

Electron Microscopic Observations of Acid-fast Bacilli in Nasal Mucosal Biopsies of Lepromatous Leprosy Patients¹

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For a long time it has been known that acid-fast bacilli can be found in the nasal excretions and/or mucosa of many leprosy patients. In the early days of leprosy work, the nose was regularly examined for the presence of leprosy bacilli, especially in lepromatous cases. It is said that nasal discharge from lepromatous and borderline cases is the most potent source of bacilli for transmission of the infection to healthy persons (⁵). A high percentage of all untreated lepromatous patients have nasal symptoms, sometimes for many years before diagnosis, which may include blockage, chronic discharge, and epistaxis (⁷). Rees (⁹) has warned that the respiratory route of infection in leprosy must be reconsidered. However, little attention is being paid to nasal examination, the main reason, as pointed out by Dharmendra (⁵), being that it does not give any useful information not already obtained from bacteriological examination of skin smears. There are not many reports concerning basic medical research of nasal mucosa in leprosy patients, and still fewer on electron microscopic observations of nasal mucosa.

The present paper is a report of electron microscopic observations on the nasal mucosa of leprosy patients, especially on the acid-fast bacilli in the mucosa.

MATERIALS AND METHODS

Leprosy patients. Four patients with previously untreated lepromatous leprosy (LL) were selected for this investigation. The Table shows some clinical and bacteriological data concerning these patients.

Tissues. Each of the small biopsies was divided into two parts. One part was used for light microscopy: biopsies were fixed in Bouin solution for routine examination by hematoxylin and eosin and Ziehl-Neelsen stains for histological sections. The other part was used for electron microscopy: fixation, dehydration, and embedding of the materials were carried out as previously described (⁶).

RESULTS

Histological observations, especially by Ziehl-Neelsen staining, were carried out to confirm the existence of acid-fast bacilli (AFB) in the material. Many AFB were observed, and they seemed to almost take the form of spindle-shaped globi (Figs. 1 and 2).

The electron microscopic feature of these bacillary clumps were the main focus of the study. They appeared to be so-called globi (Fig. 3). The fine structure of a multilaminar cell envelope was indicated in some of the bacillary cells, revealing relatively small or slender bacillary forms. As shown in Figures 4 and 5, there were 3 to 4 layers, light-dense-light or light-dense-light-dense strata on the exterior surface of the cell walls and on the cytoplasmic membrane in the interior side of the wall. Not all of the individual bacillary cells in the globi had the multilaminar envelope. This envelope was observed particularly in the bacilli which were situated as single cells in the nasal mucosa of these leprosy patients. The cytoplasmic membrane of the bacillary cells looked to be similar to that of other mycobacteria, areas showing a symmetric profile. Some cellular matter, filled with an electron-less-dense amorphous substance and surrounded with a membrane-like structure, adhered to the outermost layer of the bacillary cell envelope as demonstrated in Figure 4. The cellular matter varied in size and was dis-

¹ Received for publication on 2 January 1985; accepted for publication in revised form on 14 June 1985.

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THE TABLE. Some clinical and bacteriological data concerning the 4 Japanese lepromatous (LL) leprosy patients chosen for the present study.

	Patients			
	1	2	3	4
Sex	Male	Male	Female	Female
Age (yr)	28	36	61	56
Bacterial index				
Skin smear	5	5	4	5
Nasal smear	2	0	0	5
Nasal discharge	0	4	0	0
Nasal mucus	0	4	4	0
Skin hypersensitivity reaction				
Mitsuda	Neg.	Neg.	Neg.	Neg.
Dharmendra	Neg.	±	Neg.	Neg.

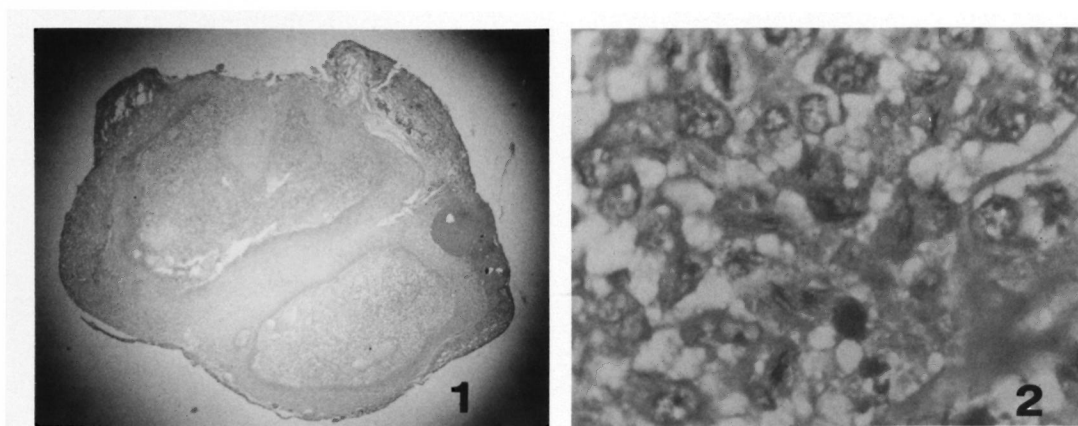
persed around the bacillus. The fine structures of the bacillary cytoplasm (Figs. 5 and 6) were not much different in comparison with the observed results of *Mycobacterium leprae* in human skin lepromas and/or *M. lepraemurium* in murine lepromas as previously reported (6).

DISCUSSION

The nasal mucosa is a highly significant site of predilection for *M. leprae*, as discussed by several workers (1,3,4). The bacillus is able to establish itself and to multiply more intensively there than in the skin. Chacko, *et al.* (2) concluded that the nasal mucosa could be one of the primary sites of involvement in leprosy. Shepard (10) presented his point of view on AFB in the nose. The acid-fast bacilli obtained in nasal washings are, in fact, *M. leprae* as evidenced by

a number of considerations, including the close correlation of the number of AFB recovered with the typical clinical manifestations of the disease, the characteristic microscopic arrangement of the bacilli in globi and packets, and their inability to grow on media for bacteria. Shepard also summarized in another paper (11) that the highest counts of leprosy bacilli were observed in patients with advanced lepromatous leprosy and nasal ulcers, and with histories of nasal observations and bleeding. Furthermore, Pedley (8) reported that the majority of untreated patients with lepromatous leprosy in Nepal discharged solid-staining leprosy bacilli through their nasal mucosa; these bacilli were present in the nasal mucus.

The nasal cavity of leprosy patients is important in relation to the dissemination of the bacilli through nasal discharges as air-



FIGS. 1 AND 2. Microscopic observations of nasal mucosal lepromas in a lepromatous leprosy patient, Ziehl-Neelsen staining (Fig. 1 = original magnification $\times 20$; Fig. 2 = original magnification $\times 800$).

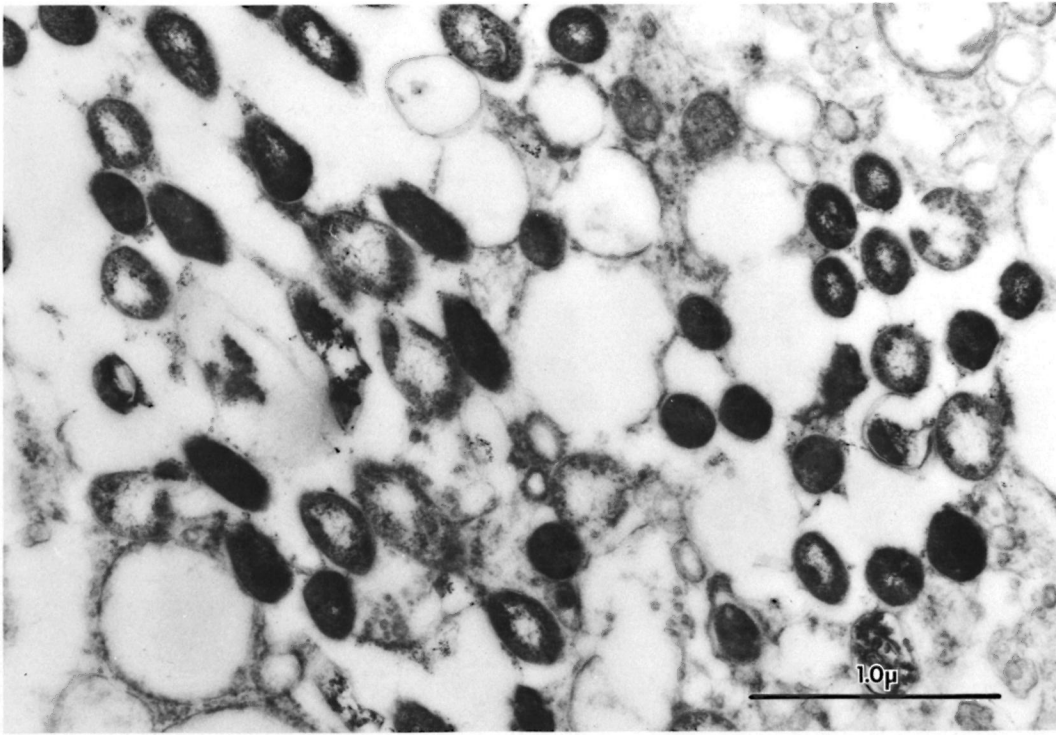


FIG. 3. Cross thin-section of a bacillary clump in the nasal mucosal leproma.

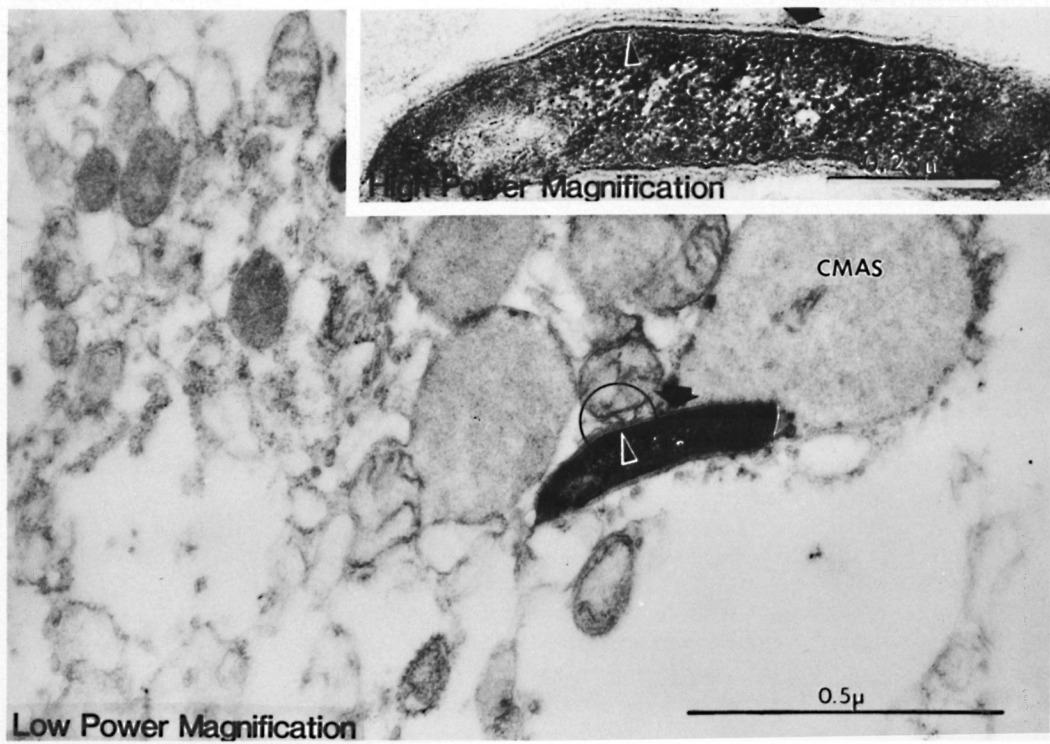


FIG. 4. Longitudinal thin-section of the bacillus situated as a single cell in a nasal mucosal leproma. The multilaminar cell envelope is very clear in the bacillary cell (see insert). CMAS = cellular matter filled with electron less-dense amorphous substance.

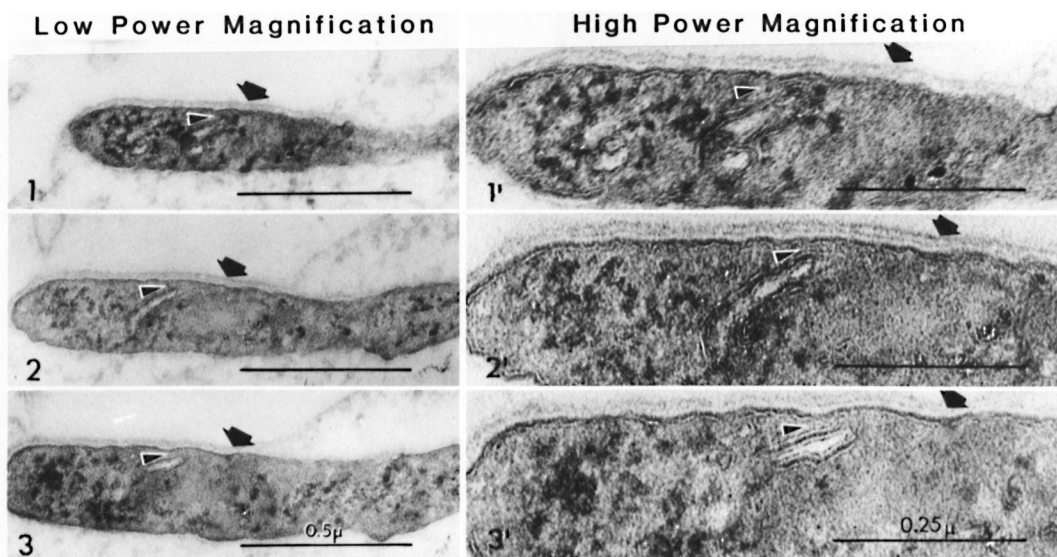


FIG. 5. Longitudinal, serial thin-sections of the bacillus in a nasal mucosal leproma showing multilaminar cell envelope of the bacillus (arrows) and intracytoplasmic membranous structure (▲).

borne droplets. However, the nature of the intranasal lesions has never been fully studied microbiologically in leprosy, and still less is known about the electron microscopic observations.

The bacilli observed here in the nasal mucosa of lepromatous leprosy patients showed that the cell envelope was very complicated

in its fine structure. The multilaminar cell envelope (indicated in Figs. 4 and 5) has never been observed in the bacillary cells of human skin lepromas, nor even in murine lepromas (6). Also, the cellular matter adhering to the bacillary cells has never been found in the skin tissue cells of leprosy patients. It may be said that these are the re-

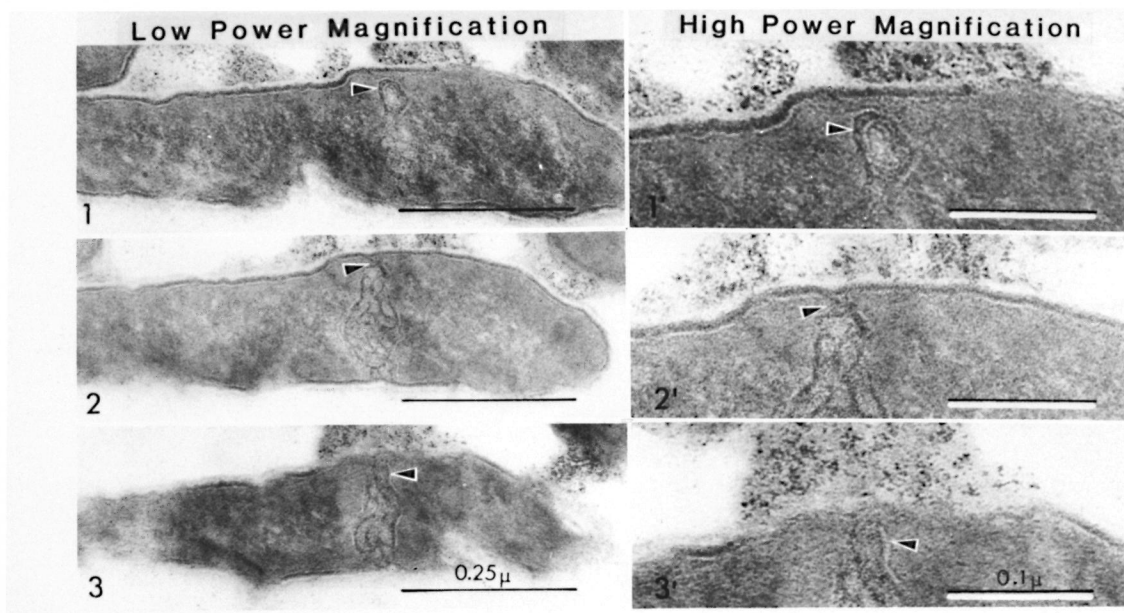


FIG. 6. Longitudinal, serial thin-sections of the bacillus in a nasal mucosal leproma showing continuity of the cytoplasmic membrane with the intracytoplasmic membranous organelles.

mains of phagocytic cells and/or cellular organelles in the tissue.

Interactions between the host cell cytoplasm and the bacilli in the nasal mucosa of lepromatous patients should not be overlooked, even in electron microscopic studies. The importance of the nasal mucosa as one of the major ports for the infection and dissemination of leprosy has been stressed by many workers in the past⁽¹²⁾. The multilaminate cell envelope observed here could be due to the reaction of the host cell cytoplasm to the bacilli. However, results are not adequate to clarify this point at present, and further study is necessary.

The interrelationships among the cytoplasmic membrane, the mesosomes, and the cytoplasm in bacillary cells seemed clearly defined by the serial thin sections (Figs. 5 and 6) in the present study.

In conclusion, it may be assumed from the electron microscopic observations that there are certain radical differences between the acid-fast bacilli in skin lepromas and the bacilli in nasal mucosal lepromas of lepromatous leprosy patients. The differences in the bacillary form in the two lesions should become clearer with additional studies.

SUMMARY

The fine structure of acid-fast bacilli in nasal mucosal lepromas of lepromatous leprosy patients was studied in ultrathin sections by the electron microscope. The cell envelope of the bacilli was the main point of these observations. The electron microscopic examination of some of the bacilli revealed the envelope to be multilaminate. Some of the cellular matter was filled with an electron-less-dense, amorphous substance, surrounded with a membrane-like structure, and was adherent to the outermost layer of the bacillary cell envelope.

RESUMEN

Se hizo un estudio al microscopio electrónico sobre la estructura fina de los bacilos ácido alcohol resistentes encontrados en los lepromas de la mucosa nasal de pacientes lepromatosos. El principal objetivo de estas observaciones fue el estudio de la envoltura celular de los bacilos. El examen de algunos bacilos reveló que esta estructura es multilaminada. Parte de la materia celular se encontró llena de un material menos electrodense, amorfo, rodeado por una estructura "mem-

branosa," y adherente a la capa más externa de la envoltura celular de la bacteria.

RÉSUMÉ

On a étudié par une méthode de sections ultra-minces à la microscopie électronique, la structure fine des bacilles acido-résistants recueillis dans des lèpres de la muqueuse nasale de malades atteints de lèpre lépromateuse. L'étude a porté essentiellement sur l'enveloppe cellulaire des bacilles. Les examens en microscopie électronique ont révélé que l'enveloppe de certains de ces bacilles était multilamellaire. Une partie du matériel cellulaire était remplie avec une substance amorphe moins dense aux électrons, entourée d'une structure semblable à une membrane, et adhérait à la couche la plus externe de l'enveloppe cellulaire des bacilles.

Acknowledgments. I wish to express my thanks to all of the staff at the National Leprosarium of Tama Zensho-en, Tokyo, who helped in this study.

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