

CHRONIC BULLOUS DERMATOSIS OF CHILDHOOD

Immunological and Ultrastructural Studies on the Melanocyte-Dermal Junction

J. P. Ortonne, D. Schmitt, and L. Jacquelin

Clinique Dermatologique and l'INSERM FRA no. 11, Hôpital Edouard Herriot, Lyon, France

Abstract. Ultrastructural study of a case of benign chronic bullous dermatosis of childhood revealed the presence of melanocytes in the base of the blister. The melanocyte anchorage mechanism is described. The absence of labelling by bullous pemphigoid antibodies on the base of the blister, where melanocytes persist, indicates that the antigens recognized by these autoantibodies do not exist in the melanocyte basal membrane-contact zones. This suggests that the chemical nature of the melanocyte dermal junction anchorage system is different from that of the hemidesmosomes.

Key words: Chronic bullous dermatosis of childhood; Melanocyte-dermal junction

INTRODUCTION

Little is known about the melanocyte-dermal junction. "Periodic dense thickenings" (1) or "dense plates" (7) along the plasma membrane of the epidermal melanocytes have been described. The function of these structures is still ill defined, though it has been suggested that they could represent the anchorage system of the melanocytes on the basal membrane and on the subjacent skin. They have also been compared to hemidesmosomes (7). By experimental dermo-epidermal cleavage, using chemical or enzymatic techniques, it is impossible to separate selectively the keratinocytes from the melanocytes. In various skin disorders which are accompanied by dermo-epidermal or intradermal separation, no independent separation of these two cellular populations has been described (6).

Ultrastructural study of a case of benign chronic bullous dermatosis of childhood revealed the presence of melanocytes in the base of the blister. Correlation with an immunological study of the same material allowed of certain observations regarding the nature of the connection between the melanocytes and the basal layer of the epidermis and the basement membrane and the subjacent skin.

MATERIAL AND METHODS

Patient

A detailed clinical and immunological study of the patient has been published elsewhere (5). The patient was a 3-year-old male child of North African origin, who had suffered from a bullous dermatosis since May 1976. The clinical, histological, immunological and ultrastructural features suggested the diagnosis of benign chronic bullous dermatosis of childhood.

Material

The immunological and ultrastructural studies were carried out on small early bullous lesions (3 mm). 6 mm punch biopsies were removed under local anesthesia (1% xylocaine). Three successive series of biopsies were taken during the evolution of the lesion. Half of each biopsy was frozen for immunological study and the other half was kept for the ultrastructural study.

Immunological study

(a) Immunofluorescence: a study of fixed autoantibodies by direct reaction with fluorescent conjugates (Institut Pasteur), i.e. anti-IgA, anti-IgM and anti-IgG on frozen sections.

(b) Immunoperoxidase (light microscopy): study of antigens of the dermo-epidermal junction using fixation of circulating autoantibodies present in bullous pemphigoid (indirect reaction on frozen sections). The bullous pemphigoid serum had a titre higher than 1/800 and was used at a dilution of 1/100. The conjugate used was a peroxidase labelled sheep anti-human immunoglobulin antiserum (Institut Pasteur).

Ultrastructural study

The biopsy specimens were fixed in 3% glutaraldehyde for 1 h, post-fixed on osmic acid for 1 h, dehydrated in alcohol, embedded in Epon and cut with a Reichert ultramicrotome. The sections were stained with uranyl acetate and lead citrate and then examined using a Hitachi HU12A electron microscope.

RESULTS

Direct immunofluorescence

In one of the three specimens a weak, linear IgA deposit was observed at the dermo-epidermal junction of the peribullous zone.

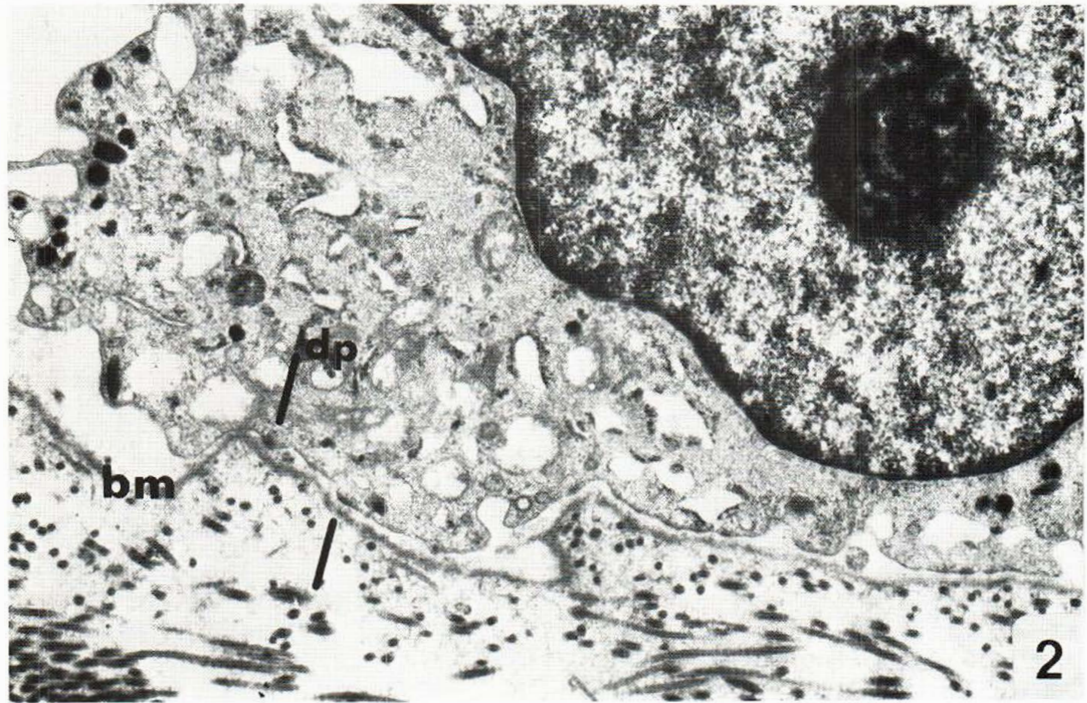
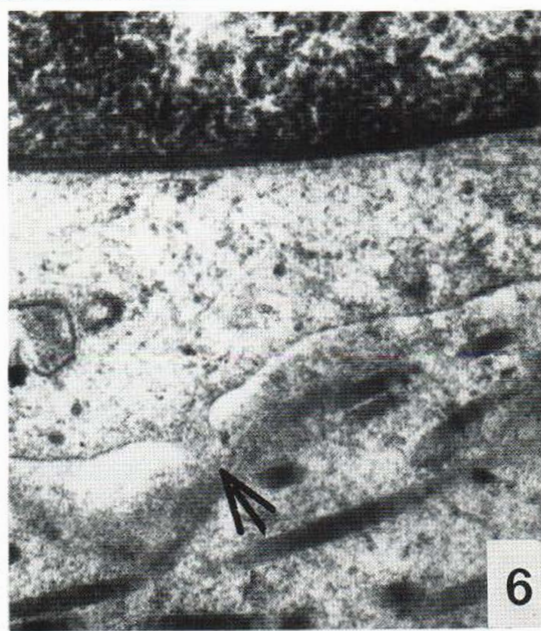
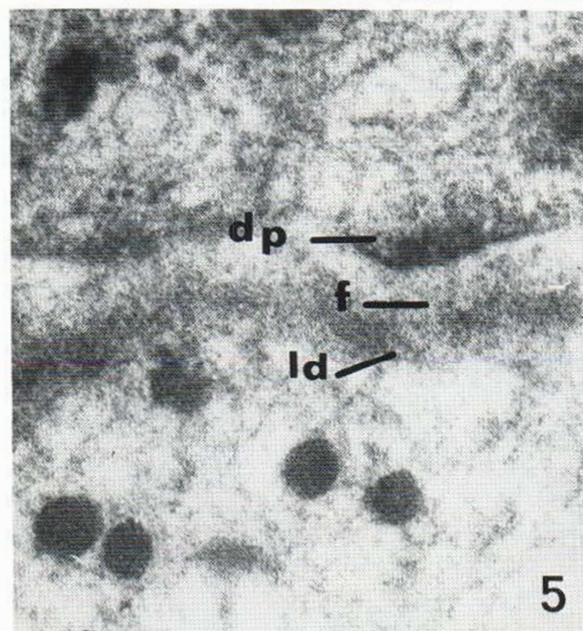
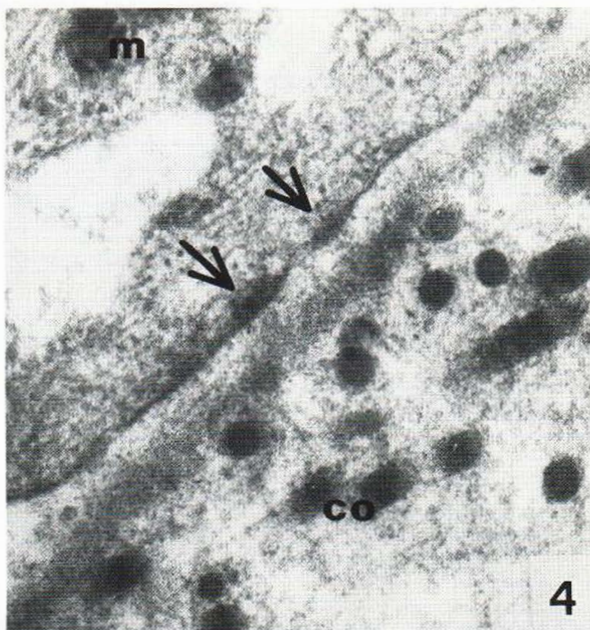


Fig. 1. Base of the blister: Melanocytes are observed still adhering to the basement membrane (*bm*); *p*: polymorphonuclear cell. $\times 3\,000$.

Fig. 2. High-power view of the contact zone between the

melanocytes and the basement membrane (*bm*). Arrows point to parietal thickenings (*dp*) along the plasma membrane of the epidermal melanocyte. $\times 20\,000$.



Figs. 3–5. High-power views of the dense thickenings or "dense plates" (dp). 3. $\times 32\,900$. 4. $\times 82\,800$; m: melanosomes; co: collagen. 5. $\times 137\,000$, dp: dense plate; f: filaments; ld: lamina densa.

Fig. 6. Contact zone between plasma membrane of the melanocyte and the lamina densa. $\times 60\,500$.

Indirect immunoperoxidase reaction (light microscopy)

The circulating autoantibodies of the bullous pemphigoid serum were only fixed in the non-separated peribullous zones. In the base and roof of

the blister no fixation of autoantibodies was observed.

Electron microscopy

Dermo-epidermal separation was observed in the lamina lucida of the basement membrane. The roof

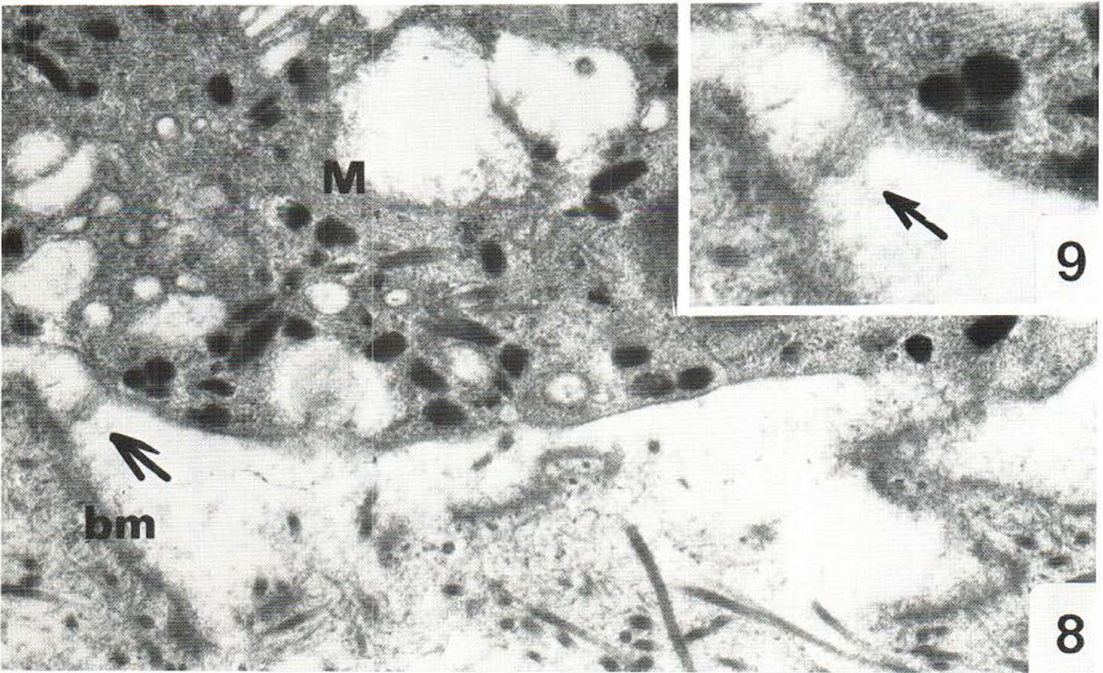
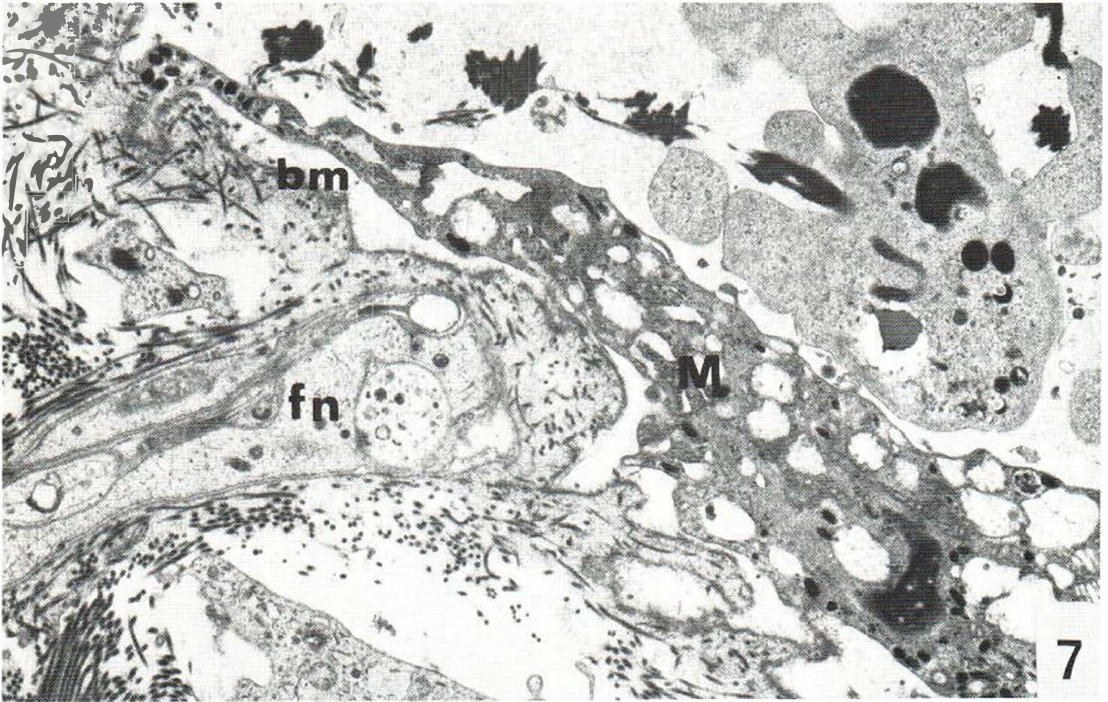


Fig. 7. Altered melanocyte (*M*) showing incipient separation: *bm* (basement membrane); *fn* (nerve ending). $\times 7\,500$.
Fig. 8. High-power view of the contact zone between an altered melanocyte (*M*) and the basement membrane (*bm*). A filamentous material is still observed. Parietal

thickenings along the plasma membrane of the melanocytes are no longer visible (arrow). $\times 32\,000$.
Fig. 9. (inset) High-power view of the filamentous material (arrow). $\times 64\,000$.

of the blister consisted of keratinocytes, with the disappearance of hemidesmosomes and associated tonofibrils. The base of the blister was superficially bordered by the lamina densa of the basement membrane. Above this, melanocytes only were observed (Fig. 1), still adherent to the lamina densa.

Few of the melanocytes showed any degenerative changes of nucleus or cytoplasm. The others contained normal numbers of melanosomes, whose size and structure had not been altered. The majority were type IV melanosomes, regularly distributed in the periphery of the melanocytes. Some of the mitochondria had a dilated appearance. The functional relationship between the cells and the basement membrane was unaltered. In particular, thickenings on the cell membrane were only observed on the inferior surface of the melanocytes and were absent from their free lateral and superior margins, whose plasma membranes otherwise appeared normal. On the other hand, thickenings of the plasma membrane, analogous to those which were observed on the inferior surface of most of the melanocytes, were occasionally found in the contact zones between adjacent melanocytes or their dendrites. The basal thickenings on melanocytes were mostly observed where the lamina lucida was seen to be of minimal thickness (Figs. 2-3). Opposite some of the thickenings, a filamentous material was observed, contrasting with the normal clear appearance of the lamina lucida. This filamentous material stretched from the plasma membrane of the melanocytes to the lamina densa (Figs. 4-5). Furthermore, where no thickenings of the melanocyte plasma membrane existed, direct contacts between the cytoplasmic membrane and the lamina densa could be seen (Fig. 6). In places, isolated melanocytic dendrites containing type IV melanosomes persisted in contact with the basal membrane. This latter usually appeared normal, but thickenings and folds occurred focally without necessarily affecting contact with the melanocytes.

Occasionally, the most severely altered melanocytes (cytoplasmic vacuolization) showed incipient separation (Fig. 7). However, at certain points a filamentous material could still be seen between the plasma membrane, which appeared to be normal, and the locally fragmented lamina densa. Frequently, in this area, the thickened zone of the plasma membrane of the melanocytes was no longer visible opposite this extracellular filamentous material (Figs. 8-9).

DISCUSSION

Thickenings of melanocyte walls have been seen previously on the inferior surface of the plasma membrane, opposite the basement membrane (1, 7), but not opposite the keratinocytes. Tonofilaments have not been observed to converge towards these zones. On the other hand, extracellular filaments have been observed between the inferior surface of the melanocytic plasma membrane and the lamina densa. The significance of these structures is not known, but it has been suggested that they could be involved in anchoring the melanocytes to the basement membrane (and the dermis). Our ultrastructural observations in general support these findings, since we have observed both wall thickenings on the inferior surface of the melanocytes and the filamentous structures joining the plasma membrane to the lamina densa. Previous studies have invariably shown an association of thickenings and extracellular filaments in relation to the inferior surface of normal melanocytes (2, 3, 8). However, our study has shown that, in places, the extracellular filamentous material and the membrane thickenings appear to be dissociated. This might suggest that these two structures react in different ways during the disease from which our patient suffered. Disappearance of the parietal thickenings as a result of pathological factors which do not affect the filamentous material, might be postulated. This view is reinforced by the fact that in early lesions the hemidesmosomes of keratinocyte membranes disappear, whereas an extracellular filamentous material, comparable to that observed opposite the thickenings of the plasma membrane of the melanocytes persists.

The observation of thickening of the plasma membrane of the melanocytes opposite the zones of contact with other melanocytes or their dendrites has not been previously recorded. The fact that the structures described above (parietal thickenings, extracellular filamentous material) were only observed in zones where the melanocytes were in contact with other structures (basal membrane or other melanocytes) and were modified at the time of bullous detachment, suggests that they are of considerable importance in the melanocyte anchorage mechanism.

There is no previous mention in the literature of the persistence of melanocytes in the base of bullous lesions. It is possible that this observation can only be made in recent lesions and there is nothing

to indicate that this phenomenon is specific to benign chronic bullous disease of childhood. Furthermore, the observation of degenerating melanocytes suggests that these cells may be in the process of separation from the basement membrane. This difference in behaviour between melanocytes and keratinocytes in the same pathological process suggests that different structures are involved in their attachments to the basement membrane. Assuming that the membrane thickenings of the melanocytes represent their anchorage structures, it is doubtful whether they can be distinguished morphologically from hemidesmosomes. It is conceivable that their chemical compositions differ—a view reinforced by our immunohistochemical observations. The autoantibodies of bullous pemphigoid fix themselves specifically to the hemidesmosomes of the keratinocytes (4). The absence of labelling by such autoantibodies on the base of the blister where melanocytes persist, indicates that the antigens recognized by these autoantibodies do not exist in the melanocyte–basal membrane contact zones. It is therefore possible to conclude that the chemical nature of the melanocyte–dermal junction anchorage system is different from that of the hemidesmosomes.

ACKNOWLEDGEMENT

We are pleased to acknowledge the expert technical assistance of Miss Danielle Germain. This work was supported in part by INSERM Grant no. 18-6-1975. 42.

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Received December 27, 1977

J. P. Ortonne, M.D.
Clinique Dermatologique
Hôpital Édouard Herriot
69374 Lyon Cédex 2
France