DOPA-STAINED MELANOCYTES IN THE MACULAR LESIONS OF EARLY LEPROSY

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ABSTRACT - *The mechanism of association of hypopigmentation and sensorial loss in a leprosy macular lesions has not been clarified yet. The biopsy of a macular lesion on the medial face of the right forearm of a fourteen-year old male leprosy patient was submitted to DOPA-staining for melanocytes, which is specific for the melanocytic tyrosinase enzyme and it is a proper method for identifying and counting these cells in the skin. A contralateral specimen of the same patient went through the same procedure as a control experiment. The specimen from the macular lesion showed a higher number of DOPA-stained melanocytes than the control fragment. Dermal melanocytes were present in high amounts in the abnormal specimen. Increased expression of tyrosinase by melanocytes in the macular lesions may reflect a positive feed-back stimulus represented by the lack of substrate tyrosine, which may in turn be utilized by the mycobacterial agent. Ultrastructural study of the normal and pathological specimens showed no significant differences in the morphological appearance of melanocytes and their melanosomes. These results suggest that the utilization of phenolic compound by the* ***Mycobacterium leprae*** *may be involved in the mechanism of hypopigmentation. A higher number of cases will be necessary to confirm this hypothesis.*

Key words: *Melanocyte, leprosy, tyrosinase, DOPA.*

INTRODUCTION

In the early stages, leprosy frequently affects both the pigmentary and peripheral nervous systems of the skin[8]. These concomitant manifestations are probably not a matter of coincidence. Melanocytes and the peripheral nervous system have the same embriological origins (neural crest)[2] and in adult life both systems seem to remain associated as indicated by the morphological relation between nerves and melanocytes in disease such as melanocytic nevi[2]. This author thinks of the perineurial cell as a melanocyte precursor in adult life, migrating from the terminal nerves to the epidermis.

Job[6] has found vacuolization, dilated endoplasmic reticulum and decreased number of mitochondria and melanosomes in the melanocytes of the macular lesions of leprosy patients. According to this author these are signs of decreased melanocyte activity in leprosy. Ischemic factors induced by vascular changes[12]

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and utilization of DO PA substrate by *Mycobacterial leprae* enzyme system[10] have already been speculated as basic factors for the cause of hypopigmented macular lesions in leprosy. The frequent association of hypo-pigmentation and loss of sensorial functions in the macular lesions of leprosy is probably based on the remaining relationship between melanocytes and the peripheral nervous system in the post-natal life A single factor brought in by the disease, could be responsible for the simultaneous pigmentary and sensory alteration.

We studied the melanocytes in one case of leprosy single macular lesion with the DOPA (dihydroxiphenylalanine) staining procedure. This reaction is based on the activity of tyrosinase upon the DOPA substrate. Tyrosinase is a melano-genesis regulating enzyme, which is present in melanosomes[5]. It converts tyrosine into DOPA, through oxidation. DOPA substrate turns into a brown-black color precipitate when exposed to the melanocytic tyrosinase, making these cells visible in the pre-fixed frozen sections as dark-brown dendritic cells in the basal layer of the epidermis[9]. Transmission electronmicroscopy for ultrastructural examination of these melanin producing cells in the skin was also utilized in this investigation.

MATERIAL AND METHODS

One white patient with a macular lesion on the right forearm was selected. The patient noticed his lesion one year before. Sensorial loss through sthesiometric examination[1] was detected. The histamin test performed in the lesion showed incomplete halo formation. The patient was submitted to a biopsy procedure of his lesion and a sample of the correspondent contralateral region was taken as control of the study after the voluntary consentment signed by the patient. The biopsy fragment was divided into three pieces. One went through conventional histopathological study for diagnostic purposes. The second was fixed with 10% formalin in phosphate-buffered saline and was frozen in liquid nitrogen. Fourteen-u-thick sections were obtained in a Reichert Cryostat (Histostat) from the frozen material and identification of melanocytes was performed with the DOPA-oxidase method. Parts of both normal and pathological specimens were cut into fragments of 1 mm^3, which were then prepared for the transmission electron microscopy study. The fragments were fixed in glutaraldehyde 2.5% (Polysciences, USA), overnight, at 4°C; post-fixed in 1% osmium tetroxide (Merck Germany) for 1h, washed in 0.2M cacodylate buffer, dehydrated in graded acetone batches; embedded in Epon, (Polysciences). One-u-thick sections of the blocks, were stained with toluidine blue for searching the specimens which contained basal portions of the epidermis, where melanocytes are usually found. Ultramicrotomy of the selected blocks was performed (Sorvall Ultramicrotome MT 6000, USA). Ultra-thin sections were laid on copper grids; contrasted with no Uranyl acetate (Merck, Germany) in methanol and in lead citrate (aqueous solution of 5.8% Tri-Sodium cytrate-2-hydrate, (Merck, Germany) and 4.4% Lead Nitrate (Merck West Germany). The grids were examined in a Zeiss Electron Microscope (FM 109, Germany) and electronmicrographies were taken with a KODAK film.

RESULTS

DOPA reaction: The biopsy specimen from the leprosy lesion showed several tyrosinase-positive melanocytes (12 cells/X40 field) in the basal layer of the epidermis, evidenced through their dark brown DOPA-stained cytoplasm (Fig.1b). Some of them were dendritic and their dendrites were seen to touch the basal surface of keratinocytes. Melanocytes were also seen in the dermis. The normal specimen however, was surprisingly totally depleted of DOPA-stained melanocytes, showing neither positive cells in the epidermis nor in the dermis (Fig.1 a).

The ultrastructural study showed melanocytes among keratinocytes, with close juxtaposition (Fig. 2a). Melanosomes were observed in both cytoplasm of melanocytes and of keratinocytes (Fig. 2b). They were constituted of membranous vesicles, containing an electrondense material. Clustered melanosomes were observed in the cytoplasm of keratinocytes. Some melanocytic dendrites containing melano-somes were seen among the basal epidermal

cells. No significant differences were noticed between the normal and pathological specimens, concerning the ultrastructural melanocytic and melanosome morphology.

DISCUSSION

The number of DOPA-stained melanocytes in the pathological specimen was strikingly higher than in the contralateral normal looking cutaneous specimen of the same patient. This finding is unexpected, since a reduction in the number of DOPA-positive melanocytes in the lesion would be much more likely due to a local decrease in the production of melanin in the hypopigmented macular lesions. However, it is necessary to confirm this result by means of a study with a higher number of leprosy patients. An alternative hypothesis for this finding is that melanin production in the leprosy macular lesions could be blocked with an accumulation of tyrosinase in the melanocytes.

We don't have a reasonable explanation for the complete absence of DOPA-positive melanocytes in the histochemical staining of the contralateral normal looking cutaneous specimen of the patient. The normal contralateral skin do contain melanocytes as these cells were seen with the ultrastructural examination, however, they were not seen with the histochemical staining.

The absence of detectable melanocyte alteration with transmission electron microscopy study is in contradiction with Job's findings[6]. The reduced number of cases of our study can explain this difference.

The result of this study suggests that hypopigmentation in this leprosy patient occurred in spite of an increased number of DOPA-positive melanocytes in the epidermis of the macular lesions. The increased expression of tyrosinase in the hypopigmented epidermis may have occurred on account of a block of melanin synthesis and subsequent accumulation of tyrosinase in these cells. A decrease in the available tyrosine for melanin synthesis could be, in a speculative way, the cause for this supposed block. This hypothesis deserves confirmation since Prabhakaran et al11 have reported oxidation of phenolic substrates by M leprae. Tyrosine could be one of them. Therefore, there could be a possibility of tyrosine utilization by the mycobacterial agent with deprivation of this substrate for melanin synthesis.

Hypopigmentation of the macular lesion in this patient was not associated with any ultrastructural alteration of melanocytes and their melanosomes in this study.

The study of mechanisms of hypopigmentation in leprosy can unveil some aspects of host-M. *leprae* relationship which are crucial for providing new approaches to the understanding of the disease.

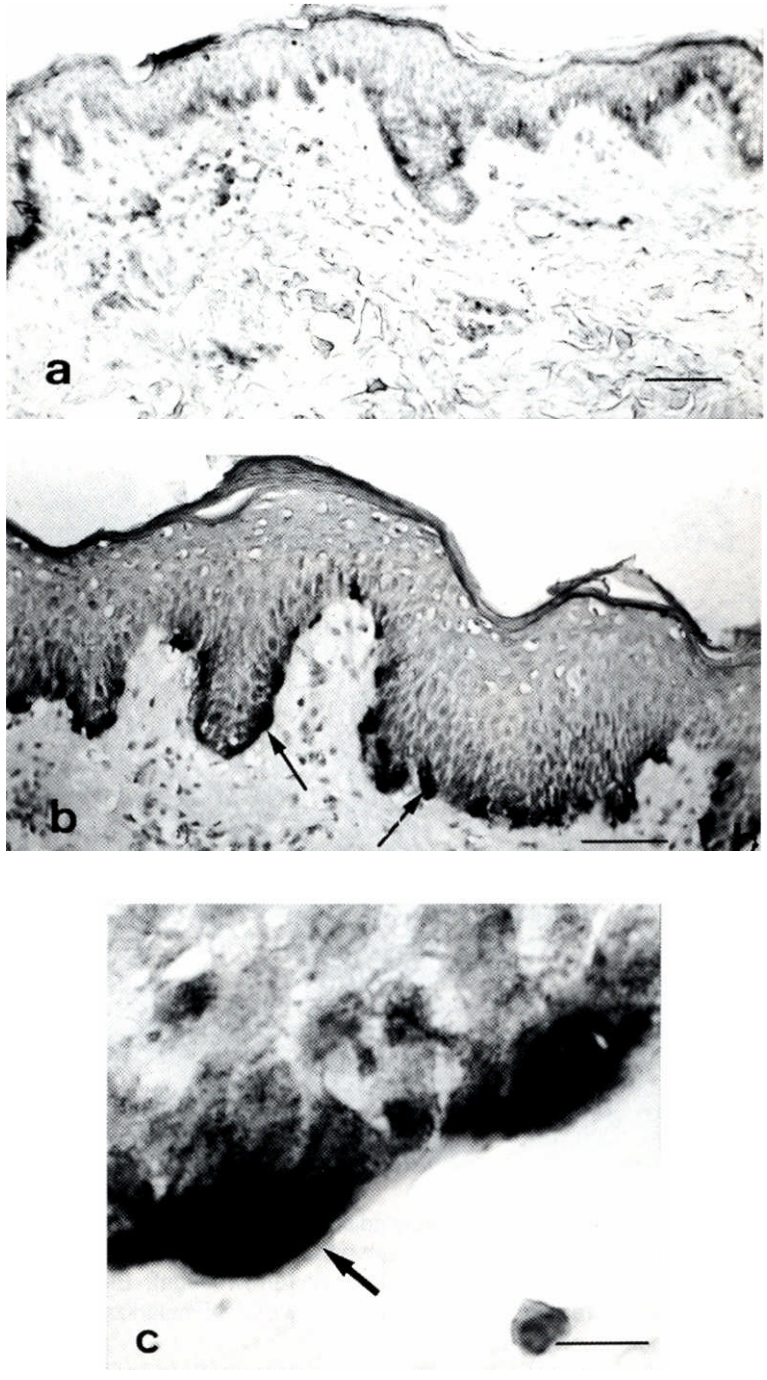

Fig. 1: (a) Normal looking skin section from the leprosy patient with no DOPA-stained melanocytes in the basal layer of epidermis. DOPA reaction. Scale bar: 50 u. **(b)** Macular skin section of the leprosy patient showing several DOPA-stained melanocytes (arrows) in the basal layer of epidermis. DOPA reaction. Scale bar: 15 u. (c) A close view of a DOPA-stained melanocyte (arrow) from the macular skin of the leprosy patient. DOPA reaction. Scale bar: 5 u.

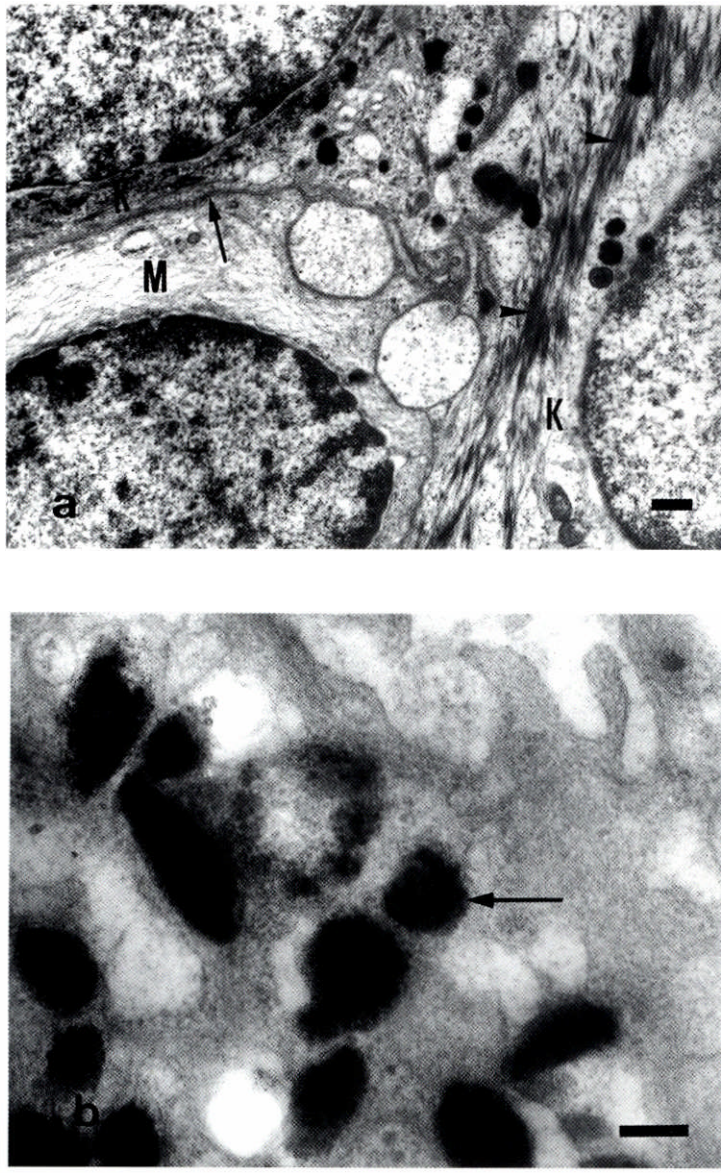

Fig. 2: (a) A melanocyte (M) between two closely adjacent keratinocytes (K) in the epidermal region of a macular skin section of the leprosy patient. Observe the juxtaposed membranes of both cells (arrow) and the keratin filaments in the cytoplasm of keratinocyte (arrowheads). Electronmicrography. Scale bar: 0.3 µ. **(b**) Melanosomes (arrow) exhibiting apparently normal melanization in a keratinocyte of a leprosy skin. Electronmicrography. Scale bar: 0.1 fit.

Resumo: *O mecanismo de hipopigmentação na hanseníase ainda não está explicado de forma definitiva. Esse estudo objetiva avaliar em termos quantitativos e qualitativos* a *população de melanócitos nas manchas hipocrômicas da hanseníase. A biópsia de um paciente de 14 anos com o diagnóstico de hanseníase "borderline" tuberculóide (BT), apresentando uma mácula hipocrômica única no antebraço direito e um fragmento da região contra-lateral correspondente do mesmo paciente foram submetidos à co/oração pelo DOPA a qual revela a enzima tirosinase que está presente em melanócitos. Os fragmentos colhidos também foram submetidos a estudo ultra - estrutural da região basal da epiderme, ocupada pelo sistema pigmentar melanocítico. O número de melanócitos corados pela DOPA foi muito maior no espécime obtido da lesão macular. No fragmento controle não havia praticamente melanócitos positivos. Havia muitos melanócitos na derme da lesão macular. O estudo ultra - estrutural não mostrou alterações morfológicas de melanócitos em nenhum dos dois espécimes. Um bloqueio da síntese de melanina no interior da mácula hipocrômica com acúmulo da enzima tirosinase foi especulado para explicar o maior número de melanócitos DOPA - positivos na pele da mácula hipocrômica do paciente hanseniano. A falta de tirosina poderia estar implicada como causa da interrupção da síntese de melanina na mácula hanseniana, já que a oxidação de compostos fenólicos pelo* ***M. leprae*** *já foi demonstrada na literatura. A ausência de alterações anatômicas do sistema pigmentar cutâneo. Um número maior de casos será necessário para confirmar esta hipótese.*

Palavras-chave: *Melanócito, hanseníase, tirosinase, DOPA.*

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