In vitro Assessment of Endothelial Cell Response to *Mycobacterium leprae*

TO THE EDITOR:

The presence of *Mycobacterium leprae* in endothelium was reported by Fite as early as 1941 ([5]). Coruh and McDougall ([4]) established it as a feature found predominantly in lepromatous leprosy patients. These observations were on dermal blood vessels. The changes in neural vessels reported by us and others ([2, 3, 7, 8]) were reactive endothelial cells (EC) and proliferation of basement membrane. Similar changes were seen in human leprosy endothelial cells in lepromatous leprosy nerves harboring the bacilli ([3]). Turkel, *et al.* ([11]) presented precise parameters to evaluate blood vessel changes. Mehta, *et al.* ([8]) applied these parameters to assess blood vessel changes in armadillo peripheral nerves from animals having been infected by three different routes: a) natural infection, b) foot pad inoculation, and c) intravenous inoculation.

ECs share morphological similarity with nerve tissues except for the junctional complexes ([1]). The blood vessels in different organs have different cell environments which may modify EC reactions. Hence, precise assessment of endothelial cell response in leprosy is not possible in *in vivo* studies.

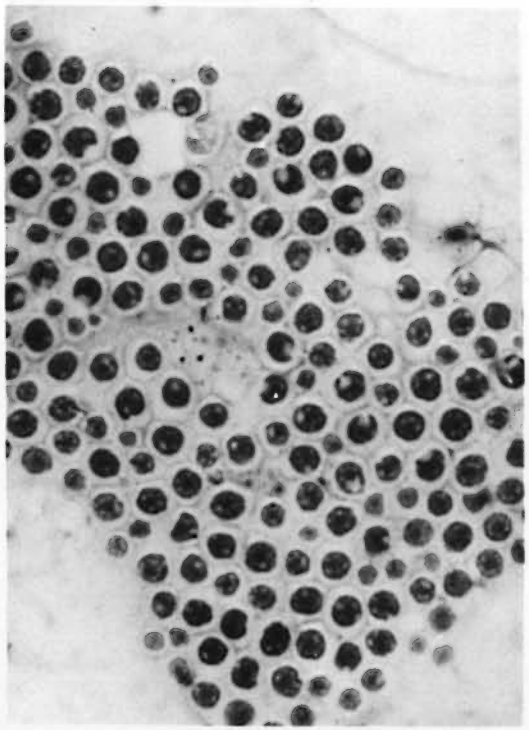

FIG. 1. Normal endothelial cells in a 3-day-old culture. Note the difference in their sizes and cell integrity (H&E ×100).

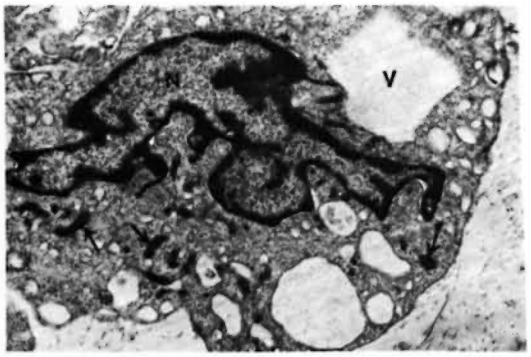

FIG. 2. Electron microphotograph of normal endothelial cells in culture. Note the rod-shaped Weibel-Palade bodies (→) (araldite embedding, uranylacetate-lead citrate stain ×8000). N = nucleus, V = vesicles.

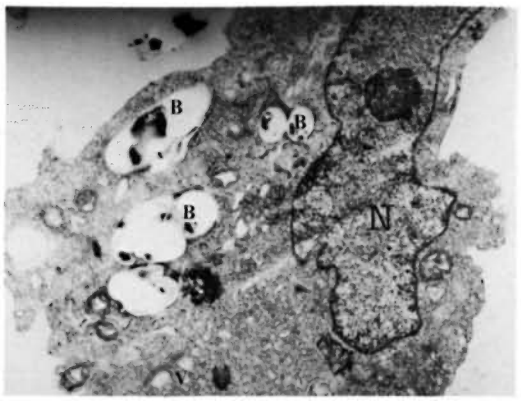

FIG. 3. Electron microphotograph of an endothelial cell infected with live bacilli (×18,000). B = bacilli, V = vacuoles, N = nucleus.

We thought it essential a) to establish the role of ECs in leprosy and b) to see whether the passage of mycobacteria and/or antigen in nerve evokes specific reactions of the endothelium or c) if the endothelial reactions seen are nonspecific passive interactions.

For *in vitro* studies, endothelial cell culture seems to be the direct method to observe the reactions ([10]). EC culture is now established as a valuable research approach to assess the pathophysiology of blood vessels ([6]).

Endothelial cell cultures were done as per the Gimbrone method ([6]) from umbilical cords. After establishing the cultures for 7–10 days, subcultures were inoculated with armadillo-derived live and irradiated leprosy bacilli (5×10^6). Controls were maintained. The phagocytic index was calculated. Cultures were observed under light and electron microscopes. A total of 45 sets of cultures were assessed.

Observations made were as follows:

Controls. A one-day culture showed a "cobblestone" pattern which after 3–4 days became polygonal in shape (Fig. 1). Confirmation of ECs was done by the demonstration of Weibel-Palade bodies (WPB) under the transmission electron microscope (Fig. 2).

ECs infected (live bacilli). Intracellular bacilli were seen. The phagocytic index was calculated as: number of bacillated cells/total number of cells counted × 100. The mean phagocytic index was 27.75% on the first day, 20.33% on the second day, and 12.66% on the third day ($p < 0.01$).

Infected (irradiated bacilli). The irradiated bacilli appeared beaded and seemed to be sticking to the cell membrane. Most of them were extracellular and not phagocytized.

EM observations. Small vesicles or plasmalemmal vesicles of different sizes were seen at the periphery of the ECs (Fig. 2). A big vacuole could be seen, indicating formation of a lumen. Free and bound ribosomes were visible. ECs were seen dividing and a few cells were forming junctional complexes.

Infected (live bacilli). There was a decrease in WPB, and increases in cell size, cytoplasmic projections, vacuoles and free ribosomes. Intracellular, well-capsulated bacilli surrounded by a clear zone were seen (Fig. 3). Two different types of cells, namely, dark and transluscent, were seen and phagolysomes were observed.

Infected (irradiated bacilli). The majority of the bacilli were extracellular. There was a gross increase in pinocytotic vesicles; rough endoplasmic reticulum and lipid droplets were seen in some cells.

In vitro endothelial cells could be studied independently, without the influence of other microenvironments which otherwise might have modified their reaction. Although the cultures were established and maturity of the cells was achieved by 7–10 days, it was necessary to confirm the identity of the ECs by the presence of WPB by electron microscopy (Fig. 2).

In *in vivo* studies, the phagocytic index of

ECs was found to be 50–100% ([4]). In the present *in vitro* study, it was 27.75% after 24 hr, and it was reduced to 12.66% on the third day. It seems that the microenvironment supports the phagocytic action of the ECs which was lost in culture.

It is reported that the phagocytic index of Schwann cells is 15.9% after 24 hr and reaches 67.2% by the third day ([9]). This suggests a high affinity of bacilli toward Schwann cells (SC). It seems that Schwann cells *in vivo* in leprous neuropathy influence phagocytic activity of ECs and both of these cells (EC and SC) seem to be complementary.

The majority of live bacilli were seen intracellularly (Fig. 3), while irradiated ones were mainly seen extracellularly. It appears that viablity of the bacillus is essential for its recognition by ECs.

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