

Mycobacterium leprae Found in Epidermal Cells by Electron Microscopy¹

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A figure of a leproma stained by the Ziehl-Neelsen method in the book entitled *Die Lepra*, written by Klingmüller (3), shows leprosy bacilli in the epidermis. Sato (11), in his book *Rai (leprosy)*, wrote that the leprosy bacillus invades the epidermal cells. Harada (1) reported that leprosy bacilli could be found in the epidermis with his modified alochrome staining method.

We have noticed leprosy bacilli in parts of the epidermis in some cases utilizing the optical microscope. However, the histopathologic sections for optical microscopy are relatively thick. Therefore, it could not be concluded with the optical microscope whether the bacilli are certainly located in the epidermal cells or whether they are attached to the surface of the epidermal tissue section. In the latter case, the bacilli and the epidermis are seen one above the other as if the bacilli are present in the epidermal cells. Leprosy bacilli attached to the sectioning knife can be transferred and adhere to other places. In fact, it can be seen in some cases that the leprosy bacilli are attached to places without any tissue or nearby epidermis (Fig. 1).

Recently we have found by electron microscopy that leprosy bacilli may be engulfed within epidermal cells. The section for electron microscopic observation is very thin. Therefore, it can be readily determined whether or not the leprosy bacilli are piled up on the epidermis.

MATERIALS AND METHODS

A leproma from a male patient, aged 65 years, having lepromatous leprosy, was excised for the study of the cultivation of leprosy bacilli. A part of the leproma was fixed with formalin without separating its epidermis and histopathologic sections were made

for optical microscopic study. The epidermis of the remainder was cut off with scissors in order to facilitate the grinding of the leproma. In order to observe the condition of the leprosy bacilli used for cultivation, a small part of the leproma from which the epidermis had been cut off was fixed with 1% osmium tetroxide in M/15 phosphate buffer (pH 7.4), dehydrated in graded ethanol and embedded in methacrylate for electron microscopy. Because the purpose of the preparation of the specimen was the electron microscopic observation of leprosy bacilli, methacrylate which can, among the various resins, best permeate leprosy bacilli in foamy cells was used. Ultrathin sections of the embedded specimen were stained with



FIG. 1. The leprosy bacilli (M) are found not only at the part occupied by the epidermal tissue, but also at the part not occupied by any tissue.

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FIG. 2. The leprosy bacilli (M) are found in an epidermal cell. D:desmosome.

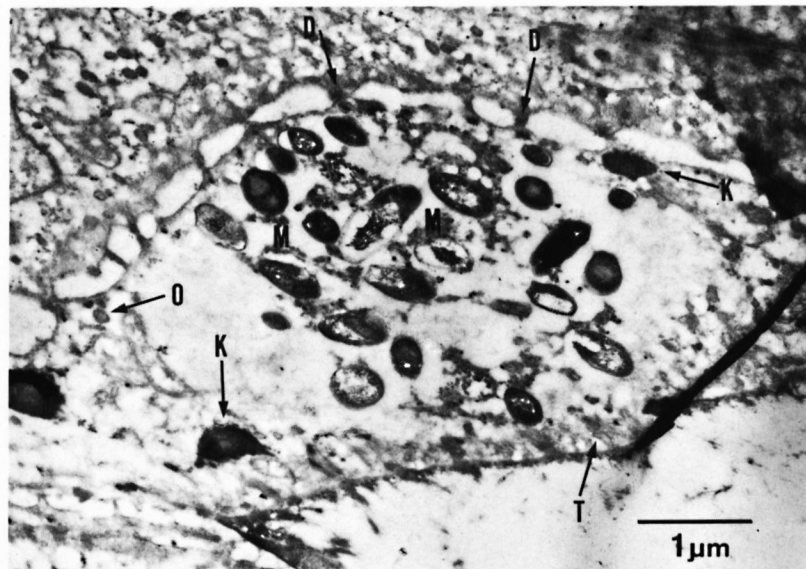


FIG. 3. Higher magnification of a portion of Figure 2. D:desmosome, which fairly loses its normal figure by being pulled by scissors; K:keratohyalin granule; M:leprosy bacilli; O:Odland body; T:tonofilament.

uranyl acetate and lead citrate and observed with an Akashi AEM-80 electron microscope.

RESULTS AND DISCUSSION

A portion of the epidermis was found still present in some specimens under electron microscopic observation. The bacilli, over

twenty of which were grouped together were found in an epidermal cell (Figs. 2, 3). Transparent zones are observed around the bacilli but there is no membrane surrounding the group of bacilli. The cell which contains the bacilli and the other cells near it are epidermal cells because they have desmosomes and tonofilaments.



FIG. 4. A foamy cell which contains leprosy bacilli (M) is observed just under the basal cell layer of the epidermis.

Desmosomes are particularly present in the stratified squamous epithelium, especially of the epidermis, but they can be found also in the other types of epithelium. In the skin, the sweat glands, sebaceous glands and epithelial hair follicles have the other types of epithelium. Nevertheless, it can be concluded from the electron microscopic findings that the epithelial tissue around the observed bacilli is not of the latter types of epithelium but is characteristic of the epidermis. The cell containing the bacilli comes in contact with adjacent cells by desmosomes, though these desmosomes are distorted by being pulled by scissors. The intercellular space between the cell containing the bacilli and the adjacent cell is widened except for bridging by the desmosomes. It is thought that the cell having the bacilli was pulled by the external force at the time of cutting off the epidermis from the leproma with scissors. The presence of desmosomes in the cell having bacilli indicates that this cell is an epidermal cell and, furthermore,

that it is not a dendritic cell such as a melanocyte or a Langerhans cell, but that it is a keratinocyte. It is also evident that the cell is not a nerve fiber extending into the epidermis. The cell having the bacilli has keratohyaline granules and the organelles which are regarded as Odland bodies. The tonofilaments in the cell are found in irregular groups. Judging from these findings, the cell containing the bacilli can be considered as a cell of the granular layer. A part of the horny layer was seen near the cell, though this is not contained in the figures presented.

Homogeneous bodies are seen in several bacilli. The bacilli found in the cell are regarded as acid-fast bacilli because all the bacilli found in the epidermis of the specimen stained with the Fite-Faraco method for optical microscopic observation were acid-fast bacilli. The epidermis was not cut off from the leproma used for the preparation of sections for optical microscopic observation. As described below, the foamy cells can be seen in contact with the epidermal basal cell layer at some places, and acid-fast bacilli are contained in these foamy structures (Fig. 4). Therefore, these acid-fast bacilli are considered to be leprosy bacilli. Since these bacilli are arranged in accordance with the contour of the foam vacuole, it cannot be held that bacilli attached to the leproma sectioning knife were transferred for attachment to this locale. Instead it is thought that leprosy bacilli in contact with the basal cells were engulfed into the keratinocyte having phagocytic activity and that the keratinocyte shifted to the upper layer as now observed. We regret that no attempt was made to cultivate the acid-fast bacilli from the epidermis in order to confirm that they were not cultivable acid-fast bacilli. This was not done because the EM finding was totally unexpected.

Generally speaking, bacteria are not found in epidermal cells. Montes and Wilborn (7) examined by optical and electron microscopy normal skin from various locations, the skin after occlusion, and the skin of comedones and intertrigo. They could not find bacteria in the epidermal cells. Lever (4) described that cocci lay extracellularly or within neutrophils in the lesion of impetigo. Pillsbury (10) wrote that the resident organisms did not occur in or between living cells of the epidermis and its appendages. Lovell

(⁵) reported that small numbers of skin bacteria were found between the most superficial cells of the horny layer, but bacteria were not present in or between the living cells of the epidermis. Montes (⁶) noted that *Corynebacterium mintissimum* could be found in the intercellular space of the stratum corneum of patients suffering from erythrasma.

The presence of pathogens in the epidermal cell may be a specific phenomenon in leprosy in which numerous bacilli are present just below the basal epidermal layer, and the young basal cells having phagocytic activity can come in contact with the pathogenic microorganisms.

The problem of how these leprosy bacilli were ingested into the epidermal cell must be considered. In lepromatous skin there is a clear sub-epidermal zone below the epidermis and this usually separates the epidermis from the leprosy bacilli. However, foamy cells having leprosy bacilli can sometimes be found just under the basal layer. Therefore, if the keratinocyte of the basal layer has phagocytic activity, it is possible that the leprosy bacilli are transferred into keratinocytes.

The phagocytic activity of keratinocytes has been fairly well studied. First, studies using tracers are briefly noted. As tracers, Nordquist *et al* (⁹) used ferritin, and Wolff *et al* (^{12,14}) used peroxidase and thorotrast. The tracers were injected intradermally and observed under the electron microscope. They penetrated into the intercellular space of the epidermis and were ingested by the keratinocytes. Wolff and his associates observed that thorotrast was stored in the vacuoles of keratinocytes and eliminated from the epidermis during the keratinization process. Next, studies on the process of transfer of melanosomes from the melanocyte which produced them to keratinocytes should be noted. Mottaz *et al* (⁸), studying the human hair, observed that melanosomes were transferred from melanocyte to keratinocyte by the phagocytic activity of the keratinocyte. Klaus (²) observed that melanosomes were ingested by keratinocytes from a projection of the melanocyte by keratinocyte heterophagy. In addition to these studies, Wolff *et al* (¹³) injected latex beads, having a diameter of 0.109 μ or 0.81 μ , into suction blisters and observed electron microscopically that the beads were engulfed into epidermal

cells. Their study also revealed that the latex beads were eliminated from the epidermis by desquamation in conjunction with the keratinized cells. Because the cell described as having leprosy bacilli is not a dendritic cell but a keratinocyte, it is held that the bacilli were transferred to the upper layer of the epidermis together with the cell and would finally have been eliminated from the epidermis. Therefore, the possibility of discharge of leprosy bacilli from the skin without ulceration should be considered.

SUMMARY

Leprosy bacilli were found in a keratinocyte of the epidermis by the electron microscopic observation of the ultrathin section of a leproma. The possibility of discharge of leprosy bacilli from the skin should be considered even if the lepromatous patient does not have any ulceration.

RESUMEN

Por microscopía electrónica de una sección ultradelgada de un leproma se encontraron bacilos de la lepra en un keratinocito de la epidermis. Debe tomarse en cuenta la posibilidad de la descarga de los bacilos de la lepra a partir de la piel, aún si el paciente lepromatoso no presenta ninguna ulceración.

RÉSUMÉ

On a découvert des bacilles de la lèpre dans un k ratinocyte de l' piderme, en utilisant des m thodes de microscopie  lectronique sur section ultra mince de l promes. On envisage la possibilit  que les bacilles de la l pre pourraient  tre lib r s de la peau, m me lorsqu'un malade l promateux ne pr sente aucune ulc ration.

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