Some new aspects of LCs in the human epidermis

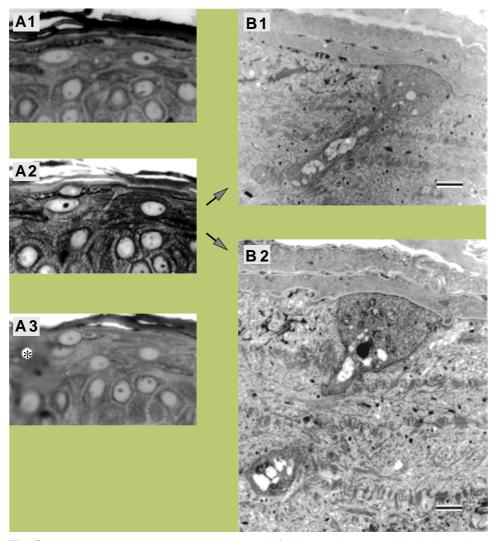


Fig. 5. The process terminal swellings in a round fashion and contacts with the horny layer, but the connection between the process and the cell body can not be confirmed. A1–3: Serial thin section.  $\times$  750 (A2 oil). A2 shows the process and terminal button, but the connection between the process and the cell body can not be found. B1 and 2: In the button, some mitochondria and vacuoles are seen, but Birbeck granules are not observed. The outlines of these buttons are not rugged.  $\times$  6,750 (bar = 1 µm).

## Morphology

The whole appearance of the button corresponds well to the image of LM. However, it is necessary to make a careful observation, because the features at the start and the end of ultrathin-sectioning differed somewhat from each other (Figs. 5B1 and B2).

## Birbeck granules and vacuoles

On one occasion, plenty of Birbeck granules (Birbeck et al., 1961) and many vacuoles of various sizes were observed in the process terminal (button) and, on another occasion, no Birbeck granules were observed at all. (Figs. 2 and 3 versus Figs. 4 and 5).

## Discussion

It is very important to start with light microscopic observation for the study of skin specimens where the view of every section changes panoramically. For a detailed examination of a specific cell or tissue in the skin, the section containing the object should first be selected by LM, then the proper object for the electron microscopic study can be carefully investigated on the ultrathin sections taken from the section viewed by LM. The author accomplished the purpose by the Thin-Section-Reembedding and Ultrathin-Sectioning (TRUS) technique (Fig. 1). This TRUS technique is simple, and moreover, trustworthy for anyone. It was used throughout the author's studies. The rate of error was less than 1%. This technique was instructed to the author from Researcher N. Shindo (Shindo et al., 1984) at the Clinical Research Institute, National Medical Center, Tokyo, Japan, and the author drew a picture of the detailed manner of this procedure in Fig. 1. The technique was called the TRUS technique by the author for brevity. In this study, because the "No Cover Glass (NCG)" object lens could not be employed in the light microscopic examination, the micrographs, except for the "oil immersion" views, were taken from the sections covered with a dry coverglass on the slide. The oil immersion micrographs were taken from the sections affixed on the surfaces of the reembedded Epon blocks. Then, a drop of water was put between the section (Epon block surface) and the coverglass in order that the section surface woul not get oil on it. The hardness of the Epon used in this study was relatively hard. The ratio of mixture A to mixture B was 3 to 7, according to Luft's method (1961). This Epon hardness seems to be the key to success.

As was stated above, in some cases a great many Birbeck granules were found in the buttons of LCs, but in the other cases were not found at all. These different results could be due to the different stage of interactions between LCs and the adjacent keratinocytes; one would be in a progressive stage of exocytosis of

Birbeck granules, while another may be in the end state of this function. Nevertheless, the exact role of the process terminals (button) and the nature of Birbeck granules remains for further study. The observations of the process terminals (having buttons) of intraepidermal LCs were achieved first by Langerhans, and succeeded by Ferreira-Marques' elaborate examination. Both Langerhans and Ferreira-Marques identified the cells by using the gold impregnation method in the human epidermis. The present author used the methylene blue staining method and identified the cells easily and precisely according to numerous studies by other workers (Breathnach, 1965; Wolff, 1972): dendritic cell shapes, a clear cytoplasm (tonofilaments free), no desmosome, indented nuclei, etc. The methylene blue staining method has the advantage of detailed description of the sections on the slide. To the large swelling of the process terminals Ferreira-Margues gave the name trompiform; the author thinks that the word knopfförmig (that is "button-shaped") by Langerhans and the word trompiform (that is "trumpet-shaped") by Ferreira-Marques are identical entities, and possibly produced from the exocytosis of Birbeck granules. Since Ferreira-Margues' time, the swellings of the process terminals of dendritic cells in the epidermis have been observed with the ATPase staining method of Bradshaw and colleagues (1963) in the human epidermis and were expressed as "buttons" or "caps", but their significance was not discussed at all.

This study describes the first time that electron microscopic examination of Langerhans' "button" has ever been done. Langerhans, who investigated this button in 1868 with a light microscope of the 19th century and recorded his findings as an important matter, was a man of insight. Ferreira-Marques who took notice of these process terminals was also a man of great ability. According to the formation of "buttons" of the process terminals of the intraepidermal LCs and their ultrastructure in the author's findings, it may be assumed that LCs play an essential role in the differentiation of the human epidermis at the process terminals. Acknowledgments: The author gratefully acknowledges the support of Emeritus Prof. Tokichi Yumoto and Prof. Yasutake Hiji who gave him the opportunity to carry out this study in the First Dept. of Pathology and then in the First Dept. of Physiology, respectively. The author further expresses his gratitude to Prof. Yasutake Hiji for the reading of this manuscript. The author also expresses his appreciation to Prof. Motoyuki Mihara for the kind help in obtaining biopsy specimens and to Dr. Toku Kanaseki for his cordial help in EM.

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