ULTRASTRUCTURAL STUDY OF THE DERMAL NERVES IN THE CUTANEOUS MACULAR LESIONS OF PATIENTS WITH EARLY LEPROSY

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ABSTRACT *- Thirteen biopsies of macular lesions of early leprosy patients were studied ultrastructurally with transmission electron microscopy (TEM). All of the biopsies displayed at least one dermal nerve partially or completely encircled by mononuclear cells in the conventional histopathological study with light microscopy The patients'diagnosis varied from indeterminate leprosy to borderline tuberculoide (BT). In the ultrastructural study, twenty-seven dermal nerve branches were found in the thirteen biopsies. Twenty dermal nerve branches in eleven biopsies were found to display no inflammatory involvement Seven nerves in seven biopsies were morphologically associated with mononuclear leukocytic cells. Four biopsies exhibited nerves with and without inflammatory involvement concomitantly. Three nerves showed morphological evidence of endoneurial fibrosis, not morphologically associated with the inflammatory process at least in the sections examined. No detectable axional and Schwann cell ultra strutural changes even in the twenty-seven nerves were found. The sensorial loss exhibited by the patients before the institution of treatment was completely reversed in eight patients after the end of multidrug therapy regimen. These findings suggest that sensory loss in the early stages of leprosy may be caused by reversible pathological mechanisms, rather than anatomical damage. It is also possible, concerning the mechanisms of nerve damage in leprosy, to speculate on the existence of a pathological process which may precede the inflammation.*

Key words*: Perypheral neuropathy, leprosy, macular lesions, indeterminate leprosy dermal nerves.*

1. INTRODUCTION

Peripheral neuropathy is the chief cause of physical disabilities and deformities found in leprosy patients. The peripheral nerves in leprosy are affected by an inflammatory infiltrate that may be composed either of epithehoid cells or by macrophages loaded with acid-fast bacilli (AFB)[8.] Leprosy neuropathy progresses with a decrease of unmyelinated[5] and myelinated fibers[3,1]. An increase of collagen deposit in the endoneurium was reported by Dastur et al[3] and in the epineurium, perineurium and endoneurium, by Junqueira et al[9].

Segmentar demyelination and axonal degeneration or atrophy were reported as morphological changes found in the peripheral nerves affected by the disease[4,3,7,19].

The pattern of trunk nerve lesion may not match with the one found in the skin. The former may have more mature granulomas, whereas the latter may present indeterminate infiltrate[13]. Furthermore, paucibacillary lesions of skin may present concomitantly with multibacillary lesions of nerves[15].

The cutaneous branches of peripheral nerves have also been studied in leprosy. Chandi and Checko[1] & reported the presence of acid-fast bacilli and of isolated macrophages within the dermal nerves of leprosy indeterminate lesions. Karanth et al[10] observed a decrease of positive neural fibers in the dermis of lepromatous and tuberculoid patients using immunohistochemical staining of nerve antigens.

The immune response elicited by the *M leprae* with recruitment of lymphocytes into the

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endoneurial compartment has also been implicated in the leprosy nerve damage. M. leprae antigens in endothelial cells, in Schwann cells and in epithehoid cells were detected by Mshana et al[12] in the nerves of leprosy. The immune response triggered followed by mononuclear cell attraction may alter the endoneurial environment, contributing to nerve damage. The level of auto-antibodies to nerve antigens in leprosy patients' sera is higher than the levels in the normal control individuals[14,20], however, no pathogenetic association could be detected with these findings. Turk[21] remarks the role of the immune response in the nerve damage of leprosy, particularly the role of tumor necrosis factor in the autoimmune demyelination[18]. Lipoarabinomanan, an antigen of the myco-bacterial wall induces the TNF production by macrophages[21], even when assayed in monocyte culture. These are evidences which favor an immunological mechanism for nerve damage in leprosy. Jacobs et al[7], however, state that the morphological characteristics of leprosy neuropathy are quite distinct from the ones exhibited by the classical, undoubtedly immunological polyneuropathies, evidences which favor pathological mechanisms other than immune ones as the main cause for neural lesion in leprosy patients.

The early pathogenic mechanism of neural lesion in leprosy is a subject for intensive discussion and speculation. This work is an ultrastructural study of the dermal nerves and their relationship with the leprosy inflammatory infiltrate in the initial cutaneous manifestations of leprosy. The concept of early leprosy according to Gupte[6] was utilized in this study and it is characterized by the presence of at least two of the following signs: macular lesions, sensorial disturbances, nerve thickening and positivity for acid-fast bacilli (AFB).

2. MATERIAL AND METHODS

Thirteen cutaneous biopsy specimens of thirteen untreated patients with early leprosy6, from the Leprosy Outpatient Service, Oswaldo Cruz Foundation, and Department of Dermatology, Antônio Pedro Hospital, Fluminense Federal University were selected for this study(TableI).

The sensorial function of the skin (thermal,

Table I - Clinical data of the patients

	Number of lesions	Sites of lesions	Paresthesia	Sensory loss	Nerve Enlargement	Acid-fast bacilli*
1	1	elbow	-	lesion	radial	+
2	1	abdomen	-	lesion	-	-
3	1	ankle	hand	lesion	ulnar	-
4	2	lower limb	-	lesions	posterior tibial	-
5	1	elbow	-	lesion	ulnar	-
6	2	upper limb	-	lesions	ulnar	-
7	1	lower limb	-	lesion, foot	-	-
8	1	upper limb	-	lesion, upper and lower limb	-	+
9	2	upper limb	-	lesions	ulnar	-
10	1	upper limb	-	lesion	-	ND
11	2	upper limb, face	-	lesions, foot	ulnar	+
12	>3	upper and lower limb	-	lesions	-	ND
13	2	back, face	hands	lesions	auricular	ND

* Patients **1, 8** and **11** had AFB-positive skin smears. Patient 1 had in addition, AFB-positive histopathological specimens.
ND: not done

touch and algic) was evaluated. Histamin test was performed and was abnormal in all of the patients. Bacillary load was evaluated with the examination of skin smears .

All patients who showed a negative AFB test were suspected to have the disease, taking into account the clinical manifestations, the abnormal response to histamin test, and the presence of inflammatory infiltrate adjacent to anexial structures and dermal nerves in the histopathological examination. The disease was confirmed in all of these patients based on the total remission of all the pre-existent lesions and of the neural symptoms at the end of a multidrug therapy for leprosy. The patients who were positive for AFB were treated with the regimen for multibacillary leprosy.

Part of the specimens obtained were processed for routine electronmicroscopical examination. Six one-mm^3-big fragments of tissue of each specimen from both control and leprosy groups were separated from the specimen and fixed in glutaraldehyde2.5% (Polysciences, USA), for 4h at 4o C; post-fixed in 1% osmium tetroxide (Merck Germany) for 24h, overnight, dehydrated in graded acetone batches; embedded in Araldite (Merck Germany). One-µm-hick sections of the blocks, stained with toluidine blue were examined in search for neural branches and nerve endings in the dermis. Sweat gland accini, hair follicles, arrector pillus muscles and dermo-epidermal junction were also selected for ultrastructural examination of the nerve endings in the adjacent dermal region. Ultramicrotomy of the fragments containing neural structures was performed (Sorvall Ultramicrotome MT 6000, USA). Ultra-thin sections were laid on copper grids; contrasted with 7% 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 35 mm film. The rest of the biopsy specimen went through conventional histological processing. Six cutaneous specimens from leprosy-free individuals, from Plastic Surgery Outpatient Service, Antônio Pedro Hospital, Fluminense Federal University were utilized as a control group for this study. They also went through the same routine procedures for histopathology and electronmicroscopy described for the leprosy patients' specimens.

3. RESULTS

All the thirteen patients selected for this investigation showed abnormal responses to sensorial function tests. The histamin test was abnormal in all of them: Three of them exhibited sensorial loss on body sites distinct from the ones occupied by the lesions (Table I). Three individuals had AFB-positive skin smears. Histopathological examination of their biopsies showed a mononuclear inflammatory infiltrate adjacent to the microvascular and anexial structures. Dermal nerve branches were also encircled by inflammatory cells. In all the biopsies, the infiltrate never occupied more than 10% of the section-area examined. Under Wade staining of the biopsy sections, AFB were detected in only one of the three patients who had positive skin smears. Leukocytes were very close to the perineurium and in two specimens, intermingled with perineurial layers. Leukocytic invasion of the endoneurium was difficult to evaluate with light microscopy.

Transmission electronmicroscopy: Twenty-seven nerves were found in the 78 Araldite embedded blocks of the 13 biopsies examined with this method. According to the involvement of nerves by inflammatory infiltrate the ultrastructural findings were classified in two groups: 1) Twenty nerves (11 biopsies) not involved by inflammation, 2) Seven nerves (six biopsies) involved by inflammation. Four biopsies exhibited nerves with and without inflammatory involvement concomitantly.

In the sections containing anexial structures (2 biopsies), we identified several nerve endings adjacent to sweat gland accini, to hair follicles and in the interstitium of arrector pillus muscle. They were represented by non-myelinated axons, devoid of encircling perineurium and just enveloped by cytoplasmic elongations of Schwann cells. These nerve endings did not show any morphological alteration.

The inflammatory infiltrate encircling the

branches of the dermal nerves was distributed according to three distinct patterns: a) it encircled the perineurial layers of the nerves (7 nerves) (Fig. 2); b) mononuclear cells were observed among the perineurial layers (2 nerves) (Fig 3); c) mononuclear cells were found in the endoneurium among neural fibers (1 nerve) (Fig. 3) (Table II). The infiltrating cells were lymphocytes and macrophages without morphological signs of activation (Fig 2 and 3). Disruption of perineurial architecture was observed in two nerves. Loose perineurial cells seemed to migrate to the endoneurial compartment and to acquire a fibroblastic appearance (Fig. 2). No AFB were found in the nerves examined by TEM. No morpho-logical alteration of axons and of Schwann cellwere detected in the neural structures examined.

A morphological appearance of increased

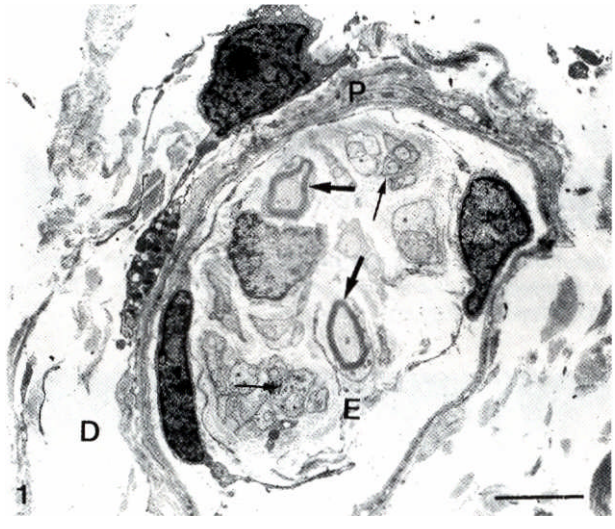

Figure 1 *- Normal morphological appearance of a dermal nerve found in the skin of a patient with early leprosy. Myelinated (thick arrows) and unmyelinated (thin arrows) neural fibers in the endoneurium (E). Perineurium (P), Dermis (D). Scale bar: 1 pm.*

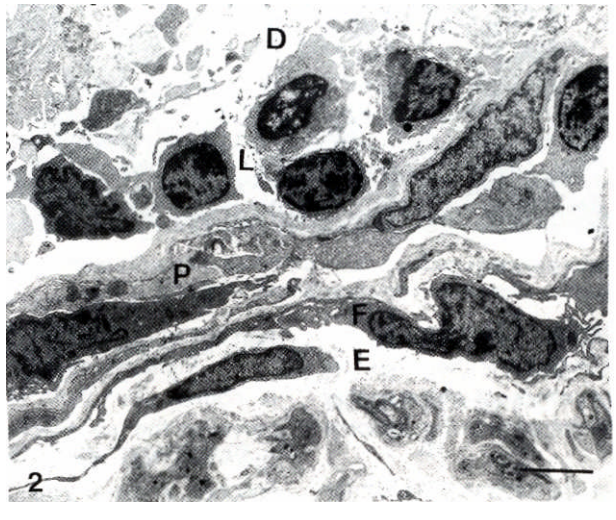

Figure 2 *- Mononuclear inflammatory cells (Lymphocytes) (L) close to the perineurial layer (P) of a dermal nerve branch. Perineurium showing architectural disruption (P). Fibroblast (F) possibly derived from loose perineurial cells. Endoneurium (E), Dermis (D). Scale bar: 0.5 pm.*

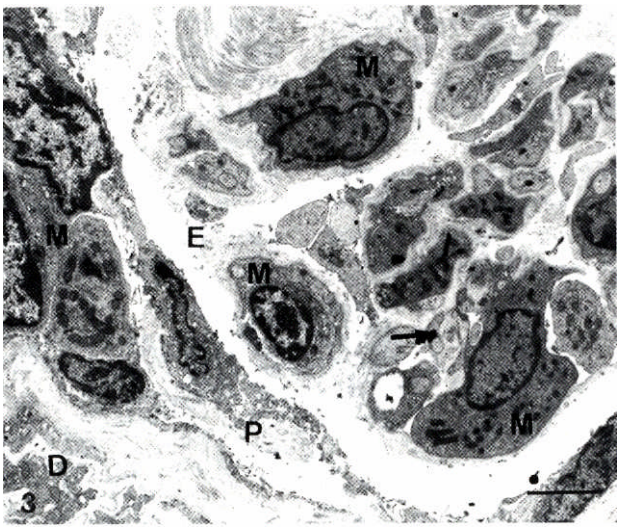

Figure 3 - Mononuclear inflammatory cells (macrophages) (M) partially encircling a dermal nerve branch. Perineurial architecture disappeared. Non-myelinated fibers (arrow) close to macrophages in the endoneurium (E). Remaining perineurial cell (P), Dermis (D). Scale bar: 0.5 um.

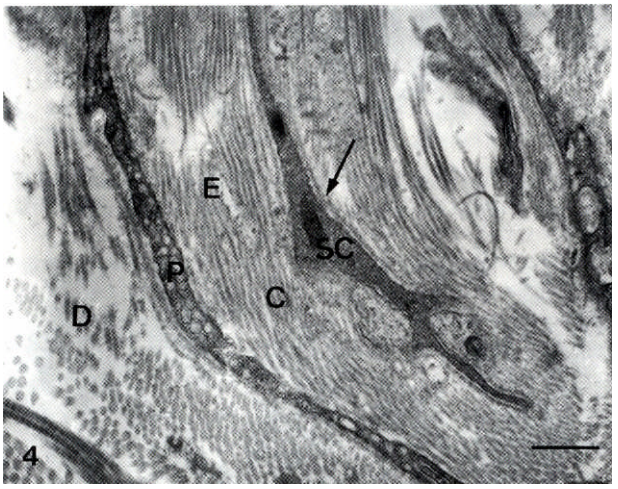

Figure 4 - increased density of endoneurial collagen fibers (C). Endoneurium (E), perineurial cell (P), dermis (D). Schwann cell cytoplasm (SC) enveloped by a basement membrane (arrow). Scale bar: 0.1 pm.

density of collagen fibers was found in the endoneurium of three nerves and they were not associated with the presence of inflammatory leukocytes in the endoneurium or in the adjacent dermis.

Eight patients of the thirteen selected for this study were examined at the end of the multidrug treatment and all of them showed recovery of their sensorial function (seven presented total recovery and one only partial).

Eight nerves were found in the specimens of normal skin: three specimens showed one nerve in the six blocks examined per each specimen, two specimens exhibited two nerves and one, three nerves. None of the histopa-thological and electronmicroscopical changes reported on the leprosy patients were detected in the sections of normal specimens.

Table II: Morphological Findings in the ultrastructural study

	Nerves found	Dermal nerves *with* inflammatory involvement	Dermal nerves *without* inflammatory involvement	Infiltration of perineurium by mononuclear cells	Endoneurial infiltration by mononuclear cells	Changes in the perineurial architecture	Morphological evidence of endoneurial fibrosis
1	3	1	2	0	0	0	1
2	1	0	1	0	0	0	0
3	1	0	1	0	0	0	0
4	3	1	2	0	0	0	0
5	1	1	0	1	1	1	0
6	1	0	1	0	0	0	0
7	3	2	1	1	0	1	0
8	2	0	2	0	0	0	1
9	1	1	0	0	0	0	0
10	2	0	2	0	0	0	1
11	1	0	1	0	0	0	0
12	3	1	2	0	0	0	0
13	5	0	5	0	0	0	0
total	27	7	20	2	1	2	3

4. DISCUSSION

The striking discrepancy in the detection of nerves involved by inflammatory infiltrate, with light microscopy and with electronmicroscopy was due to the higher number of nerves observed with the former method. Histopathology with light microscopy renders the detection of peripheral nerve branches easier, permitting the visualization of a higher number of neural structures in a wide section.

Perineurium seems to be the first barrier to refrain the leukocytes from invading the endoneurial compartment. Pearson and Weddell 16 characterized fibrosis and cell proliferation as mechanisms of perineurial thickening in leprosy. In two nerves, disruption of perineurial structure associated with perineurial leukocytic infiltration was found. The cohesive architecture of perineu-rium may be disrupted after the contact between inflammatory cells and perineurial cells, followed by the modification of their adhesive properties and subsequent lack of cohesion among them.

Fibrosis is a frequently reported alteration in advanced leprosy neuropathy[3,9] but its patho-genesis and role in leprosy nerve degeneration has not been clarified yet. A morphological evidence of perineurial cell migration to the endo-neurial compartment was shown in one nerve (Fig. 2). Perineurial cell contribution to endoneurial fibrosis after the loss of cohesiveness and migration of these cells to the endoneurium is morphologically suggested in this study.

The recovery of sensorial function by the

Table III - Quantification of Nerves and inflammatory involvement

	Number of nerves	Number of biopsies*
1) ***Absence*** **of inflammatory involvement**	20	11
2) ***Presence*** **of inflammatory involvement**	7	6

*Four biopsies were found to have nerves with inflammatory involvement and without inflammatory involvement concomitantly.

selected patients at the end of treatment and the absence of anatomical neural fiber damage in all the nerves examined in this study may be an indication that the disturbances caused by the inflammatory cells upon the peripheral neural fibers in the early stages of leprosy are reversible. These sensorial disturbances might occur on account of microenvironment modification of the perineurial and of the endoneurial compartment by the inflammatory process.

The triggering mechanisms which attract leukocytes to the nerves and the role of the inflammatory infiltrate in the axonal degeneration and in the segmentar demyelination observed in leprosy neuropathy[4,3,7,19] have to be clarified yet. These subjects therefore, are relevant for the research on the pathogenesis of leprosy neuropathy.

RESUMO - *Treze biópsias de lesões maculares de pacientes com hanseníase em fase inicial foram submetidas a estudo ultra-estrutural com microscopia eletrônica de transmissão. A microscopia ótica dessas biópsias havia evidenciado previamente envolvimento inflamatório de nervos cutâneos. 0 diagnóstico clínico dos casos variou entre hanseníase indeterminada ou hanseníase "borderline tuberculóide". Ao exame ultra-estrutural, 27 nervos foram encontrados nas treze biópsias. Em vinte nervos de 11 biópsias não foram encontrados envolvimento inflamatório, nem qualquer outro tipo de alteração morfológica. Sete nervos de sete biópsias estavam morfologicamente associados com infiltração leucocftica mononuclear que se localizava predominantemente em tomo do nervo, permeando o perineuro ou invadindo o endoneuro. Em quatro biópsias foram encontrados nervos com envolvimento inflamatório e concomitantemente nervos sem infiltrado inflamatório. Três nervos mostraram evidências morfológicas de fibrose endoneural não associada à presença de células inflamatórias, pelo menos nas secções examinadas. Não foram observadas alterações ultra-estruturais de fibras nervosas (axônio e célula de Schwann). Ao fim do tratamento, os pacientes exibiram recuperação da hipoestesia nas manchas hipocrômicas. A ausência de lesão de fibras neurais pelo infiltrado inflamatório, o encontro de alterações restritas aos componentes acessórios do nervo (perineuro) associados à recuperação da sensibilidade pelos pacientes ao final do tratamento sugerem que as lesões neurais nas fases iniciadas da hanseníase são ocasionadas por processos patológicos reversíveis e não por lesão anatômica de fibras neurais. Podemos ainda especular que pode existir um processo patológico independente e paralelo 6 inflamação no mecanismo de lesão neural da hanseníase.*

Palavras-chave: *Neuropatia periférica, hanseníase, lesões maculares, hanseníase indeterminada, nervos dórmicos.*

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