INTERNATIONAL JOURNAL OF LEPROSY Volume 50, Number 2
Printed in the U.S.A.

Ultrastructure of the Dermal Microvasculature in Leprosy[1]

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Human infection with *Mycobacterium leprae* may be associated with the presence of the organism in the endothelium of small dermal vessels ([12]). The report by Joelsohn in 1893 and cited by Fite in his review is evidently the initial mention of this phenomenon ([12]). As reviewed by Coruh and McDougall, previous light microscopic studies have shown an intraendothelial location for the bacteria, a feature found predominantly in lepromatous leprosy patients. Using serial sections of skin, they concluded that endothelial bacillation was universal in patients with lepromatous disease ([9]). Endothelial bacillation appears to be particularly well developed in the lesional and perilesional skin of patients exhibiting Lucio's phenomenon and may be of importance in the pathogenesis of this reaction ([20]).

The ultrastructural features of leprosy have been the subject of study for many years ([2, 4, 8, 13, 14, 15, 17]). Much attention has been paid to the ultrastructure of the lepra cell and the fate of phagocytized organisms. Boddingius has studied the ultrastructure of the small endoneurial vessels ([5, 6]). However, the ultrastructural features of the dermal microvasculature and its response to the chronic infection of leprosy have not been emphasized. Stimulated by the apparent intraendothelial location of *M. leprae* at the level of light microscopy, we sought information concerning the ultrastructural appearance of endothelial bacillation and other changes in the cutaneous vessels.

[1] Received for publication on 2 April 1981; accepted in revised form on 7 January 1982.

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MATERIALS AND METHODS

Eighteen cases of active leprosy were included in the study (Table 1). There were 11 males and seven females, ranging in age from 14 to 66 years. Fourteen of the 18 patients were from Mexico, 2 were from Viet Nam, 1 from Thailand, and 1 from Guatemala. Ten patients were untreated at the time of biopsy, and two were in relapse from three to six months after stopping dapsone therapy. Routine paraffin sections prepared with hematoxylin-eosin and Fite stains were reviewed. Using clinical and histologic criteria, the patients were grouped according to the classification of Ridley and co-workers ([21, 22]). There were 14 cases of lepromatous (LL) leprosy, 1 of borderline lepromatous (BL) leprosy, and 3 of borderline tuberculoid (BT) leprosy. Of those patients with lepromatous leprosy, four had erythema nodosum leprosum (ENL) and four had Lucio's phenomenon.

Punch biopsies were taken from lesional skin in all except three cases: in three LL patients with Lucio's phenomenon, clinically normal skin from the upper outer arm was sampled. Immediately after each biopsy, the tissue was placed in Karnovsky's fixative ([16]), fixed for 24 hr, post-fixed with osmium tetroxide, processed, and embedded in epon. One-micron-thick sections were cut and stained with toluidine blue for light microscopy. After trimming, thin sections were cut and stained with uranyl acetate and lead citrate for electron microscopic examination. Multiple epon blocks were examined from each patient. Vessels were selected for ultrastructural study within areas of light microscopic changes.

The perivascular inflammatory infiltrate and the changes in the dermal microvasculature were evaluated in each case by light and electron microscopic examination (Table 2). The degree of each change was graded on a scale of 0 to 3+.

TABLE 1. *Patient population.*

Patient	Sex	Age	Nationality	Class
1	F	28	Mexican	LL[a]
2	F	38	Mexican	LL[b]
3	M	66	Vietnamese	BT
4	F	42	Mexican	LL[b]
5	M	35	Mexican	LL
6	M	14	Thai	BL
7	M	23	Mexican	LL[b]
8	F	32	Mexican	LL
9	M	43	Vietnamese	LL[b]
10	M	34	Mexican	LL
11	M	56	Mexican	LL
12	M	50	Mexican	LL
13	F	30	Mexican	BT
14	M	50	Mexican	LL[a]
15	F	26	Mexican	LL[a]
16	F	60	Guatemalan	BT
17	M	32	Mexican	LL[a]
18	M	23	Mexican	LL

[a] Patient with Lucio's phenomenon.
[b] Patient with erythema nodosum leprosum.

RESULTS

Phagocytized *M. leprae* were present within the endothelial cells in all except one of the lepromatous cases. They were absent or only a very few were found in the endothelium in biopsies of borderline (BT and BL) patients. Organisms were most frequently encountered in material from patients with Lucio's phenomenon. As previously reported, intraendothelial bacteria were found within membrane bound vacuoles, presumed to be of lysosomal origin, and not free within the cytoplasm ([6]) (Fig. 1). Bacteria were either single or multiple within the vacuoles and occasionally fragmented, degenerating bacteria and a variety of lysosomal residual dense bodies, similar to those within lepra macrophages, were found.

Endothelial hypertrophy and swelling were consistently found in all the small dermal vessels examined, varying from a moderate degree in the majority of patients to marked swelling in five. This swelling frequently resulted in marked compromise of the vessel lumens. In half the cases occasional very large, swollen endothelial cells were encountered. These cells were very pale and translucent with widely dispersed, normal appearing organelles (Fig. 2). In some small vessels in which intraendothelial organisms were found, endothelial hypertrophy and basal lamina changes were minimal.

The endothelial cell surfaces were usually very irregular. Small, plump bleb-like extensions were frequently seen on the luminal side and numerous, elongated, circumferential abluminal cytoplasmic processes were found. These abluminal processes were usually associated with extensions of the basal lamina. The pericytes were also usually hypertrophied and they, too, had thin, elongated cytoplasmic processes which extended in a similar cir-

TABLE 2. *Summary of ultrastructural findings.*

	Lepromatous cases						Borderline cases	
	LL		ENL		Lucio's		BL	BT
Number of patients	4	2	3	1	2	2	1	3
Treatment	no	relapse	yes	no	yes	no	no	no
Perivascular Inflammation								
Mast cells	2+	1+	1+	2+	3+	3+	0	1+
Lymphocytes	3+	2+	3+	2+	3+	3+	3+	3+
Macrophages	3+	2+	3+	3+	2+	3+	3+	2+
Vascular Changes								
Endothelial swelling	2+	2+	2+	3+	3+	2+	3+	2+
Increased cytoplasmic processes	2+	2+	2+	2+	2+	2+	3+	1+
Increased basement membrane	2+	2+	2+	2+	2+	2+	3+	1+
Cytoplasmic vacuoles	2+	2+	2+	3+	1+	2+	3+	1+
Intraendothelial organisms	1+	few	few	0	2+	2+	1+	0
Translucent endothelial cells	+	−	−	+	−	+	+	+

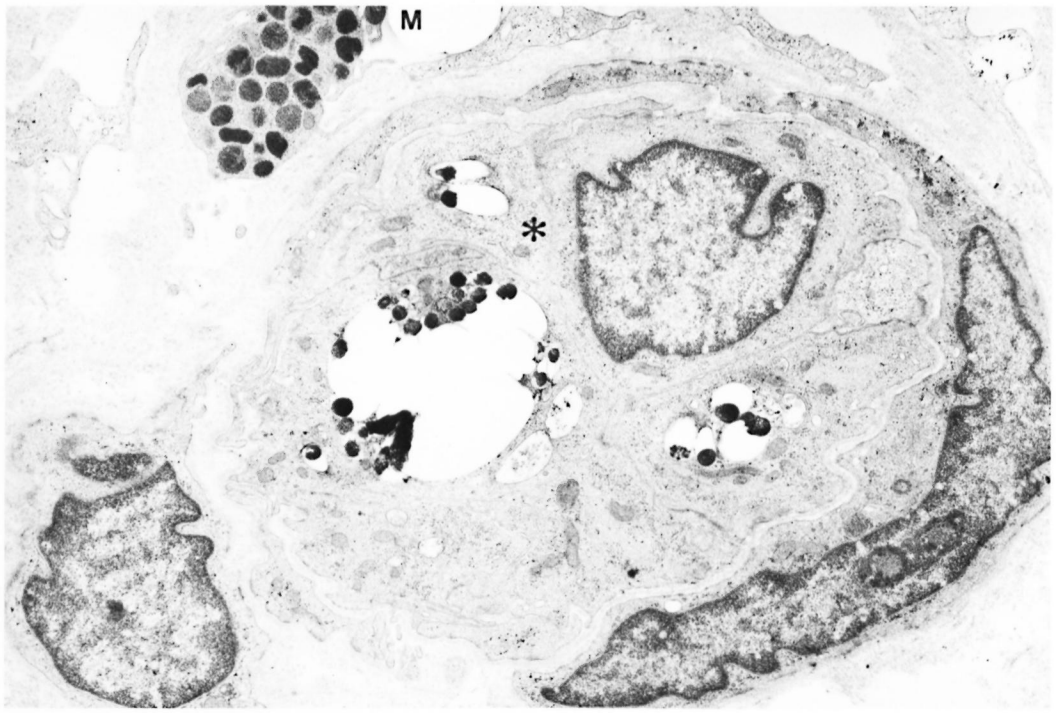

FIG. 1a. Intraendothelial organisms (*) and floccular appearing basal lamina are seen in this biopsy from a patient with Lucio's phenomenon. Several vacuoles containing organisms, some artefactually disrupted, are found within these swollen endothelial cells. A fragment of a mast cell (M) is seen nearby. (Original magnification ×3600.)

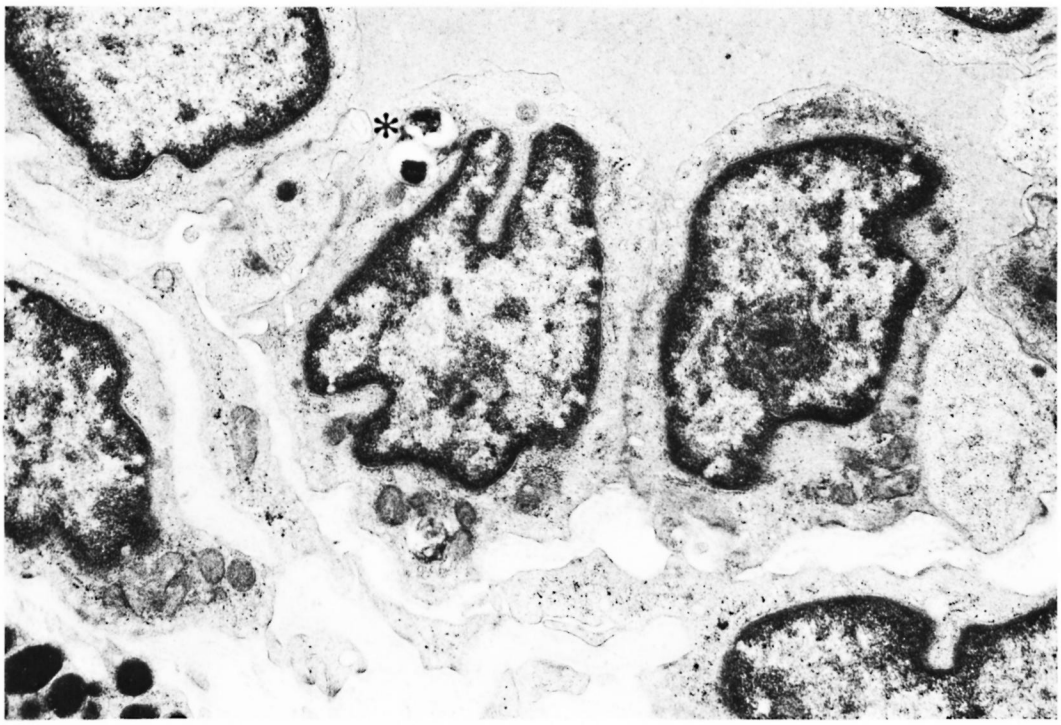

FIG. 1b. Higher magnification illustrating bacteria (*) within vacuoles of an endothelial cell. (Original magnification ×5100.)

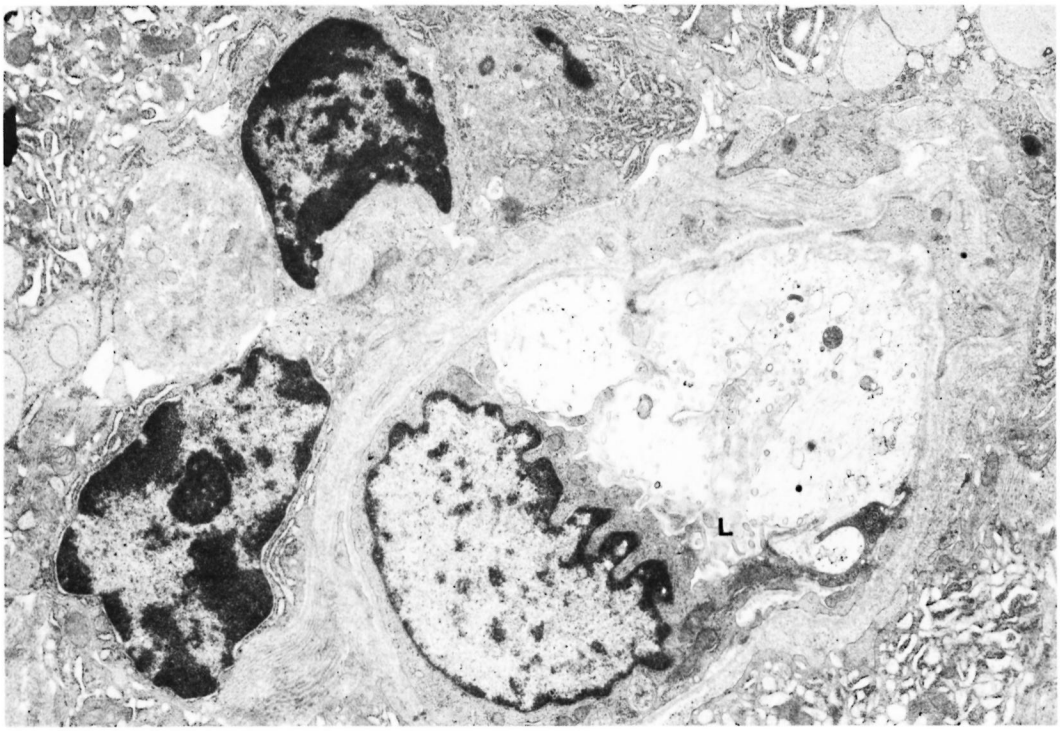

FIG. 2. A swollen, translucent endothelial cell and another darker hypertrophied endothelial cell are seen, severely compromising the vascular lumen (L). (Original magnification ×3900.)

cumferential fashion around the small vessels. The basal lamina surrounding the vessels was frequently multilayered and reduplicated, arranged in many concentric lamellae. Collagen fibers were common around the vessels, but they were absent between the multiple thin layers of basal lamina (Fig. 3). These endothelial and basal lamina changes were present in all 18 biopsies. These changes were most pronounced in the group of lepromatous cases, and least prominent in the borderline tuberculoid patients. In the biopsies from patients with Lucio's phenomenon the basal lamina had a slightly different character and appeared more amorphous and floccular and less distinctly lamellar (Fig. 1).

Pinocytotic vesicles and tiny vacuoles were commonly found in the endothelial cells just beneath the plasma membrane. These endothelial vacuoles were numerous in all patients except two with Lucio's phenomenon. They were seen in the abluminal cytoplasmic extensions as well as along the luminal surface.

The dermal inflammatory infiltrate consisted predominantly of lymphocytes, mast cells and mononuclear phagocytes ([1,27,28]). Lymphocytes diffusely infiltrated the dermis and were closely associated with the small dermal vessels, often abutting the periphery of the vascular basal lamina. Plasma cells and polymorphonuclear leukocytes were rare or absent in the material examined. Mast cells were prominent in the inflammatory infiltrate in lepromatous patients, especially untreated ones, and were most numerous in the patients with Lucio's phenomenon. The mast cells were also usually found in a perivascular distribution and in close proximity to lymphocytes (Fig. 4). Macrophages containing phagocytized organisms and a wide variety of intracytoplasmic lysosomal residual dense bodies were common around the small vessels ([23]). Phagocytized organisms were found only within membrane bound vacuoles, not free within the cytoplasm.

DISCUSSION

The changes found in the dermal microvasculature in these biopsies from patients with a variety of types of leprosy are similar to those described by Boddingius in her

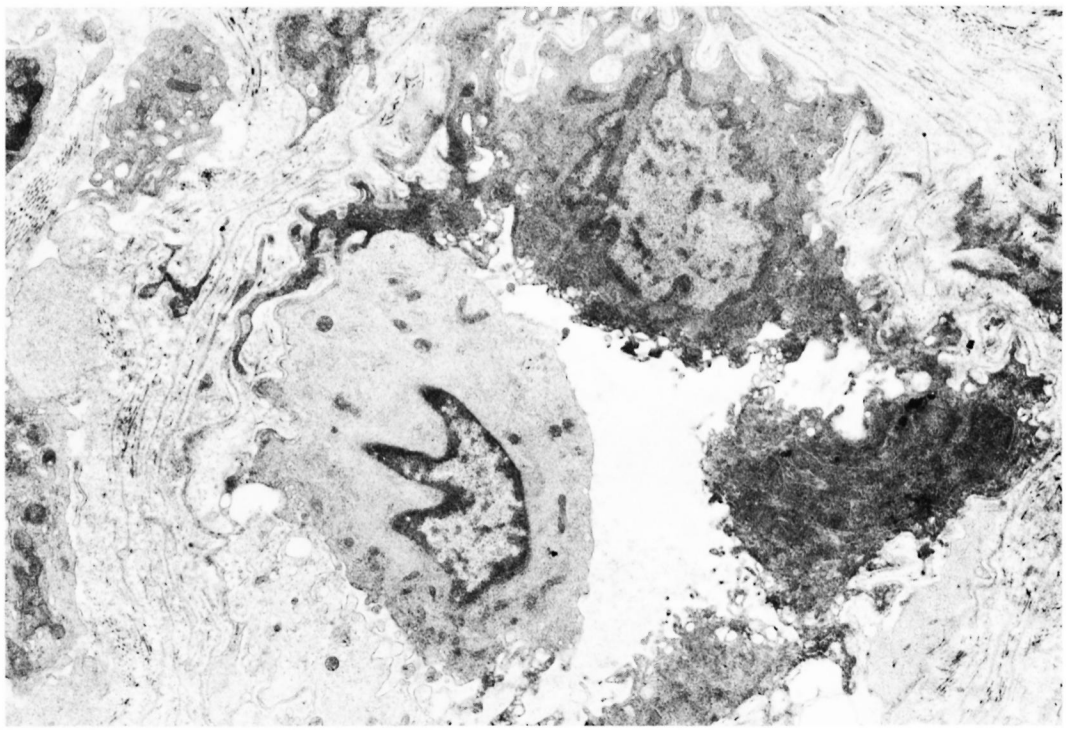

FIG. 3. Endothelial vacuolization and luminal and abluminal cytoplasmic processes with associated basal lamina reduplication are seen in this small vessel. (Original magnification ×3600.)

study of the endoneurial vessels in leprosy ([5,6]). Intraendothelial organisms, endothelial hypertrophy and swelling, prominent vacuolation, and numerous cytoplasmic processes were all findings noted in the endoneurial vessels ([5,6]). With the exception of endothelial bacillation, these microvasculature changes are most likely nonspecific reflections of the chronic inflammatory process ([7]).

Bacilli within the endothelium of dermal capillaries are a common finding in leprosy ([17]). When studied by light microscopy, organisms can be found in 50% to 100% of small dermal vessels ([9]). Intraendothelial organisms are not seen in tuberculosis, are rarely found in tuberculoid forms of leprosy, and are most common in lepromatous patients, especially those with Lucio's phenomenon ([9,20]). Endothelial bacillation was most common in the patients with Lucio's reaction in our study as in previous reports ([20]) and appeared to be equally well developed in both clinically normal and perilesional skin.

Endothelial activation, hypertrophy, proliferation, and swelling are typical of the microvasculature response to injury ([11,25]). Endothelial hypertrophy has been described in the vessels of the peripheral nerves in leprosy ([5,6]), and earlier light microscopic studies have reported endothelial proliferation and protrusion with luminal compromise in dermal vessels in leprosy ([9]). The swollen translucent endothelial cells seen have been described in skin biopsies of hypersensitivity induced allergic contact dermatitis ([11]) and again in the endothelium of small endoneurial vessels in leprosy ([5,6]).

The basal lamina of vessels is derived from the endothelial cells and pericytes and is produced within their endoplasmic reticulum ([3,10,18]). In the small dermal vessels in chronic inflammation the basal lamina appears reduplicated. It would appear that the multiple layers result as the cytoplasmic processes extend outwards, laying down additional layers of associated basal lamina. Perhaps the small vacuoles seen within these processes are related to the produc-

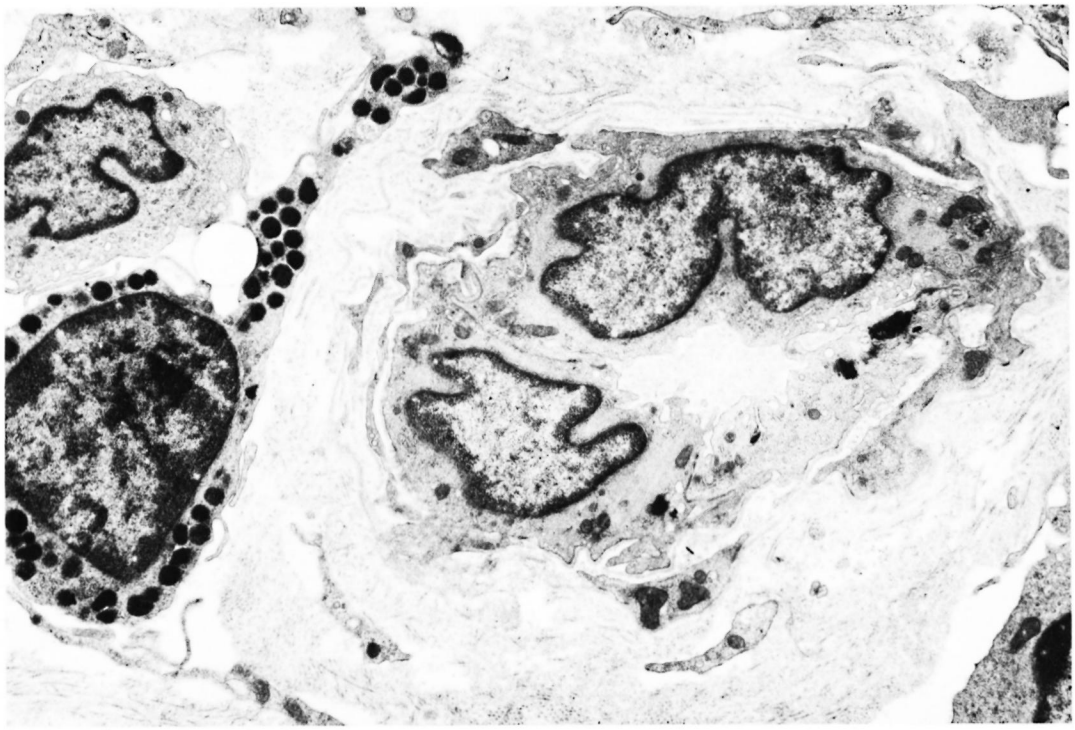

FIG. 4. This small dermal capillary has hypertrophied endothelial cells compromising its lumen. A mast cell is seen nearby. (Original magnification ×5700.)

tion of basal lamina [5, 6]. In the descriptions of the endoneurial vessels in leprosy, endothelial projections were more often encountered in lepromatous than in tuberculoid forms [5, 6], a difference not noted in our study of the dermal microvasculature.

Concentric perivascular cuffing by lymphocytes and macrophages is typically present in the dermis in all types of leprosy [21]. The large mast cell component of the perivascular inflammatory infiltrate observed in our study has not been widely appreciated. An infiltrate of plasma cells and mast cells has been recently reported in chronic dermal ENL among New Guinean highlanders [24]. Although mast cells have been described to be part of the hypersensitivity cellular response [11], their role in leprosy is unknown. The mast cells' perivascular location and close association with lymphocytes and macrophages may be related to a possible role in the modulation of the immune response [26, 29].

Features of the ultrastructure of the Lucio lesion and the specific ENL lesion were not successfully delineated in our study. Previous reports of the morphology of ENL and Lucio's phenomenon have described a polymorphonuclear infiltrate and a vasculitis in the former and ischemic necrosis in association with endothelial proliferation in the latter [19, 20, 24, 29]. These changes were not found in our material, perhaps due to sampling skin away from these specific lesions or because comparatively mild or early lesions were biopsied.

SUMMARY

Infection with *M. leprae* may lead to the presence of the organism within the dermal vascular endothelium, a phenomenon most pronounced in lepromatous leprosy. In order to study the ultrastructural features of the dermal microvasculature in leprosy, biopsies from 18 patients with lepromatous (14), borderline lepromatous (1) and borderline tuberculoid (3) leprosy were examined. Four patients with Lucio's phenomenon and four with erythema nodosum leprosum were included. The ultrastructural changes in the dermal microvasculature included endothelial swelling and hy-

pertrophy, increased endothelial and pericytic cytoplasmic processes, and pronounced basal lamina reduplication. Occasional large, pale, endothelial cells with widely dispersed organelles were encountered. Phagocytized, membrane-bound intraendothelial organisms were found, similar in appearance to those within dermal macrophages. The predominantly perivascular dermal inflammatory infiltrate consisted of lymphocytes, macrophages and mast cells. The observed ultrastructural changes in the dermal microvasculature are similar to those previously described in the endoneurial vessels. While reflecting nonspecific responses of the dermal microvasculature in chronic inflammation, the findings support a possible role of the small dermal vessels in the chronic nature of the host's response to infection with *M. leprae*.

RESUMEN

La infección con el *Mycobacterium leprae* puede conducir a la localización del microorganismo dentro del endotelio vascular dérmico, un hallazgo que es más común en la lepra lepromatosa. Con objeto de estudiar las características ultraestructurales de la microvasculatura dérmica en la lepra, se examinaron biopsias de 14 pacientes con lepra lepromatosa, uno con lepra cercana (borderline) a la lepromatosa y 3 con lepra cercana a la tuberculoide (borderline tuberculoide). En el estudio se incluyeron 4 pacientes con fenómeno de Lucio y 4 con eritema nodosa leproso. Los cambios ultraestructurales en la microvasculatura dérmica incluyeron engrosamiento endotelial e hipertrofia, incremento en el número de procesos citoplásmicos endoteliales y pericíticos, y una pronunciada reduplicación de la lámina basal. Ocasionalmente se encontraron células endoteliales grandes, pálidas y con organelos muy dispersos. También se encontraron organismos fagocitados intraendotelialmente adheridos a membrana, similares en apariencia a aquellos encontrados dentro de macrófagos dérmicos. El infiltrado dérmico inflamatorio, predominantemente perivascular, consistió de linfocitos, macrófagos y mastocitos. Los cambios ultraestructurales observados en la microvasculatura dérmica son similares a aquellos previamente descritos en los vasos endoneurales. A la vez que estos hallazgos reflejan una respuesta no específica de la microvasculatura dérmica a la infección crónica, sugieren un posible papel de los pequeños vasos sanguíneos en la naturaleza crónica de la respuesta del huésped a la infección con el *Mycobacterium leprae*.

RÉSUMÉ

L'infection par *Mycobacterium leprae* peut conduire à la pénétration de cet organisme dans l'endothélium vasculaire du derme, phénomène qui est le plus marqué dans la lèpre lépromateuse. En vue d'étudier les caractéristiques de l'ultra structure microvasculaire du derme dans la lèpre, on a examiné des biopsies prélevées chez 18 malades, dont 14 atteints de lèpre lépromateuse, un de lèpre dimorphe, et 3 de lèpre tuberculoïde. Quatre malades souffrant du phénomène de Lucio, ainsi que quatre autres malades présentant un erythème noueux lépreux, ont été également étudiés. Les modifications de l'ultrastructure microvasculaire de derme comprenaient un gonflement et une hypertrophie de l'endothélium, un accroissement du nombre des organelles cytoplasmiques de l'endothélium et du pericytium, de même qu'une duplication marquée de la membrane basilaire. On a observé de ci de là des cellules endothéliales pâles et de grande dimension, avec des organelles largement dispersées. On a également mis en évidence des organismes à l'intérieur de l'endothélium, phagocytés, et associés à la membrane, dont l'apparence était semblable à celle présentée par les organismes trouvés à l'intérieur des macrophages du derme. L'infiltrat inflammatoire dermique, surtout périvasculaire, consistait en lymphocytes, en macrophages et en mastocytes. Les modifications de l'ultrastructure microvasculaire au niveau du derme étaient semblables à celles qui ont été décrites auparavant dans les vaisseaux de l'endoneurium. Ces observations, tout en reflétant les réponses no spécificques de l'appareil microvasculaire du derme lors d'une inflammation chronique, indiquent également que les petits vaisseaux du derme pourraient jouer un rôle dans la nature chronique de la réponse de l'hôte à l'infection par *Mycobacterium leprae*.

REFERENCES

1. Adams, D. O. The granulomatous inflammatory response: a review. Am. J. Pathol. **84** (1976) 164–191.
2. Aquino, T. I. and Skinsnes, O. K. Pathobiologic significance of the subcellular organelles of leprae cells. Int. J. Lepr. **38** (1970) 134–148.
3. Ashton, N. Vascular basement membrane changes in diabetic retinopathy. Brit. J. Ophthal. **58** (1974) 344–366.
4. Bishop, F. W., Suhrland, L. G. and Carpenter, C. M. A comparative study by electron microscopy of the morphology of *Mycobacterium leprae* and cultivable species of *Mycobacteria*. Int. J. Lepr. **16** (1948) 361–366.
5. Boddingius, J. Ultrastructural changes in blood vessels of peripheral nerves in leprosy neuropathy. I. Tuberculoid and borderline leprosy patients. Acta Neuropathol. **35** (1976) 159–181.
6. Boddingius, J. Ultrastructural changes in blood vessels of peripheral nerves in leprosy neuropathy. II. Borderline, borderline-lepromatous and lepromatous leprosy patients. Acta Neuropathol. **40** (1977) 21–39.

7. CASLEY-SMITH, J. R. The fine structure of the microvasculature in inflammation. Bibl. Anat. **17** (1979) 36–53.
8. CHANDI, S. M. and JOB, C. K. The early cellular response to *M. leprae*: an ultrastructural study. Lepr. India **50** (1978) 344–351.
9. CORUH, G. and MCDOUGALL, A. C. Untreated lepromatous leprosy: histopathologic findings in cutaneous blood vessels. Int. J. Lepr. **47** (1979) 500–511.
10. DASTUR, D. K. and DAVE, U. P. Ultrastructural basis of the vasculopathy in and around brain tuberculomas: possible significance of altered basement membrane. Am. J. Pathol. **89** (1977) 35–49.
11. DVORAK, A. M., MIHM, M. C. and DVORAK, H. F. Morphology of delayed-type hypersensitivity reactions in man. II. Ultrastructural alterations affecting the microvasculature and the tissue mast cells. Lab. Invest. **34** (1976) 179–191.
12. FITE, G. L. The vascular lesions of leprosy. Int. J. Lepr. **9** (1941) 193–202.
13. IMAEDA, T. Electron microscopic analysis of the components of lepra cells. Int. J. Lepr. **28** (1960) 22–27.
14. IMAEDA, T. Electron microscopy: approach to leprosy. Int. J. Lepr. **33** (1965) 669–683.
15. JOB, C. K. *Mycobacterium leprae* in nerve lesions in lepromatous leprosy: an electron microscopic study. Arch. Pathol. Lab. Med. **89** (1970) 195–207.
16. KARNOVSKY, M. J. A formaldehyde-gluteraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. **27** (1965) 137A.
17. NISHIURA, M. The electron microscopic basis of the pathology of leprosy. Int. J. Lepr. **28** (1960) 357–399.
18. PIERCE, G. B., MIDGLEY, A. R. and RAM, J. S. The histogenesis of basement membranes. J. Exp. Med. **117** (1963) 339–349.
19. QUISMORIO, F. P., REA, T., CHANDOR, S., LEVAN, N. and FRIOU, G. Lucio's phenomenon: an immune complex deposition syndrome in lepromatous leprosy. Clin. Immunol. Immunopathol. **9** (1978) 184–193.
20. REA, T. H. and RIDLEY, D. S. Lucio's phenomenon: a comparative histologic study. Int. J. Lepr. **47** (1979) 161–166.
21. RIDLEY, D. S. Histologic classification and the immunological spectrum of leprosy. Bull. WHO **51** (1974) 451–465.
22. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity: a five group system. Int. J. Lepr. **34** (1966) 255–273.
23. RIDLEY, M. J., BADENOCH-JONES, P. and TURK, J. L. Ultrastructure of cells of the mononuclear-phagocyte series (MPS) across the leprosy spectrum. J. Pathol. **130** (1980) 223–227.
24. RIDLEY, D. S., REA, T. H. and MCADAM, K. P. W. J. The histology of erythema nodosum leprosum. Variant forms in New Guineans and other ethnic groups. Lepr. Rev. **52** (1981) 65–78.
25. SCHOEFL, G. I. Studies on inflammation: III. Growing capillaries: their structure and permeability. Virchow Arch. Pathol. Anat. **337** (1963) 97–141.
26. SOTER, N. A. Necrotizing vasculitis of the skin. In: *Dermatology in General Practice*. Fitzpatrick, T. B. and Eisen, A. eds. New York: McGraw Hill Book Co., 1979, pp. 548–554.
27. SPECTOR, W. G. and LYKKE, A. W. J. The cellular evolution of inflammatory granulomata. J. Pathol. **92** (1966) 163–177.
28. WEINER, J., LATTES, R. G., and SPIRO, D. An electron microscopic study of leukocyte emigration and vascular permeability in tuberculin sensitivity. Am. J. Pathol. **50** (1967) 485–521.
29. WEMAMBU, S. N. C., TURK, J. L., WATERS, M. F. R. and REES, R. J. W. Erythema noduosum leprosum: a clinical manifestation of the Arthus phenomenon. Lancet **2** (1969) 933.