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Lymphatics in leprosy: relationship to elastic fibres and observations following intra-lesional injections of colloidal carbon

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Summary An investigation of skin lymphatics in leprosy has been undertaken. Examination of 62 skin biopsies from 31 patients with various classifications of leprosy has revealed dilated initial lymphatics within granulomas of lepromatous leprosy, but no significant abnormalities in non-lepromatous disease or in non-granulomatous skin. Colloidal carbon injected intra-lesionally failed to appear within granulomas, but could be seen in lymphatics in non-granulomatous dermis. Elastic fibres were also absent within granulomas. AFB were clearly identified within endothelial cells of initial lymphatics. We suggest lymphatic malfunction may be compartmental, existing only within the granulomas and not in the surrounding normal appearing dermis.

Introduction

The lymphatic system in the skin has two main functions, to clear protein, particulate matter including bacteria, and excess fluid from the interstitial spaces and to channel locally presented antigenic material to the regional lymph nodes where it can stimulate an appropriate immune response. In doing this, it works in partnership with the macrophage and provides the pathways along which many of the cells of the immune system, e.g. T lymphocytes and Langerhans cells, travel.

There is some evidence for impairment of both functions in leprosy. Older textbooks refer to elephantiasis in leprosy, and lymphoedema is well recognized in lepromatous leprosy. Hansen himself wrote that 'the lymph glands are permeable in leprosy but penetration is evidently more difficult, for the lymph vessels leading to them are dilated.' Raasch *et al.*,[2] using lymphangiography, showed no obstruction to lymphatic flow, but Carayon *et al.*[3] claimed nerve involvement in leprosy was due to lymphatic blockage. Klingmuller[4] commented on bacilli inside (lymphatic) 'endothelial lined slits' and quoting Deycke,

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stated that lymphatics are a favourite site for leprosy bacilli. The role of lymphatics in the clearance of bacteria is debated, and continues to be discussed.[5]

The role of the immune response in determining the course of the disease in each patient cannot be disputed. What determines a strong cell-mediated response in tuberculoid cases compared with a lack of response in lepromatous forms remains unclear. The route of infection is a determinant in the swaying of the balance between tolerance and immunity and may be the first and most important determining factor for unresponsiveness in lepromatous leprosy (LL).[6] In searching for a cause, most workers have sought answers from cellular immunology, while the role of lymphatic pathways has been neglected. The observations of Turk and Waters[7] and almost all other investigators concentrated on the cellular implications of the changes in lymph node morphology rather than any effect they might have had on the physiological function of the lymphatics supplying the node.

Our hypothesis was that the dermal lymphatic function is disturbed in leprosy. This could explain both the oedema often observed in tissues affected by leprosy and the altered immune function. To investigate skin lymphatics in leprosy, histopathological studies were undertaken.

The aims of this observational study were to identify (i) any morphological changes to dermal lymphatics in lesional skin, and (ii) any abnormalities in the distribution of colloidal carbon, the implication being that trafficking routes to and within initial lymphatics might be impaired.

Materials and methods

Thirty-one male patients were selected from those attending the Dermatology Outpatients Clinic, Baranas Hindu University, Varanasi, India Only those patients who were considered untreated were entered into the study. The lepromatous classification was established by clinical findings, multiple slit-skin smears, lepromin test and skin biopsy. Patients with disease ranging from sub-polar lepromatous (LLs) to tuberculoid (U) were seen (U, 4 patients; BT, 10; BB, 2; BL, 6; LLs, 9).

Skin biopsies were taken under local anaesthesia by standard techniques, fixed in 10% formalin and embedded in paraffin wax. Consecutive 5 μm sections were stained with haematoxylin and eosin (H&E), the Fite-Faraco method for *M. leprae*[8] and orcein for elastic fibres. Two biopsies were obtained from each patient, one from lesional skin (thigh, arm, buttock, trunk and lower leg) and a control biopsy from non-lesional skin from an equivalent site on the contralateral side of the body. In lepromatous cases, a second biopsy was taken from an equivalent site on the contralateral side, as a control biopsy was not possible. Prior to removal, the biopsy site was inoculated sub-epidermally with 0·03 ml of 1·0% colloidal carbon to create a bleb, a technique used by Casley-Smith and Florey (1961)[9] (Table 1). The colloidal carbon is inert, like Indian ink, and has been used for skin marking for decades, for example, marking out radiotherapy fields. At some sites, the injection was intentionally made deeper into the dermis. Following injection, the majority of patients were subjected to gentle local massage for up to 60 min in an attempt to maximize clearance of the colloidal carbon via the lymphatics. The carbon depot was excised in all patients and the biopsy processed for classification. Ethical permission was granted through The Banaras Hindu University.

Table 1. Leprosy biopsies to study clearance of colloidal carbon. (Contra-lateral biopsies were also taken from each patient)

	Leprosy classification				
	TT	BT	BB	BL	LL
Trunk	2	4	1	4	4
Arm	2	6	1	2	5
Total	4	10	2	6	9

Results

'NORMAL' BIOPSIES

H&E

Except in the biopsies from LL patients, the control biopsies were completely normal on H&E stain. Few lymphatics were seen in normal skin, in which the identification of dermal lymphatics is difficult because they often collapse *ex vivo* and cannot be seen on an H&E section. Placing the biopsy under stretch can overcome this effect.[10]

Orcein

As expected from previous studies, lymphatics were best visualized by acid orcein stain in the sub-papillary dermis in the vicinity of the superficial vascular plexus. In slightly dilated lymphatics, valves could be seen (in contrast to blood vessels at this level in the dermis, which do not possess valves). An elastic fibre envelope was observed around all lymphatics, but not around blood microvessels (Figure la).

Carbon

A depot of colloidal carbon was visualized in the sub-papillary dermis or deeper in the mid to lower reticular dermis as intended. As carbon particles migrated, so they tended to 'line up' in rows, presumably in pre-lymphatic tissue channels where they were clearly associated with elastic fibres (Figure 2). Carbon particles were seen migrating towards and finally surrounding initial lymphatics some distance away from the main depot (Figure 3). Massage, while known to increase local lymph flow,[11] did not obviously alter the pattern of carbon distribution. Even after massage, deeper injections failed to demonstrate any upward passage of carbon to superficial initial lymphatics, suggesting competent valve function and no dermal backflow. However, entry into lymphatics at this level is suboptimal.

LEPROSY BIOPSIES

H&E

Between granulomatous areas, initial lymphatics in the superficial plexus appeared morphologically normal (Figure 1), whereas within the granuloma, where blood vessels are

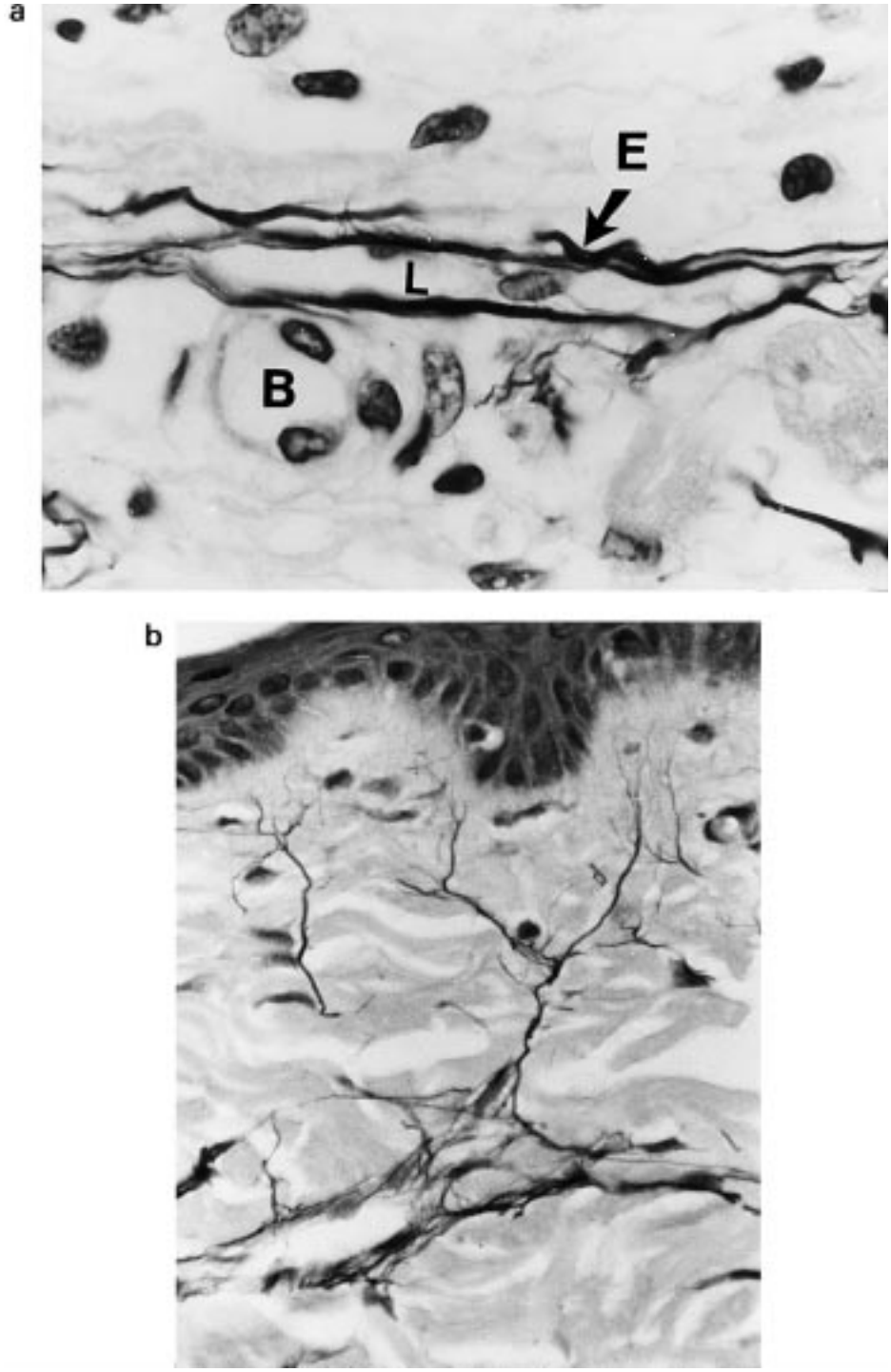

Figure 1. Control skin: (a) Normal initial lymphatic (L) in upper dermis surrounded by elastic fibres (E) with adjacent blood vessel (B) that is not surrounded by elastic fibres. (b) Lymphatics surrounded by elastin fibres in the upper dermis showing elastin fibres extending to the epidermis. Stain, orcein; magnification, ×200.

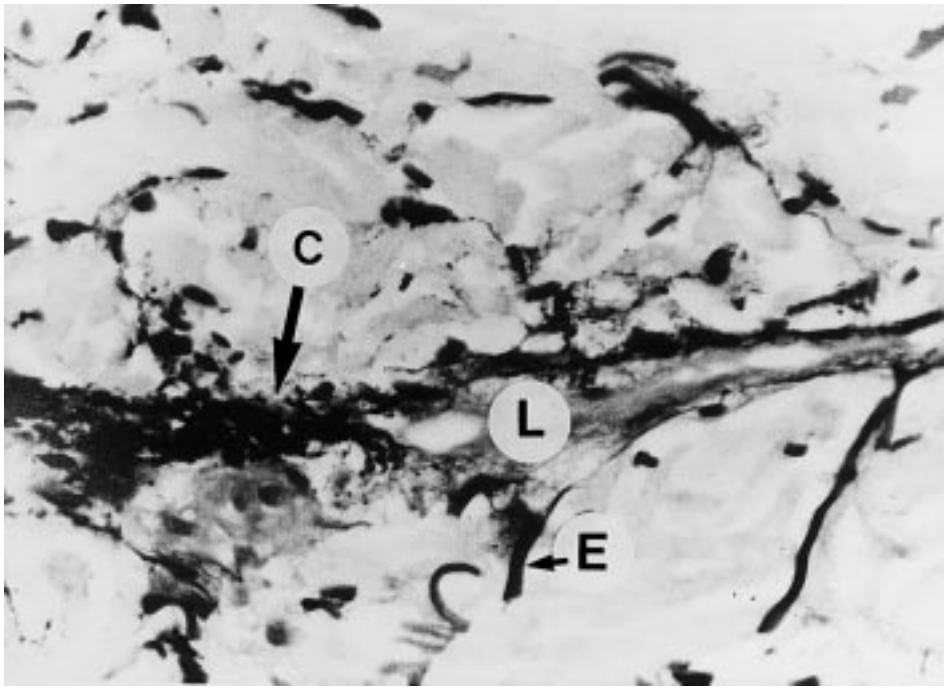

Figure 2. Control skin: carbon particles (C) associated with elastic fibres. E = uninvolved elastic fibres. L = lymphatic lumen. Stain, orcein; magnification × 700.

plentiful, lymphatics could not be identified. No differences across the leprosy spectrum were noted except in lepromatous leprosy, where many dilated lymphatics were seen at the edge of the granuloma (Figure 4). In the deep dermis, lymphatics were equally difficult to recognize within granulomas.

Orcein

The striking abnormality was the total absence of elastic fibres within areas of lepromatous infiltrate, but retention of a normal elastic fibre network in the surrounding normal connective tissue. Correspondingly, initial lymphatics lost their rim of elastic fibres except in interpathological areas where they appeared normal.

Carbon

In the lesional biopsies, no significant deposits of carbon could be detected within granulomas, but carbon was abundantly seen surrounding the perimeter of such areas (Figure 5). The severity of the inflammation did not seem to influence the distribution of the carbon. Particles were seen in deep dermis and subcutis irrespective of the density of lepromatous infiltration.

Fite-Faraco

Leprosy bacilli were identified, sometimes in profusion, i.e. globi in the endothelial cells of initial lymphatics (Figure 6).

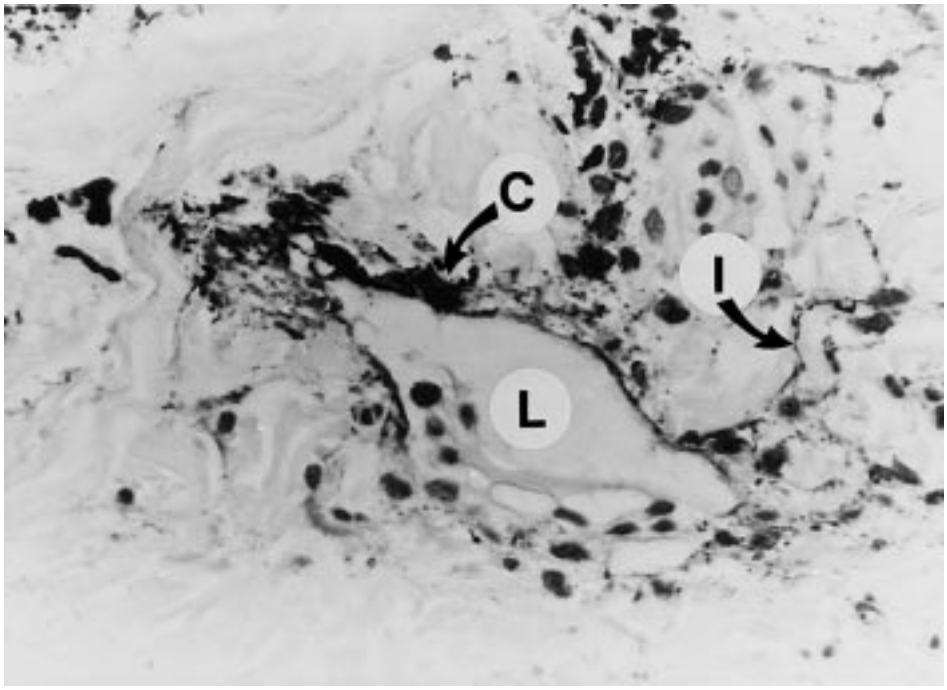

Figure 3. Control skin: carbon particles migrating (I) towards and outlining (C) an initial lymphatic (L). Stain, haematoxylin and eosin, magnification, ×700.

Discussion

Although dilatation of the lymph vessels in leprosy has been recognized since Hansen's observations in 1895,[1] surprisingly few detailed studies have been undertaken on lymphatic involvement in leprosy. Dilatation of lymphatics occurs in response to an increased load, or when the lymphatic vessels malfunction. The purpose of this paper is to add to the story the role of elastin, which we believe is a preferential route into the lymphatics, and which is destroyed by the granuloma that is the hallmark of leprosy. The role of elastin and the lymphatic was first described by Unna[12] and the relationship was further explored by Mortimer et al.,[13] Gerli *et al.*,[14,15] Poggi *et al.*[16] and Solito *et al.*[17] These studies established, in the skin, the importance of the elastin fibre in the wall of the lymphatic. Parish *et al.*[18] argued that elastases may effectively protect lymphatics from entry of harmful bacteria by the destruction of elastin. The loss of elastic fibres within inflammatory areas is not a new observation. Unna[12] commented on their loss 'where leprous new growth is established', but its significance in relation to initial lymphatics has not been appreciated. Elastic fibres appear removed or destroyed, and not simply pushed aside by an expanding leprous infiltrate. Elastic fibres surround normal initial lymphatics in the skin and are believed to be important for the normal function of such vessels.[13] The loss of elastic fibres probably influences the way lymphatics respond to tissue movement and thereby the entry of material into lymphatics. The observation that carbon particles are associated with elastic fibres and are similarly absent within the granulomatous infiltrate is of further interest. Absence of

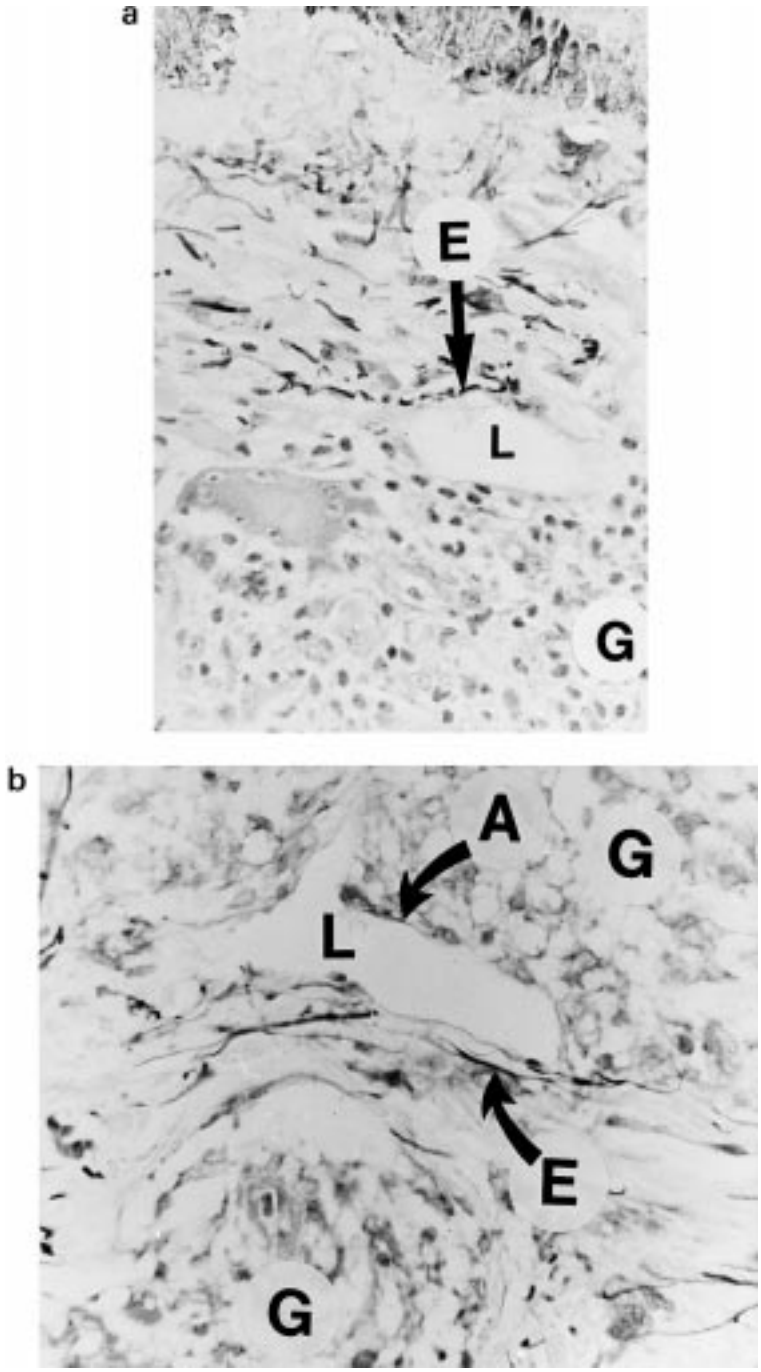

Figure 4. (a) Dilated initial lymphatic (L) at upper edge of lepromatous granuloma (G) showing loss of elastic fibres at border with the granuloma and their presence in uninvolved tissue (E). Stain, orcein; magnification, ×430. (b) The initial lymphatics (L) lose their 'elastic fibre envelope' (A) within the lepromatous granuloma (C). The remains of the 'elastic fibre envelope' are just visible (E) along the non-granulomatous edge of the lymphatic. Stain, orcein; magnification, ×650.

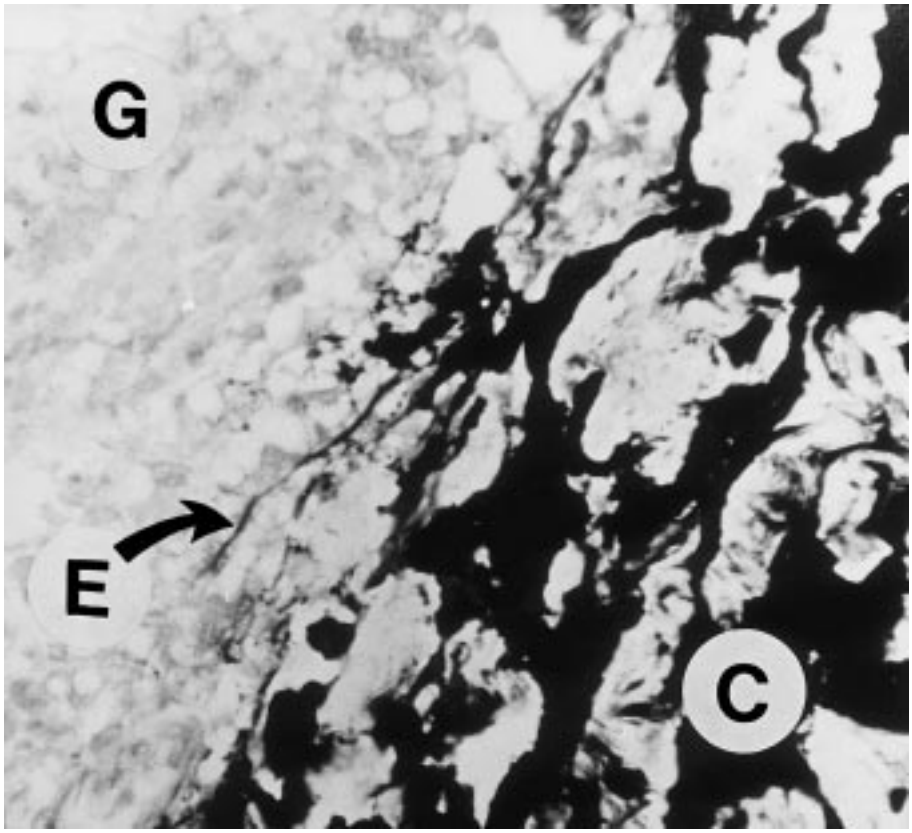

Figure 5. Carbon particles (C) are excluded from the lepromatous granuloma (G). Elastic fibres (E) can be seen terminating at the edge of the granuloma. Stain, orcein; magnification, ×430.

elastic fibres and injected carbon within granulomata suggest an alteration in the way initial lymphatics clear material, and an interruption in the normal pathways carrying information to the lymphatics.

The route by which antigen gains access to the biological system has a major effect on the nature of the immune expression as well as the components of the immune system.[19,20] The renewed interest in immune deficiency of AIDS has also stimulated discussion of the role of lymphatic massage, the most effective way to stimulate flow into and along lymphatics. Ironson *et al.*[21] were able to increase blood stream natural killer cell cytotoxicity, soluble CD8 and the cytotoxic subset of CD8 cells by massage therapy. There is also the discussion of the origin of Kaposi's sarcoma and its absence from certain sites not involved in leprosy, such as the brain and bone marrow.

The question as to whether lymphatic failure can contribute to immunological tolerance, and whether failure of the lymphatics is important in leprosy, has been debated for more than 100 years. Hoggan[22] concluded, 'Although leprosy causes changes in the appearance of lymphatics ... these changes are merely secondary, and the lymphatics themselves have no share in the causation of the disease.' In the first great dermatopathological textbook,

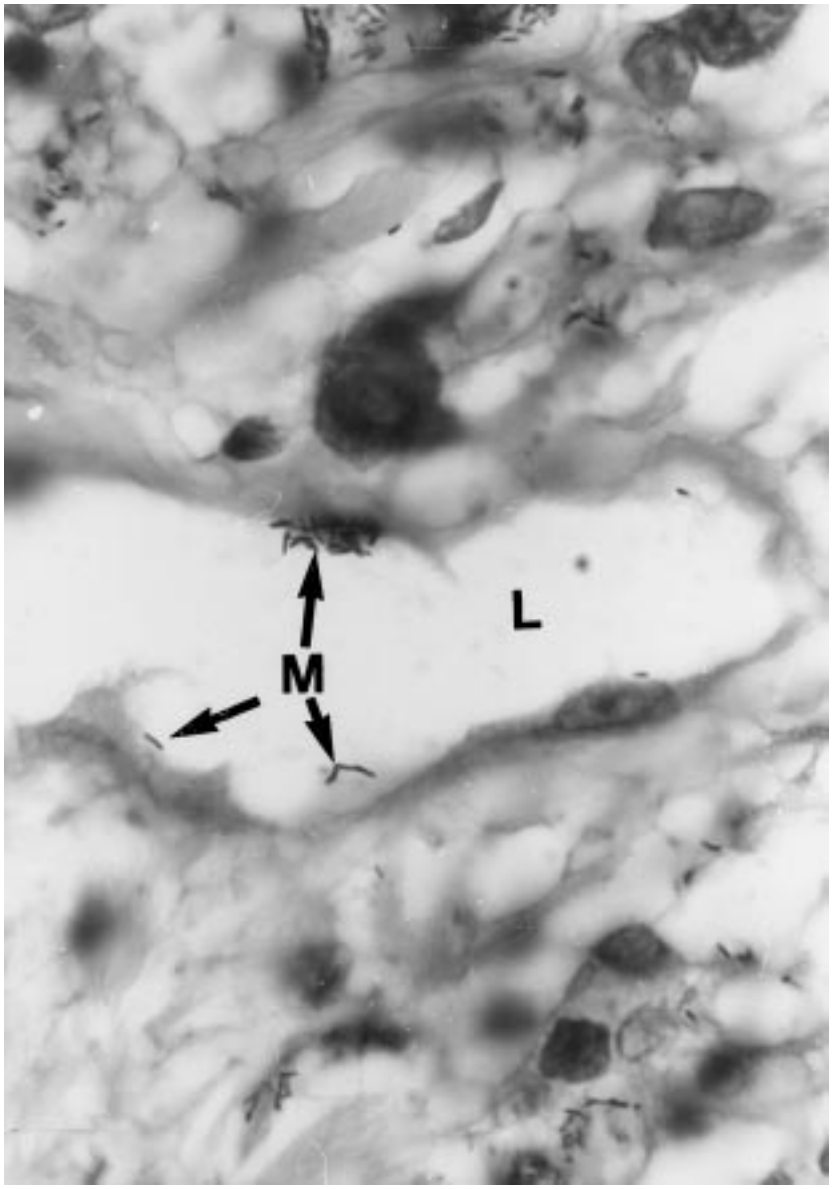

Figure 6. *M. leprae* (arrows) present in endothelial cells of initial lymphatics (L). Stain, modified Fite-Faraco; magnification, ×1800.

Unna[12] stated, 'We may define the leproma of the skin as a diffuse granuloma, whose peculiarity consists on the one hand in its limitation to the connective tissue elements, and especially to the lymphatic system of the skin, and on the other hand in the enormous growth of organisms, whose number far exceeds anything they are accustomed to find in other infectious diseases.' Shepherd *et al.*,[23] using female laboratory mice, found that injection of *Mycobacterium leprae* in an arachis oil suspension intradermally produced the greatest

sensitization, while injection by the intravenous route and to a lesser degree the intraperitoneal route tended to produce tolerance. This opened up the possibility that bypassing the lymphatics could lead to tolerance, in the same ways as tolerance to chrome was shown by Polak[24] to develop as a result of direct passage of chrome into the blood stream rather than through the lymphatic system.

Our own studies of technetium colloid (99 TMC) clearance from the skin[11] in some 30 patients studied in the Section of Skin & Venereal Disease, Banaras Hindu University, showed no absolute failure in transfer of macromolecules from the skin to the lymph node in patients with leprosy (unpublished observation) but the lymphatics between granulomata are normal and we could not exclude the possibility of clearance via the blood stream through cutaneous lympho-venous shunts.

The small world of the granuloma is rich in vasculature. Jones[25] showed factor VIII positivity in many vessels, but he was unable to demonstrate any lymphatics, features very similar to adipose tissue. A fat globule is well vascularized, but lymphatics are confined to peripheral areas. Ryan and Mallon[19] have argued that such systems are designed to have the same effect as Shepherd[23] had shown, namely diversion away from lymph nodes, direct flow into the blood vascular system and consequent immune tolerance. Vascular endothelial uptake of bacteria, and vascular circulation, rather than the lymphatic route, is a system that bypasses cell recognition in the lymph node.

This study has confirmed the value of acid orcein stain and pre-biopsy injections of colloidal carbon for investigating skin lymphatics. In normal skin the migration of carbon particles following massage suggests a route towards nearby initial lymphatics via pre-lymphatic tissue pathways that are associated with the elastic fibres. Superficial deposits of carbon drain to initial lymphatics and subsequently downwards into deeper dermis whereas the reverse does not hold true. No carbon particles could be identified superficial to deep dermal deposits.

Bacillary invasion of larger lymphatic trunks in lepromatous leprosy has been known for many years.[4] Coruh and McDougall[25] demonstrated bacilli in the endothelium of cutaneous blood vessels. We can add to this by reporting the presence of leprosy bacilli, including globi, in endothelial cells of initial lymphatics. This is a clear sign of peripheral lymphatic failure: normal lymphatic endothelium contributes to complete clearance and shows little or no features of phagocytosis. In order to generate a normal immune response, antigen must be directed to the regional lymph node via the afferent lymphatic. Any factors that interfere with this route thereby redirecting antigen, for example, via the bloodstream or via the enhanced vasculature within the granuloma, may alter immunological response and so induce tolerance. Shunts within the lymph node may have a similar effect.

Involvement of lymph nodes in both tuberculoid and lepromatous leprosy is well described but how much of this is a result of dissemination of the disease from the periphery, e.g. skin, via the lymphatics is unclear. On the other hand, how such lymph node involvement influences peripheral lymphatic flow and pathology is not clear, even in such major and well-studied lymphatic disorders as lymphatic filariasis. In onchocerciasis, elastin is also destroyed, and there is some evidence that onchocerciasis favours lepromatous leprosy.[26] McDougall,[27] in a review of the records of the Leprosy Study Centre, describes how 13,498 sections were examined: of these, 7000 biopsies which were originally classified as having ‘indeterminate leprosy’ or ‘simple macular cases’ revealed microfilariasis as the only pathology.

In conclusion, there are theoretical reasons to re-examine the role of lymphatic vessels

in leprosy. Observations on the distribution of injected carbon in response to initial lymphatics, loss of the elastic fibre framework surrounding the lymphatic, dilatation of initial lymphatics and the identification of leprosy bacilli within lymphatic endothelium in LLs support the view that dermal lymphatics are compromised by the process of leprosy. The problem may be compartmental, with normal lymphatic function existing between granulomata. Dilated tissue channels and initial lymphatics in lepromatous leprosy could mean overfilling or decreased failure to empty, and until we have better techniques for investigation, the role of lymphatics in disease processes such as leprosy remains poorly understood.

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