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An Electron Microscopic Study on Macrophages and Lymphocytes in Lepromatous and Borderline Leprosy[1]

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The clinical pattern of leprosy depends on the response of the host to the organism *Mycobacterium leprae.* In well-developed cellular immunity, the clinical picture corresponds to tuberculoid leprosy; without cellular immunity, the clinical pattern is that of lepromatous leprosy. Between these two polar forms lies the remaining leprosy spectrum: borderline leprosy as well as the borderline lepromatous (BL) and borderline tuberculoid (BT) forms ([13]).

Corresponding to this classification, characteristic histologic alterations occur ([14]). In tuberculoid leprosy, the macrophages differentiate into epithelioid cells and giant cells. Nests of epithelioid cells can be surrounded by small lymphocytes, thus forming a "tubercle." In lepromatous leprosy (LL), on the other hand, the macrophages cannot differentiate into epithelioid cells.

Ultrastructural studies have been made on the mononuclear cell series throughout the spectrum of leprosy from LL to BT ([14, 15]). In the present study, some further remarks concerning macrophage development in the leprosy spectrum and some observations concerning the interaction of macrophages and lymphocytes are reported.

MATERIALS AND METHODS

Patients. The study was carried out on biopsies from 6 patients with lepromatous leprosy (2 from Ethiopia, 3 from India, and 1 from Laos) and from 3 patients with BL and BB leprosy (1 from Turkey, 1 from Ethiopia, and 1 from Laos). Each diagnosis was confirmed by clinical examination and by skin biopsy.

Biopsy. Skin biopsies were taken under local anesthesia. Immediately after the biopsy, the material was fixed in 5% glutaraldehyde in 0.1% phosphate or cacodylate buffer (ph 7.2) for 4–12 hr. The material was then washed in buffer and sent to the Ernst-Rodenwaldt Institut in Koblenz, Germany.

Preparation for electron microscopy. The material was contrasted and postfixed in a solution of 2% osmium tetroxide and 1% potassium dichromate for 2–3 hr. After dehydration in graded alcohols and propylene oxide, embedding was performed in Durcopan®. Ultrathin sections were contrasted with lead citrate and uranyl acetate. Some probes were contrasted en bloc in 70% alcohol with 2% phosphotungstic acid and 1% uranyl acetate.

RESULTS

Lepromatous leprosy. In all biopsies the leprosy bacilli were found under the epidermis, not only in connective tissue cells but also extracellularly (Fig. 1).

Near the cutaneous vessels macrophages are found which still resemble monocytes (Fig. 2). These macrophages contain an eccentric nucleus with an irregular surface and some nucleoli. These cells have only some membranous folds, and only occasionally may contain mycobacteria. These cells can show cell division, during which the phagocytic activity seems to be reduced. At greater distances from the cutaneous vessels the macrophages contain lysosomes. The ectoplasm extends to form pseudopodia and membranous folds; the ergastoplasm now is seen more distinctly (Fig. 3) and the nucleoplasm contains inclusion bodies (spheridia).

These macrophages phagocytose mycobacteria by: a) Attachment phase: The my-

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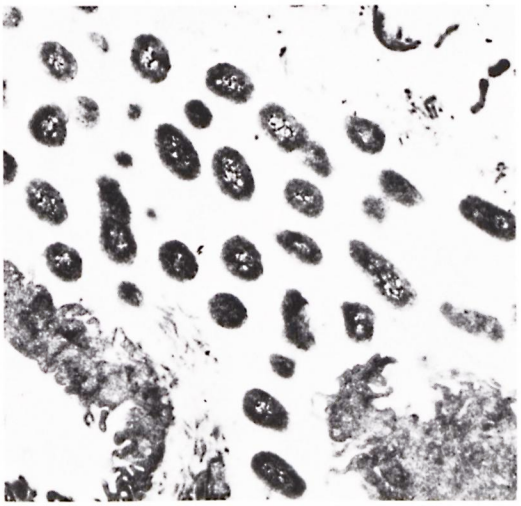

FIG. 1. Extracellular bacilli in the interstice (original magnification ×25,000).

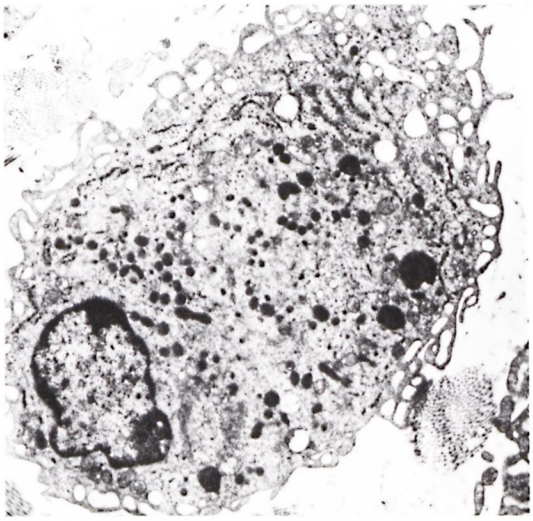

FIG. 3. Activated macrophage with distinct ergastoplasm (original magnification ×9000).

cobacteria lie between the lamellipodia of the macrophages and are encircled by them (Fig. 4). In this part of the cytoplasm the organelles disappear and the cytoplasm appears homogenous with fine filaments or granules. b) Ingestion phase: The pseudopodia fuse behind the bacteria, engulfing several bacteria at the same time. The cytoplasm around the newly developed phagosome remains homogenous. Then the phagosome with several mycobacteria is transported into the cell body.

The mycobacteria inside the macrophages look viable. With the continuation of the phagocytic process several phagosomes may fuse to form larger vacuoles, and the mycobacteria multiply until the vacuoles completely fill out the ectoplasm (Fig. 5). In some cases the cell membrane ruptures, setting the bacteria free into the interstitium. Obviously this happens in connection with bacterial overload.

Borderline lepromatous leprosy. Lym-

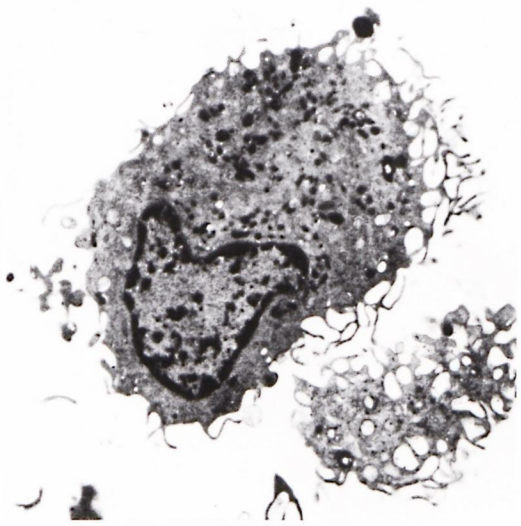

FIG. 2. Macrophage in early stage of activation (original magnification ×10,000).

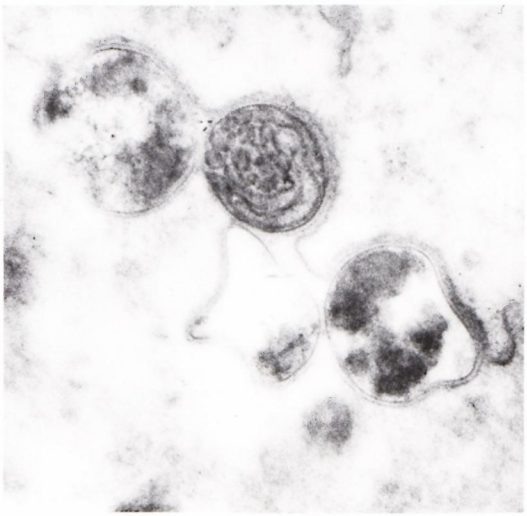

FIG. 4. Beginning invagination of surface of a macrophage after attachment of mycobacteria (original magnification ×60,000).

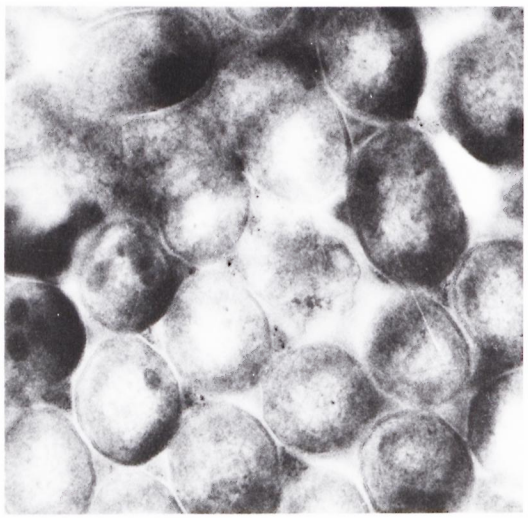

FIG. 5. Vacuole inside macrophage completely filled by mycobacteria (original magnification ×50,000).

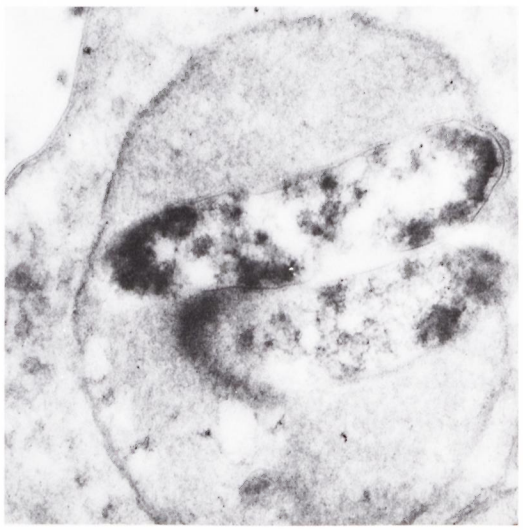

FIG. 7. Two mycobacteria inside vacuole showing disintegration of cellular contents; cell envelope preserved (original magnification ×50,000).

phocytes enter the tissue through the vessel walls. Small lymphocytes are found with a round nucleus nearly completely filling out the cell, without a rough endoplasmic reticulum (ER), and with only some mitochondria. Large activated lymphocytes are also found, containing nucleoli in the nucleus, a great number of ribosomes and polyribosomes, and exhibiting multiple folds of the cell membrane.

Mycobacteria disintegrate inside the macrophages (Figs. 6 and 7); simultaneously, greater numbers of lymphocytes appear. The disintegration of the mycobacteria seems to be rather slow; several disintegration stages can always be observed simultaneously. With the disintegration of the mycobacteria, the macrophages themselves more often show degenerative changes: at first, deposition of fatty vacuoles, then myelin and deposition of osmiophilic sub-

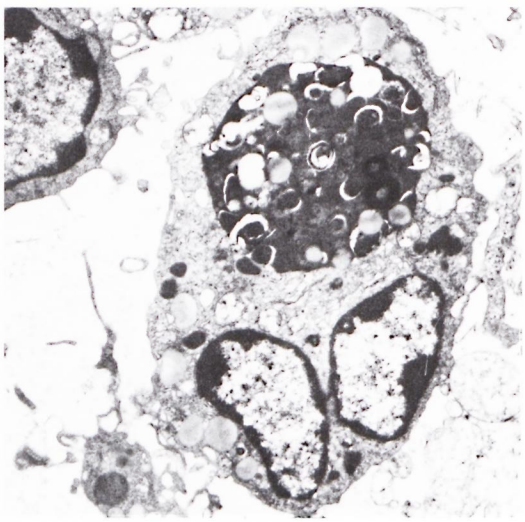

FIG. 6. Macrophage with large vacuole containing remnants of mycobacteria; fatty vacuoles can also be seen (original magnification ×15,000).

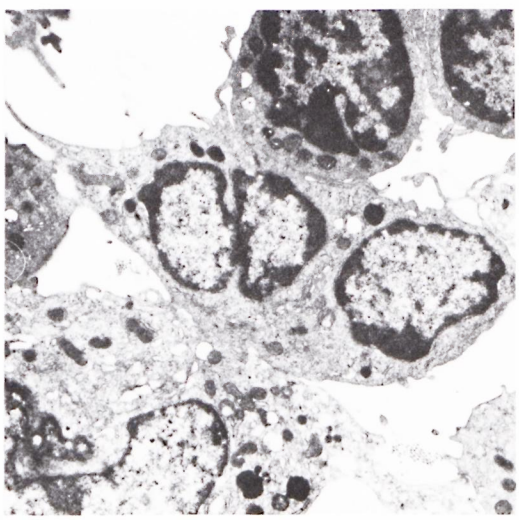

FIG. 8. Contact between macrophage and lymphocyte (original magnification ×8000).

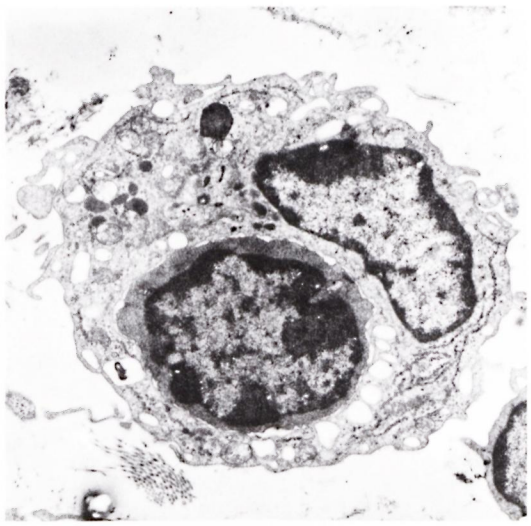

FIG. 9. Macrophage and lymphocyte in close apposition (original magnification ×18,000).

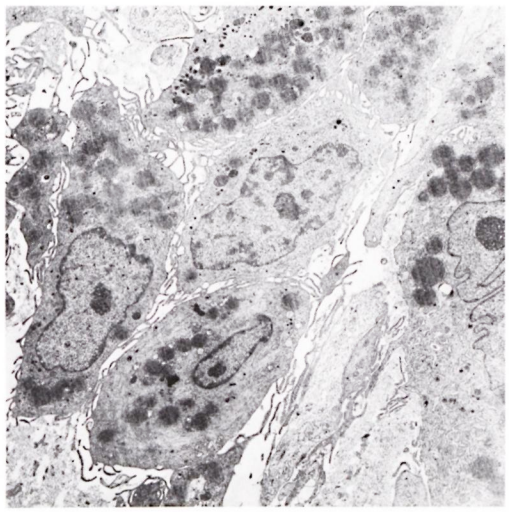

FIG. 11. Accumulation of macrophages with increased volume (original magnification ×5000).

stances, and lamellar folds of membranes in the cytoplasm.

The small as well as the larger lymphocytes closely adjoin the macrophages (Fig. 8). Sometimes the macrophages seem to surround the lymphocytes (Fig. 9). Inside the lymphocytes no mycobacteria can be found. Some plasma cells also are seen in this stage.

BB leprosy. The granulomas develop out of accumulations of macrophages. In the beginning some lymphocytes may lie within the accumulation of macrophages (Fig. 10), but a distinct wall of lymphocytes around the granuloma does not appear. Only a rim of macrophages encircles the granuloma. These macrophages contain some organelles only. Neighboring cells often are indented into each other. Mycobacteria are predominantly found in these macrophages; toward the center of the granuloma the macrophages contain less mycobacteria.

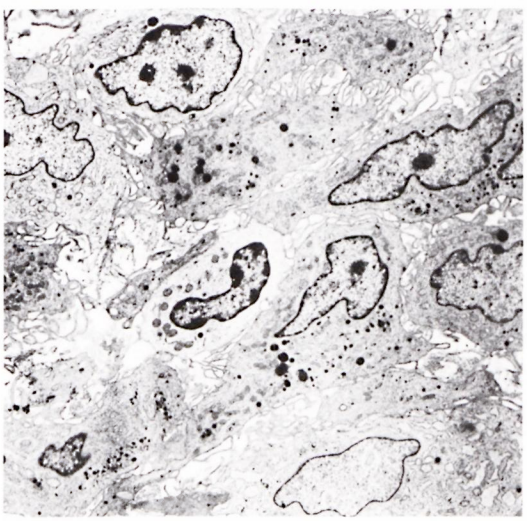

FIG. 10. Accumulation of macrophages with large lymphocyte in center (original magnification ×5000).

In the center the macrophages adopt the characteristics of epithelioid cells. The development of the epithelioid cells can be recognized by their ultrastructural characteristics, especially by their dense cytoplasm and their membranous folds. At first the macrophages increase in volume (Fig. 11). The number of lysosomes decreases; tonofilaments increase in number. Many Golgi complexes and abundant mitochondria can be seen. Additionally, the pattern of chromatin inside the nucleus changes; the nuclear material becomes more dense and many nucleoli can always be seen.

Fibrocytes near the granulomas show an increased activity with a greater cell volume and an increase in their ER. An increased formation of collagen fibers results.

Some granulomas also contain giant cells (Fig. 12). Their electron microscopic structure shows their derivation from epithelioid cells and macrophages: many mitochondria, well-developed ER, clearly distinguished tonofibrils. The nuclei are mostly

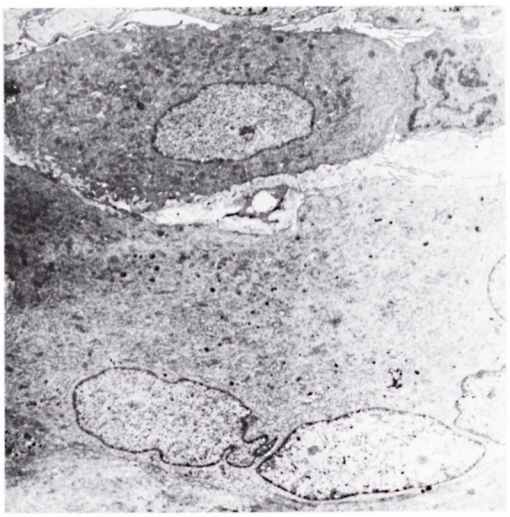

FIG. 12. Epithelioid and giant cell in apposition (original magnification ×5000).

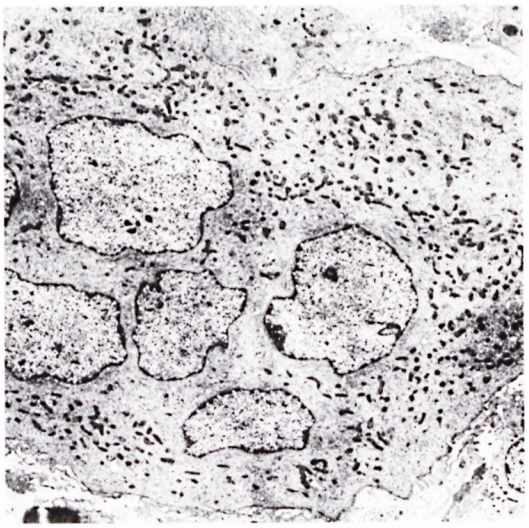

FIG. 13. Giant cell with centrally situated nuclei (original magnification ×5000).

situated in the center (Fig. 13); larger cells also may show a peripheral position of the nuclei. Indentations of the nuclei cannot be observed. Mycobacteria are rarely found inside the giant cells. In the center of the granulomas the giant cells can show fatty degeneration. Sometimes vessels are seen within the granulomas but they are always obliterated.

DISCUSSION

The results show that *M. leprae* is propagated in the skin, through the interstitital tissue and also via the cutaneous vessels. Around the vessels the macrophages are concentrated and activated; this finding is supported by other studies ([7, 8, 10, 17]). This mobilization happens at first without lymphocytes, in the sense of a nonspecific defense mechanism. The accumulation of macrophages seems to be due to some chemotactic substances of the mycobacteria. The extensive phagocytosis of mycobacteria also occurs without lymphocytes. Often even several mycobacteria are ingested simultaneously. In leprosy, phagocytosis also can be seen with macrophages that are already filled with bacteria. These cells correspond to the Virchow cells or lepra cells of light microscopy ([2, 4, 6, 11]). A fusion of phagosomes and lysosomes could not be observed.

Lymphocytes get into the lesions by leaving the venules. In addition to small lymphocytes, blasts can also be seen. These lymphocytes get into close contact with the macrophages. Obviously the lymphocytes induce some sort of activation in the macrophages, thus leading to a more intense disintegration of the mycobacteria. Differentiation of the lymphocytes in leprosy lesions with immunological methods has shown a predominance of T lymphocytes ([5]). Some plasma cells are also seen. Secretory cells of plasmacytoid appearance with much rough endoplasmic reticulum arranged in concentric lamellae were described by Ridley, *et al.* in borderline lepromatous leprosy ([15]). The function of these cells remains unclear, although it is known that humoral immunity in leprosy seems to be well preserved ([3]).

In borderline leprosy granulomas are formed ([7, 9, 16, 18]). The formation of the granulomas is analogous to that in other diseases ([1]). Macrophages differentiate into epithelioid cells which can develop into giant cells. These giant cells still have many organelles, an indication of an active metabolism. The giant cells are believed to be the result of a reaction to harmful substances. Possibly they may present antigens in a permanently harmless form to induce immune responses. Very rarely could we find mycobacteria inside giant cells.

In summary, our results concerning the mononuclear cell series are comparable to the results of Ridley, *et al.* ([15]). Contrary to their findings, we could see a greater activation of the macrophages with increased numbers of lysosomes and mitochondria and with a disintegration of the mycobacteria only when lymphocytes were also present. Obviously, the development of the phagocytic mononuclear cell series is partly a consequence of macrophage-lymphocyte interactions.

SUMMARY

We describe how macrophages are activated and phagocytose mycobacteria in lepromatous leprosy. The differentiation of macrophages into epithelioid cells and into giant cells in borderline leprosy is shown. Close apposition between macrophages and lymphocytes is seen in those areas where mycobacteria disintegrate inside macrophages.

RESUMEN

Se describe como se activan los macrófagos en la lepra lepromatosa y como fagocitan micobacterias. Se muestra como ocurre la diferenciación de macrófagos en células epitelioides y en células gigantes en la lepra intermedia. En aquellas áreas donde las micobacterias se desintegran dentro de los macrófagos, se observa una íntima aposición entre macrófagos y linfocitos.

RÉSUMÉ

On décrit la manière dont les macrophages sont activés, et selon laquelle la phagocytose des mycobactéries se déroule au cours de la lèpre lépromateuse. On illustre aussi la différenciation des macrophages en cellules épithélioides et en cellules géantes au cours de la lèpre dimorphe. Ces études ont permis de constater une juxtaposition étroite entre les macrophages et les lymphocytes dans les régions où les mycobactéries se désintègrent à l'intérieur des macrophages.

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