

An Electron Microscopic Study of Lymphatics in the Dermal Lesions of Human Leprosy¹

Ashok Mukherjee, Radhey S. Misra, and Wayne M. Meyers²

Involvement of regional lymph nodes in both lepromatous and tuberculoid leprosy is seen quite frequently (^{4, 5, 9, 10, 14}). The histopathological changes in lymph node lesions are similar to those in the skin (⁴). While these observations indicate a regular flow of bacterial antigens/bacteria through the lymphatic system, there is as yet no description of the changes in the dermal lymphatic capillary network around the granuloma. The present study describes the fine structural changes occurring in these vessels in leprosy lesions.

MATERIALS AND METHODS

Skin biopsies were collected from eight lepromatous (LL and BL) and eight tuberculoid (TT and BT) leprosy patients. The tissues were immediately fixed in Trump's fixative (4% formal-1% glutaraldehyde in phosphate buffer, pH 7.2) for 24 hr and then processed for embedding in Spurr's resin after postfixing in 1% osmium tetroxide. Initially, 1- μ m semithin sections were cut and stained with 1% toluidine blue in borax for scanning and identification of sections with lymphatic vessels. Selected blocks were then final trimmed, and ultrathin sections (gold to silver) were cut using glass knives and double contrasted with uranyl acetate and Reynold's lead citrate. Finally, the sections were examined under a JEOL 100 CXII electron microscope.

The patients included in the study were attending the Leprosy Clinic at the Department of Dermatology, Safdarjang Hospital, New Delhi, India. There was no history of previous treatment for leprosy in any of the

patients. None of the cases, lepromatous or tuberculoid, showed any features of type 1 or type 2 reactions at the time the biopsy was taken.

RESULTS

Light-microscopic examination of the 1- μ m semithin sections showed that initial lymphatics could definitely be identified in the subpapillary dermis in these sections. The lymphatics had irregularly shaped lumina and thin walls consisting of a single layer of endothelial cells without a well-defined basement membrane or pericytes being seen around them (Fig. 1). Using these criteria, lymphatics were identified within the granuloma and in the dermal collagen around the granulomas in the lepromatous lesions. In the tuberculoid lesions, the lymphatics were identified only in the peri-granulomatous areas. In both groups, lymphatics in the sections were predominantly located on the edge of the granuloma. The lumen was seen in all of these lymphatics, and appeared to be dilated in some of them.

Electron microscopy of ultrathin sections cut from the same blocks confirmed that the structures were lymphatic vessels. In the lepromatous lesions, the lymphatic vessels usually had slit-like lumina. The lining cell cytoplasm was thin along the length of the vessel, while at the corners the cytoplasmic mass increased and numerous slender processes projected into the lumen. Mitochondria, lysosomes, lipid droplets, pinocytotic vesicles, and rough endoplasmic reticulum formed the bulk of the cytoplasm in these areas (Figs. 2 and 4). Large lipid droplets were present in many lymphatic lining cells (Fig. 2) and also occasionally in cells lying in the lumen of the lymphatic vessel. Lymphatic endothelial cells with intra-cytoplasmic bacilli were rare, seen in only one of the seven lepromatous biopsy specimens (Fig. 4). In another lepromatous patient, a histiocyte with a bacillus in its cytoplasm was seen in the lymphatic lumen (Fig. 3).

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² A. Mukherjee, M.D., Assistant Director, Institute of Pathology-I.C.M.R., P.O. Box 4909, Safdarjang Hospital, New Delhi 110029; R. S. Misra, M.D., Dermatologist, Safdarjang Hospital, New Delhi 110029, India. W. M. Meyers, M.D., Ph.D., Chief, Division of Microbiology, Armed Forces Institute of Pathology, Washington, D.C. 20306, U.S.A.

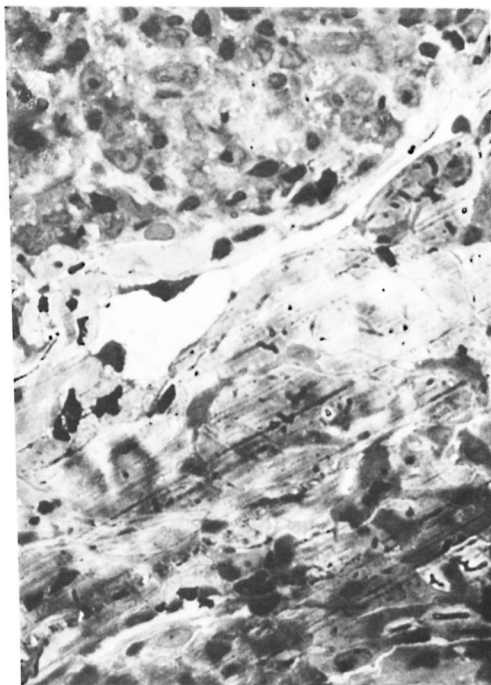


FIG. 1. Photomicrograph of lymphatic vessel adjacent to dermal granuloma in BT leprosy (2 μ m Epon-embedded section stained with toluidine blue $\times 1000$).

Lymphocytes (Fig. 2) and histiocytes (Fig. 3) lying beneath the lining cell or in the intercellular junctional areas between two lymphatic endothelial cells were frequently observed in all specimens. Intact elastic fibers (Fig. 3) and anchoring filaments (Fig. 6) were identified along the abluminal aspect of the lining cells in all of the peri-granulomatous and in some of the intra-granulomatous lymphatics.

The tuberculoid lesions showed identifiable lymphatic vessels in the peri-granulomatous collagen only. Here, too, some vessels had slit-like lumina (Fig. 5), while others showed dilated and irregularly shaped lumina. The lymphatic endothelial cells in these lesions had fewer cytoplasmic organelles than those seen in lepromatous lesions. Lipid vacuoles were absent, and lysosomes were only seen in occasional cells. Lymphocytes traversing the lymphatic endothelium were frequently seen however. Bacilli were not found in the lymphatic endothelial cells in any of the eight tuberculoid cases studied.



FIG. 2. Electron micrograph of lymphatic vessel from LL lesion. Lipid droplets (L), mitochondria and lysosome are seen in lymphatic endothelial cell cytoplasm; lymphocyte (Ly) traversing lymphatic wall; *M. leprae* in lower left corner ($\times 14,000$).

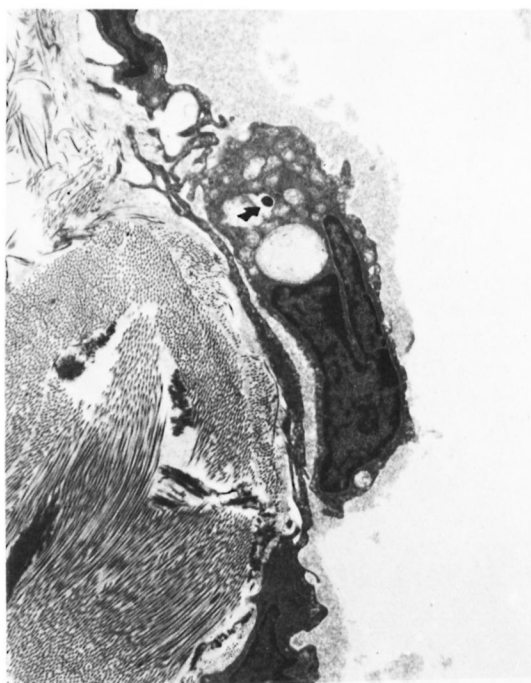


FIG. 3. Electron micrograph of a lymphatic vessel from a lesion of lepromatous leprosy (LL). A macrophage containing a bacillus (◆) in its cytoplasm lies in the lymphatic lumen just over a point of overlap of two adjoining lymphatic endothelial cells. Elastic fibers lying in the immediate perilymphatic area are also seen ($\times 9000$).



FIG. 4. Electron micrograph of a lymphatic lining cell in a lesion of lepromatous leprosy (LL) showing a uniformly stained bacillus (◆) with an electron transparent zone around it lying in a cytoplasmic vacuole. Degenerated organisms (▶▶) are also seen in the same cell. L = vessel lumen ($\times 23,400$).

DISCUSSION

This study demonstrates an actively functioning terminal lymphatic system in the cutaneous lesions of leprosy, as shown by activated and hypertrophied lymphatic endothelial cells, the presence of lipid vacuoles, and movement of inflammatory cells across the lymphatic wall.

The involvement of the lymphatic system in the clearance of lipidic material from the granuloma, especially in the bacilliferous lesion, is of importance in view of the fact that lipidic antigens of *Mycobacterium leprae* have been shown to be involved in immunostimulation (¹). The movement of cells, both lymphocytes and histiocytes, through the lymphatic system is a regular function of the skin lymphatics. The large number of cells between lymphatic endothelial cells in both tuberculoid and lepromatous cases further emphasizes the con-

tinued and increased functioning of the skin lymphatics in leprosy.

Surprisingly, only a few of the lymphatic endothelial cells in the lepromatous lesions contained bacilli in their cytoplasm. Vascular endothelial cells, on the other hand, were much more heavily bacillated in all eight cases. Earlier reports (^{2, 3, 12, 15}) have shown consistent endothelial cell bacillation in blood capillaries in and around lesions. It seems that the blood vascular endothelial cell offers a better environment for bacillary growth than the lymphatic endothelial cell. The higher pO_2 in the vascular endothelial cells may be a factor responsible for better growth of the microaerophilic *M. leprae*. Thus, compared to the blood vascular system, the lymphatic pathway seems a minor route for dissemination of *M. leprae*.

The few reports on the subject of skin lymphatics (^{7, 8, 11, 13}) indicate that elastic fi-

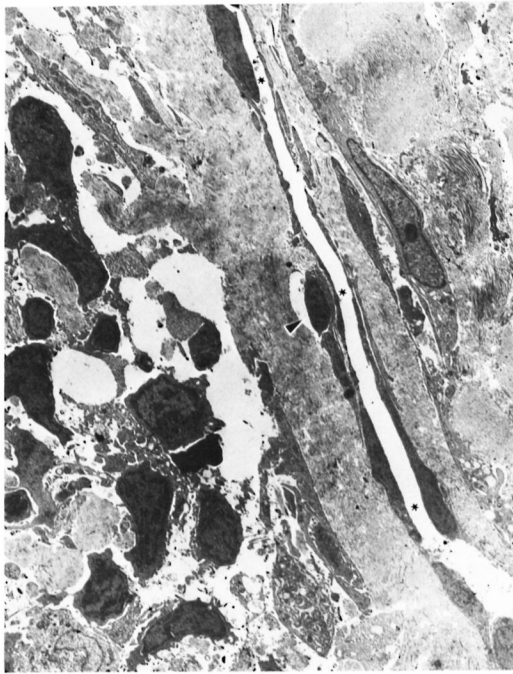


FIG. 5. Electron micrograph of a peri-granulomatous lymphatic (* marks the vessel lumen) from a lesion of borderline tuberculoid (BT) leprosy. Lining cell is indented by a lymphocyte (▶) crossing the lymphatic wall. Cells comprising the granuloma are seen to the left and below the lymphatic vessel ($\times 2200$).



FIG. 6. Electron micrograph of a lymphatic lining cell from a lesion of lepromatous leprosy (LL). Cytoplasm contains numerous pinocytotic vesicles and abluminal surface of the cell shows a sheaf of anchoring filaments in continuation with the plasma membrane ($\times 35,600$).

bers and anchoring filaments are of primary importance in the functioning of the terminal lymphatic vessels. Both structures were seen in the lymphatics in the peri-granulomatous areas and in a few of the intra-granulomatous lymphatics in lepromatous lesions. The absence of intra-granulomatous lymphatics in tuberculoid lesions is of interest. It may be noted in this connection that in tuberculoid granulomas the reticulin framework is of an open type with large gaps toward the center of the granuloma, while in lepromatous lesions the reticulin fibers are more uniformly distributed throughout the lesion (6). The absence of the reticulin framework in the inner parts of the granuloma along with the destruction of the elastic fibers could lead to the disappearance of the intra-granulomatous lymphatics in the tuberculoid cases. The lymphatic function, however, continues to be maintained by the vessels at the granuloma edge which show movement of inflammatory cells through them.

SUMMARY

The dermal lymphatic vessels in lepromatous and tuberculoid leprosy lesions were studied by light- and electron-microscopy. In the lepromatous patient, lymphatic vessels were seen in both intra- and peri-granulomatous areas. The lymphatic lining cells contained lipid droplets, lysosomes, and numerous pinocytotic vesicles. Cells bearing bacilli were only occasionally seen. In the tuberculoid cases, lymphatic vessels were seen only along the edges of the granulomas and the lining cells were less prominent. Inflammatory cells, both lymphocytes and histiocytes, were found traversing the walls of lymphatic vessels in both groups of patients. The results of the study confirm the continued and increased functioning of the lymphatic drainage system in dermal leprosy lesions, and indicates that it may be a major route for the clearance of lipids from the lipid-rich bacilliferous lesions in the lepromatous patient. The lymphatic pathway

appears to be a minor pathway for the dissemination of *Mycobacterium leprae* in comparison with the blood vascular system.

RESUMEN

Utilizando las microscopías de luz y electrónica, se estudiaron los vasos linfáticos dérmicos en las lesiones de la lepra lepromatosa y tuberculoide. En las lesiones lepromatosas se observaron vasos linfáticos en las áreas intra- y perigranulomatosas. Las células de revestimiento linfático contuvieron gotas de lípidos, lisosomas, y numerosas vesículas pinocíticas. Sólo ocasionalmente se observaron células con bacilos. En los casos tuberculoideos, los vasos linfáticos sólo se observaron en los bordes de los granulomas y las células de revestimiento fueron menos prominentes. En ambos grupos de pacientes se observaron células inflamatorias (linfocitos e histiocitos) atravesando las paredes de los vasos linfáticos. Los resultados del estudio confirman el continuo funcionamiento del sistema de drenaje linfático en las lesiones de la lepra, e indican que ésta puede ser una importante ruta de depuración de los lípidos de las lesiones lepromatosas bacilíferas ricas en lípidos. Comparado con el sistema vascular sanguíneo, el sistema linfático representa sólo una ruta menor de disseminación del *Mycobacterium leprae*.

RÉSUMÉ

On a étudié par la microscopie optique et par la microscopie électronique les vaisseaux lymphatiques du derme dans des lésions de lèpre lépromateuse et de lèpre tuberculoïde. Chez le malade lépromateux, on a observé des vaisseaux de lymphatiques tant dans les zones intragranulomateuses que dans les zones périgranulomateuses. Les cellules du revêtement lymphatique contenaient des gouttelettes de lipide, des lysosomes, et de nombreuses vésicules pinocytotiques. Ce n'est que rarement qu'on a relevé la présence de cellules contenant des bacilles. Dans les cas de lèpre tuberculoïde, on a noté la présence de vaisseaux lymphatiques seulement le long des bords des granulomes; les cellules de revêtement étaient moins visibles. Dans les deux groupes de malades, des cellules inflammatoires, consistant en lymphocytes et histiocytes, traversaient les parois des vaisseaux lymphatiques. Les résultats de cette étude confirment la persistance et l'augmentation du fonctionnement du système de drainage lymphatique dans les lésions dermiques de la lèpre; ils indiquent également que ce système pourrait constituer chez le malade lépromateux une voie importante pour l'évacuation des lipides des lésions bacillifères. Le réseau lymphatique semble jouer un rôle moindre que le système vasculaire sanguin dans la dissémination de *Mycobacterium leprae*.

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