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Ultrastructure of Leprous Phlebitis[1]

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Blood vessel involvement and endothelial bacillation in leprosy was initially noted by Joelson in 1899 ([8]) and described in detail in the works of Fite ([6]) and Mitsuda ([11]). More recently Coruh and McDougall ([4]) in a light microscopic study showed the constant presence of bacilli in dermal capillary endothelial cells in untreated lepromatous leprosy (LL) patients. At the ultrastructural level, Boddingius ([2]) demonstrated bacilli in the neural capillary endothelium; while Turkel, *et al.* ([15]) found bacilli in the endothelial cells in 5 out of 6 LL patients in an ultrastructural study of the dermal microvasculature around the leprosy granulomas. Other features noted by Turkel, *et al.* were the swelling of capillary endothelial cells, the reduplication of the basal lamina, and evidence of increased pinocytotic vesicle formation, all of which were interpreted as nonspecific changes of a chronic inflammatory state.

Our earlier studies ([12, 13]) on lesions in the subcutaneous medium-sized veins in LL patients showed specific venous involvement in 33 out of 36 LL patients. The venous lesion was characterized by a panphlebitis with predominant intimal involvement. Early lesions showed mild intimal thickening and foamy transformation of the lumen lining cells; while in later lesions the lumen was compromised by a progressive increase in intimal thickness, ultimately leading to complete lumen occlusion.

Bacilli were seen in the intimal lumen lining cells, the macrophages in the infiltrate, and in occasional smooth muscle cells. This histological report is now followed by an ultrastructural study of the venous lesions in lepromatous leprosy with particular reference to the changes seen in the bacillated cells.

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MATERIALS AND METHODS

Six LL patients, 3 males and 3 females between the ages of 35 and 55 years attending the Safdarjang Hospital Leprosy Clinic, were included in the present study. The duration of disease ranged from 6 months to 5 years, and no patient had any history of previous treatment for leprosy. One of the patients had a history of a recent episode of erythema nodosum leprosum (ENL). Clinically obvious phlebitis ([12]) not being seen in any of the patients, one of the subcutaneous veins on the forearm or dorsum of the hand was selected for biopsy and a 1 cm long vein segment along with a piece of skin was resected under local anesthesia. After immediate transfer to 4% Formol glutaraldehyde ([10]), the vein segment was cut open along its long axis, laid flat, and diced into 1–2 mm cubes under a dissecting microscope such that each cube included a portion of the intima. Following primary fixation for 6 hr in Formol glutaraldehyde, the tissues were post-fixed in 1% OsO_4 for 1 hr at 4°C, dehydrated through ethanol, and embedded in Araldite after clearing in propylene oxide. Semithin 1 μm sections were cut and stained with toluidine blue and blocks with adequate portions of intima selected. Ultrathin sections, cut with glass knives from the selected blocks, were double-stained with lead citrate and uranyl acetate and viewed under a JEOL 100 CX II electron microscope at 80 kV.

RESULTS

A light-microscopic examination of 1 μm plastic sections showed changes of early (Grade I) leprous phlebitis in all six cases. Bacilli were seen in the luminal cells in five cases, with two of these showing foamy transformation of the intimal cells. The adventitia was infiltrated by small lepromatous granulomas in all six cases, and the media showed bacilli in occasional smooth muscle cells in four patients. Luminal occlusion was less than 30% in all six patients.

On electron microscopy, the intimal layer was seen to consist of a single row of irreg-

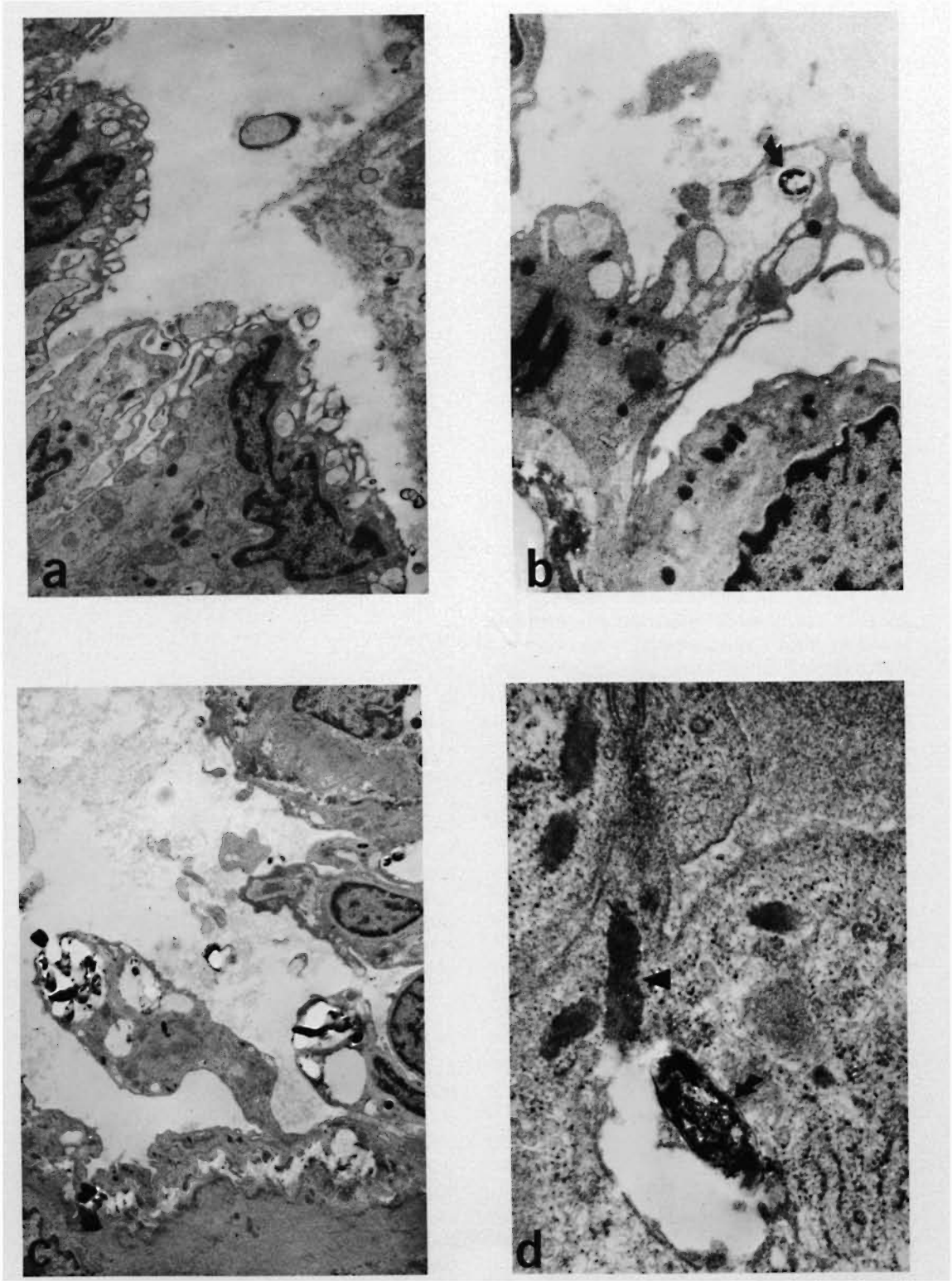

FIG. 1. Endothelial cells showing: **a)** increased pinocytotic vesicle formation along the luminal surface; **b)** formation of a pseudopod containing a single organism (⬟) on the luminal surface; **c)** a pseudopod containing a cluster of *M. leprae*; and **d)** the presence of Weibel-Palade (▲) bodies along with *M. leprae* (⬟) in the cytoplasm (original magnifications = **a)** ×11, 300; **b)** ×32,000; **c)** ×7,000; **d)** ×48,000).

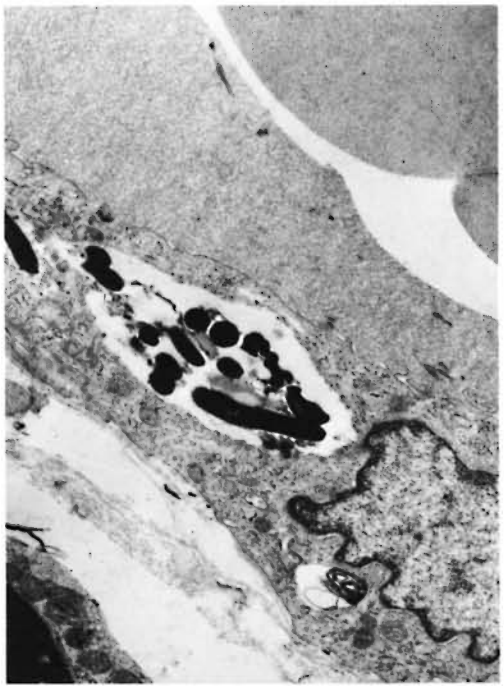

FIG. 2. An endothelial cell with a large cluster of *M. leprae* with a well-developed electron-transparent zone and membrane structures in the cytoplasm (×18, 300).

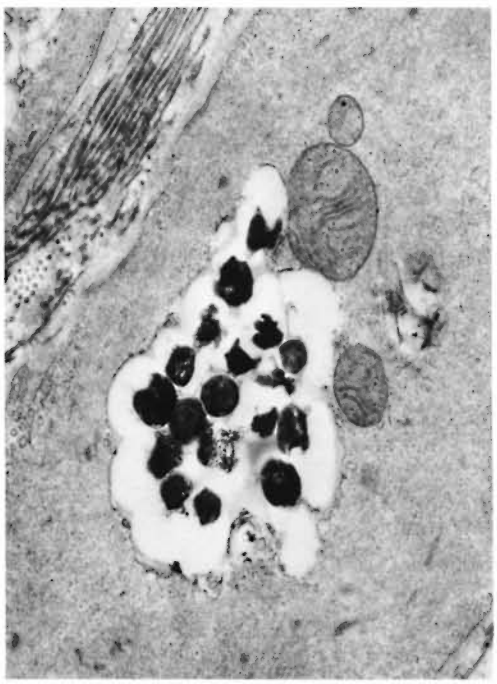

FIG. 3. Smooth muscle from the media containing a cluster of *M. leprae* with a well-developed electron-transparent zone (×19,000).

ularly cuboidal cells separated from the subjagent smooth muscle tissue by a layer of collagen and elastic fibers. The intimal cells showed an indistinct basement membrane on the abluminal aspect, while the luminal surface generally showed a few blunt projections and moderate pinocytotic vesicle formation. One of the biopsies, however, revealed markedly increased pinocytotic vesicle formation along the luminal aspect (Fig. 1a). The two cases with foamy transformation seen on light microscopy showed pseudopod formation on the intimal surface with either a single organism (Fig. 1b) or clusters of several organisms (Fig. 1c) at their ends. The cytoplasm of the intimal cells contained several mitochondria, pinocytotic vesicles, free ribosomes with a few heterophagosomes, multivesicular bodies, and stacks of 100 Å filaments running across the cell. The rough endoplasmic reticulum (RER) was sparse and seen in short chains, while the Golgi zone was poorly developed and could not be identified in many of the cells.

Tubular inclusions representing the specific endothelial cell bodies described by Weibel and Palade ([16]) were identified in several bacillated (Fig. 1d) and nonbacillated luminal cells.

In the five cases with bacillated endothelial cells, *Mycobacterium leprae* were seen in almost all of the endothelial cells in the sections studied. The number of organisms present in each cell varied from one organism (Fig. 1d) to clusters of several organisms (Fig. 2). Single organisms showed minimal or absent electron-transparent zones (ETZ) around them. The larger clusters (Fig. 2), however, had well-developed zones of electron-transparent material and membrane structures around the bacilli. Both solid and degenerated organisms were seen within the clusters. No lysosomes could be identified in any of the endothelial cells.

Bacilli were seen in the smooth muscle cells in 4 of the 5 cases with endothelial bacillation. Here also, the number of bacilli ranged from a solitary organism to multibacillary clusters (Fig. 3). However, the number of muscle cells with bacilli were few in each case. The organisms were always found in the central or paranuclear part of the cell along with the mitochondria and RER, with the periphery of the cell being

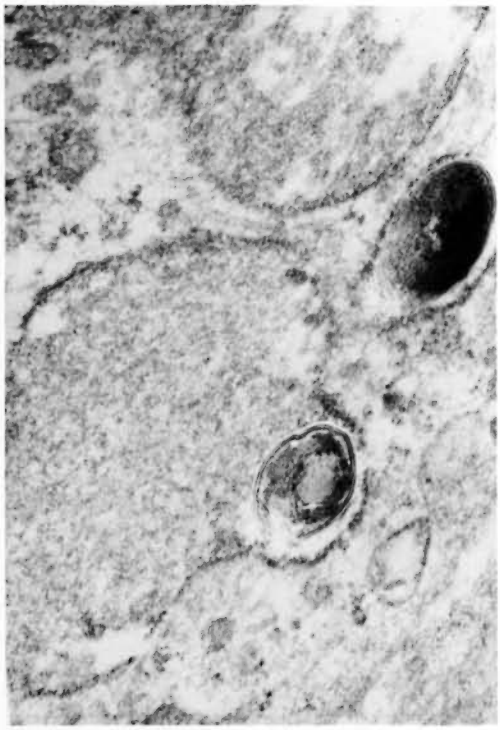

FIG. 4. *M. leprae* with phagolysosome formation in a macrophage from the media (×60,000).

occupied by the myofilaments. Both solid and nonsolid forms were seen and the ETZ was well developed. An interesting feature was the consistent maintenance of the shape and internal arrangement of the muscle cells and integrity of all the intracytoplasmic organelles with no evidence of any reaction on the part of the muscle cell to the contained organisms.

Macrophages containing organisms were seen in the medial and the adventitial layers in two cases. In both, the bacillated macrophages had large irregular nuclei with prominent nucleoli, well-developed rough endoplasmic reticulum, and prominent Golgi zones. Organisms were present in membrane-bound vacuoles with the formation of secondary phagolysosomes (Fig. 4) in some of the vacuoles. In one case, a macrophage was seen penetrating the basement membrane and invaginating the abluminal plasma membrane of an endothelial cell. A concentric membranous body (Fig. 5) was seen in one of the plasma cells in the adventitia.

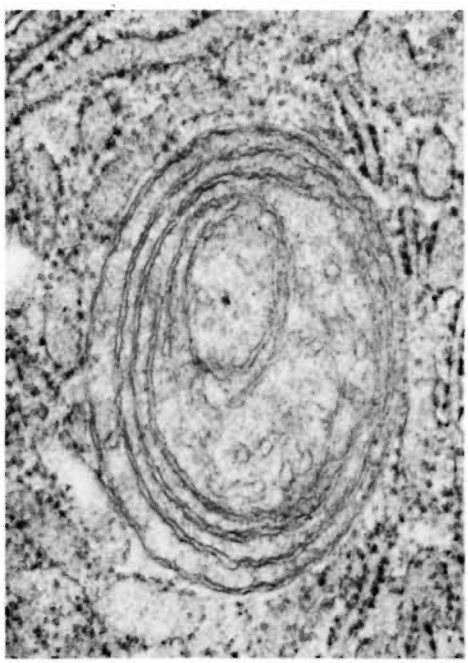

FIG. 5. Concentric membranous body surrounded by rough endoplasmic reticulum in a plasma cell in the adventitia (×64,000).

DISCUSSION

Most workers now accept hematogenous dissemination as the main mode of spread of the bacilli within the body in lepromatous leprosy. Leprosy, however, is an exception to the other systemic bacterial diseases like tuberculosis and syphilis inasmuch as bacilli can regularly be demonstrated in the blood ([5, 9]) and in the lumen lining cells of the blood vessels, particularly in the untreated LL patients. It was not clear, however, whether these bacillated cells were endothelial in origin or were derived from pavemented bacilli-laden monocytes from the blood. The presence of the specific endothelial cell granule—the Weibel-Palade ([16]) body—in these cells along with other characters, e.g., the formation of right junctions in overlap areas, the presence of multivesicular bodies, and stacks of 100 Å ([14]) filaments, quite clearly establishes the endothelial origin of the bacillated, lumen lining cells.

Persistent bacillemia and endothelial bacillation both indicate a regular process of release/uptake of bacilli from/to the blood. In fact, the formation of bacilli-filled pseu-

dopods on the luminal surface of the endothelial cells in two cases probably demonstrates the process of bacillary release into the vascular lumen. It is not clear, however, whether the bacilli are released by exteriorization of the bacilli-filled vacuole or by sequestration of the portion of the cell containing the vacuole by separation at the narrow neck of the pseudopod. This form of exophagocytosis does not seem to have been described in endothelial cells earlier ([3]), the usual mechanism of cellular transport in these cells being carried out through micropinocytotic vesicles. Similar bacilli-laden projections were seen extensively along the luminal surface in our earlier histopathological studies ([13]), indicating that the pseudopod formation may be one of the major modes of bacillary release.

The large numbers of *M. leprae* found in the endothelial cells and the fact that they can be seen as a single, solid bacilli as well as in clusters of several solid and nonsolid organisms indicate a process of growth and multiplication of *M. leprae* in the endothelial cell similar to that occurring in macrophages and Schwann cells. In this context, it is interesting to note two further considerations: a) no lysosomes were found in the endothelial or smooth muscle cells with bacilli, and b) although there is preliminary evidence ([1]) for the involvement of endothelial cells in antigen processing and T-cell activation, there is as yet no evidence that the endothelial or smooth muscle cell, or the growth of *M. leprae* in either cell, is influenced by any of the effector mechanisms of the cell-mediated immune system.

Bacillated muscle cells did not show any structural change or reaction to the bacilli present in them. Further, in our earlier histopathological studies ([13]) none of the muscle cells containing *M. leprae* had any inflammatory cells in their vicinity. These findings support the concept that the smooth muscle in the blood vessel wall and at other sites, e.g., the dartos and arrectores pilorum muscles, could be protected sites for the growth of "persister" organisms.

A well-developed, electron-transparent zone was seen around the larger bacillary clusters in all three types of cells studied. Of these, one was an activiated phagocytic cell; another, an endothelial cell with membrane changes and the third, a smooth muscle cell which showed no reaction to the presence of intracellular bacilli. The presence of a similar ETZ around the larger clusters in all three cell types once again supports the purely bacterial origin of this zone and indicates that its formation is independent of cellular reactions or defense mechanisms. Macrophages in the media and adventitia showed evidence of secondary phagolysosome formation around some *M. leprae* groups, although all of the cases were LL. This feature has been seen in several dermal-lesion macrophages also, and is strong evidence for the absence of an evident phagosome-lysosome fusion defect in lepromatous leprosy. An unusual finding was the presence of a concentric membranous body in a plasma cell in the media. This ultrastructural abnormality has been seen in a variety of conditions ([7]) but, to the best of our knowledge, does not seem to have been reported in human or experimental leprosy. It is believed to be due to aberrant RER development, and may be related to hyperfunction of the humoral immune system in LL.

SUMMARY

An ultrastructural study on vein biopsies from six lepromatous leprosy patients was carried out. The results showed that a) the lumen-lining bacillated cells were endothelial in origin due to the presence of specific Weibel-Palade endothelial cell granules; b) endothelial cells released *Mycobacterium leprae* into the lumen by exophagocytosis; c) *M. leprae* were able to grow and multiply in the endothelial and smooth muscle cells; and d) smooth muscle cells did not show any evidence of reaction due to the presence of *M. leprae* in their cytoplasm.

RESUMEN

Se hizo un estudio sobre la ultraestructura de las venas en biopsias de 6 pacientes lepromatosos. Los resultados mostraron que a) las células con bacilos que se encontraron recubriendo el lumen de los vasos fueron de orígen endotelial porque contuvieron gránulos de Weibel-Palade, especificos de las células endoteliales; b) las células endoteliales liberaron *Mycobacterium leprae* hacia la circulación venosa por un proceso de exofagocitosis; c) el *M. leprae* fue capaz de crecer y multiplicarse en las células endoteliales y en las células del músculo liso y d) las células del músculo liso no

mostraron ninguna evidencia de reacción debida a la presencia del *M. leprae* en su citoplasma.

RÉSUMÉ

On a mené une étude ultrastructurelle des biopsies veineuses de 6 malades atteints de lèpre lépromateuse. Les résultats ont montré a) l'origine endothéliale des cellules bacillaires qui tapissent la lumière des veines, par suite de la présence de granules cellulaires endothéliaux spécifiques du type Weibel-Palade; b) la libération de *Mycobacterium leprae* par les cellules endothéliales dans la lumière par un mécanisme d'exophagocytose; c) la capacité de *M. leprae* de croître et de se multiplier dans les cellules endothéliales et dans celles des muscles lisses; d) l'absence de tout signe d'une réaction due à la présence de *M. leprae* dans le cytoplasme des cellules des muscles lisses.

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