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Attachment and Detachment of Human Epidermal Melanocytes

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Human epidermal melanocytes can leave their basal position following topical application of sodium lauryl sulphate. They thereby undergo changes which strongly indicate that the dense plates along their cytomembrane constitute a mechanism for attachment to the basal lamina. These detached melanocytes move rapidly to, or are transported to, more superficial levels of the living epidermis. They are probably not desquamated, but disintegrate within the stratum spinosum. It was remarkable that one melanocyte was found that broke the basal lamina, showing that melanocytes are capable of migrating, under certain conditions. A special fixation method which exposes the cytomembranes and allows the demarcation of cells and their finest cytomembrane protrusions was used to demonstrate that keratinocytes often bulge into the innermost spinous layer and become attached to the basal lamina only via more or less thin cytoplasmic protrusions. This is in contrast to the routine textbook description of a basal layer that consists of a strict single row of cuboid or columnar keratinocytes. ***Key words: Hemidesmosomes; Basal lamina; Sodium lauryl sulphate; Intercellular space.***

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The epidermal melanocytes are confined to the basal layer but the nature of the mechanism that allows them to maintain in their position is a controversial topic. The present study demonstrates that topical application of sodium lauryl sulphate can cause melanocytes to leave their basal position, thereby undergoing changes which strongly indicate that the dense plate with its anchoring filaments at the basal aspect of such cells represents a mechanism for attachment.

MATERIALS AND METHODS

Three series of experiments were performed. In the first experiment, patch-tests (Finn chambers, Epitest Ltd) with either 50 μl 1% sodium lauryl sulphate (SLS) in distilled water or distilled water only were applied to the skin of the upper back of 3 healthy adults for 4, 8 and 24 hours.

In the second experiment, 50 μl 0.5% SLS solution or distilled water was applied on volar forearm (same individuals, except for one who had to be replaced) for 8 h a day on 3 consecutive days.

The volunteers participating in these experiments are designated 'group I volunteers', below.

In the third experiment, 3 different adult volunteers ('group II volunteers' below) were patch-tested with 1% SLS for 24 h on the volar forearm in the same manner, but the test solution was prepared from a different batch of SLS.

Punch biopsies (3 mm) from the test areas and normal skin were taken without local anesthesia after removal of the last dressing and were fixed in 2.5% glutaraldehyde dissolved in cacodylate buffer iso-osmolar to blood (0.16 M). The specimens were then treated according to a protocol reported elsewhere (1) that allows processing within a few hours. About 200 serial sections were taken from each biopsy; the sections were distributed on 10 grids and viewed in an electron microscope. The number of melanocytes observed in the section series varied between 8 and 19.

RESULTS

Normal skin

The general morphology of the epidermis seen by electronmicroscopy was consistent with that seen in recent studies based on the iso-osmolar fixation principle (2). The width of the space (lamina lucida) between the melanocytes and the basal lamina was similar to that of the keratinocytes. The space showed slight, discrete and irregularly distributed widenings. There were numerous plaques of varying size and irregularly distributed along the melanocytic cytomembrane facing the basal lamina. These plaques were distinguished by an increased electron density of the plasma membrane, especially of the inner leaflet, and of a thin zone of subjacent cytoplasm that was roughly 3 times as thick as the unit membrane. Moreover, the parts of the lamina lucida corresponding to the plaque areas showed fine filaments traversing the lamina lucida. This filamen-

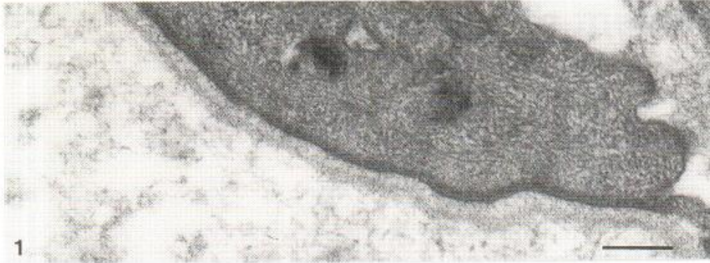

Fig. 1. SLS-exposed skin (24 h). A much enlarged but structurally otherwise normal attachment zone of a melanocyte. There are numerous filaments in the lamina lucida, and the cytomembrane and subjacent cytoplasm show a high electron density. Scale bar, 0.25 μm.

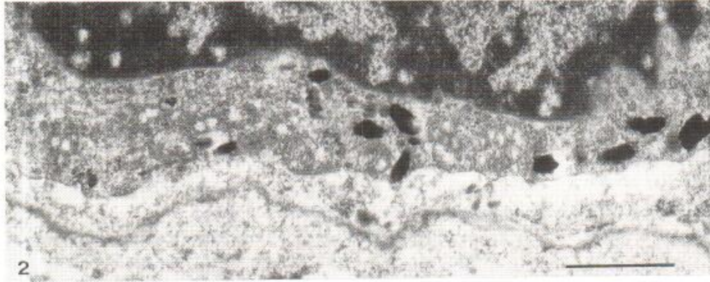

Fig. 2. SLS-exposed skin (24 h). Part of a melanocyte facing the basal lamina. The intercellular space is increased, indicating early detachment and there are no dense plaques present. Scale bar, 0.5 μm.

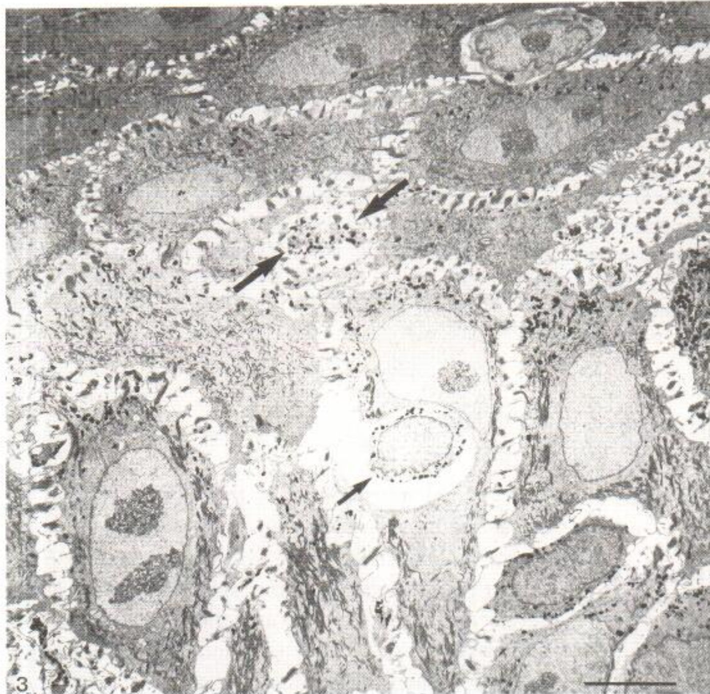

Fig. 3. SLS-exposed skin (24 h). A detached shrunken melanocyte *(small arrow)* in a slightly spongiotic mid-spinous layer. *Large arrows* point to cytoplasmic remnants containing melanosomes. Scale bar, 5 μm.

tous arrangement was thus strikingly similar to the filamentous arrangement of the keratinocytic hemidesmosomes, although the keratinocytic filaments were clearly more numerous and easy to observe. The parts of the basal lamina facing the melanocytes had a uniform electron density similar to that seen between the hemidesmosomes of the keratinocytes.

The iso-osmolar fixation prevents cellular swelling (2) and makes it possible to observe and demarcate all cytomembrane protrusions. Several keratinocytes had the bulk of their cell bodies and nuclei located in the layer above the stratum basale, and had contact with the basal lamina only via slender protrusions provided with hemidesmosomes. Quite a few melanocytes were superimposed by such a keratinocytic cell body and were surrounded by its protrusions to

the basal lamina. Another – not uncommon – arrangement was that two and sometimes three cell bodies of basal keratinocytes bulged up into the second cell layer and enveloped melanocytes in an arcade-like fashion. It could also be seen that the basal lamina was not entirely covered by epithelial cell membranes, but that tiny areas of the basal lamina were exposed to the epidermal intercellular space.

Patch-tested skin

Simple occlusion with water did not produce any ultrastructural changes in keratinocytes and melanocytes.

The results from group I volunteers were as follows. In the *short-term experiments,* 4 h of SLS exposure produced no changes in the epidermis and the melanocytes had a normal appearance, with one exception. In one section series, a melanocyte had detached, as evidenced by a slightly enlarged space between the basal lamina and its basal cytomembrane which, moreover, had lost its electron-dense plaques and the anchoring filaments. At 8 h of exposure, some inflammatory signs were obvious. The intercellular space was thus slightly widened and, in 2 cases, several exocytic cells appeared in the basal part of the epidermis. The melanocytes in the section series lacking exocytic cells appeared unaffected. In one biopsy, 3 out of 8 (and in another biopsy 5 out of 10) melanocytes had detached and were found in the lower spinosum or mid-spinosum. These cells had a normal appearance with a seemingly normal elaborate dendritic tree.

The 24 h of exposure and the repeated treatment with SLS induced dramatic changes in the melanocyte system, in addition to pronounced spongiosis and the appearance of numerous exotytic cells.

Several melanocytes were strictly confined to the basal layer and had a normal subcellular appearance. A few of these had, however, a greatly reduced contact surface towards the basal lamina and their cell bodies, including the nuclei, protruded above the basal cell layer. Remarkably, nearly the whole basal cytomembrane had acquired the characteristics of the dense plates (Fig. 1). Some other melanocytes remained in the basal layer but had completely detached; that is, the basal extracellular space was slightly but clearly enlarged (Fig. 2) and the basal plasma membrane was devoid of electron-dense plates and anchoring filaments. Still other cells were encountered at more superficial levels. Most of the nuclei from cells found at superficial levels were conspicuously small and the melanosome-containing cytoplasm had few organelles and was reduced to a thin perinuclear rim without dendritic processes (Fig. 3). Scattered remnants of cytoplasmic material containing melanosomes but lacking a limiting membrane were found among the uppermost keratinocytes (Fig. 3).

The basal lamina was covered by cells, except for tiny areas as in normal skin. This observation shows that the space left by detached melanocytes is rapidly occupied by keratinocytes.

The group II volunteers showed no signs of the detachment of melanocytes described above for group I. However, it was noteworthy that one of the SLS-exposed biopsies had a melanocyte and a Langerhans cell together breaking the basal lamina.

The granular and corneal layers appeared normal in both groups I and II and contained no non-keratinocytic cells or cell material, with the exception of occasional dendrites penetrating the granular layer and belonging to activated Langerhans' cells (cf 3).

DISCUSSION

It is generally accepted that the hemidesmosomes of the basal keratinocytes are special attachment devices but are only one component in a complex epidermal-dermal adherence mechanism (4). There is less unanimity concerning the mechanism that allows the desmosome-lacking Langerhans cells and melanocytes to maintain their positions in the epidermis against the stream of keratinocytes (5). Cell to cell adhesion controlled by changes in the cytoskeleton, the 'stick and grip' concept of Rees et al. (6), has been suggested (5) as one such mechanism.

The situation regarding Langerhans cells has recently become much clearer. The Langerhans cells were shown to be able to migrate out of the epidermis within hours (7) and rapidly rearrange their positions within the epidermis (8, 9). This motility, and the fact that the intercellular space (2, 3, 10) of the human epidermis is very wide, are two prerequisites to explain how these cells maintain their position (cf 3).

Odland (11) reported the occurrence of 'dense plates' on the melanocyte cytomembrane facing the basal lamina and suggested that the plates could have an attachment function. His finding was later confirmed in more extensive and detailed studies by Tarnowski (12) and Briggaman & Wheeler (4). Both

ιudies revealed, however, certain structural differences between the keratinocytic hemidesmosomes and the electron-dense plates of the melanocytes. This finding led the authors to suggest that the designation 'dense plate' be retained until more was known about the function of these plates.

The present study has confirmed Tarnowski's observations of discrete electron-dense plates along the melanocyte membrane abutting the basal lamina and has shown that the plates differ from the hemidesmosomes of the keratinocytes in the following respects. They are less electron dense, lack intracytoplasmic converging filaments and have far fewer anchoring filaments traversing the lamina lucida. Moreover, there is no sub-basal dense plaque such as there is beneath the hemidesmosomes of the keratinocytes.

This is also the first report of experimentally induced detachment of melanocytes. The results seem to be of significance for delineating the function of the dense plates. Thus, a decreased contact surface between melanocytes having their cell bodies in an abnormal, erect position, reaching over the basal layer and the basal lamina led to a pronounced enlargement of the dense plates. This enlargement could indicate a compensatory phenomenon in the initial stage of detachment. All detached melanocytes had lost their dense plates. This was also seen in those melanocytes which had recently become detached, judging by the fact that they were still situated near the basal lamina and did not exhibit any other structural sign of cell damage. These findings support the probability that the dense plates represent an attachment device.

The special fixation procedure used in this study revealed that quite a few melanocytes are enfolded by basal keratinocytes. It cannot be excluded that this remarkable arched position of one or more keratinocytes helps keep melanocytes confined to the basal layer. This arrangement, however, cannot have a major function in this respect if it has a function at all, since several melanocytes were not thus surrounded by basal keratinocytes.

The unresponsiveness with respect to melanocyte detachment in group II may seem puzzling. However, it is well-known that the reaction pattern to SLS varies widely between individuals (13), test sites and repeated tests (14, 15). Also two different batches of SLS were used for the two groups. The commercially available SLS is of a relatively low grade of purity and it cannot be excluded that contaminants participated in inducing the detachment process.

It is also puzzling that when the SLS solution produced detachment, many melanocytes did not exhibit any structural changes at all. A recent fluorescence and electron microscopic study of the short-term effects of SLS on the human epidermis has shown that several reactive events occur in a scattered manner in the test area (16). One such event is the presence of damaged Langerhans' cells which are found among undamaged Langerhans' cells.

Surprisingly, detached melanocytes could be seen in a mid-spinous position as soon as after 8 h and become even more superficial after 24 h. Obviously, this cannot be explained as passive transport induced by the slow stream of keratinocytes to the surface, but indicates active transport. It is not known whether transport is executed by the melanocytes themselves before they are devitalized, or by passive flow in the intercellular spaces generated by the keratinocytes. The phenomenon in any event supports the view that the keratinocytes in the living epidermis are not closely packed as is commonly believed but are separated by large interstices. Another finding of interest in this context was that of a melanocyte breaking the basal lamina, which indicates that under certain conditions these cells are capable of migration.

The frequent melanocyte detachment following exposure of the skin to SLS made it possible to elucidate another point: the ultimate fate of dying non-keratinocytes. The finding of highly shrunken cells and cytoplasmic debris in the upper spinous layer but no trace of such material in outer strata leaves little doubt that such cells do not desquamate but disintegrate within the living epidermis.

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