Immunoelectron microscopical demonstration of S-100 protein in epidermal Langerhans cells

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ABSTRACT

In our previous immunohistochemical studies for S-100 protein in malignant melanoma and pigmented nevi, we frequently observed S-100 protein-positive dendritic cells in the suprabasal layer of the epidermis, being assumed to be Langerhans cells. However, the direct evidence of the presence of S-100 protein in those cells has not been verified yet. In this study, we demonstrated S-100 protein in Langerhans cells of human epidermis by the immunoelectron microscopic method. S-100 protein will serve as an important marker for Langerhans cells.

Epidermal Langerhans cells have been identified by the presence of Birbeck granules on electron microscopic observation (1) or by positive histochemistry for ATPase, acid phosphatase and non-specific esterase (14). Furthermore, Langerhans cells possess Fc and C3 receptor as well as Ia antigen on its cell surface (11, 12). These results strongly suggest that Langerhans cells belong to a macrophage subpopulation (14). Skin graft and bone marrow transplantation experiments indicated that Langerhans cells originated from bone marrow (5). Recently, Langerhans cells are considered to be morphologically, histochemically and immunologically similar to interdigitating cells in the paracortical area of many lymphoid organs (10, 14). Furthermore, Birbeck granules were found in proliferating large mononuclear histiocytosis X cells by electron microscopy, and histiocytosis X has been thought to be a pathological condition of Langerhans cells (9).

S-100 protein was first isolated by Moore (6) in 1965 and was found to be present mainly in glial cells in the central nervous system and in Schwann cells in the peripheral nervous system (3). Recently, the amino-acid sequence of S-100 protein was determined by Isebo et al. (4). As a result of biochemical analysis, S-100 protein was classified as a calcium-binding protein such as calmodulin and troponin C. However, the true function of S-100 protein in nervous tissue is still obscure.

In order to verify the presence of S-100 protein in epidermal Langerhans cells, we carried out light and electron microscopic immunohistochemistry and conventional electron microscopy on the surgical material from a patient with solar keratosis. In immunohistochemistry, we used two kinds of anti-S-100 protein antibodies, which were raised in New Zealand white rabbits. One was anti-S-100 protein rabbit IgG (9 mg/ml) made by the method of Uemura et al. (15). Another was obtained by the methodology of Isebo et al. (4). This antiserum was purified to obtain monospecific IgG by affinity chromatography using CNBr-activated Sepharose 4B coupled with purified bovine S-100 protein.

Surgically resected skin from a patient with solar keratosis was fixed in cold 10% buffered formalin (pH 7.0) overnight for immunohistochemistry, and in 2.5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.2) for 1.5 h at 4°C for conventional electron microscopy. Glutaraldehyde fixed specimens were postfixed with 2% osmium tetroxide and processed for electron microscopic observation. Paraffin sections (3
μm thick) from 10% buffered formalin fixed materials were stained for S-100 protein by peroxidase anti-peroxidase (PAP) method described elsewhere (7). After 10% buffered formalin fixation, some specimens were washed overnight in PBS (pH 7.2) containing 5% sucrose for immunoelectron microscopy. Sucrose and glycerol concentrations were gradually raised to 20% and 10%, respectively. Cryostat frozen sections 8 μm thick were placed glutaraldehyde-treated gelatin glass slides and were immediately immersed in cold PBS containing 5% sucrose. After treating with 10% normal goat serum for 30 min at room temperature, the sections were covered with monospecific anti-S-100 rabbit IgG (16 μg/ml) solution containing 2% normal goat serum for overnight at 4°C and one hour at room temperature. After washing with PBS, the sections were treated with 20 times diluted horseradish peroxidase-labelled anti-rabbit IgG (Fab fragment) goat serum (Medical and Biological Laboratories, Japan) for 2 h at room temperature. After washing enough with cold PBS, these sections were fixed with 2.5% glutaraldehyde for 15 min. Then, DAB reaction was carried out for 15 min at room temperature (DAB solution: 0.05 M ammonium acetate-citric acid buffer, pH 5.6, containing 20 mg/ml of 3,3'-diaminobenzidine tetrahydrochloride and 0.005% H2O2). The sections were postfixed with 2% osmium tetroxide for 30 min and processed for electron microscopic study.

By PAP method, S-100 protein was demonstrated in the dendritic cells in the suprabasal layer of the epidermis. Brown benzidine product accumulated much more in the cytoplasm than in the nuclei. S-100 protein-positive cells extended long cytoplasmic processes among keratinocytes. There were also S-100 protein-positive cells in the upper dermis, where chronic inflammation was present. Besides, another kind of S-100 protein-positive cells were often observed in the basal layer of the epidermis, which contained not only less immunoreaction product for S-100 protein than that of S-100 protein-positive cells in the suprabasal layer of the epidermis, but also contained melanin pigments.

Fig. 1 Human epidermis (solar keratosis) stained for S-100 protein. By indirect immunoelectron microscopic method, Langerhans cells were found to be positive for S-100 protein. Compare the immunoreactivity for S-100 protein of Langerhan cells with that of melanocyte (M). Slightly stained with uranyl acetate. ×1,700
Indirect immunoelectron microscopy also demonstrated S-100 protein-positive cells extending long cytoplasmic processes among keratinocytes, whose features were almost the same as those seen by peroxidase anti-peroxidase immunohistochemistry (Fig. 1). Higher magnification revealed that S-100 protein-positive cells contained irregularly shaped nuclei and abundant cytoplasm. S-100 protein was diffusely distributed in the cytoplasmic matrix and weakly in the nucleus. Many mitochondria, Golgi complexes and endoplasmic reticula were devoid of S-100 protein reaction product (Fig. 2). Detailed observation disclosed the presence of many rod-shaped granules in the cytoplasm which lacked immunoreaction product for S-100 protein. The average size of these granules was 387±61 Å in diameter. These rod-shaped granules contained central linear density which was more clearly visible, if stained with uranyl acetate (Fig. 3, a and b).

In controls using anti-S-100 protein rabbit IgG absorbed with purified S-100 protein, no immunoreaction product was observed in the sections of light and electron microscopic immunohistochemistry.

Conventional electron microscopic study confirmed the results of immunoelectron microscopy. Many Langerhans cells were observed not only in the epidermis, but also in the upper dermis. These cells contained characteristic convoluted nuclei and many round mitochondria, endoplasmic reticula and well-developed Golgi complexes in the cytoplasm. Centrioles, lysosomes, lipid droplets and occasional melanin granules were also found in its cytoplasm. Birbeck granules were easily detected in close proximity to Golgi complexes (Fig. 4), which were rod-shaped and contained linear striated lamellar area midway between two limiting membranes (Fig. 3c). The average diameter of these granules was 430±50 Å.

Our previous light microscopic immunohistochemical study demonstrated the presence of S-100 protein in epidermal Langerhans cells (7). Furthermore, interdigitating cells in T cell-dependent areas of various lymphoid organs and histiocytosis X cells were also S-100 protein-

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![Fig. 2 Langerhans cells by indirect immunoelectron microscopy for S-100 protein. There are many Birbeck granules in the cytoplasm, which are devoid of S-100 protein immunoreaction product (arrows). Langerhans cell is in contact with degenerating cells (N). Slightly stained with uranyl acetate. ×18,500](image-url)
positive (8). By immunoelectron microscopy, Takahashi et al. confirmed that S-100 protein-positive cells in human lymph nodes with non-specific lymphadenitis and dermatopathic lymphadenitis showed morphologic characteristics similar to interdigitating cells (13). In this study, S-100 protein-positive dendritic cells in the epidermis contained Birbeck granules in their cytoplasm so that the identity of S-100 protein-positive dendritic cells and Langerhans cells was confirmed. In light microscopic immunohistochemistry for S-100 protein, melanocytes are usually S-100 protein-negative or slightly positive (7). Melanocytes differ from Langerhans cells with regard to S-100 protein immunohistochemistry, its location in the epidermis and the morphology.

Recently, Langerhans cells and interdigitating cells have been regarded as a macrophage subpopulation (14). There are many common characteristics between those cells and macrophages. However, S-100 protein is not present in phagocytic macrophages (8, 13). In a recent experiment using hybridoma antibodies, Langerhans cells express surface antigens reacting with OKT 6 (anti-thymocyte) and OKI 1 (anti-Ia) (2). The absence of OKT 6 reactivity with peripheral blood monocytes indicates differences between Langerhans cells and phagocytic macrophages.

In conclusion, our results disclosed that S-100 protein was distributed in epidermal Langerhans cells and would be a useful marker for Langerhans cells and probably, interdigitating cells and histiocytosis X cells.

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Note Added in Proof: The S-100 protein has recently been identified in human epidermal Langerhans cells by D. Cocchia et al. (Nature 294, 85–87, 1981).

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Fig. 3 a: Rod-shaped Birbeck granule contained linear midway areas. Immunelectron microscopy. Slightly stained with uranyl acetate. ×100,000. b: Racket-shaped Birbeck granule. Immunelectron microscopy. Slightly stained with uranyl acetate. ×100,000. c: Rod and racket-shaped Birbeck granules by routine electron microscopy. ×100,000.
Fig. 4 Characteristic Langerhans cells are easily found by the transmission electron microscopy. Note many Birbeck granules in close apposition to Golgi complex. Doubly stained with uranyl acetate and lead citrate. ×8,850

basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. J. Invest. Dermatol. 37, 51–64
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