An Ultrastructural Study of Dermal Nerves in Early Human Leprosy[1]

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Several studies in the past have established that leprosy is characterized by inflammatory lesions in the skin and nerves ([11, 14, 19, 24]). *Mycobacterium leprae* may be found in nerves as far centrally as the satellite cells and the inter-vertebral and sympathetic ganglia ([15]), but preferentially involve and damage cutaneous terminal portions or more proximal and superficially located segments of nerve trunks ([12, 18]). These latter portions have in common a short perpendicular distance from the skin surface and a relation to joints, bony prominences or fibro-osseous tunnels, with consequent exposure to greater trauma or compression and a lower tissue temperature than elsewhere ([4–6, 13]).

The route and mode of entry of *M. leprae* into nerves has remained uncertain. It has long been considered that infection is initiated in dermal nerve endings ([15, 23]) with proximal spread from one Schwann cell to the next or by transaxonal transport ([3]). Pearson and Weddell have drawn attention to the frequent occurrence of perivascular inflammatory cell infiltrates in the epineurium and perineurium ([22]) with decreasing cellular density centripetally (toward the core of the nerve). These could represent the sites of emergence of inflammatory cells and *M. leprae* from the blood stream and mark the path taken by bacilli in their passage into the endoneurium. In order to explore this possibility and also since electron microscopic studies of dermal nerves in early human leprosy are few ([17]), the present study was undertaken.

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MATERIALS AND METHODS

Fifteen previously untreated patients with leprosy, classified clinically as indeterminate (Ind.) = 4 patients, borderline-tuberculoid (BT) = 6 patients, borderline lepromatous (BL) = 3 patients and lepromatous leprosy (LL) = 2 patients, were selected for skin biopsy. All of these patients had symptoms of the disease for a period of 1 year or less, and were clinically judged to have the disease in an early form.

A wedge biopsy of skin was taken from each patient, across the edge of a selected skin lesion, including the dermo-subcutaneous junction. It was immediately bisected, and one half was further divided in two drops of ice cold 3% or 5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 to yield a slice of skin 1–2 mm thick which was immersed in 5 ml of the same fixative at 4°C for 30-60 min. The tissue was then cut into roughly 1 mm^3 pieces and returned to the fixative at 4°C for up to 1 week.

The second half of the skin biopsy was fixed in 10% Formalin, processed to paraffin embedding, and sectioned for light microscopy. In those cases where paraffin sections showed unequivocal changes of leprosy, the corresponding glutaraldehyde-fixed tissue pieces were osmicated and processed to yield 1 μm araldite sections. These sections were screened, and the blocks that contained nerve twigs and suitable lesions were selected for electron microscopy. In this manner, blocks of skin tissue from the biopsies of 7 patients (2 with Ind. leprosy, 2 with BT, 2 with BL, and 1 with LL) were examined. Sections for electron microscopy were stained with uranyl acetate and lead citrate, and examined with a Philips EM 201 electron microscope.

RESULTS

Light microscopy

In paraffin sections and 1 μm araldite sections of all the biopsies, there were focal

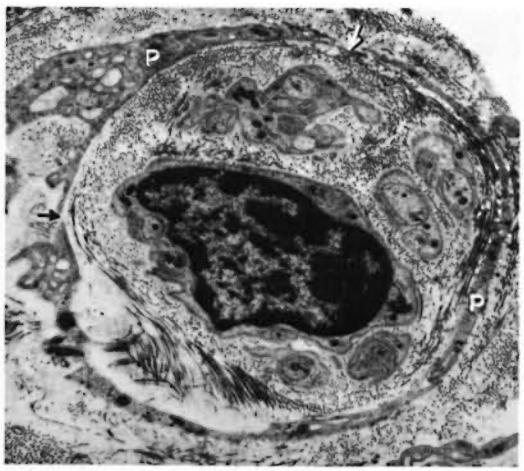

FIG. 1. Indeterminate leprosy skin showing an upper dermal nerve with a single layered perineurium (P) having inter-cellular gaps (arrow) (×12,150).

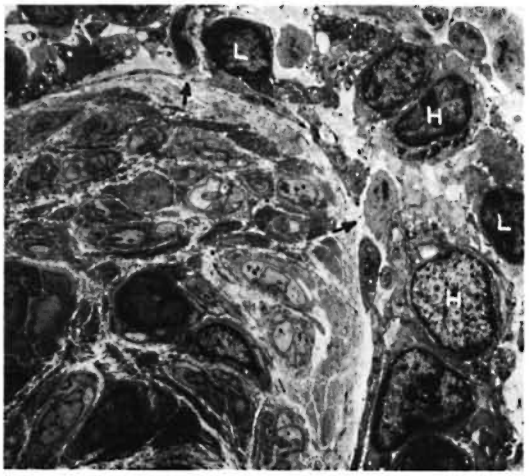

FIG. 2. Indeterminate leprosy skin showing a dermal nerve with a fenestrated perineurial covering. Epineurial lymphocytes (L) and histiocytes (H) are located near gaps (arrows) in the perineurium (×3350).

collections of inflammatory cells in the dermis, centered upon blood vessels, nerves, arrector pili muscles, sweat glands, and hair follicles. The biopsies were separable into distinct groups (Ind., BT, BL, and LL) based on the nature of the inflammatory cellular infiltrate and the number of acid-fast bacilli present.

Electron microscopy

Indeterminate leprosy (cases 1 and 2). Uninflammed nerves were more easily found in these cases than in the other cases. In the deep papillary region of the dermis, there were very small nerves consisting of a few unmyelinated axons partially ensheathed by Schwann cell cytoplasm and enclosed within the basement membrane of the latter. There were associated bundles of collagen but no enclosing lamina of perineurial cells. Nerves from evidently deeper dermal levels consisted of a few more unmyelinated axons bundled together by Schwann cell cytoplasm and its basal lamina, associated with collagen fibers and a single layer of perineurial cells (Fig. 1). These cells were separated from one another in foci by gaps of varied widths. Larger nerves had a greater number of axons which were still mostly unmyelinated with a well formed perineurium composed of about two to four laminae of perineurial cells. Inflammatory infiltrates around nerves consisted of lymphocytes and a few histiocytes. A few lymphocytes were present at the thresholds of gaps between adjacent perineurial cells (Fig. 2). Rarely, a macrophage containing intracytoplasmic *M. leprae* was present in close proximity to a superficial nerve without a complete perineurial covering (Fig. 3). In one instance, a terminal nerve fiber was infiltrated by a macrophage which had wedged itself between two unmyelinated axons and the covering Schwann cell.

Borderline tuberculoid leprosy (cases 3 and 4). *M. leprae* were not detected in a careful examination of several grids. The inflammatory cells consisted predominantly of lymphocytes and epithelioid cells in the epineurial region. Lymphocytes and histiocytes were also seen in the endoneurium between nerve fibers and also around and beneath the perineurial sheath. A few histiocytic cells had enfolded nerve fibers within cytoplasmic processes.

Borderline lepromatous leprosy (cases 5, 6, and 7). *M. leprae* were widely distributed within endothelial cells, pericytes, nerves, smooth muscle, and macrophages. The dominant inflammatory cell of infiltration was the macrophage. *M. leprae* were far more abundant in the epineurial region of nerves than in the endoneurium (Fig. 4), and the vast majority of them were within macrophages. A few macrophages laden with *M. leprae* were seen to infringe upon the perineurial sheath (Fig. 5), which appeared loos-

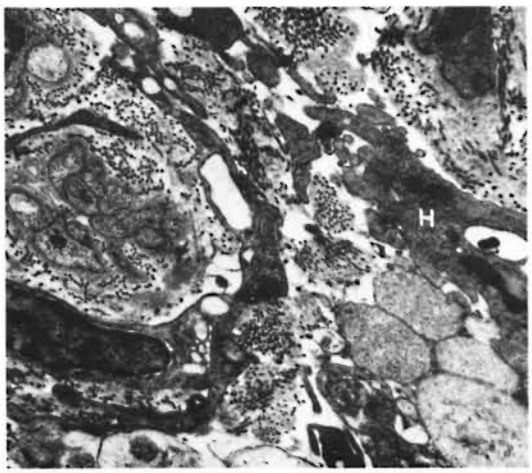

FIG. 3. Indeterminate leprosy skin showing an upper dermal nerve with an adjacent histiocyte (H) containing a leprosy bacillus (×21,900).

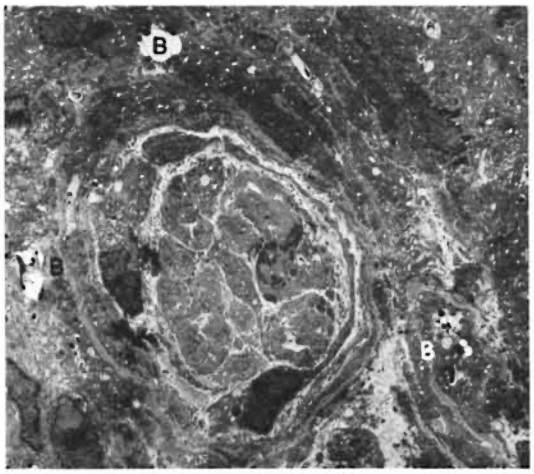

FIG. 4. Lepromatous leprosy skin. Several leprosy bacilli (B) are present in the epineurium while there are none in the endoneurium (×3850).

ened. A few perineurial cells contained *M. leprae*. Occasional bacilli were present in a very few Schwann cells ensheathing unmyelinated axons but not in those related to myelinated axons. One myelinated axon with degenerative features as seen in early Wallerian degeneration was present. Apparently unaltered unmyelinated axons being engulfed by macrophages were seen infrequently. Occasional phagocytic cells had insinuated themselves within the investing Schwann cell basement membrane and caused separation of the enclosed axon from the ensheathing Schwann cell cytoplasm (Fig. 6). Vascular basement membrane proliferation was rarely encountered, and there was no significant increase in intraneural collagen. Intra-axonal bacilli and endoneurial colonies of *M. leprae* were not seen.

DISCUSSION

The location of *M. leprae* in the skin *is* usually marked by focal aggregates of inflammatory cells and, characteristically, these are found in relation to nerves, neurovascular bundles, and arrector pili muscles ([23, 24]). The involvement of neurovascular bundles is a striking feature of all stages and types of leprosy. This, and the disseminated nature of the lesions, suggests a hemic mode of spread of *M. leprae* to the skin. Their egress from the vasculature may take place via fenestrated capillaries or venules ([7, 9]) aided by minor trauma to the skin.

There is a rich plexus of nerves in the skin which, in the superficial dermis, consists of unmyelinated axons partially ensheathed in the cytoplasmic processes of a terminal Schwann cell without a perineurial covering ([8, 9]). It is conceivable that invasion of nerves is initiated in this relatively unprotected zone and then ascends proximally. Alternatively, a transperineurial route of neural infection has been proposed by some workers ([10, 21]).

As observed in this study, greater concentrations of inflammatory cells and bacilli may be found in the epineurium than in the endoneurium. This probably represents an early stage of the emergence of bacilli from epineurial blood vessels, or the vascular components of neurovascular bundles in the skin, with inflammatory infiltration of the perineurium and endoneurium occurring subsequently.

The perineurium, particularly its inner lamellae, and the endoneurial blood vessels act as barriers, keeping tracer molecules from entering the endoneurium ([1, 20]). It is probable that the former barrier may be breached due to chronic inflammation in the adjacent epineurium, permitting transit of macrophages laden with bacilli ([2]) into the subjacent endoneurium. This is suggested by the loosening and separation of perineurial cells seen by Imaeda and Convit ([17]) and

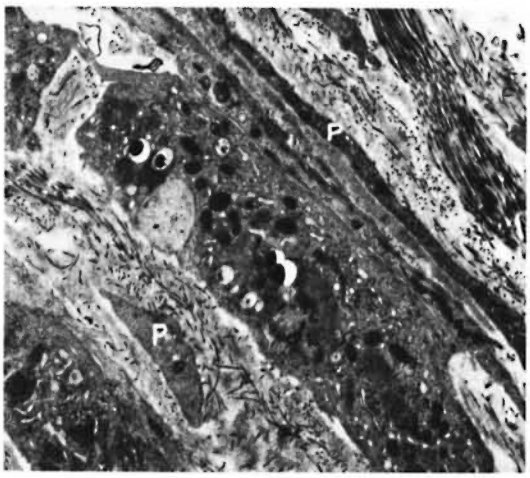

FIG. 5. Lepromatous leprosy skin. A bacillated macrophage has infiltrated the perinerium (P) of a dermal nerve (×9685).

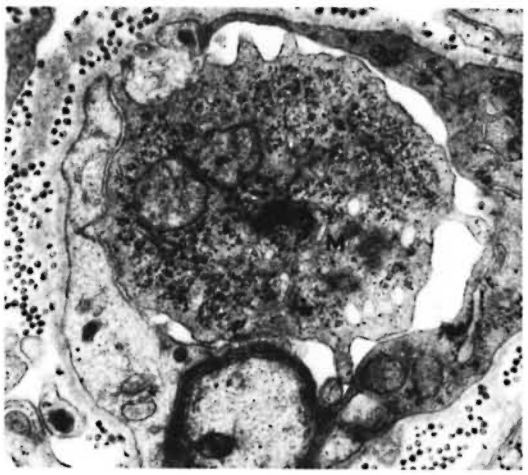

FIG. 6. Borderline-lepromatous leprosy skin. A macrophage (M) has entered the basement membrane envelope of a Schwann cell and separated it from two unmyelinated axons related to it (×43,225).

also in this study. Pearson and Weddell have described inflammatory cells passing out of blood vessels in the "perineural zone," entering the endoneurium via the perineurium ([22]). What chemotactic or other factor induces the directional movement of macrophages bearing *M. leprae* toward the endoneurium is not known. It might occur incidental to attempts at repairing focal damage in the perineurium that develops from chronic epineurial inflammation. It is also possible that infection of perineurial cells by *M. leprae* may spread into successive layers of the perineurial sheath until the endoneurium is finally invaded ([10]), since perineurial cells are known to phagocytose *M. leprae* ([2]).

The inflammatory cellular infiltrate in leprous neuritis could be construed as a response to the infecting organism or to the products of endoneurial elements damaged by *M. leprae*. In Wallerian degeneration, the cellular infiltrate of hematogenous origin is derived from endoneurial vessels and not from across the perineurium ([16]). It is, therefore, likely that the transperineurial infiltration of inflammatory cells toward the core of nerves in leprosy is not primarily a response to damaged endoneurial elements. If infection of nerves takes place exclusively in an ascending manner from their peripheral terminations, it is more likely that the resultant cellular infiltrate would be derived from damaged endoneurial blood vessels rather than from across a more distant and better preserved perineurial barrier.

It is known that nerves are most densely bacillated with *M. leprae* at sites of predilection characterized by a superficial course beneath the skin ([18]). These locations permit greater exposure to trauma and a lower tissue temperature than deeper locations ([4–6, 13]). If these factors are important, then intervening portions where the nerves are buried under muscle masses would not be conducive to the growth, multiplication, and spread by contiguity of *M. leprae*. An exclusively ascending intraneural infection would also not easily explain the frequent finding of focal epineurial infiltrates of inflammatory cells around nerve trunks in biopsy material.

Cutaneous lesions in leprosy most often develop in areas of the skin that are exposed to all sorts of trauma. By facillitating the emergence of *M. leprae* from the circulation, trauma may have a role in the initiation of lesions in the skin around epineurial vessels of cutaneous nerve branches and in more proximal, superficially located segments of nerve trunks.

SUMMARY

Skin biopsies from the cutaneous lesions of seven patients with indeterminate, BT, BL, and LL leprosy of less than 1 year's duration were examined by light and elec-

tron microscopy. Inflammatory cells, which marked the location of *Mycobacterium leprae* in bacilliferous cases (BL and LL) were most frequently and consistently found in relation to dermal blood vessels, neurovascular bundles, nerves, arrector pili muscles, and skin adnexa. The number of bacilli and inflammatory cells in the epineurium was in great excess of those in the perineurium and endoneurium. Perineurial infiltration by lymphocytes and bacillated macrophages was seen to occur through gaps between the constituent cells of a loosened and sometimes proliferated perineurium. Bacillation of Schwann cells and associated inflammation in the endoneurium was minimal. *M. leprae* were identified in endothelial cells, arrector pili muscles, macrophages and Schwann cells. At this stage, inflammatory destruction of nerve fibers was not encountered.

It is concluded that *M. leprae* which are extruded from the circulation into the epineurium (or perineurium) may be carried in inflammatory cells across the perineurium which is loosened and rendered permeable to inflammatory cells as a consequence of chronic inflammation in the adjacent epineurium. This is suggested as a very probable route for *M. leprae* to enter nerves.

RESUMEN

Por microscopía de luz y electrónica, se examinaron las biopsias de lesiones de 7 pacientes con lepra indeterminada, BT, BL, y LL, todos ellos con menos de 1 año de evolución. Las células inflamatorias, las cuales marcaron la localización del *Mycobacterium leprae* en los casos bacilíferos (BL y LL), se encontraron consistentemente asociadas a vasos sanguíneos dérmicos, fibras neurovasculares, nervios, músculos piloerectores y anexos de la piel. El número de bacilos y de células inflamatorias en el epineurio fue más abundante que el encontrado en el perineurio y en el endoneurio. Fue evidente la infiltración perineurial por linfocitos y macrófagos con bacilos en los espacios observados entre las células constituyentes de un perineurio laxo y a veces proliferativo. En el endoneurio, la presencia de bacilos en las células de Schwann y la inflamación asociada fueron mínimas. Los *M. leprae* fueron encontrados en las células endoteliales, en los músculos piloerectores, en los macrófagos y en las células de Schwann. En este estadío no se encontró destrucción inflamatoria de las fibras nerviosas. Se concluyó que los *M. leprae* que pasan de la circulación al epineurio (o al perineurio) pueden ser acarreados por las células inflamatorias a través del perineurio que se torna laxo y permeable como consecuencia de la inflamación crónica en el epineurio adyacente. Se sugiere como muy probable esta ruta de entrada del *M. leprae* a los nervios.

RÉSUMÉ

On a examiné par la microscopie optique et par la microscopie électronique des biopsies cutanées prélevées au niveau des lésions de la peau chez 7 malades atteints de lèpre indéterminée, BT, BL, et LL, d'une durée de moins d'un an. Les cellules inflammatoires, qui caractérisaient la localisation de *Mycobacterium leprae* chez les cas bacillifères (BL et LL), étaient plus fréquemment et de manière plus régulière observées à proximité des vaisseaux sanguins du derme, des faisceaux neurovasculaires, des nerfs, des muscles arrecteurs des poils et des annexes cutanées. Le nombre de bacilles et de cellules inflammatoires dans lépineurium, était très fortement supérieur a celui noté dans le périneurium ou dans l'endoneurium. On a observé que l'infiltration périneurale par des lymphocytes et par les macrophages chargés de bacilles se produisait à travers les interstices libres séparant les celules, celles-ci constituant un périneurium lâche et parfois en prolifération. La colonisation des cellules de Schwann par les bacilles, de même que l'inflammation qui y est associée dans l'endoneurium, étaient minimales. *M. leprae* a été identifiée dans les cellules endothéliales, dans les muscles arrecteurs des poils, dans les macrophages et dans les cellules de Schwann. On n'a pas noté à ce stade de destruction inflammatoire des fibres nerveuses. On en conclut que les bacilles qui passent du système circulatoire vers l'épineurium ou vers le périneurium peuvent être transportées dans les cellules inflammatoires à travers l'épineurium, car celui-ci est rendu lâche et perméable aux cellules inflammatoires par suite de l'inflammation chronque de l'épineurium adjacent. Ces constatations suggèrent que c'est là la route la plus problable empruntée par *M. leprae* pour pénétre dans les nerfs.

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