Electron microscopy of dendritic cells in the human corneal epithelium

A modified Masson's ammoniated silver nitrate stain

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Dendritic cells in the human corneal epithelium were studied by electron microscopy with an ammoniated silver nitrate stain, and were compared with those in the human conjunctival epithelium. Electron microscopy showed that the former cells, as well as the latter cells, possess characteristic cytoplasmic granules which show a specific affinity for the stain.

It has been suggested that the nature of dendritic cells in the corneal epithelium is a melanocyte of neural crest origin, but no cytochemical evidence is yet available.1, 2 Recently, it has been reported by Mishima and co-workers3 that an ammoniated silver nitrate stain, modified for use in electron microscopy, produces a highly selective metallic deposit not only in melanin granules but also in cytoplasmic structures which are essential to melanin formation but contain neither tyrosinase activity nor a chemical precursor of melanin. This report describes the fine structures of dendritic cells in both human corneal and conjunctival epithelium, stained with the reduced silver nitrate stain.

Materials and methods

Specimens for this study were taken from an eye of an 83-year-old Japanese woman. Pieces of corneal tissues were dissected from nonpigmented area of the central cornea, and pieces of conjunctival tissues were excised from the upper region of the bulbar conjunctiva. Staining was carried out according to the method described by Mishima and co-workers:1 (1) excised tissues were fixed 1 to 2 hours in cold buffered 5 per cent formalin and then rinsed twice in distilled water; (2) they were then incubated 30 to 40 minutes in a 10 per cent ammoniated silver nitrate solution at 58° C., followed by 15 minutes' rinsing in distilled water; (3) tissues were then immersed 1 to 2 minutes in a solution of gold chloride, sodium thiosulfate, and ammonium sulfocyanide, followed immediately by 4 to 8 minutes in a 6 per cent sodium thiosulfate solution and then by 10 minutes' washing in running water. After the staining, the tissues were fixed an additional one hour in cold buffered 1 per cent osmium tetroxide and embedded in a water-soluble aliphatic polyepoxide, Durcupan, as described by Kushida.4 Thin sections were cut on a JUM-ultramicrotome and were mounted on formvar-coated grids. The sections were then examined without any additional electron staining with a Hitachi model HU-11A-electron microscope.

Observations

The corneal epithelium (Figs. 1 to 4). In the corneal epithelium, only dendritic cells reacted positively to the ammoniated silver nitrate stain, while any epithelial cells or nerve fibers showed no affinity for the stain. In the dendritic cells, metallic deposits produced by the reaction were localized only in cytoplasmic granules and were never found in the nucleus, the mitochondria, or the cell membrane. The granules ranged from 0.1 to 0.2 μ in diameter and were elliptical or spherical in shape,
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being singly located. There were two classes of granules observed: one was less dense to electrons and had numerous fine metal particles with diameter of 5 mμ, while the other was comparatively electron dense, and the metal particles seen in it, about 10 mμ in diameter, were larger in size and smaller in number than those of the former granules. The characteristic granules were found not only in dendritic cells in the basal layer but also in the cells in the suprabasal layer.

The conjunctival epithelium (Figs. 5 and 6). In the conjunctival epithelium, both dendritic cells and epithelial cells reacted positively to the stain. As shown in Fig. 5,
Figs. 2 and 3. Portions of the dendritic cell shown in Fig. 1 at higher magnification. In the cytoplasm, two types of granules are seen. One ($G_1$) has numerous fine metal particles, while the other ($G_2$) contains sparse coarse particles. Nucleus ($N$); epithelial cell ($EC$). ($\times45,000$.)
Fig. 4. Electron micrograph of a portion of a high-level dendritic cell in the human corneal epithelium, stained with the reduced silver nitrate stain. Metal particles are present in a granule (G1) but they are never found in mitochondria (M). Nucleus (N). (×45,000.)

Fig. 5. Electron micrograph of portion of a dendritic cell in the human conjunctival epithelium, stained with the reduced silver nitrate stain. Two types of granules (G1 and G2) identical with those of corneal cells are found in the cytoplasm. Nucleus (N); epithelial cell (EC). (×45,000.)
the dendritic cells also contained characteristic granules, quite similar in structure to those of corneal cells. In addition to this type of granules, the former cells frequently contained together melanin granules with high electron density and a few large metal particles. In the epithelial cells, metallic deposits were found only in or around individual melanin granules composing melanin inclusion bodies (MIB) react positively to the stain. Individual melanin granules (G3) usually contain a few large metal particles. Nucleus (N). (×45,000.)

Discussion

Mishima and colleagues have demonstrated that the ammoniated silver nitrate stain shows a remarkable degree of specificity, and that the size of metal particles in granules has a close relation to degrees of maturation of melanin granules. It is well known that, in the human conjunctival epithelium, dendritic cells synthesize melanin and transmit it to epithelial cells. Therefore, it can be naturally expected that, in the conjunctiva, dendritic cells contain melanin granules of various developing stages whereas epithelial cells have only melanin granules of the last stage. The result obtained from the present study is well in accordance with this expectation. The former cells frequently contain mature melanin granules with a few large metal particles in addition to two different types of granules with more numerous, finer metal particles, while the latter cells have only the mature melanin granules.

On the other hand, dendritic cells in the central part of the human corneal epithelium, as shown by the previous study, normally contain nonpigmented granules.
but possess no mature melanin granules. After the reduced silver nitrate staining, however, the cells also do possess characteristic granules which show a specific affinity for the stain, but epithelial cells have no such granules. The characteristic granules are approximately similar in size, shape, and site to the nonpigmented granules. In addition, the metal particles seen in the granules are quite similar in structure to those in the early stages of melanin granules observed in conjunctival dendritic cells. These findings strongly reinforce the conclusion, obtained from the previous study, that dendritic cells in the human corneal epithelium are a melanocyte which can form cytoplasmic structures essential to melanogenesis but cannot deposit melanin within it, probably because of a disturbance in the enzyme formation.

REFERENCES