Acta Derm Venereol (Stockh) 1991; 71: 108–112

Ultrastructural Changes In Darier's Disease Induced by Ultraviolet Irradiation

M.-A. HEDBLAD, T. NAKATANI and H. BEITNER

Department of Dermatology, Karolinska Hospital, Stockholm, Sweden

A male patient with clinically and histopathologically verified Darier's disease and a history of deterioration after sun exposure was irradiated on uninvolved skin with 25, 50 and 75 J/cm^2 of UVA once a week for five weeks. He also received 3 and 5 times his individually established MED of UVB. Since no signs of keratosis follicularis were detected one week after the last irradiation, he was then exposed to 10 times his MED with UVB, whereupon clinically characteristic lesions of keratosis follicularis developed. One week after this exposure, biopsies were taken from the UV-induced lesions and processed for light and transmission electron microscopic investigation.

Light microscopy revealed suprabasal lacunae, corps ronds and grains. In the electron microscope, gaps in the basal lamina beneath the suprabasal lacunae were also observed, through which cytoplasmic processes of lymphocyte- and fibroblast-like cells and basal keratinocytes protruded. Parts of keratinocytes with disruptive cell membranes were localized in the gaps of the basal lamina. Also, multiplication of the basal lamina was frequent. *Key words: UVA/UVB provocation; Keratosis follicularis; Basal lamina; Herniation of keratinocytes.*

(Accepted September 10, 1990.)

Acta Derm Venereol (Stockh) 1991; 71: 108–112.

H. Beitner, Department of Dermatology, Karolinska Hospital, S-104 01 Stockholm, Sweden.

Several reports have characterized the ultrastructural changes of Mb Darier, especially dyskeratosis and acantholysis (1–7). The histopathological alterations have been observed mainly in the keratinocytes (kc) . The basal lamina (BL) at the floor of the suprabasal lacunae has been described as continuous (1) and adhering to basal keratinocytes with semidesmosomes (1–7). Mann & Haye (3) have shown that a breakdown of the BL occurs in Darier's disease, and in two recently published papers (8, 9) in particular, the changes of the BL were described.

The purpose of the present study was to investigate whether changes in the BL occurred in skin with clinically characteristic lesions of Mb Darier induced by ultraviolet irradiation.

MATERIAL AND METHODS

Patient

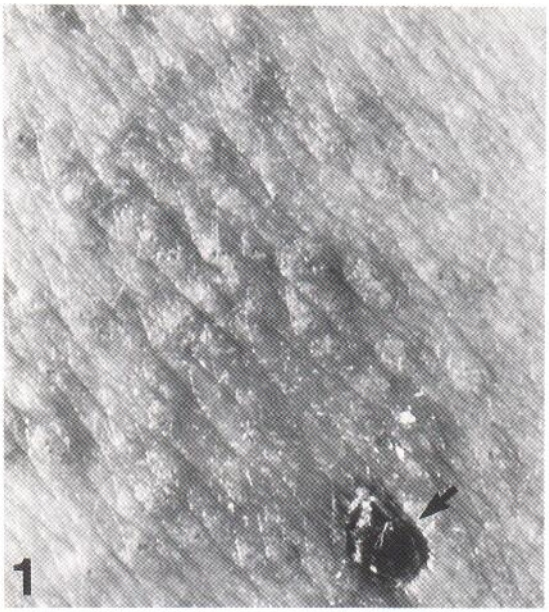
A twenty-eight-year-old male with extensive Darier's disease since puberty, exacerbated by sunlight despite his skin type, III, and his minimal erythema dose (MED) of 27.2 mJ/cm^2, was investigated. At the time of the experiment he had erythematous, papular and keratotic lesions on the neck, chest and upper back.

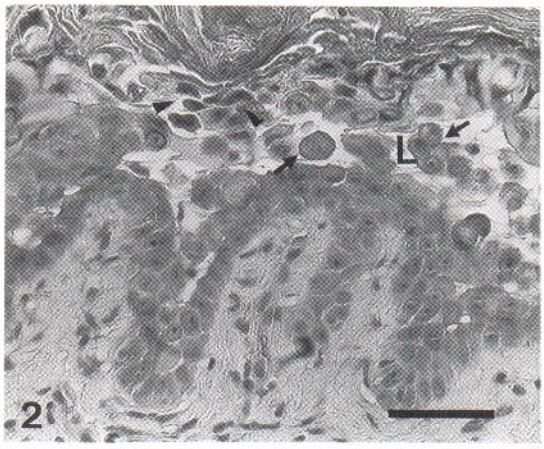
Light source

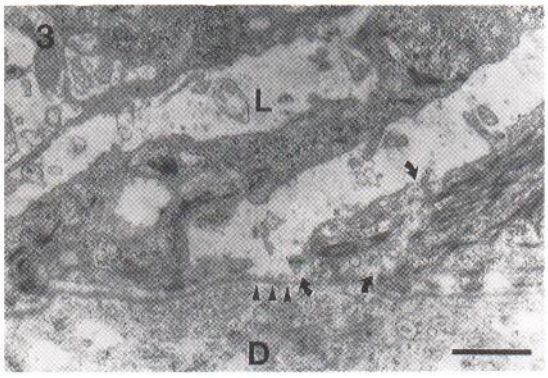
An Osram high pressure xenon arc lamp (XBO 150 W) in a Zeiss Microscope lamp housing with quartz collector was used to produce a round, uniformly bright spot, 1.5 cm in diameter. The exposure distance was 15 cm from the side of the abdomen of the patient to the filter-holder. A Schott WG 295 filter was used and the UVB intensity of the beam was 1.8 mW/cm^2. As source for the UVA provocations a glass-filtered UVA-sun 3000 lamp (Mutzhas International AG, Switzerland) was used. The skin was exposed at a distance of 30 cm and the intensity of the lamp was 43 mW/cm^2.

UV-provocation and tissue preparation

After establishing the patient's MED, an uninvolved part of the skin was exposed to 3 and 5 times his MED once a week, during 5 weeks. Simultaneously on another location the patient was exposed to 25, 50 and 75 J/cm^2 of UVA. One week after this 5-week period, a non-exposed uninvolved part of the skin on the side of the abdomen was irradiated with a single exposure of UVB 10 times the patient's MED value. One week after UV exposure 4 mm-punch biopsies were taken from skin irradiated with a single exposure of 10 times MED and from unexposed normal skin (control). One part of the biopsy from irradiated skin was fixed in 10 % formalin and processed for light microscopy. The other part was fixed with 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4°C overnight, rinsed in the same buffer, postfixed with 2% Osmium tetroxide for one hour, dehydrated in acetone, and embedded in Spurr. 500 Å ultra thin sections were cut on an LKB ultramicrotome, collected on net grids, and double stained with uranyl acetate and lead citrate. The sections were examined under a JEM-100 S transmission electron microscope.







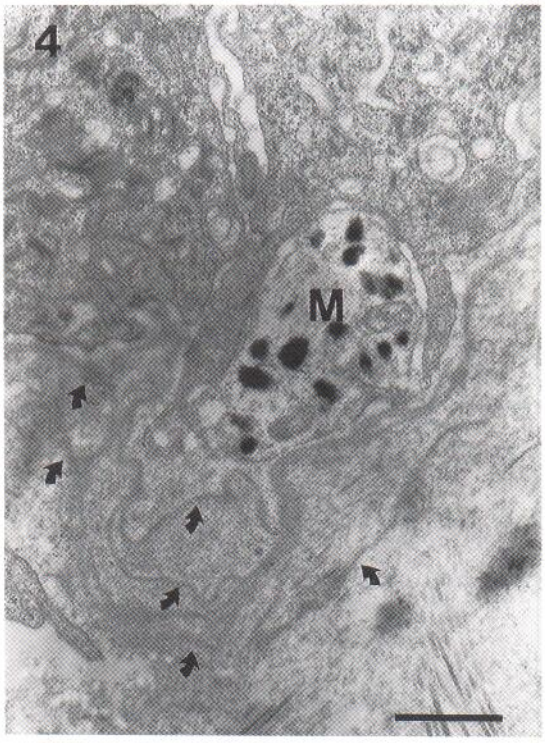


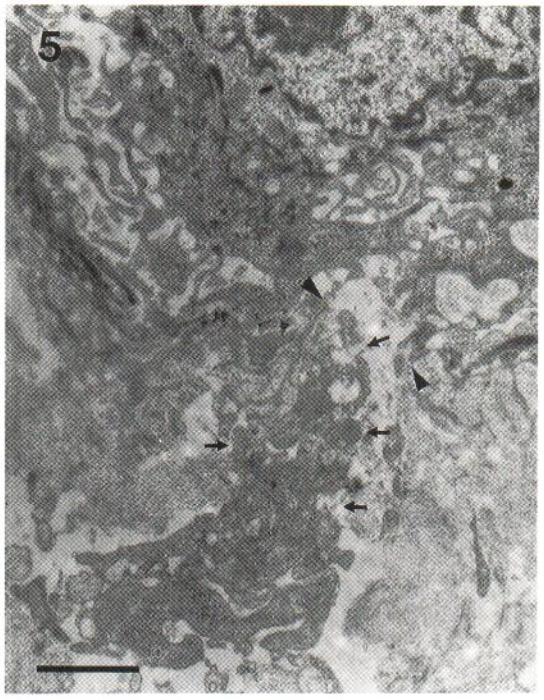
Fig. 1. Lesions of keratosis follicularis one week after exposure to UVB ten times MED. The *arrow* indicates the punch biopsy scar. – *Fig. 2.* Light-microscopic findings of keratosis follicularis induced by UVB. Hematoxylin-eosin-stained section shows suprabasal lacunae (L) with acantholytic cells (*arrows*) and grains (*arrow heads*) in the granular layer. Bar = 20 μm (× 950). – *Fig. 3.* Basal lamina (*arrow heads*) uncovered with keratinocytes and disruption (*arrows*) of cytoplasmic membrane of keratinocytes. L = suprabasal lacunae. D = dermis. Bar = 1,0 μm (× 21 700). – *Fig. 4.* Multiplication of basal lamina (*arrows*). M = melanocyte. Bar = 1,0 μm (× 22 400).

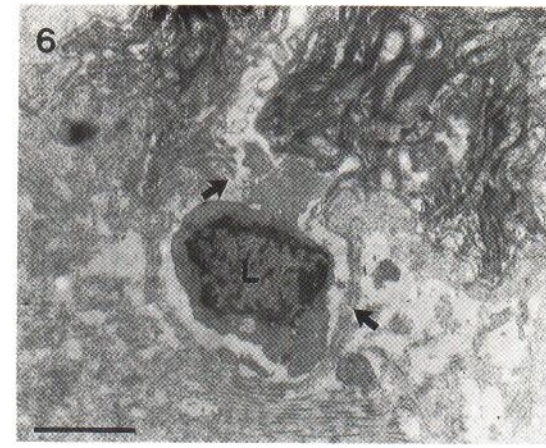
RESULTS

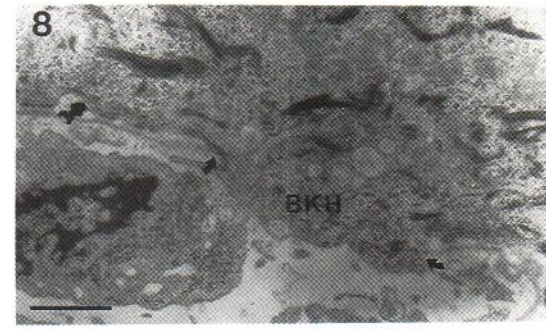
Clinical and light microscopic findings

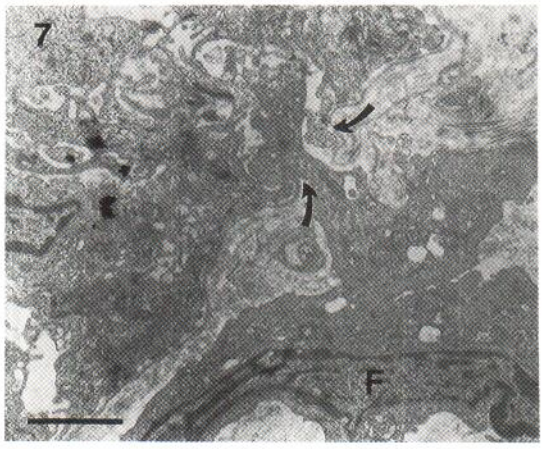
UVB provocation performed with 3 and 5 multiples of MED once a week for 5 weeks did not induce clinical Darier's disease, and neither did UVA provocation with 25, 50 and 75 J/cm^2 at the same intervals over 5 weeks.

One week after a single exposure to 10 MED of UVB, lesions with characteristic keratotic papules were observed (Fig. 1). The light-microscopic examination revealed characteristic features such as su-









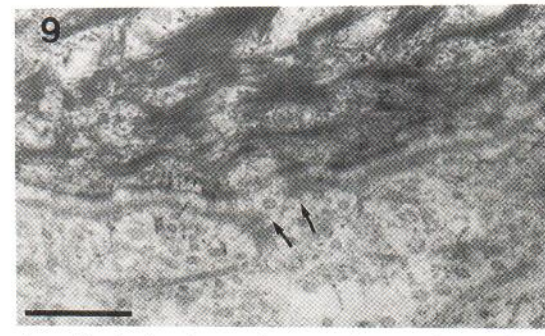


Fig. 5. Note gap in basal lamina (*arrows heads*) and disruption of cytoplasmic membrane and exposed cytoplasmic organellae (*arrow*) in area of suprabasal lacunae. Bar = 2 μm (× 12 200). – *Fig. 6.* A lymphocyte-like cell (L) penetrating the gap (*arrows*) in the basal lamina into the suprabasal lacunae. Bar = 2 μm (× 14 300). – *Fig. 7.* A cytoplasmic process of a fibroblast-like cell (F) protrudes into the lacunae through the gap (*arrows*) in the basal lamina. Bar = 2 μm (× 13 000). – *Fig. 8.* A representative basal keratinocyte herniation (*BKH*). Arrows show contact between a keratinocyte and the basal lamina. Bar = 1 μm (× 21 700). – *Fig. 9.* A micro-gap in the basal lamina beneath a keratinocyte. Bar = 0.5 μm (× 59 000).

prabasal detachment of the spinous layer forming lacunae containing acantholytic cells. In supraposition, corps ronds and grains embedded in a hyperkeratotic horny layer were seen (Fig. 2).

Transmission electron microscopy

The suprabasal lacunae, corps ronds and grains shown with light microscopy were also detected with ultramicroscopy. However, additional lesions in the form of remarkable changes of the BL beneath the suprabasal lacunae were observed with electron mi-

croscopy. The BL beneath the widened intercellular space between basal keratinocytes at the floor of the lacunae was not covered with basal cells but directly faced to the lumen of lacunae (Fig. 3).

Gaps or focal discontinuites in the BL were observed in some areas (Figs. 5–9), while the BL in other areas remained continuous. Large gaps in the BL were situated beneath the widened intercellular space between basal cells. Parts of the membrane of basal keratinocytes were degenerated and cytoplasmic organellae protruded into the lacunae and the dermis. These cell portions seemed to drop from the lacunae into the dermis through the gap in the BL (Fig. 5). Dermal lymphocyte- and fibroblast-like cells penetrated the BL through the gap of the epidermal lacunae (Figs. 6, 7). Also, cytoplasmic processes from basal keratinocytes protruded through gaps in the BL into the dermis (Fig. 8). The terminals of the gaps in the BL were in close contact with the stem of the basal keratinocyte herniation. The phenomenon illustrated in Fig. 5 occurred more commonly than the basal keratinocyte herniation. Micro-gaps were also observed in the BL (Fig. 9). Gaps in the BL were only observed in areas where suprabasal lacunae appeared. In UV-exposed skin without lacunae or in the control skin, these gaps were not seen.

Multiplications of BL were observed (Fig. 4). Elastic fibre-like materials were present among the multiplicated BL and occasionally a part of cytoplasmic processes of basal cells remained.

DISCUSSION

The patient investigated in this study developed lesions characteristic of Darier's disease after a single UVB dose of 10 times MED. This high UVB exposure was performed after serial exposures to 3 and 5 times the UVB MED once a week for 5 weeks and after UVA provocation had, to our surprise, failed to elicit a similar response. Previous reports by Baba & Yaoita (10) suggest that lesions of keratosis follicularis cannot be induced by single UVB-exposures, but rather by repeated sub-erythemal doses. However, Penrod et al. (11) and Heyl (12) showed that single exposures of erythema-producing UV irradiation did elicit lesions with features of Mb Darier. In our experience it is possible, but rather difficult, to develop keratosis follicularis in uninvolved skin with a single UVB exposure. The necessary UVB dose seems to be several magnitudes of the MED and to depend on individual variations. Since the biopsies in this case were taken one week after the UVB provocation, most of the UVB-induced erythema and other signs of UV-induced inflammation had disappeared. Also during this period the lesions characteristic of Mb Darier had developed.

The ultrastructural changes that we observed with TEM could either be a result of the unphysiologically high UVB exposure or a feature of keratosis follicularis. In previous papers discussing ultrastructural changes due to UVB irradiation (13-18), similar changes of the BL, in the form of the gaps reported in this paper, have not been observed. However, gaps and multiplication of the BL, and herniation of basal keratinocytes, have been observed by others investigating the ultrastructure of Darier's disease (8,9). In addition, in our study we observed the gaps in the BL only in the area where the suprabasal lacunae appeared, even though the whole specimen had been exposed to 10 times MED of UVB. This further supports the conclusion that the changes observed in the BL and in basal keratinocytes are predominantly the result of the induced keratosis follicularis.

The gaps in the BL are formed by the herniation of basal keratinocytes as described in psoriasis (19, 20), or are due to the penetration of the mesenchymal cells as in allergic contact dermatitis (21). Moreover, we observed that parts of keratinocytes with disrupted cell membranes were situated on the BL in the large gaps.

It has been suggested that the multiplication of the BL is caused by the synthesis of BL in epithelial cells as a result of injury and as a protective response on the part of injured cells (22). The present finding could thus be a result of the UV-induced trauma.

In conclusion, our findings support the observation by others that gaps of the BL in close connection with the suprabasal lacunae and the herniation of basal keratinocytes are connected with Darier's disease, while the multiplication of the BL is most probably the result of the UV injury.

ACKNOWLEDGMENTS

This investigation was carried out in the electron microscope laboratories at the Department of Pathology, Karolinska Hospital, with the valuable support of Associate Professor Romuald Wroblewski. The skilful technical assistance of Kerstin Bruce is gratefully acknowledged. We are also indebted to Associate Professors Anne-Marie Ros and Björn Lagerholm, Department of Dermatology, Karo-

linska Hospital, for their generous support and valuable suggestions.

REFERENCES

1. Charles A. An electron microscope study of Darier's disease. Dermatologica 1961; 122: 107–115.
2. Caulfield JB, Wilgram GF. An electron-microscope study of dyskeratosis and acantholysis in Darier's disease. J Invest Dermatol 1963; 41: 57–65.
3. Mann PR, Haye KR. An electron microscope study on the acantholytic and dyskeratotic processes in Darier's disease. Br J Dermatol 1970; 82: 561–566.
4. Gottlieb SK, Lutzner MA. Darier's disease: An electron microscopic study. Arch Dermatol 1973; 107: 225–230.
5. Biagini G, Costa AM, Laschi R. An electron microscope study of Darier's disease. J Cutan Pathol 1975; 2: 47–49.
6. Sato A, Anton-Lamprecht I, Schnyder UW. Ultrastructure of dyskeratosis in Morbus Darier. J Cutan Pathol 1977; 4: 173–184.
7. Mesquita-Guimarâes J, Mesquita-Guimarâes I. Cellular differentiation in Darier's disease. Ultrastructural aspects. J Submicrosc Cytol 1984; 16: 387–394.
8. Heng MCY, Kloss SG. Basal keratinocyte herniations in Darier's disease. J Am Acad Dermatol 1985; 13: 307–310.
9. El-Gothamy Z, Kamel MM. Ultrastructural observations in Darier's disease. Am J Dermatopathol 1988; 10: 306–310.
10. Baba T, Yaoita H. UV radiation and keratosis follicularis. Arch Dermatol 1984; 120: 1484–1487.
11. Penrod JN, Everett MA, McCreight WG. Observations on keratosis follicularis. Arch Dermatol 1960; 82: 367–370.
12. Heyl T. Sunlight and Darier's disease. Br J Dermatol 1971; 85 (Suppl. 7): 57–59.
13. Nix TE, Nordquist RE, Scott JR, Everett MA. Ultrastructural changes in stratum corneum induced by ultraviolet light. J Invest Dermatol 1964; 43: 301–317.
14. Nix TE, Nordquist RE, Scott JR, Everett MA. Ultrastructural changes induced by ultraviolet light in human epidermis: Basal and spinous layers. J Invest Dermatol 1965; 45: 52–64.
15. Nix TE, Nordquist RE, Scott JR, Everett MA. An ultrastructural study of nucleolar enlargement following ultraviolet irradiation of human epidermis. J Invest Dermatol 1965; 45: 114–118.
16. Wilgram GF, Kidd RL, Krawczyk WS, Cole PL. Sunburn effect on keratinosomes. A report with special note on ultraviolet-induced dyskeratosis. Arch Dermatol 1970; 101: 505–519.
17. Hönigsmann H, Wolff K, Konrad K. Epidermal lysosomes and ultraviolet light. J Invest Dermatol 1974; 63: 337–342.
18. Olson RL, Gaylor J, Everett MA. Ultraviolet-induced individual cell keratinization. J Cutan Pathol 1974; 1: 120–125.
19. Cox AJ. The dermal-epidermal junction in psoriasis. J Invest Dermatol 1969; 53: 428–435.
20. Heng MCY, Kloss SG, Kuehn CS, Chase DG. The sequence of events in psoriatic plaque formation after tape-stripping. Br J Dermatol 1985; 112: 517–532.
21. Komura J. Some ultrastructural observations on the dermoepidermal junction. Effect of electron stains (phosphotungstic acid, lanthanum) and in vitro trypsin digestion. Acta Derm Venereol (Stockh) Suppl 1973; 73: 111–120.
22. Pierce GB, Nakane PK. Basement membranes. Synthesis and deposition in response to cellular injury. Lab Invest 1969; 21: 27–41.