University of Wurzburg Wurzburg, Germany

-M. Ishaque, Ph.D.

Armand Frappier Institute Montreal, Canada

REFERENCES

- EDWARDS, U., ROGALL, T., BLOCKER, H., EMDE, M. and BOTTGER, E. C. Isolation and direct nucleotide determination of entire genes; characterization of a gene coding for 16S ribosomal RNA. Nucl. Acid Res. 19 (1989) 7843–7853.
- FRANZBLAU, S. G. Oxidation of palmitic acid by *Mycobacterium leprae* in an axenic medium. J. Clin. Microbiol. 26 (1988) 18–21.
- 3. ISHAQUE, M. and STICHT-GROH, V. Investigations into the growth of *Mycobacterium leprae* in a medium with palmitic acid under different gaseous environments. Microbios **75** (1993) 171–179.

- 4. KATO, L., SZEJTLI, J. and SZENTE, L. Water-soluble complexes of C_{14} and C_{16} fatty acids and alcohols in media for cultivation of leprosy-derived psy-chrophilic mycobacteria. Int. J. Lepr. **62** (1994) 75–87.
- TESKE, A., WOLTERS, J. and BOTTGER, E. C. 16S rRNA nucleotide sequence of Mycobacterium leprae: phylogenetic position and development of DNA probes. FEMS Microbiol. Lett. 89 (1991) 231– 238.
- WEISBURG, W. G., BARNS, S. M., PELLETIER, D. A. and LANE, D. J. 16S Ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173 (1991) 697-703.
- WHEELER, P. R., BULMER, K. and RATLEDGE, C. Enzymes for biosynthesis *de novo* and elongation of fatty acids in mycobacteria grown in host cells: *Mycobacterium leprae* competent in fatty acid biosynthesis? J. Gen. Microbiol. **136** (1990) 211–217.

Mycobacterium leprae in the Epidermis: Ultrastructural Study I

TO THE EDITOR:

Mycobacterium leprae is an intracellular bacterium which is located mainly in the dermal and subcutaneous regions of the skin. In the skin lesion, there is known to be a clear zone separating the epidermis from the granuloma in both borderline lepromatous (BL) and lepromatous (LL) leprosy. In our present study, we examined the skin biopsy sample from a male BL patient, 51 years old, under the electron microscope. The sample was prepared with routine procedures for electron microscopy. Ultrathin sections of the specimen were stained in saturated uranyl acetate and lead citrate separately before they were examined in a Phillips CM10.

The samples revealed some bacilli in the epidermal cells. Just above the basement membrane of the epidermis, there were several cells containing *M. leprae* in their cytoplasm without any membrane structure around the bacilli (Fig.1). These bacilli looked free in the cytoplasm of the epidermal cells (Fig.2). The cells in which the bacilli are located are the typical epidermal cells, having tonofilaments (T) and melanosomes (M) in their cytoplasm and also des-

mosomes (D) at the junction of each of the cells. Beneath the basement membrane, there is a cell filled with the bacilli (Fig.3). This cell has well developed dendrites on its surface.

There have been reports $({}^{2, 6})$ about the epidermal localization of *M. leprae* in lepromatous leprosy. In their report, Okada, *et al.* (6) suggested that the bacilli can be phagocytized by the keratinocyte, but they did not mention where the bacilli meet the keratinocyte.

There are several kinds of dendritic cells in the dermis, such as melanocytes, Langerhans cells, and Merkel cells. These cells can move from the dermis to the epidermis and among these cells, Langerhans cells can move up and down through the basement membrane. These cells also have some phagocytic abilities. According to the reports from Cramer (1) and Hulley, et al. (3), melanocytes migrate up from the dermis into the epidermis, not only in normal development, but also during normal tissue maintenance. Also, Klaus (4) showed the transfer of melanosomes from melanocytes to keratinocytes. With all of these previous reports we suggest that the dendritic cell(s)

63, 1

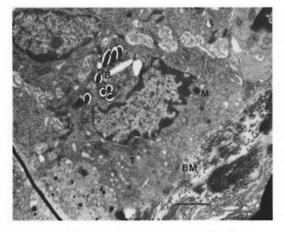


FIG. 1. *M. leprae* (B) in epidermal cells. $T = ton-ofilament; M = melanosome; D = desmosome; BM = basement membrane. (Bar = 2 <math>\mu$ m)

in the dermis can take up (or get) the bacilli and transfer them to the keratinocyte by the same method as that used in the transfer of melanosomes from melanocytes to keratinocytes ($^{4, 5}$).

The possibility that the dendritic cell can transfer the bacilli to the epidermis and the identity of the dendritic cell need more verification. We also found a bacillus in a Langerhans cell (unpublished data) of a LL patient's epidermis.

With the progress of keratinization, the bacilli will go up to the keratin layer and eventually be removed from the skin. The question is whether the bacilli are alive or

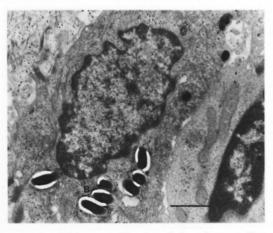


FIG. 2. Higher magnification of a keratinocyte. (Bar = $1 \mu m$)

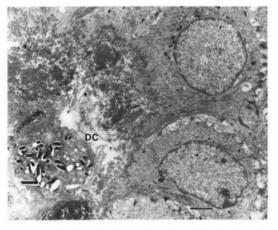


FIG. 3. *M. leprae* in a dendritic cell in the dermal region. DC = dendritic cell. (Bar = $2 \mu m$)

not when they leave the skin. The significance of the existence of M. *leprae* in the epidermis is not as yet explained, but more information about the epidermal bacilli should give us the answer.

Finally, this study suggested several possibilities in the progress of leprosy: 1) A dendritic cell can transfer the bacilli to the epidermis although the mechanism of uptake of the bacilli by the dendritic cell and the keratinocytes is not known. 2) The bacilli can be discharged from the skin of the patients, although the condition of the bacilli is not yet known.

Young-Hoon Seo, Ph.D.
Wook Cho, M.D.
Hae-Young Choi, M.D.
Yong-Ma Hah, M.D., Ph.D.

Institute for Leprosy Research Korean Leprosy Control Association Anyang, Korea

-Sang-Nae Cho, D.V.M., Ph.D. Department of Microbiology College of Medicine Yonsei University Seoul, Korea

Reprints requests to Dr. Young Hoon Seo, The Institute for Leprosy Research, Korean Leprosy Control Association, Anyang P.O. Box 27, Kyunggi-do 430–600, Korea.

Acknowledgment. This work was supported by the Institute for Leprosy Research, Korean Leprosy Control Association. Our special thanks to Mrs. Kyung Hee Hwang and Mr. Dong Yong Chung for their excellent technical support in the electron microscopy work.

REFERENCES

- CRAMER, S. F. The origin of epidermal melanocytes; implications for the histogenesis of nevi and melanomas. Arch. Pathol. Lab. Med. 115 (1991) 115-119.
- HARADA, K. A modified alochrome procedure for demonstrating mycobacteria in tissue sections. Int. J. Lepr. 45 (1997) 49-51.
- HULLEY, P. A., STANDER, C. S. and KIDSON, S. H. Terminal migration and early differentiation of melanocytes in embryonic chick skin. Dev. Biol. 145 (1991) 182-184.
- KLAUS, S. N. Pigment transfer in mammalian epidermis. Arch. Dermatol. 100 (1969) 756-762.
- MOTTAZ, J. H. and ZELICKSON, A. S. Melanin transfer: a possible phagocytic process. J. Invest. Dermatol. 49 (1967) 605-610.
- OKADA, S., KOMURA, J. and NISHIURA, M. Mycobacterium leprae found in epidermal cells by electron microscopy. Int. J. Lepr. 46 (1978) 30–34.

Detection of AFB in Tuberculoid Biopsies

TO THE EDITOR:

The diagnosis of tuberculoid leprosy is made with a good margin of security since the characteristic clinical findings are associated with histological features of tuberculoid granulomatous reaction with involvement and fragmentation of the dermal nerves. The diagnosis becomes more certain and definitive if Mycobacterium leprae are detected, usually in dermal nerves or in the remains of nerve fibers. In this sense, the frequency of detection of acid-fast bacilli (AFB) in biopsies of patients with tuberculoid leprosy is underestimated. Indeed, there is a consensus concerning the rarity of AFB in these cases, figures not exceeding 7% of cases $(^2)$.

In a review of the archives from the Department of Pathology of the Instituto Lauro de Souza Lima (Bauru, Brazil) between 1980 and 1992, we came across the following data:

Tuberculoid leprosy [TT according to Ridley and Jopling's (⁴) criteria]

Biopsies with AFB	415	(37.7%)
Biopsies without AFB	685	(63.3%)
Total	1100	(100%)

Reactional tuberculoid leprosy (TTs according to Ridley)

Biopsies with AFB	102 (71.3%)
Biopsies without AFB	43 (28.7%)
Total	145 (100%)

We used Faraco-Fite staining (1, 3) and on each slide we put the largest possible number of sections. The section close the slide edge with the identification label is exhaustively examined. At the same time, dermal nerves and the remains of nerve fibers, if present, are localized. If AFB are found in this section, the search is concluded. If not, AFB are searched for exhaustively in the dermal nerves and/or fragments. If we increase the number of slides to be examined, the frequency of AFB found also increases, although this procedure is not viable as a routine.

-Raul N. Fleury, M.D., Ph.D.

Assistant Professor of Pathology University of São Paulo/Bauru Director, Department of Pathology

-Cristina M. Aranda, M.D.

Dermatologist Instituto Lauro de Souza Lima P. O. Box 62 Bauru, SP, Brazil 17.0001–970

REFERENCES

- FITE, G. L., CAMBRE, P. J. and TURNER, M. H. Procedure for demonstrating lepra bacilli in paraffin sections. Arch. Pathol. 43 (1947) 624–625.
- LEVER, W. F. and SCHAUMBURG-LEVER, G. Histopathology of the Skin. 7th edn. Philadelphia: J. B. Lippincott Co., 1990, p. 256.
- RIDLEY, D. S. Skin Biopsy in Leprosy. Basle: CIBA-GEIGY, 1984, pp. 14, 15, 42.
- 4. RIDLEY, D. S. and JOPLING, W. H. Classification of leprosy according to immunity; a five-group system. Int. J. Lepr. **34** (1966) 255–273.