Acta Derm Venereol (Stockh) 1989; 69: 407–409

Circular and Branched Birbeck Granules and Cytomembrane Blebbing in Langerhans' Cells after Dithranol Irritation

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Mild dithranol irritation of healthy human skin has a stronger effect on the fine structure of Langerhans' cells (LC) than on that of other epidermal cells, causing mitochondrial enlargement and disruption of the cristae of LCs. With a stronger dithranol irritation, LCs were even more affected resulting in circular and branched Birbeck granules (BG) and blebbing of the LC cytomembrane. More often than is normal, BGs were contiguous with the LC cytomembrane. Electron microscopic observations indicated that blebbing and the abnormal BG formation were associated phenomena, in accordance with the hypothesis that BGs are endocytotic organelles formed from the cytomembrane.

Key words: Electron microscopy; Irritant contact dermatitis; Anthralin; Mitochondria; Endocytosis; Free radicals; Cell membrane; Psoriasis.

(Accepted April 7, 1989.)

Acta Derm Venereol (Stockh) 1989; 69: 407–409.

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It has earlier been shown that the Langerhans' cells (LC) are more susceptible than the keratinocytes when the skin is mildly irritated with dithranol (1–4). When the lowest irritant concentration of dithranol yielding a clinically detectable erythema was used, the mitochondria of LCs were seen to be enlarged with distorted cristae, while the keratinocytes remained largely unaffected (1, 3). Here the effect of a stronger dithranol irritation on LCs is reported.

MATERIAL AND METHODS

Irritant contact dermatitis was provoked on the backs of 5 healthy volunteers with freshly mixed dithranol 0.1% in pet. or 0.1% Psoradrate® (Rhone-Poulenc, France) containing 0.1% dithranol, using the Finn Chamber® occlusion technique. This gave macroscopically an erythematous reaction with moderate-to-considerable edema that persisted for 2–4 days.

Punch biopsies (total numbers in parentheses) were taken from irritant patch tests as follows: at the end of occlusion at 24 h (6) and at 2 days (6), 3 days (2), 4 days (6), 5 days (2) and 10 days (2) after challenge. Non-occluded, uninvolved skin from the contralateral back skin served as control. Finn-Chamber® occlusion for 24 h with Psoradrate®-base (kindly provided by Rhone-Poulenc, France) was used as another control. The control biopsies were taken at the end of occlusion. The biopsies were fixed in phosphate-buffered (0.1 M, pH7.2–7.3) 2.5% glutaraldehyde, (total osmolality about 530 m0sm/kg H_2O) for 2 h or longer at 4°C and postfixed in 1% osmium tetroxide. The specimens were processed in routine manners (4) and viewed with a Jeol CX 100 electron microscope equipped with a side-entry goniometer ($\pm 60°$ tilting range), operated at 60 or 80 kV. A more detailed description of the material and methods has been published elsewhere (5).

RESULTS AND DISCUSSION

Virtually all LCs were affected. Changes in LCs were seen throughout the observation period, i.e. from removal of occlusion (24 h) up to 10 days after challenge. Mitochondrial enlargement and disruption of cristae as reported earlier (1) were prominent. In the more mildly affected LCs, there was a parallel activation of other organelles: a prominent Golgi complex with an increased number of coated and non-coated vesicles, widened endoplasmic reticulum containing fine-granular protein-like substance, many lipid droplets and lysosomes, and cytomembrane folds were seen.

In more strongly affected LCs, circular and branched BGs were formed (Figs. 1–2). In these LCs the cell membrane was focally totally disrupted, resulting in outpouring of cytoplasm into the intercellular space (Fig. 1). The cytomembrane disruption was confirmed by tilting the specimen. LC cytomembrane pseudopodia (6) or microvilli (7) were not frequent, but many membrane-bound bodies (blebs) were seen apposed to or in the immediate vicinity of LCs (Figs. 1–2). Serial sections showed that the blebs were not

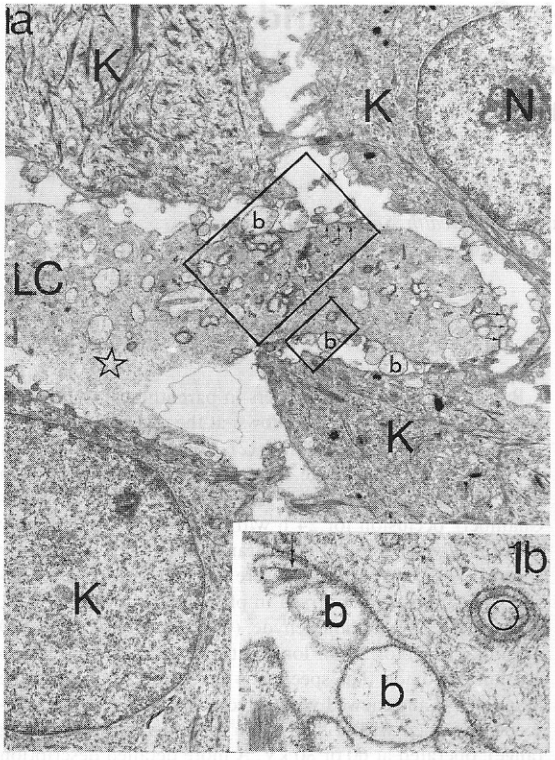

Fig. 1. Severely damaged Langerhans' cell dendrite (*LC*) amongst well preserved keratinocytes (*K*), one of which has a prominent nucleolus (*N*). The cytomembrane of the LC is partly missing (*star*). Note the large number of blebs (*b*) around the LC. Arrows denote areas where BGs are apposed to blebs. The boxed areas are seen at higher magnification in Figs. 1 *b* and 2. (1 *b*) Blebs (*b*), one of which contains a BG (*double-headed arrow*). Circle shows circular BG. 48 h after dithranol challenge (*a*, ×5 900; *b*, ×26 400).

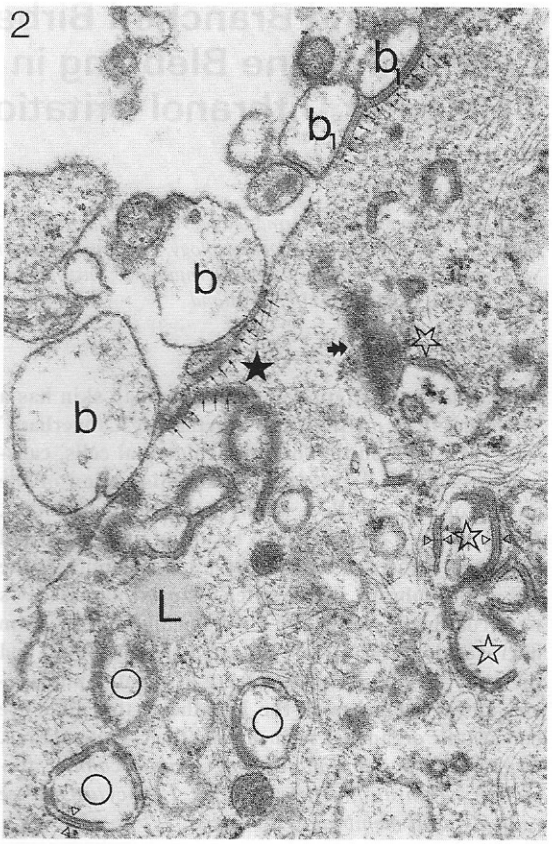

Fig. 2. Higher magnification of boxed area in Fig. 1 *a*. Note the blebs (*b*, b_1) and their apposition to 'tangential' BGs (*arrows*). The trilaminar unit membranes of the BG seem to be formed from the cytomembrane of the LC (*arrow*) and the unit membrane of the bleb (b_1). Some of the BGs (*solid star, double arrow*) are blurred because the plane of their discs has an unfavourable angle towards the beam. Circles denote circular BGs and open stars branched BGs. The trilaminar unit membrane of the BG (*arrowhead*) and the striated central lamina are visible. Note the numerous fibrils in the cytoplasm. *L*, opaque lipid droplet. ×26 400.

protrusions of LCs. BG profiles were seen between blebs and LCs in such a way that the trilaminar unit membranes of the BG consisted of the cytomembrane of the LC and the unit membrane of the bleb (Fig. 2). This could indicate that bleb and BG formation were associated phenomena. Occasionally BGs were encountered inside these blebs (Fig. 1 *b*), indicating that they originated from LCs. 'Tangential' BGs associated with blebs, were also seen after milder dithranol irritation (2).

When the plane of the BG had an unfavourable angle towards the beam and was cut *en face*, BGs were blurred, and the disc and grid structure of BGs (8) was seen. BGs did not have a clathrin-type coat (7). Their unit membrane was trilaminar (Fig. 2) as in normal BGs, but otherwise they showed highly uncharacteristic features: they were branched, circular and longer, and continuity with the cytomembrane was more frequent than is normal (Figs. 1–2). The insides of circular BGs (as seen in section) were in most cases devoid of the type of filament that was otherwise plentiful in the cytoplasm of LCs (Fig. 2). LCs with circular or branched BGs were observed 24 and 48 h after challenge, but not in later biopsies. Circular or branched BGs or cytomembrane blebbing were not observed in control biopsies.

The current knowledge of the effects of irritants and toxic substances on LCs is fragmentary, but it has

been shown that some substances, e.g. dithranol have a specific affinity for LCs, while others have a stronger effect on other epidermal cells (3). Dithranol is widely used in the treatment of psoriasis, but its exact antipsoriatic action is poorly understood. Dithranol generates free radicals, becomes rapidly associated with cell membranes and is a mitochondrial inhibitor (9). As shown earlier, the mitochondria of LCs are especially sensitive to dithranol (1, 3) and the present findings of changes in BGs tend to reflect a cytomembrane effect. However, no indications that BGs are derived from mitochondria (10) or desmosomes (11) could be detected. Interestingly, BGs in histiocytes in histiocytosis X show resemblance to the BGs reported here after dithranol treatment, being often both contiguous with the cytomembrane and having anastomoses and branches (12). This may indicate that the endocytic activity of histiocytes (LC-like cells) in histiocytosis X is higher than in normal LCs.

The present findings after dithranol irritation correspond morphologically in many respects to those reported after digitonin and sodium lauryl sulphate exposure (13, 14), indicating that branching, circular and tangential BGs are formed because dithranol has a direct effect on the cytomembrane of LCs. The LCs with branched and circular BGs seemed to be so severely damaged that it is improbable that BGs would have been formed in the Golgi area and transported to the cytomembrane with fusion of the cell membranes (exocytosis) (see 12). The present observations are in accordance with the view that BGs (after dithranol treatment) are endocytotic organelles formed from the cell cytomembrane, as first suggested by Hashimoto & Tarnowski (12, 15).

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