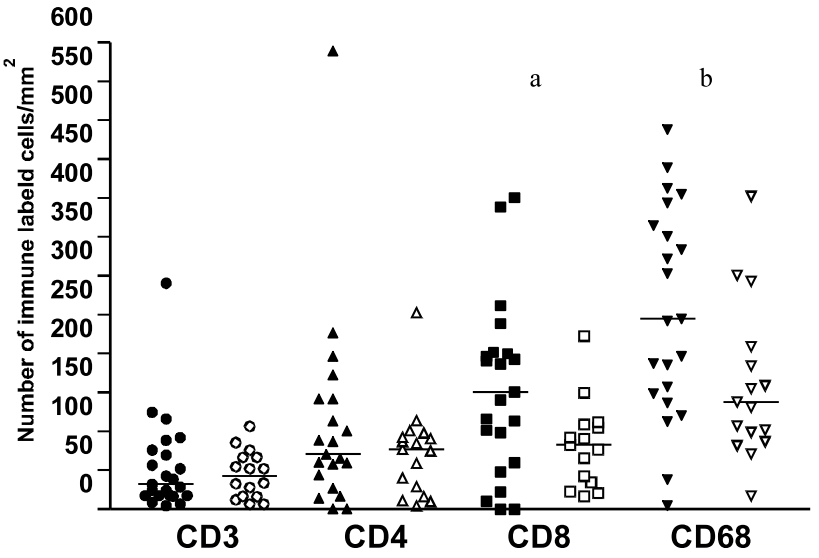


Fig. 3. Population of CD3+ lymphocytes; CD4+ and CD8+ lymphocytes and macrophages (CD68+) in the tissue reaction of actinomycetoma (solid symbols) and eumycetoma (empty symbols). Bar-median; (a) $P = 0.02$; (b) $P = 0.01$ (Mann–Whitney test).

when both mycetoma groups were compared with each other ($P > 0.05$) (Fig. 5).

4. Discussion

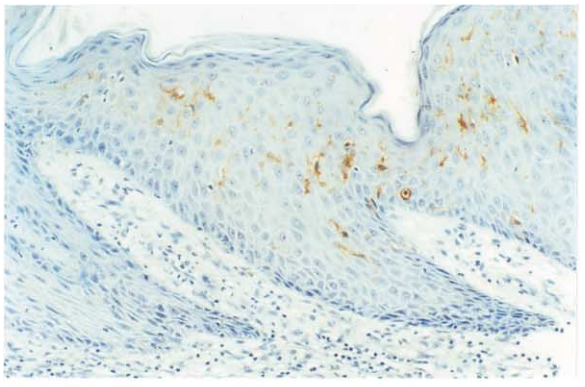
Epidemiology, clinical aspects, pathology and treatment of mycetoma have been well addressed in medical literature (Lacaz, 1981; Castro et al., 1993; McGinnis, 1996; Salinas-Carmona, 2000). Immunopathological aspects of tissue reaction have not been well characterized. Little is known about the immuno-phenotype of cell elements of mycetoma tissue reaction. There is only one report addressing the phenotype of the cellular elements in tissue reaction of ACM (El Hassan et al., 2001).

The present study demonstrates that lymphocyte population in mycetoma tissue reaction is composed of both CD4 and CD8 phenotype T lymphocytes. Macrophages also participate in the inflammatory reaction. We did not observe a well organized stratification of the different immunolabeled cells in mycetoma inflammatory foci as described by El Hassan et al. (2001). All but one tissue specimen of each group exhibited type I reaction as proposed by Fahal et al. (1995). T lymphocytes, CD4+ and CD8+ T lymphocytes, as well as macrophages were observed around the neutrophil zone and also intermingled with these cells. ACM lesions exhibited a higher number of CD8+ T lymphocytes and macrophages than EUM. B lymphocytes were not seen in tissue reaction of either mycetoma group. B lymphocytes do not participate in the so-called SALT of normal skin (Streilein, 1983; Bos et al., 1987).

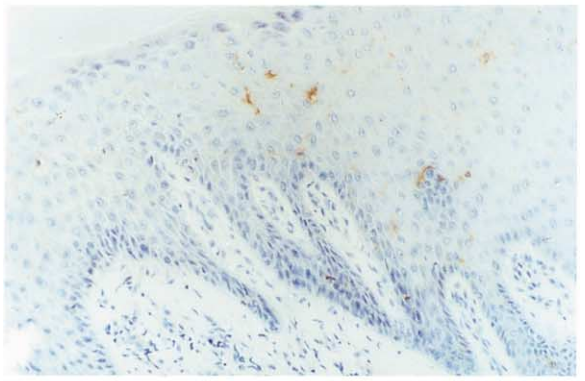
A T lymphocyte defect has been proposed in the pathogenesis of experimentally induced mycetoma (Mahgoub, 1978). Disseminated infection was described following *Nocardia* inoculation in T cell deficient mice. On the other hand, mycetoma has been obtained by inoculation of animals without lymphocyte defects (Beaman and Scates, 1981; Salinas-Carmona et al., 1999).

In chromoblastomycosis, considered by Zaias (1978) as a minimycetoma, the lesion is considered to be controlled by phagocytes (neutrophils and macrophages) although this cellular defense mechanism is inefficient in eliminating the fungi; the disease chronically evolves and is difficult to treat (Esterre et al., 1993).

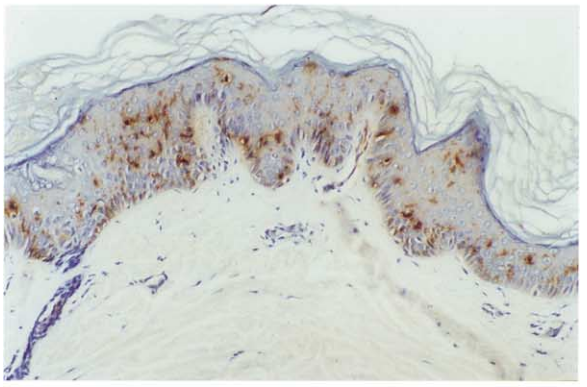
T lymphocytes with a predominance of helper phenotype were demonstrated in skin and lymph node in paracoccidioidomycosis and these cells are considered to be actively involved in the disease



(A)



(B)



(C)

Fig. 4. Epidermal LC demonstrated by CD1a antibody. (a) Actinomycetoma lesion. Note few LC with short dendrites between keratinocytes in the spinous layer of epidermis (× 630 original magnification). (b) Eumycetoma lesion. Very few LC in the spinous layer of epidermis (× 630 original magnification). (c) Control skin. Numerous LC with anastomozing dendrites between spinous layer keratinocytes (× 400 original magnification). Immunohistochemistry technique SABC complex.

process (Moscardi-Bacchi et al., 1989). T lymphocytes with helper/inducer phenotype were also the predominant cells observed in the site of cytoplasmic *Paracoccidioides brasiliensis* antigen intradermal inoculation (Marques et al., 1993).

Actinomycotic mycetomas respond better to chemotherapy than eumycotic mycetomas (McElroy et al., 1992). We observed that ACM lesions exhibit a higher number of both CD8+ lymphocytes and macrophages than EUM lesions. CD8+ T lymphocytes are related to cytotoxic mechanisms against intracellular pathogens. On the other hand, they have been demonstrated as cytotoxic T lymphocytes against schistosomal egg antigen and able to produce interferon gamma in a murine experimental model (Pancrè et al., 1999). Macrophages secrete many cytotoxic factors which enable them to kill parasites without ingesting them and also to act as killer cells through antibody dependent cell-mediated cytotoxicity (Roitt et al., 1998). The data obtained in the present study might explain the better response to therapy ACM presents, i.e. a more efficacious immune response is present in ACM than in EUM.

The role of LC in cutaneous infectious diseases has been well studied in leishmaniasis. These cells can be both target and nest of parasites in the murine experimental model (Dompmartin et al., 1988; Tapia et al., 1989). Moll (1993) considered LC as part of the protective mechanisms towards *Leishmania*.

There are few papers that discuss the role of LC population in cutaneous deep mycosis. Morphologically altered LC, presenting short dendritic processes were described in paracoccidioidomycosis. A reduction in number of LC was also noted (Gimenez et al., 1987). S100 protein positive cells were demonstrated next to the granuloma in experimental paracoccidioidomycosis and were interpreted as participating in the process of T lymphocytes activation with a possible role in the formation of granuloma (Moscardi-Bacchi et al., 1989). Sandoval et al. (1996) demonstrated an even distribution of S100 protein positive dendritic cells in epidermis and dermis of paracoccidioidomycosis skin lesions. These authors were not able to demonstrate *P. brasiliensis* antigen in S100+ cells

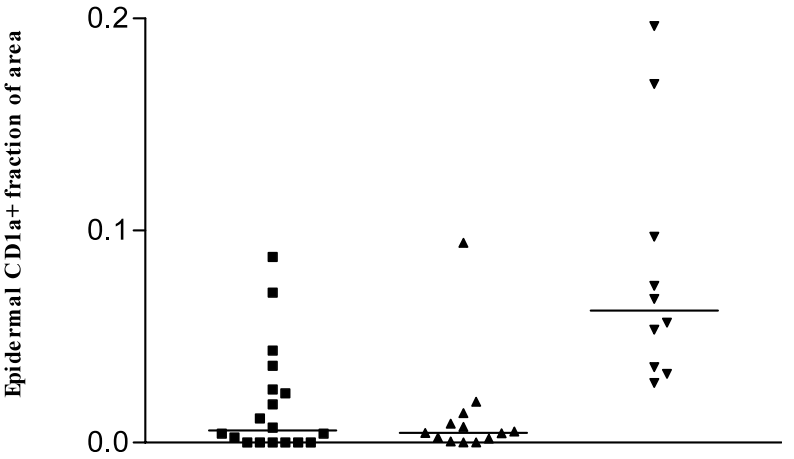


Fig. 5. Epidermal CD1a+ fraction of area in actinomycetoma (■), eumycetoma (▲) and control group (▼). Bar-median; control group > actinomycetoma and eumycetoma groups ($P = 0.004$; Kruskal–Wallis test).

using the double labeling immunohistochemical technique. On the other hand, Esterre et al. (1993) observed the same number of CD1a+ cells in cutaneous lesions of chromomycosis and in normal skin control group.

LCs play a pivotal role in the cellular immunity mechanisms. Their function includes antigen internalization, processing and presentation to T lymphocytes. In the skin they are involved in the mechanisms of immune surveillance against infectious agents.

We have been able to demonstrate that epidermal LC were morphologically altered and decreased in number in mycetoma when compared with normal skin, but El Hassan et al. (2001) observed normal number and morphology of epidermal LC in overlying ACM epidermis when compared with normal skin. The cutaneous surface depleted of LC may allow unprocessed parasite antigens to gain access to the circulation, playing a role in the development of immune tolerance. The markedly reduced number of LC found in both ACM and EUM lesions may reflect immune tolerance and/or a depressed cellular immune response secondary to the infection as proposed for paracoccidioidomycosis (Gimenez et al., 1987). The degenerative aspects and LC numerical reduction in mycetoma epidermis could also be related to chronic exposition to ultraviolet B radiation, as described by Bacci et al. (1998). Mycetoma patients are chronically exposed to sunlight because of their job activities in rural areas (Fahal and Hassan, 1992). Another explanation could be LC migration from skin to draining lymph nodes in order to promote T lymphocytes activation (Holíková et al., 2001).

We demonstrated that T lymphocytes, either with CD4+ or CD8+ phenotypes, participate in tissue reaction in mycetoma lesions. Although mycetoma occurs as a consequence of inoculation of the agent into the skin, it is not generally followed by systemic dissemination. T lymphocytes observed in skin mycetoma foci may be involved in the immunopathological mechanisms preventing disease dissemination, although it is not sufficient to resolve the local infection.

Acknowledgements

This work was supported by Conselho Nacional de Pesquisas-CNPq grant (141375/98-1). We thank Carla Pagliari and Elaine Raniero Fernandes for immunohistochemical procedures.

References

Ashworth, J., Turbitt, M.L., Mackie, R., 1986. The distribution and quantification of Langerhans cell in normal human epidermis. Clin. Exp. Dermatol. 11, 153–158.

Bacci, S., Romagnoli, P., Streilein, J.W., 1998. Reduction in number and morphologic alterations of Langerhans cells after UVB radiation in vivo are accompanied by influx of monocytoid cells into the epidermis. J. Invest. Dermatol. 111, 1134–1139.

Beaman, B.L., Scates, S.M., 1981. The role of L-forms of *Nocardia caviae* in the development of chronic mycetomas in normal and immunodeficient murine models. Infect. Immun. 33, 893–907.

Bieber, T., Ruig, J., Braun-Falco, O., 1988. Comparison of different methods for enumeration of LC in vertical cryosections of human skin. Br. J. Dermatol. 118, 385–392.

Bos, J.D., Zonneveld, I., Das, P.K., Krieg, S.R., Van Der Loos, C.M., Kapsenberg, M.L., 1987. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in the normal human skin. J. Invest. Dermatol. 88, 569–573.

Castro, L.G.M., Belda, W., Jr, Salebian, A., Cucé, L.C., 1993. Mycetoma: a retrospective study of 41 cases seen in São Paulo, Brazil, from 1978 to 1989. Mycoses 36, 89–95.

Conde, C., Mancilla, R., Fresan, M., Ortiz-Ortiz, L., 1983. Immunoglobulin and complement in tissues of mice infected with *Nocardia brasiliensis*. Infect. Immun. 40, 1218–1222.

Dompmartin, A., Healy, A.T., Nacy, C.A., Hauser, C., Meltzer, M.S., et al., 1988. *Leishmania major* infects and replicates within murine epidermal LC. J. Invest. Dermatol. 91, 404.

El Hassan, A.M., Fahal, A.H., Ahmed, A.O., Ismail, A., Veress, B., 2001. The immunopathology of actinomycetoma lesions caused by *Streptomyces somaliensis*. Trans. R. Soc. Trop. Med. Hyg. 95, 89–92.

Esterre, P., Peyrol, S., Sainte-Marie, D., Pradinaud, R., Grimaud, J.A., 1993. Granulomatous reaction and tissue remodelling in the cutaneous lesion of chromomycosis. Virchows Arch. A. Pathol. Anat. Histopathol. 422, 285–291.

Fahal, A.H., Hassan, A.M., 1992. Mycetoma. Br. J. Surg. 79, 1138–1141.

Fahal, A.H., El Toum, E.A., El Hassan, A.M., Mahgoub, E.S., Gumaa, S.A., 1995. The host tissue reaction to *Madurella mycetomatis*: new classification. J. Med. Vet. Mycol. 33, 15–17.

Gimenez, M.F., Tausk, F., Gimenez, M.M., Gigli, I., 1987. Langerhans' cells in paracoccidioidomycosis. Arch. Dermatol. 123, 479–481.

Holíková, Z., Hercogová, J., Plzáki, J., Smetna, K., 2001. Dendritic cells and their role in skin-induced immune responses. J. Eur. Acad. Dermatol. Venereol. 15, 116–120.

Hsu, S.M., Raine, L., Fanger, H., 1981. Use of avidin–biotin–peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 577–580.

Key, M.E., Phillips, T., 1996. Catalysed signal amplification (CSA). An enhanced immunohistochemical staining method based on biotinyl tyramide. J. NIH Res. 8, 72.

Lacaz, C.S., 1981. Distribuição geográfica dos micetomas no Brasil. Ann. Bras. Dermatol. 56, 167–172.

Mahgoub, E.S., 1978. Experimental infection of athymic nude New Zealand mice with mycetoma agents. Sabouraudia 16, 211–216.

Mahgoub, E.S., Gumma, S.A., Hassan, A.M., 1977. Immunological status of mycetoma patients. Bull. Soc. Pathol. Exo 70, 48–54.

Marques, M., Moscardi-Bacchi, M., Marques, S., Franco, M., 1993. Immunohistochemical characterization of mononuclear cells in delayed hypersensivity reactions to *Paracoccidioides brasiliensis* (paracoccidioidin test). Mycopathologia 124, 7–11.

McElroy, J.A., Prestes, C.A., Su, W.P.D., 1992. Mycetoma: infection with tumefaction, draining sinuses, and "grains". Cutis 107, 107–110.

McGinnis, M.R., 1996. Mycetoma. Dermatol. Clin. 14, 97–104.

Moll, H., 1993. Epidermal Langerhans cells are critical for immunoregulation of cutaneous leishmaniasis. Immunol. Today 14, 383–387.

Moscardi-Bacchi, M., Soares, A., Mendes, R., Marques, S., Franco, M., 1989. In situ localization of T lymphocytes subsets in human paracoccidioidomycosis. J. Med. Vet. Mycol. 27 (3), 149–158.

Pancrè, V., Delacre, M., Herno, J., Auriault, C., 1999. Schistosomal egg antigen-responsive CD8 T-cell population in *Schistosoma mansoni* infected BALB/c mice. Immunology 98, 525–534.

Roitt, I., Brostoff, J., Male, D., 1998. Immunology. In: Cell-Mediated Immune Reactions. Mosby International Limited, London, UK, pp. 121–138.

Salinas-Carmona, M.C., 2000. *Nocardia brasiliensis*: from microbe to human and experimental infections. Microbes Infect. 2, 1373–1381.

Salinas-Carmona, M.C., Torres-Lopez, E., Ramos, A.I., Licon-Trillo, A., Gonzalez-Spencer, D., 1999. Immune response to *Nocardia brasiliensis* antigens in experimental model of actinomycetoma in BALB/c mice. Infect. Immun. 67, 2428–2432.

Sandoval, M.P., Brito, T., Sotto, M.N., Santos, R.T., Franco, M., 1996. Antigen distribution in mucocutaneous biopsies of human paracoccidioidomycosis. Int. J. Surg. Pathol. 3, 181–188.

Streilein, J.W., 1983. Skin-associated lymphoid tissues (SALT): origins and functions. J. Invest. Dermatol. 80, 12S–16S.

Tapia, F.J., Cárceres-Dittmar, G., Acuña, L., Mosca, W., 1989. Epidermal Langerhans cells in infectious diseases. Histol. Histopathol. 4, 499–508.

Yousif, M.A., Hay, R.J., 1987. Leucocyte chemotaxis to mycetoma agents—the effect to the antifungal drugs griseofulvin and ketoconazole. Trans. R. Soc. Trop. Med. Hyg. 81, 319–321.

Zaias, N., 1978. Chromomycosis. J. Cutan. Pathol. 5, 155–164.