An Ultratsructural Study of Blood Vessels in Peripheral Nerves of Leprosy Patients: Blood Vessels in Peripheral Nerves

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Leprosy is a chronic infectious disease caused by Mycobacterium leprae affecting the skin and nerves. Histologically, nerves in the tuberculoid leprosy are infiltrated by paucibacillary epithelioid cell granulomas containing large numbers of lymphocytes while the nerves of lepromatous leprosy comprise of macrophages loaded with M. leprae along with some lymphocytes diffusely distributed among them (1). The lymphocytes and macrophages infiltrating the nerve granulomas have been characterized (2, 3). Besides the macrophages, in the lepromatous nerves Schwann cells also contain M. leprae and Schwann cell degeneration has also been documented (4, 5, 6, 7). M. leprae organisms have been found in the endothelial cells of cutaneous blood vessels of lepromatous leprosy suggesting the involvement of blood vessels the pathogenesis of lesions (8, 13). However not much information is available on blood vessels in nerves in leprosy. For example, a marked invasion of blood vessels as a possible cause of nerve damage in leprosy has been suggested (9, 10, 11, 12). In the present communication, ultrastructure of endoneural blood vessels in the tuberculoid and lepromatous leprosy has been carried out to understand some of the mechanisms leading to nerve damage in leprosy.

MATERIALS AND METHODS

Reagents used: Glutaraldehyde; Spurr resin (TAAB Laboratory Equipment, UK); Osmium tetraoxide (John Matthey Chemicals Ltd., UK); Phosphate buffered saline (PBS) pH 7.2; 1% Toludine blue, Uranyl acetate (E Merck, India); Lead citrate.

Nerve Biopsies: Six patients of leprosy (3 BT/TT & 3 LL) have been studied. The patients were selected from the out-patient clinic of the Institute. Peripheral nerves from these patients were

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<th>Type</th>
<th>Duration</th>
<th>Status</th>
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<tr>
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<td>BT</td>
<td>3 months</td>
<td>Thickened</td>
</tr>
<tr>
<td>PW</td>
<td>BT</td>
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<tr>
<td>VR</td>
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<td>6 months</td>
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<tr>
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<td>LL</td>
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<td>Thickened</td>
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<tr>
<td>PR</td>
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</tr>
<tr>
<td>NA</td>
<td>LL</td>
<td>2.5 years</td>
<td>Thickened</td>
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Table 1 Selection of patients
biopsied (Table 1). One part of the biopsy specimen was fixed in formal-Zenker's fluid and processed for histopathology. The other part was used for ultrastructural studies.

**Tissue Preparation for Electron microscopy:** Small pieces of the biopsy were fixed overnight in cold 2.5% glutaraldehyde in PBS. Subsequently, the tissue was washed in PBS, post fixed in 1% osmium tetroxide and again washed in PBS. The tissues were dehydrated in different grades of alcohol (30%, 70%, 80%, 90% & 95%, absolute alcohol and propylene oxide). The tissues were subsequently embedded in spurr's resin at 4°C and blocks were made at 70°C. Ultrathin sections were cut in an ultramicrotome (MT2, Porter blum, USA), and stained with uranyl acetate & lead citrate. The sections were observed in an electron microscope H-300 (6).

**RESULTS**

Nerve biopsies from six patients of leprosy (3 BT/TT BI=0 & 3LL BI=3+) classified as per Ridley-Jopling criteria were included in the study.

**Tuberculoid Leprosy (BT/TT):** Semithin sections of peripheral nerves of tuberculoid leprosy stained with toluidine blue showed the presence of endoneural blood vessels and myelinated fibres.

![Fig. 1 Semithin section of peripheral nerve of BT leprosy showing endoneural blood vessels (BV) and myelinated fibres (MF). Toluidine blue stain (original X 800).](image1)

Ultrastructural examination of endoneural blood vessels revealed the hypertrophy of endothelial cells with enlarged nucleus (Fig 2 & 3).

![Fig. 2 Ultrathin section of peripheral nerve of BT leprosy showing the multilayering and finger like protrusions of the basement membrane (BM) of endothelial cells (EC). Note closed lumen (L) of blood vessels and the hypertrophy of EC. Uranyl acetate and Lead citrate (original X 10,000).](image2)
These cells were partly separated from the neighbouring cells and were extending into the lumen, and lumen of blood vessel was often closed. The basement membrane of endothelial cells contained many folds and finger-like protrusions.

**Lepromatous Leprosy (LL):** In contrast, the endoneural blood vessels of lepromatous nerves had an open lumen Fig. 4, 4a, 4b, & 5.
Though these cells did not show hypertrophy, still they contained finger like protrusions. Many *M. leprae* organisms were seen inside the endothelial cells. The bacilli which appeared intact were surrounded by electron transparent zone. It also appeared that the membrane of endothelial cells ruptured into the lumen allowing the mycobacteria to move freely into circulation where they may be phagocytosed by monocytes.

**DISCUSSION**

Nerve involvement and damage is one of the primary manifestation of leprosy (1). Invasion of endoneural blood vessels by the organism could possibly be a contributing factor towards nerve involvement and damage in leprosy. The endoneural blood vessels play a vital role in the supply of nutrients to the nerves and in the maintaining of normal Schwann cell metabolism and normal functional status of nerve (3,12). Hence the study of endoneural blood vessels in leprosy becomes important. The morphology and characteristics of endoneural blood vessels and the bacteria inside the endothelial cells cannot be defined by light microscopy satisfactorily and needs study by ultrastructural techniques. In the present communication, we report our observations on the ultrastructural changes in the blood vessels of nerves of patients with tuberculoid and lepromatous leprosy.
Semithin sections of peripheral nerves stained with toluidine blue showed the presence of endoneural blood vessels and myelinated fibres. Ultrastructural examination of these endoneural blood vessels showed hypertrophy of endothelial cells with many folds and finger like protrusions in the basement membrane. The hypertrophied endothelial cells had also blocked the lumen of these vessels hampering the endoneural blood circulation. This could affect the nutrition and metabolism of the nerve. Observation similar to ours have been recorded in tuberculoid nerves by other workers (8). In contrast, blood vessels of lepromatous nerves had open lumen and there was no hypertrophy of endothelial cells. The endothelial cells contained many M. leprae organisms. These organisms appeared to be intact and so were probably viable. Further the membrane of endothelial cells may rupture into the lumen thus releasing bacilli freely into circulation. Other workers have demonstrated similar ultrastructural changes in lepromatous leprosy and also the presence of M. leprae in the endothelial cells (4,5,9,10,11,13).

It is possible that immunological mechanisms may also play some role in causing damage to the blood vessels as noticed by ultrastructure. In this context, it has been documented that high proportions of activated T cells are found in the dermal and neural granulomas of tuberculoid leprosy. They were predominantly CD4+ cells which were diffusely distributed into granuloma and also surrounding blood vessels. Further, it has been found that the endothelial cells expressed HLA DR antigens (2). Endothelial cells have also been shown to be involved in antigen presentation (14). This then may lead to an effective immunological interaction between T lymphocytes and endothelial cells possible resulting in the damage of blood vessels and killing of bacilli as observed in tuberculoid leprosy. In comparison, in lepromatous leprosy since there is breakdown of endothelial cells containing M. leprae, the bacilli moves freely into circulation. Here they may be phagocytosed by monocytes. As there is a constant exchange of cells from blood to tissues, the nerves may be infiltrated by macrophages containing bacilli resulting in the formation of macrophage granulomas in the nerves and thus contributing to its pathogenesis. The bacilli however are not destroyed as there is a paucity of T cells in the granulomas of these nerves even though the endothelial cells are HLA DR positive (2).

**SUMMARY**

Ultrathin sections of nerves of tuberculoid and lepromatous leprosy were examined in an electron microscope for changes in endoneural blood vessels. In the tuberculoid nerves, hypertrophy of endothelial cells was the most prominent feature. This was to such an extent that the lumen of blood vessel was often closed. Endoneural blood vessels showed multilayers seperated by collagen and ground substances. In contrast, in the lepromatous nerves, there was no hypertrophy of endothelial cells in the blood vessels and the lumen of the vessels was open. M. leprae were seen within the endothelial cells and these organisms were intact and probably viable. These observations suggest a possible involvement of endoneural blood vessels which may contribute to nerve damage in leprosy.

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