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Light and Electron Microscopic Study of Peripheral Nerve Damage in Patients with Lepromatous Leprosy (LL) and Borderline Lepromatous Leprosy (BL)

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ABSTRACT

Cutaneous branches of radial nerves in patients with lepromatous leprosy (LL) and borderline lepromatous (BL) were studied by light and electron microscopy. Foamy macrophages were found more or less in the nerve fibers of all leprosy patients and distributed in the epineurial, perineurial and endoneurial areas. In the endoneurium, the foamy macrophages were mainly located in the subperineurial and perivascular spaces. Vacuolated Schwann cells were also found in the nerve fasciculus. In electron microscopy, these foamy macrophages and vacuolated Schwann cells contained numerous small dense materials, irregular in size and shape, considered to be degenerated and fragmented mycobacterium leprae. These dense materials were found also in the cytoplasm of vascular endothelial cells. These findings suggest that mycobacteria enter into the endoneurium via the blood vessels. In our present study, on the other hand, it was very difficult to find the intact mycobacteria in the cytoplasm of the foamy macrophages, Schwann cells or endothelial cells, as well as in the Ziehl-Neelsen staining of paraffin sections. The disappearance of intact bacilli in our present study might have been caused by multi drug therapy.

The myelinated nerve fibers were degenerated and disappeared in variable degrees. Degenerative changes of the myelin sheath developed from the outer layer to the inner layer with disarrangement of the lamellar structure. These findings were different from myelin destruction of peripheral nerves in Wallerian degeneration. The degenerative changes of the myelin sheath are caused by degeneration and destruction of Schwann cells in leprosy patients.

Fibrosis surrounding myelinated and unmyelinated nerve fibers, i.e, periaxonal fibrosis, was found to a greater or lesser extent in the endoneurium.

In the present study, it is still unclear whether the periaxonal fibrosis was due to necrosis of the Schwann cells by infection of mycobacteria or to an autoimmune mechanism such as antiperipheral nerve antibody. However, lamellated concentric fibrosis surrounding regenerative myelinated and unmyelinated nerve fibers with the disappearance of mycobacteria suggests that degenerations and regenerations of nerve axons were repeated during clinical cause. These findings indicated that autoimmune mechanisms play an important role in the pathogenesis of periaxonal fibrosis.

Key words: *Leprosy, Peripheral nerve, Biopsy, Ultrastructure*

Leprosy is a disease which presently affects mostly developing countries, with an estimated 1.5 million patients world wide[26)]. Nerve damage leading to permanent disability is the major hazard in patients with leprosy. The nerve damage is an essential feature of the clinical manifestations and pathology of leprosy. It results from interactions between M. leprae and host cells within nerves.

Leprosy manifests itself as a spectrum from lepromatous to tuberculoid forms and affects skin

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and nerve. The number, distribution, appearance and bacillary load of the skin lesion are used as the main criteria for classification of the disease[15].

Nerve granulomas may be found at all points across the leprosy spectrum. At the lepromatous end the granuloma is of the typical macrophage type, whereas at the tuberculoid end the granuloma is more like that associated with delayed hypersensitivity[19].

The nature of the cellular infiltrate, particularly the cells of the macrophage-monocyte system in neural granulomas in leprosy, has been studied using electron microscopy[2,3,20]. However, the precise mechanism of bacterial entrance into the endoneurium, destruction of the myelin sheath and pathogenesis of the endoneurial fibrosis, especially periaxonal fibrosis, still remain unknown.

In this study, cutaneous branches of radius nerves were obtained from 10 leprosy patients with LL and BL to clarify the inflammatory process, especially the localization and proliferation of mycobacterium leprae, behavior of the foamy macrophages and Schwann cells, changes of myelin sheath, and the possible mechanism of periaxonal fibrosis, by light and electron microscopy.

PATIANTS AND METHODS

Radial cutaneous nerves of 10 leprosy patients, 6 male and 4 female, in "Daya" Leprosy Hospital Ujung Pandang, Indonesia, were biopsied and observed by light and electron microscopy.

The ages of these patients ranged from 8 to 60 years. According to the Ridley-Jopling classification, as shown in Table 1, 5 patients were classified as having lepromatous leprosy (LL) and the other 5 as having borderline lepromatous leprosy (BL). These patients all had the multibacillar type and were treated with multiple drug therapy (MDT) with/without Prednisolon. The duration of the disease ranged from 2 to 18 years.

Nerve biopsies were taken from cutaneous branches of the radial nerve under local skin anesthesia. The nerve tissues obtained were divided into 2 pieces. One of them was fixed for electron microscopy in 2.5% glutaraldehyde solution buffered with cacodylate at pH 7.4 immediately after biopsy. These nerve tissues were then post-fixed in 1% buffered OsO4 solution at pH 7.4, dehydrated in graded alcohols and embedded in epox resin. Semi-thin sections were cut with an Ultramicrotome (Reihert-Jung Ultracut E) equipped with glass knives, and stained with methylane blue. Thin sections were cut with the same Ultramicrotome equipped with a diamond knife, stained with uranyle acetate and lead citrate and observed in a JEM-1200EX electron microscope. The other piece of nerve tissue was also fixed in 10% formaline solution and embedded in paraffin. Cut sections were stained with hematoxilin-eosin, PAS and Ziehl-Neelsen's solutions.

RESULTS

1) Clinical findings

In LL and BL cases, as shown in Table 1, papules and nodules were recognized in the skin of all patients. Ulceration of skin was seen in 2 of 5 LL, and 3 of 5 BL patients. Paresthesia was noticed in all LL and BL patients. Neuritis was seen in 3 LL and 3 BL patients, madarosis in 2 LL

Table 1. Clinical findings and types in leprosy of 10 leprosy patients.

No	Age	Sex	Duration	Skin lesion	Sensory nerve	Motor nerve	Treatment	Type
1	8	M	3 y	Papule Nodule	Paraesthesia Neuritis	Normal	MDT	LL
2	19	M	6 y	Papule Nodule	Paraesthesia Neuritis	Muscle atrophy Claw hand	MDT	LL
3	60	M	12 y	Papule Nodule Ulcus (Bil. feet)	Paraesthesia Neuritis Saddle nose Madarosis	Normal	RFT	LL
4	22	F	10 y	Papule Nodule	Paraesthesia Saddle nose Madarosis	Left hand webs Muscle atrophy	MDT	LL
5	32	F	13 y	Ulcer (Left foot)	Paraesthesia	Muscle atrophy	MDT	LL
6	17	M	3 y	Papule Nodule	Paraesthesia Neuritis	Muscle atrophy	MDT	BL
7	23	M	2 y	Papule Nodule	Paraesthesia	Normal	MDT	BL
8	60	M	5 y	Papule Nodule Ulcus	Paraesthesia Neuritis Madarosis Hyperpigment.	Normal	MDT	BL
9	20	F	8 y	Ulcer (right hand)	Paraesthesia Lagophthalmus (right)	Muscle atrophy	MDT	BL
10	25	F	10 y	Papule Nodule Ulcus	Neuritis	Muscle atrophy	RFT	BL

MDT: Multi drug therapy
RFT: Release from treatment

Table 2. Light microscopic findings of cutaneous radial nerves of 10 leprosy patients.

No	Age	Sex	Type	Duration	Foamy Cell	Inflammatory Cell Infiltration		Fibrosis		Destruction of Myelinated Nerve Fiber
						Endoneurial	Epineurial	Endoneurial	Epineurial	
1	8	M	LL	3 y	+++	+++	+++	+++	+++	+++
2	19	M	LL	6 y	++	++	++	++	+++	++
3	60	M	LL	12 y	+	±	±	++	++	++
4	22	F	LL	10 y	++	+	+	++	++	++
5	32	F	LL	13 y	++	+	+	++	++	++
6	17	M	BL	3 y	+	±	+	++	++	+
7	23	M	BL	2 y	++	++	++	+++	++	+++
8	60	M	BL	5 y	++	+	+	++	+	++
9	20	F	BL	8 y	±	±	±	+	++	+
10	25	F	BL	10 y	+	±	+	++	+	++

Foamy Cell: almost disappeared ±; small number +; moderate number ++; many +++
Inflammatory Cell Infiltration: almost disappeared ±; small number +; moderate number ++; many +++
Fibrosis: slight +; moderate ++; marked +++
Destruction of Myelinated Nerve Fiber: slight loss +; moderate loss ++; completely absent +++

and 1 BL patient, and a saddle nose in 2 LL patients. A webbed hand was observed in 1 LL patient, and a claw hand in another LL patient. Muscle atrophy was found in 3 LL and 3 BL patients.

2) Light microscopic findings

Histological observations were carried out in paraffin sections stained with H-E and epon semithin sections stained with methylane blue. As shown in Table 2, foamy cells appeared to a greater or lesser extent in the epineurium, perineurium and endoneurium of the radial nerves in all cases of LL and BL patients. However, the foamy cells tended to be more numerous in patients with a short duration of the disease. These foamy cells were distributed mainly in the subperineurial and perivascular spaces of the endoneurium, as well as in the epineurial connective tissues (Fig. 1). In the Ziehl-Neelsen staining, it was very difficult to recognize any mycobacterium leprae in these foamy cells.

Infiltration of inflammatory cells, such as lymphocytes and plasma cells, was variable in degree in the endoneurial, perineurial and epineurial tissues of the nerve fibers in all LL and BL patients. The inflammatory cell infiltration, however, was more intense in the epineurial tissues than in the endoneurial tissues (Fig. 2). The inflammatory change of these nerve fibers was less intense in patients of over 10 years duration.

Fibrosis of the epineurial and endoneurial tissues was distinctly recognized in all leprosy patients. In the endoneurial tissue, fibrosis was usually remarkable in the subperineurial, periaxonal and perivascular areas (Fig. 3, 4). The degree of endoneurial fibrosis was intimately related to the destruction of myelinated nerve fibers (Fig. 4).

The remaining myelinated nerve fibers were variable in diameter and more or less degenerated. The myelin sheath was usually irregular in shape and showed thickening, thinning, splitting or granulation (Fig. 4). The nerve axons surrounded by these degenerated myelin sheaths were frequently vacuolated.

3) Electron microscopic findings

Numerous histiocytic cells having many large vacuoles were recognized in the epi- and endoneurial spaces of the nerve fibers in all LL and BL patients. The vacuoles of these cells usually contained many small dense materials, irregular in size and shape, suggesting degenerated and fragmented mycobacteria (Fig. 5). In large magnification, some of these dense materials were partially surrounded by membranous structures (Fig. 6). Intact mycobacteria were very difficult to find in this study.

The Schwann cells of the myelinated nerve fibers were usually degenerative and contained numerous vacuoles, some of which contained small dense materials resembling fragments of mycobacteria (Fig. 7). The nucleus of these degenerative Schwann cells frequently showed pyknotic changes with aggregation of heterochromatin and an increase in electron density (Fig. 7).

The structure of the myelin sheath was damaged to a varying extent. In some myelinated fibers, the outer layer of the myelin sheath was disorganized, and the lamellar structure was irregular in arrangement due to the separation, fragmentation or adhesion of lamellar elements (Fig. 8). In these myelin sheaths, the lamellar structure of the inner layer was still preserved, but the inner layer was usually collapsed and distorted. The Schwann cells had already disappeared in these fibers, while the axoplasm still remained. The degree of outer layer destruction was variable in each myelin sheath. There were also some myelinated fibers in which the myelin

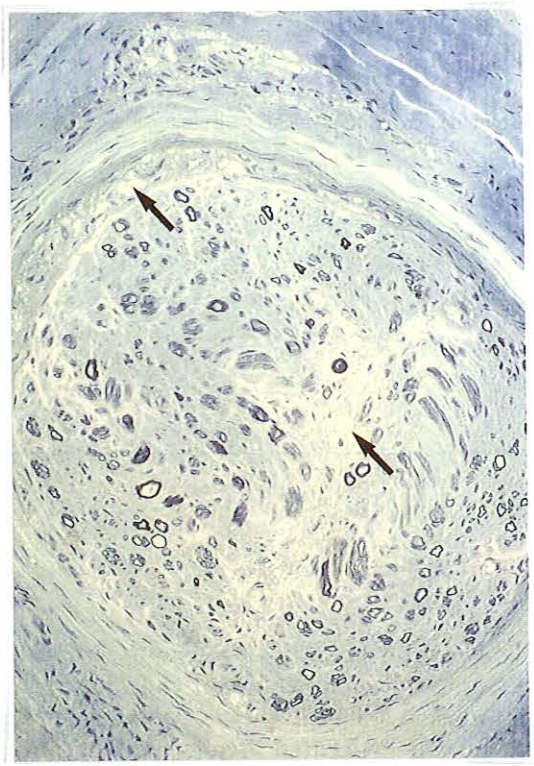

Fig. 1. The foamy macrophages (arrows) are recognized mainly in the subperineurial and perivascular spaces of the endoneurium, as well as in the epineurial connective tissues. Case No. 4 (LL), epon semithin section with methylane blue stain. ×200

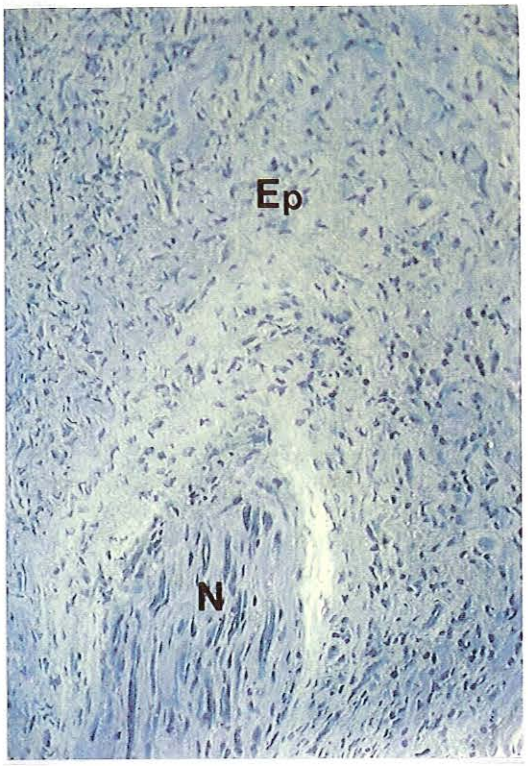

Fig. 2. Inflammatory cell infiltration and fibrosis are more intense in the epineurial area (Ep) than in the endoneurial area (N). Case No. 2 (LL), methylaneblue stain. ×200

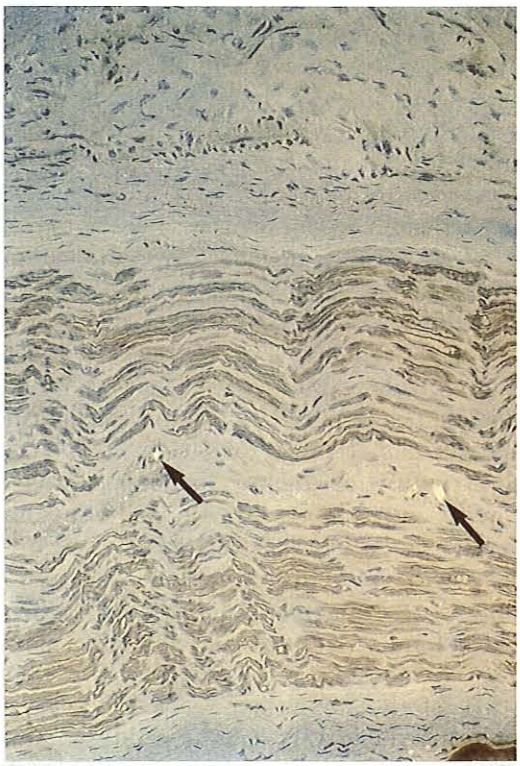

Fig. 3. Perivascular fibrosis with proliferation of foamy macrophages is distinct in the endoneurial tissue. Arrows: blood vessels. Case No. 6 (BL), methylane blue stain. ×200

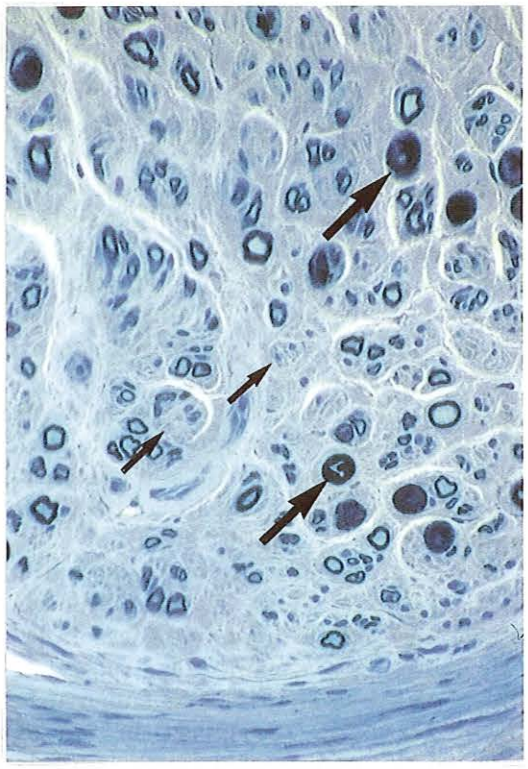

Fig. 4. Myelinated nerve fibers are decreased in number and the myelin sheath shows degenerative changes, thickening, thinning or splitting (large arrows). Intraneurial fibrosis, especially periaxonal fibrosis (small arrows), is also distinct. Case No. 10 (BL), ×400

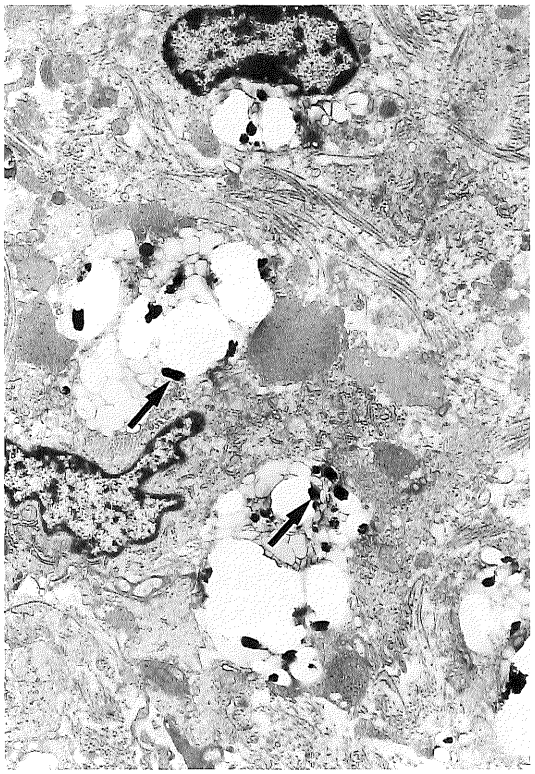

Fig. 5. Fine structure of foamy macrophages. Many electron dense materials suggesting degenerated mycobacteria (arrows) are observed in the cytoplasmic vacuoles. Case No. 7 (BL), ×5,600

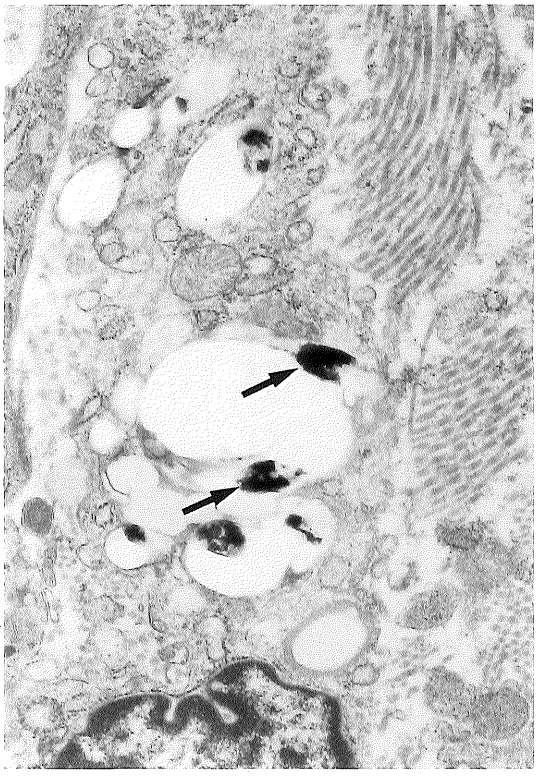

Fig. 6. Large magnification of electron dense materials (arrows) in the vacuoles of a macrophage. Case No. 7 (BL), ×18,000

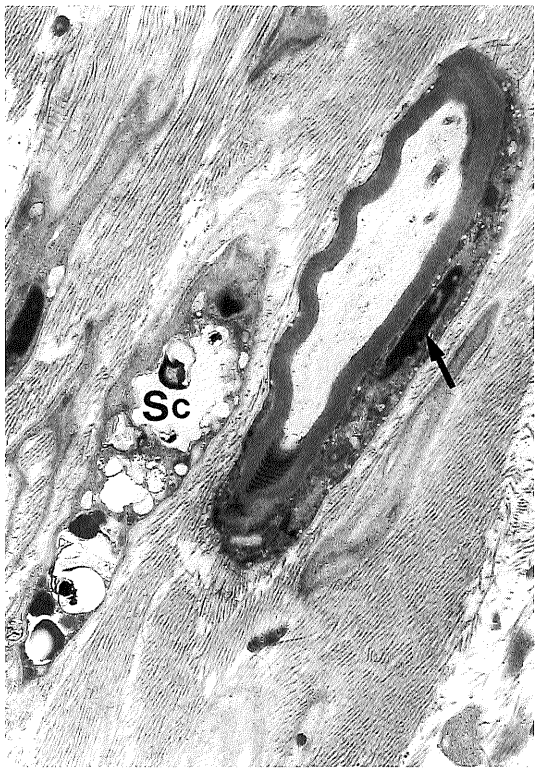

Fig. 7. Degenerative Schwann cells (Sc) showing foamy changes. The Schwann cell nucleus (arrow) shows picnotic change. Case No. 6 (BL), ×7,000

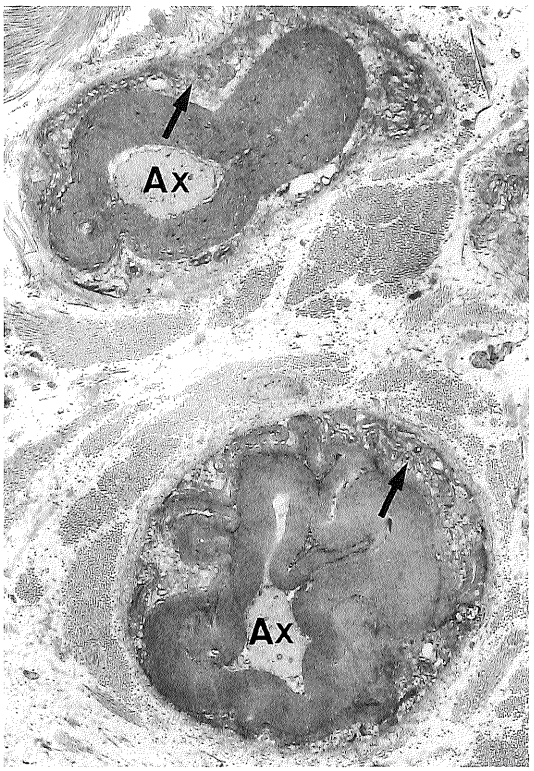

Fig. 8. The outer layer of the myelin sheath is degenerated and lammelar elements are disarranged and disorganized (arrows). Ax: nerve axon. Case No. 6 (BL), ×4,000

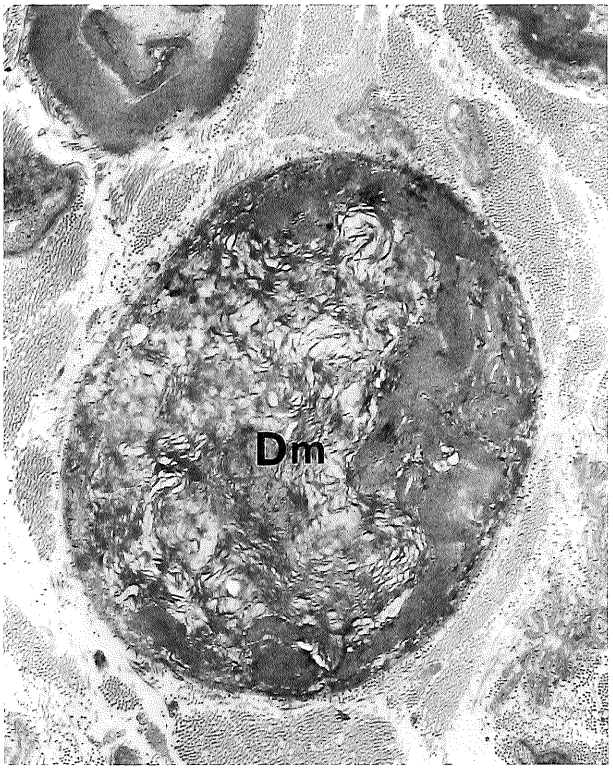

Fig. 9. All layers of the myelin sheath (Dm) are degenerated with marked disorganization of the lamellar structure. The nerve axon can not be seen in this severely damaged nerve fiber. Case No. 6 (BL), ×6,400

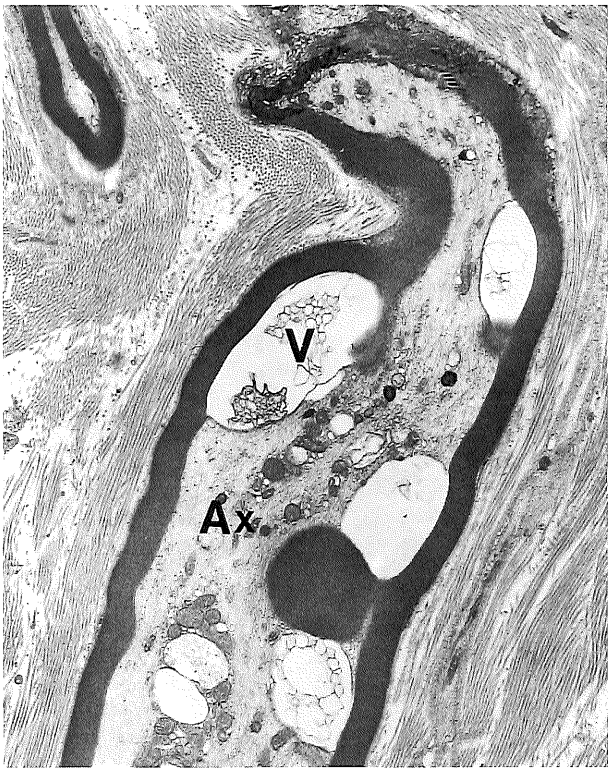

Fig. 10. Vacuolar changes (V) can be seen in the axoplasm (Ax) of a myelinated nerve fiber. Case No. 6 (BL), ×6,000

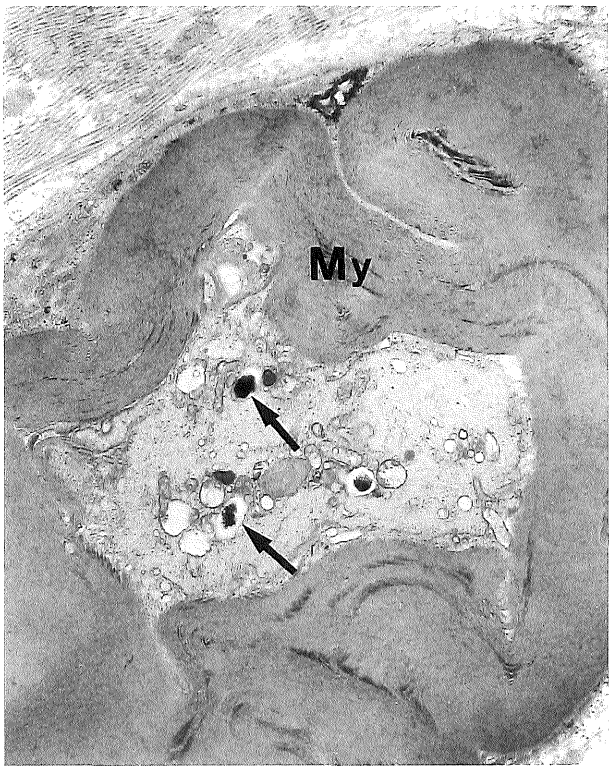

Fig. 11. Electron dense materials (arrows) are also found in the axoplasm of the myelinated nerve fiber. My: myelin sheath. Case No. 9 (BL), ×8,000

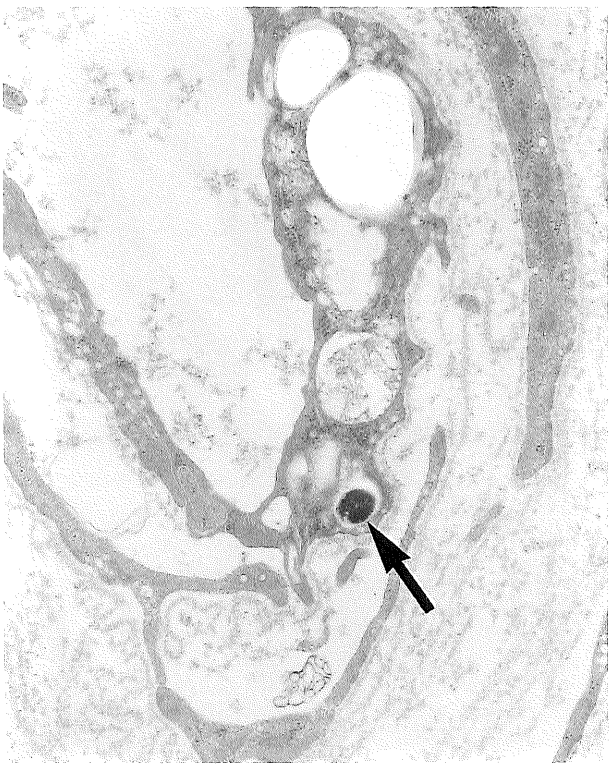

Fig. 12. Vascular endothelial cells frequently contain electron dense materials (arrow). Case No. 5 (LL), ×8,000

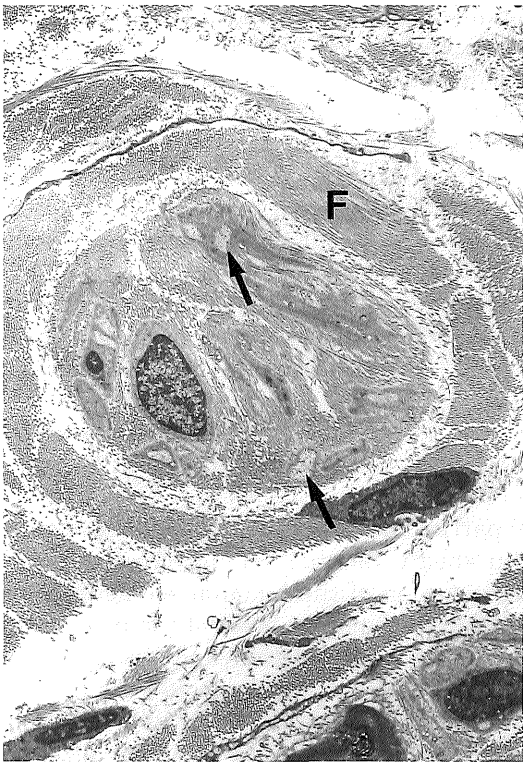

Fig. 13. Marked endoneurial fibrosis (F) is seen in a patient with a long duration of disease. The collagen fibers showed a concentric arrangement surrounding the central unmyelinated nerve fibers (arrows). Case No. 10 (BL), ×3,700

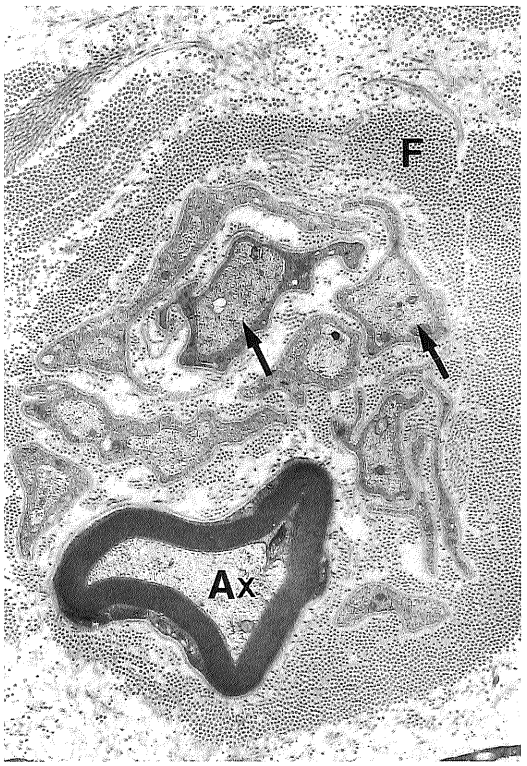

Fig. 14. Unmyelinated nerve axons (arrows) surrounded by the cytoplasmic processes of Schwann cells are abundant in the periaxonal fibrotic areas. Ax: axoplasm of myelinated nerve fiber. Case 10 (BL), ×8,000

sheath was completely destroyed (Fig. 9). All lamellar elements were disorganized and conglomerated in these myelinated fibers with the disappearance of the axoplasm.

The axoplasm of the myelinated fibers was frequently vacuolated and contained an increasing number of lysosomes (Fig. 10). In rare instances, small dense materials like those observed in the histiocyte and Schwann cell cytoplasm were found in the axonal cytoplasm of the myelinated nerve (Fig. 11). In addition, the small dense materials considered to be degenerated mycobacteria were also found in the cytoplasm of the vascular endothelial cells and pericytes (Fig. 12).

Endoneurial fibrosis was found in various degrees in all LL and BL patients. In patients with a long duration of the disease, endoneurial fibrosis was remarkable and almost all the myelinated nerve fibers had disappeared. As shown in Fig. 13, however, collagen fiber formation was frequently concentric with several layers. Nerve axons surrounded by Schwann cell cytpolasm could be found only in the central core of the fibrotic area. These unmyelinated nerve fibers were regenerative branches (sprouts) of remaining nerve fibers. Small myelinated nerve fibers were occasionally recognized near the regenerative unmyelinated fibers in the fibrotic areas (Fig. 14). In the cases having marked endoneurial fibrosis, it was very difficult to find mycobacteria in the Schwann cell cytoplasm surrounding the nerve axons.

DISCUSSION

It is now well accepted by many investigators that the clinical pattern of leprosy depends on the immune response of the individual against mycobacterium leprae[4,9,13,18,23]. Lepromatous leprosy (LL) appears to occur in patients without cell-mediated immunity for the mycobacterium, and tuberculoid leprosy (TT) in patients with well developed immunity. Between these two types of leprosy, there are several types of borderline leprosy: borderline lepromatous (BL), borderline borderline (BB) and borderline tuberculoid (BT). Meyers and his co-workers (1988)[14] reported the fact that there were changes in classification toward the tuberculoid end (TT) of the leprosy spectrum in patients initially classified as LL or as BL with treatment of a mixture of autoclaved mycobacterium leprae and BCG.

In our present study, cutaneous branches of radial nerves were obtained by biopsy from leprosy patients (LL and BL) with morbidity of various durations and observed by light and electron microscopy.

A variable number of foamy cells was found in the endoneurium and perineurium as well as in

the epineurium of nerve fibers obtained from patients with LL and BL. However, the number of foamy cells did not show a distinct difference between patients with LL and BL. In the endoneurium, the foamy cells were accumulated predominantly in the perivascular and subperineural areas. By electron microscopy, numerous dense materials, irregular in size and shape, were recognized in the cytoplasmic vacuoles of the foamy macrophages. Vacuolated Schwann cells also contained same dense materials in the cytoplasmic vacuoles. These dense materials had the same appearance as those reported by Dastur and his co-workers (1973)[3] and were considered to be degenerated and fragmented M. leprae. In our present study, it was very difficult to find intact bacilli either in the Schwan cells, or in the macrophages. The degeneration and destruction of M. leprae might have resulted from treatment by anti-mycobacterial drugs or cell-mediated immune reaction. Haimanot and his co-workers (1984)[8] reported that 3 of 8 LL and BL patients with a long duration of therapy showed few or no mycobacteria in the tissue sections of the skin and nerve. In our cases, mycobacteria were also difficult to identify in the foamy cells by acid fast staining. These results indicate that the degenerated and fragmented mycobacteria are deprived of the character of acid-fastness.

It is now well known that M. leprae have an affinity to the Schwann cells although the entry route of the M. leprae into the nerve fibers still remains uncertain. As has been reported by Pearson and Ross (1975)[16], however, three possible ways were considered for the entry of mycobacteria into the nerve fibers: (1) by phagocytosis of Schwann cells in the upper dermis, (2) by penetration of the perineurium, and (3) via the endoneurial blood vessels. Another idea put forward has been that the infection of M. leprae would be initiated in dermal nerve endings with proximal spread by Schwann cell infection or by transaxonal transport[1]. Finlayson and his co-workers (1974)[6] previously reported that Schwann cell infection by mycobacteria led to extensive focal myelin breakdown and severe fibrosis with axonal loss, and concluded that there was no evidence of significant bacterial transport within the nerve fibers or in the extracellular space. On the other hand, Dastur and his co-worker (1973)[3] demonstrated that the mycobacteria proliferated in the cytoplasm of the Schwann cells and vascular endothelial cells, and suggested that both a Schwann cell passage as well as a vascular dissemination of the bacilli probably occur in all types of leprosy. In rare instances, fragments of degenerated M. leprae have been found in the axoplasm[7,11]. Job (1970)[11] demonstrated that the mycobacteria found in the axoplasm were surrounded by a double membrane, and suggested a possibility that the mycobacteria were in tongues of the Schwann cell cytoplasm invaginating into the axoplasm. Although the mode of entry of mycobacteria into the nerve axons is still uncertain, there is another possibility that M. leprae enter into the nerve axons following necrosis of infected Schwann cells and degradation of the myelin sheath. In our present study, the endothelial cells of endoneurial and perineurial vessels frequently contained mycobacterial fragments. In addition, macrophages containing degenerated M. leprae accumulated around the endoneurial blood vessels. These findings suggest that M. leprae enter into the endoneurium via blood vessels, and thus support the third hypothesis of Pearson and Ross (1975)[6].

Of particular interest is the morphogenesis of myelin sheath destruction. In our present study, disorganization of the lamellar elements was first recognized in the outer layer of the myelin sheath, following the degeneration and disappearance of the Schwann cell. Axoplasm could not be found in the myelinated nerve fibers in which the myelin sheath was completely disorganized and destroyed. These findings indicate that the myelin sheath destruction initiated from degeneration and necrosis of the Schwann cell and progressed from the outer to inner layer with loss of the nerve axon. This type of myelin destruction was different from that in Wallerian degeneration of peripheral nerves.

A decrease in the number of myelinated nerve fibers and endoneurial fibrosis were distinct in the peripheral nerve of all leprosy patients[10,12,16]. Each surviving myelinated and unmyelinated nerve fiber was usually surrounded by a thick layer of collagen fibers. By electron microscopy, many small unmyelinated nerve fibers, irregular in size and shape, were found in a thick layer of collagen fibrils surrounding a myelinated nerve fiber. These small nerve axons surrounded by the Schwann cell cytoplasm were considered to be newly formed, regenerated nerve axons. Lamellar and concentric periaxonal fibrosis was frequently observed in the case of severe endoneurial fibrosis. However, regenerated nerve axons were found only in the core fibrotic area. Mycobacteria could not be found in these severe fibrotic areas. These findings suggest that the newly formed nerve fibers were repeatedly damaged without the existence of mycobacterium leprae.

The pathogenesis of the concentric periaxonal fibrosis is still unclear. It has been repeatedly reported by many investigators that the Schwann cell is a target cell of M. leprae and easily infected by these bacilli[2,11,12,16,17,25]. It is obvious that the degeneration and destruction of the Schwann cells by infection of bacilli are followed by destruction of the myelin sheath and periaxonal inflammation. The periaxonal inflammation might be a cause of

proliferation of collagen fibers, i.e., periaxonal fibrosis. On the other hand, Shetty and his co-workers (1985)[13] previously reported that a patchy segmental demyelination of the peripheral nerve fiber was induced in mice by subcutaneous injection of whole serum of leprosy patients (BT, BL). They also demonstrated that normal mouse nerve extract with live and killed M. leprae produced degenerative changes of the non-myelinated fibers in the mouse nerve. These facts indicate that the M. leprae has also an adjuvant like activity. Thomas & Mukherjee (1990)[24] reported that all 258 sera of leprosy patients showed positive for antineural IgG antibodies. On the other hand, Eustis Turf and his co-workers (1986)[5] showed 40% positivity in the sera of leprosy patients by means of indirect immunofluorescence. Desikan and his co-workers (1994)[23] recently demonstrated 9% positivity. These facts suggest that humoral immunity plays a role in the destruction of the nerve fibers and the pathogenesis of concentric periaxonal fibrosis in leprosy patients.

Inflammatory cells such as lymphocytes and plasma cells infiltrated to a greater or lesser extent in and around the nerve fibers. These inflammatory changes tended to be less intense in patients with a short duration of therapy than in those with long term therapy. However, there was no distinct difference in the intensity of inflammation between patients with LL and those with BL. Recently, some investigators reported that inflammatory cytokines such as TNF α were elevated in the serum of multibacillar leprosy patients suffering from neuritis as well as from reverse reaction (RR) or erythema nodosum leprosum (ENL)[21,22]. Moreover, CD3 and CD4 positive T lymphocytes accumulated in the inflammatory areas of ENL[21]. These findings indicate that the nerve fibers of leprosy patients might be damaged by such inflammatory cytokines as TNF α, but the role of cytokines in nerve fiber damage must be further investigated.

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