Electron-Microscopic Study of Negative Mitsuda Reaction in Nine-Banded Armadillos and Lessons to be Learned

TO THE EDITOR:

A negative Mitsuda reaction in a leprosy patient indicates the absence of a cell-mediated immune response to *Mycobacterium leprae* and, therefore, either the presence of generalized lepromatous disease or a tendency to progress toward it. In a normal person it indicates the inability to destroy *M. leprae* and, therefore, he/she is liable to become infected if exposed and, when infected, more likely to develop lepromatous leprosy ([3]). It is not yet clear whether this anergy to *M. leprae* is inherent or acquired ([2]). There have been several histopathological studies of the Mitsuda reaction ([4, 6, 8, 11]), but an electron-microscope study, especially of the negative reaction, is not found in the literature. In this study we describe the histopathologic and electron-microscopic appearance of the negative Mitsuda reaction in nine-banded armadillos and discuss its significance.

Three known lepromin-negative armadillos were chosen. An armadillo-derived, autoclaved, saline suspension of 1.7×10^8/ml *M. leprae* was prepared and 0.1 ml of the suspension was intradermally injected into the abdominal skin of the armadillos. The injected site was circled with a skin-marking pencil. It is well known that at 21 days the inflammatory cells present at a negative lepromin site are very few, widely dispersed in the dermis, and are occasionally difficult to find. Therefore, it was decided to biopsy the lepromin site on the 10th day using a 5-mm punch.

The biopsy tissues obtained were bisected; one half was fixed in 10% buffered, neutral formalin and processed for paraffin sections (5 μm sections were made); one section was stained with hematoxylin and eosin (H&E) and the other with a modified Fite's technique for acid-fast bacilli (AFB) ([5]). The other half of the tissue was cut into 1-mm cubes and fixed in 5% glutaraldehyde at 4°C for 4 hr, washed with sodium cacodylate buffer at pH 7.3, postfixed in 1% osmium tetroxide, processed for embedding in Spurrs resin (Polysciences, Inc., Warrington, Pennsylvania, U.S.A.), and ultrathin sections were made for examination under a Philips EM.410 electron microscope.

Histopathologic examination showed no significant change in the epidermis. In the dermis there were several scattered spindle-shaped, fibroblast-like macrophages with their long thin processes between collagen bundles distributed in a large area of the section. Also present were small focal collections of macrophages having a pink granular cytoplasm (Fig. 1). There were no recognizable plasma cells. Lymphocytes were very scanty. Neutrophils were absent. The acid-fast stain showed granular bacilli

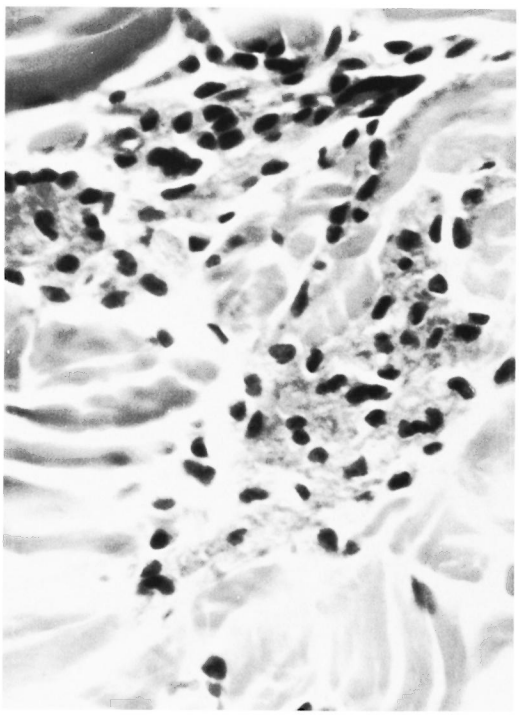

FIG. 1. Photomicrograph showing small focal collections of macrophages with abundant granular cytoplasm in the dermis (H&E ×400).

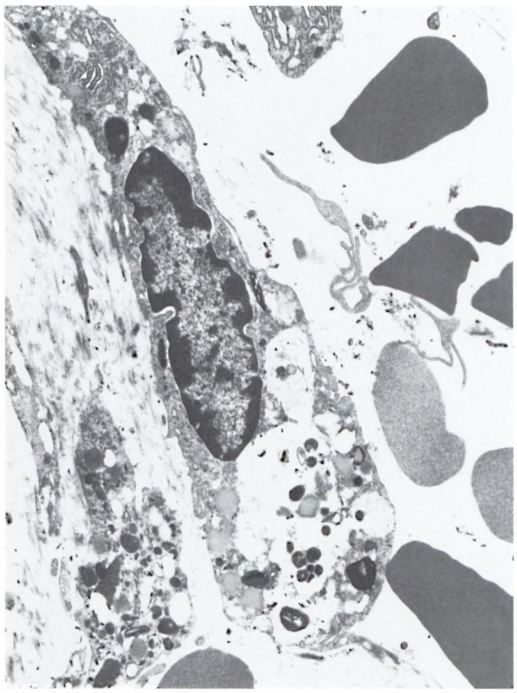

FIG. 2. Electronmicrograph of a spindle-shaped macrophage lying between collagen bundles containing many *M. leprae* (×7000).

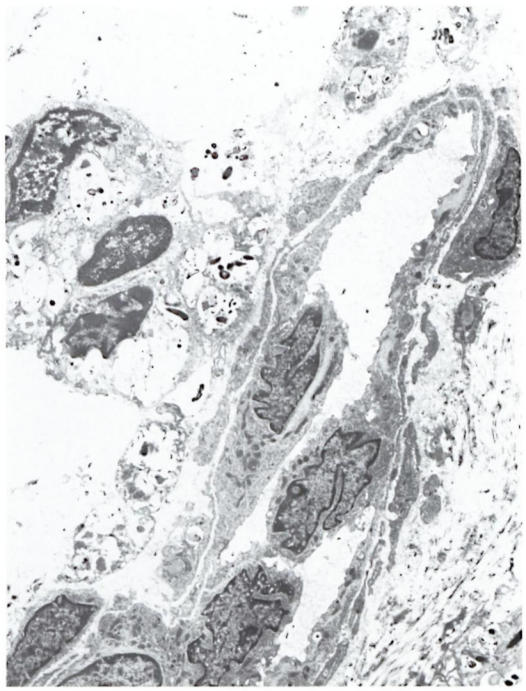

FIG. 3. Electronmicrograph of a blood vessel surrounded by macrophages containing *M. leprae* (×7000).

in clumps inside macrophages. Many organisms were lying free in tissue spaces and a few were present in endothelial cells of capillaries.

An electron-microscopic study yielded very interesting and informative findings. Several thin, elongated macrophages with long processes in between collagen bundles containing clumps of *M. leprae* were seen (Fig. 2). There were also many macrophages around and in close apposition to several small lymph and blood vessels (Fig. 3). All of them had many intracellular *M. leprae* with an accumulation of electron-transparent material around them. Macrophages containing *M. leprae* were found inside a few dilated lymph channels and occasional blood vessels (Figs. 4 and 5). The endothelial cells lining the capillaries and lymph vessels also showed *M. leprae* (Fig. 5). Many fragmented *M. leprae* were seen lying free in the interstitial spaces of the dermis. No plasma cells were identified, and lymphocytes were very rare.

When 1.6×10^7 *M. leprae* were injected intradermally into a lepromin-negative subject only a minimal inflammatory reaction was initiated which on the 10th day consisted almost entirely of a small number of macrophages, some scattered and others in small focal collections in the dermis. The blood monocytes that came into the site ingested as much of the bacilli as possible, migrated back into the lymph and blood vessels, and were carried away to regional lymph nodes and other remote organs. It was observed that the macrophages had not phagocytized all of the organisms injected because a sizable amount of bacilli were still seen lying free in interstitial spaces. In a study evaluating the efficacy of a vaccine, the sites which gave an earlier negative Mitsuda response turned positive many months later following the administration of an effective vaccine, thus showing the persistence of *M. leprae* antigen at the injected site for many months ([1]).

It is quite possible that in lepromin-negative armadillos the macrophages have a genetic defect similar to that discovered in murine macrophages ([10, 12]) and, therefore, they were unable to secrete T-cell-amplify-

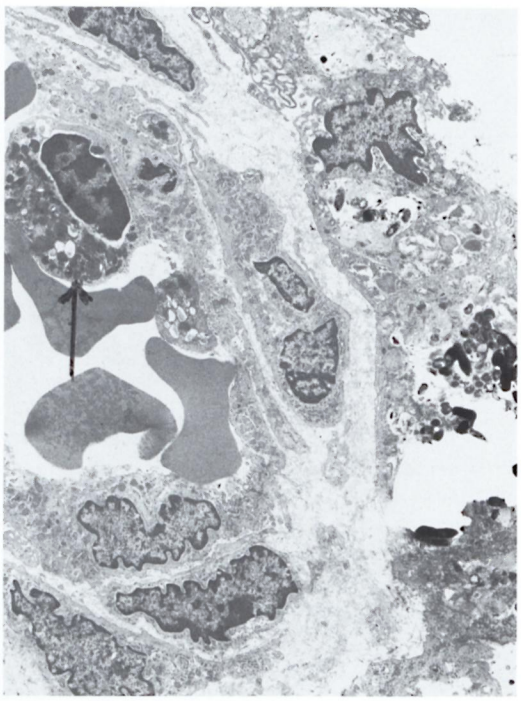

FIG. 4. Electronmicrograph of a blood vessel showing in its lumen a macrophage (↑) with several *M. leprae* (×7000).

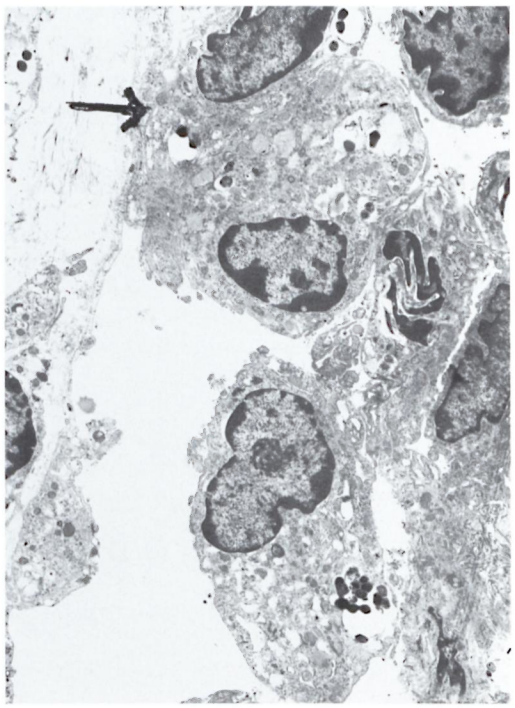

FIG. 5. Electronmicrograph of a lymphatic vessel with macrophages in its lumen. An endothelial cell (→) and the macrophages contain many *M. leprae* (×7000).

ing monokines such as interleukin-1 and tumor necrosis factor-alpha. Defective macrophage function in lepromatous disease has been pointed out by many other workers ([7-9]). Hardly any lymphocytes were found at the injected site. It is obvious that there were no cytokines either to stimulate the recruitment of inflammatory cells or to immobilize them at the site and to aid in the formation of a protective granuloma.

The fact that some portion of the killed bacilli were left behind lying free in the tissue even on the 10th day without being handled by phagocytes, gives credence to the thesis that if *M. leprae* get in, in a susceptible host they will be exposed to and enter parenchymal cells like endothelial cells, as shown in this study, and also in Schwann cells and smooth muscle cells even at the early stage of infection. If the infected person develops immunity, he may develop a tuberculoid lesion at the site of entry where the organisms persist in interstitial tissue and parenchyma cells. Failure to develop immunity will result in lepromatous disease.

Study of the cytokine kinetics of the negative lepromin reaction in normal human subjects will yield valuable results to clarify the immunologic defect in lepromatous leprosy.

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