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INVESTIGATIVE REPORT

Immunohistochemical Examination of P-cadherin in Bullous and Acantholytic Skin Diseases

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Autoimmune blistering diseases (pemphigus vulgaris, pemphigus foliaceus, bullous pemphigoid, dermatitis herpetiformis) and certain genodermatoses with acantholysis (Darier-disease, Hailey-Hailey disease) have different aetiological factors, but all result in bulla formation and/or in acantholysis. Cadherins are Ca^{++}-dependent cell-cell adhesion molecules which play an important role in the cellular connection between normal cells. P-cadherin is involved in the selective adhesion of epidermal cells, and is expressed only on the surfaces of the two basal layers. We examined the expression of P-cadherin in some autoimmune bullous skin diseases and Darier's disease using immunohistochemistry and found P-cadherin to be strongly upregulated. We believe the upregulation is compensatory to the primary pathophysiological events in the various bullous dermatoses.
Key words: Darier's disease; dermatitis herpetiformis; epidermal cell adhesion; pemphigoid; pemphigus; upregulation.

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Cell adhesion molecules are significant for the cellular connection of normal cells, for the maintenance of specific organ architecture as morphogenetic regulators and for their importance in malignancy (1, 2). They can be divided into two groups, calcium-dependent, termed "cadherins", and calcium-independent ones (3). A third large family comprises: (a) adhesion molecules of the immunoglobulin gene family, (b) integrins, (c) catenins, (d) selectins, and (e) CD44 molecules.

Cadherins are a multigene family of Ca^{++}-dependent homophilic (cell-cell) adhesion molecules that are membrane glycoproteins with a single transmembrane domain. Classification of the cadherin superfamilies is based on the homology of their extracellular domain (4). Among the classical cadherins, E- (epithelial) and P- (placental) cadherins are involved in the selective adhesion of epidermal cells. E-cadherin is expressed on the cell surfaces of all epidermal layers, whereas P-cadherin is expressed only on the surfaces of the basal and most immediate suprabasal cells of the epidermis (Fig. 1) (5–7). T-cadherin was recently detected on the basal cell layer of human and mouse skin, but its physiologic and pathologic role in the skin needs further investigation (8). Normal melanocytes express E- and P-cadherin (9, 10). E- and P-cadherins are found in the adherent or desmosomal junctions, which in the epidermis are desmoglein 1, desmoglein 3 and desmocollin (11).

Identification of the desmosomal cadherins created a new way for studies on the pathogenesis of autoimmune bullous diseases, especially when the identity between pemphigus vulgaris (PV) antigen and desmoglein 3 was verified (12–16). Antibodies in PV and pemphigus foliaceus (PF) are directed towards desmoglein 3 and desmoglein 1, and are mostly IgG (mainly IgG4 subclass) (17–19). In bullous pemphigoid (BP) the hemidesmosome is the target (20–22). The arrangement of desmoglein 3 and desmoglein 1 in the epidermis explains the site of acantholysis and bulla formation, which is intraepidermal in PV (with involvement of the oral mucosa), subcorneal/superficial in PF and subepidermal in BP (23). Darier's disease (DD) is an autosomal dominantly inherited genodermatosis characterized by abnormal keratinization and acantholysis (24). Mutations in ATP2A2, which encodes the Ca^{++} transporting sarco/endoplasmic reticulum pump type 2 isoforms (SERCA2), cause DD (25). In DD there is a genetically determined weakness in the desmosomes, while in autoimmune

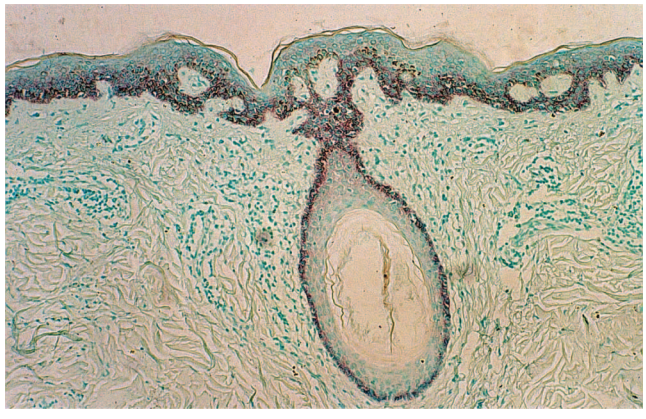

Fig. 1. P-cadherin expression in normal skin (chromogen: etilcarbazol).

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blistering diseases desmosomes are normal until autoantibodies bind to disrupt them (26).

MATERIAL AND METHODS

Skin biopsies from 12 patients with PV, 2 patients with PF, 20 with BP, 2 patients with DH and 2 patients with DD were studied. The diagnoses were established by the characteristic clinical picture, family history and histopathological findings.

Mouse monoclonal antibodies against P-cadherin (clone: 56) were purchased from Transduction Laboratories (Lexington, KY, UK).

Immunohistochemistry was performed using an avidin-biotin-peroxidase technique (Streptavidin, Biotinylated Horseradish Peroxidase and Mouse Immunoglobulins/Biotinylated were purchased from DAKO A/S, Denmark). A heat-induced antigen retrieval method was applied. Deparaffinized 4-μm-thick sections were placed in prewarmed 0.1 M citrate buffer, pH 6.0 for 2 min in a pressure cooker. To block non-specific background we used normal rabbit serum. The primary antibody at a dilution of 1:50 was incubated overnight at 4°C in a humid chamber. As secondary antibody, we applied biotinylated mouse immunoglobulins at a dilution of 1:400. As chromogen, either diaminobenzidin or etilcarbazol was applied. Sections were counterstained with haematoxylin. Normal perilesional skin was used as a positive control. This showed intense P-cadherin immunoreactivity along the cell-cell contacts of basal keratinocytes (Fig. 1).

RESULTS

Immunostaining with the antibody to P-cadherin demonstrated immunoreactivity not only on the cell membrane of basal and suprabasal layers of epidermis in lesional skin of PV and PF, but expression of P-cadherin was observed in the epidermal layers of the top of the bulla (Fig. 2). In BP and DH there was expression of P-cadherin in the lower layers of the subepidermal bulla. In DD the acantholytic epidermis expressed P-cadherin as well (Fig. 3).

DISCUSSION

In the present study, we observed immunoreactivity of P-cadherin not only on the surface of the two basal layers of keratinocytes, but also of suprabasal layers in the lesional skin in PV, PF, BP, DH and DD indicating upregulation of P-cadherin. Our findings suggest that the upregulation of P-cadherin is common in both the autoimmune bullous skin diseases (PV, PF, BP, DH) and DD, which is an inherited acantholytic disease.

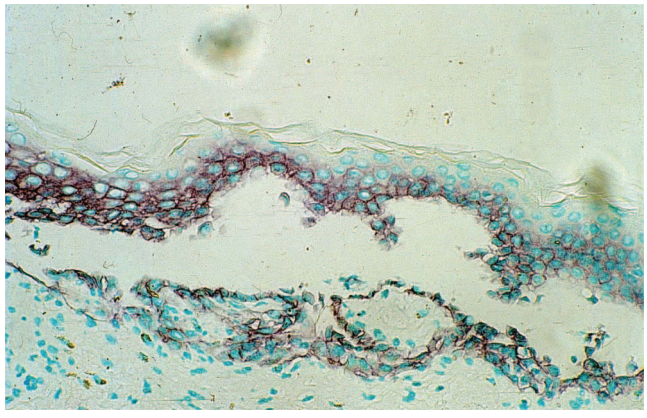

Fig. 2. Upregulation of P-cadherin in pemphigus vulgaris (chromogen: etilcarbazol).

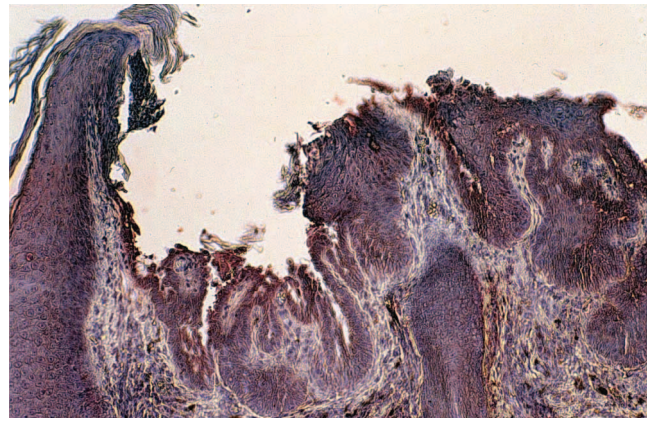

Fig. 3. Upregulation of P-cadherin in Darier's disease (chromogen: diaminobenzidin).

Our results are surprising because, logically, we expected that cessation of cell-connection during bulla formation/acantholysis would mean destruction and disappearance of the adhesion molecules. Hakuno et al. (30) were the first to describe the upregulation of P-cadherin in pemphigus (PV: $n=2$, PF: $n=2$), Hailey-Hailey disease ($n=4$) and DD ($n=3$). We had similar results among more patients and more types of bullous skin diseases.

Several hypotheses may be raised to explain our observation. One is that P-cadherin is associated with proliferation and differentiation and is therefore upregulated in the lesional skin (27). Another hypothesis is that abnormal Ca^{++} concentration in the keratinocytes may be associated with the upregulation of P-cadherin shown in DD, because mutations in the genes coding for Ca^{++} pumps are reported to be responsible for acantholysis (28).

Dissociation of intra- and extracellular domains of desmosomal cadherins and E-cadherin was found in Hailey-Hailey disease and DD (29). However, E-cadherin is expressed throughout the whole epidermis, unlike P-cadherin, which is restricted to the basal two layers. The 'proteolysis model' can be identical in the case of P-cadherin, as the extracellular domains of cadherins are cleaved from the cell surface by proteolysis during acantholysis, but the cytoplasmic domain remains at the membrane and in association with the cytoskeleton anchored by catenins. During immunohistochemical staining this cytoplasmic/intracellular domain of cadherin may remain sufficient to bind the antibodies, which results in immunopositivity. Another explanation is the incomplete destruction of the molecules: certain conformational changes in cadherins mask

the extracellular epitopes which will be recognized by the antibodies.

Hakuno et al. (30) raised the possibility of involvement of P-cadherin's upregulation in the pathomechanism of both the autoimmune blistering diseases and the inherited blistering diseases. We presume that upregulation of P-cadherin is not playing a causal role in the pathophysiology, but it might be a compensatory consequence at the end of the whole process. As synergism between P-cadherin and desmoglein 3 was already demonstrated *in vivo*, it suggests a cooperative/compensatory effect of these adhesion molecules (31). Similarly, as Amagai et al. (12) stated, we can support the fact that the autoimmune blistering diseases and the acantholytic genodermatoses are diseases of cell adhesion. Further studies are required to elucidate the pathomechanism of upregulation of P-cadherin in blistering/acantholytic skin diseases. This may carry a parallel to expression of P-cadherin in high-grade breast cancer (32). To investigate that question, upregulation of P-cadherin in blistering/acantholytic skin diseases could serve as a model for the incohesiveness of tumour cells.

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