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Keratin Expression in Epidermolysis bullosa simplex (Dowling–Meara)

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The Dowling–Meara variant of epidermolysis bullosa simplex (EBS) is characterized microscopically by clumping of the keratin tonofilaments within areas of incipient blistering, thereby raising the possibility that an abnormality of the keratin cytoskeleton might underlie blister formation in this condition. In order to investigate keratin expression in Dowling–Meara EBS, the staining profile of a panel of antikeratin monoclonal antibodies was examined in perilesional skin from 5 affected subjects, using a standard immunoperoxidase technique. Normal labelling characteristics were demonstrated by antibodies identifying keratins of the basal and suprabasal compartments of normal interfollicular epidermis and of simple epithelia (keratin 19). In addition, a keratin expressed in hyperproliferative epidermal states was shown to be absent. The results suggest that the profile of keratin synthesis is normal in Dowling–Meara EBS and that the tonofilament clumping may be, therefore, the result of a post synthetic modification of keratin molecules. (Received June 1, 1987.)

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Epidermolysis bullosa simplex (EBS) is a debilitating, and occasionally lethal, inherited bullous disorder, with several clinically distinct sub-types (1), in which blisters arise by lysis of the basal epidermal cells (2) via an unknown mechanism. Acantholysis may be an occasional feature (3). The Dowling–Meara variant of EBS is characterized by herpetiform clustering of intra-epidermal blisters and a marked degree of palmoplantar hyperkeratosis (4–6). Pronounced clumping of the epidermal tonofilaments within areas of incipient blister formation is considered to be a specific structural feature of this form of EBS (7, 8), suggesting that an abnormality of the keratin cytoskeleton might underlie the cytolytic blistering process. In order to examine this possibility, therefore, the staining characteristics of the epidermis in Dowling–Meara EBS with a variety of antikeratin monoclonal antibodies was studied.

MATERIALS AND METHODS

Tissue samples

Perilesional skin was obtained from 5 adults with the typical clinical features of Dowling–Meara EBS. In the families of 4 of these cases, one of whom was an original patient of Dowling & Meara (4), there were no other affected members, but the fifth had a daughter with the same condition. In all subjects, cleavage by lysis of the basal epidermal cells and the presence of tonofilament clumping within the lower epidermis was confirmed by light (Fig. 1) and electron microscopy (Fig. 2). Examination of frozen skin by a direct immunofluorescence technique to detect in vivo bound immunoglobulin and complement was negative in 3 patients and demonstrated weak granular staining of the basement membrane zone with anti-IgM in the remaining 2.

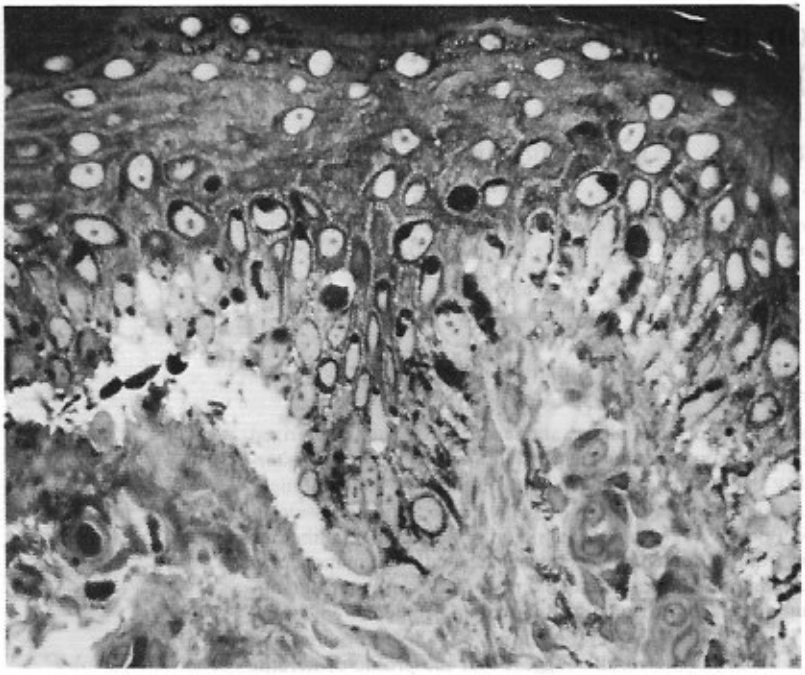

Fig. 1. Light micrograph of a 1 μm thick resin-embedded section showing tonofilament clumping within cells of the basal layer and lower stratum spinosum of perilesional skin in Dowling–Meara epidermolysis bullosa simplex (Huber stain, original mag. ×90.)

Antibodies

A panel of monoclonal antibodies (Table I) was employed, distinguishing 3 major compartments in normal adult human skin: basal keratinocytes (LH6 and LH8), suprabasal keratinocytes (LHP1, LHP2 and LHP3) and simple epithelium (LP2K). Full characterization of these antibodies, however, remains incomplete. LH8 was made to keratin 14 (MW 50 K daltons), and the identical distribution in

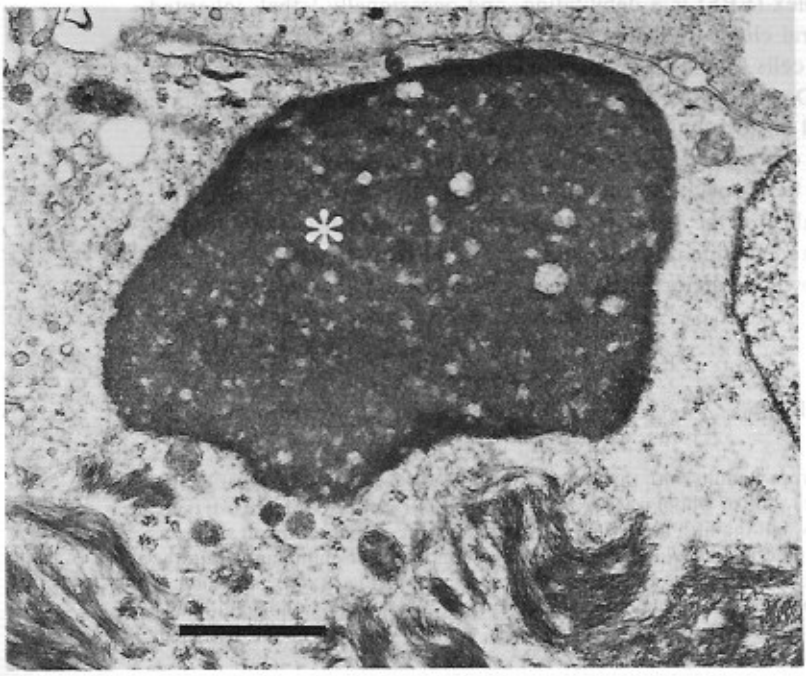

Fig. 2. Electron micrograph demonstrating an aggregate of tonofilaments (*) in close approximation to normal tonofibrils in Dowling–Meara epidermolysis bullosa simplex. Calibration bar = 1 μm.

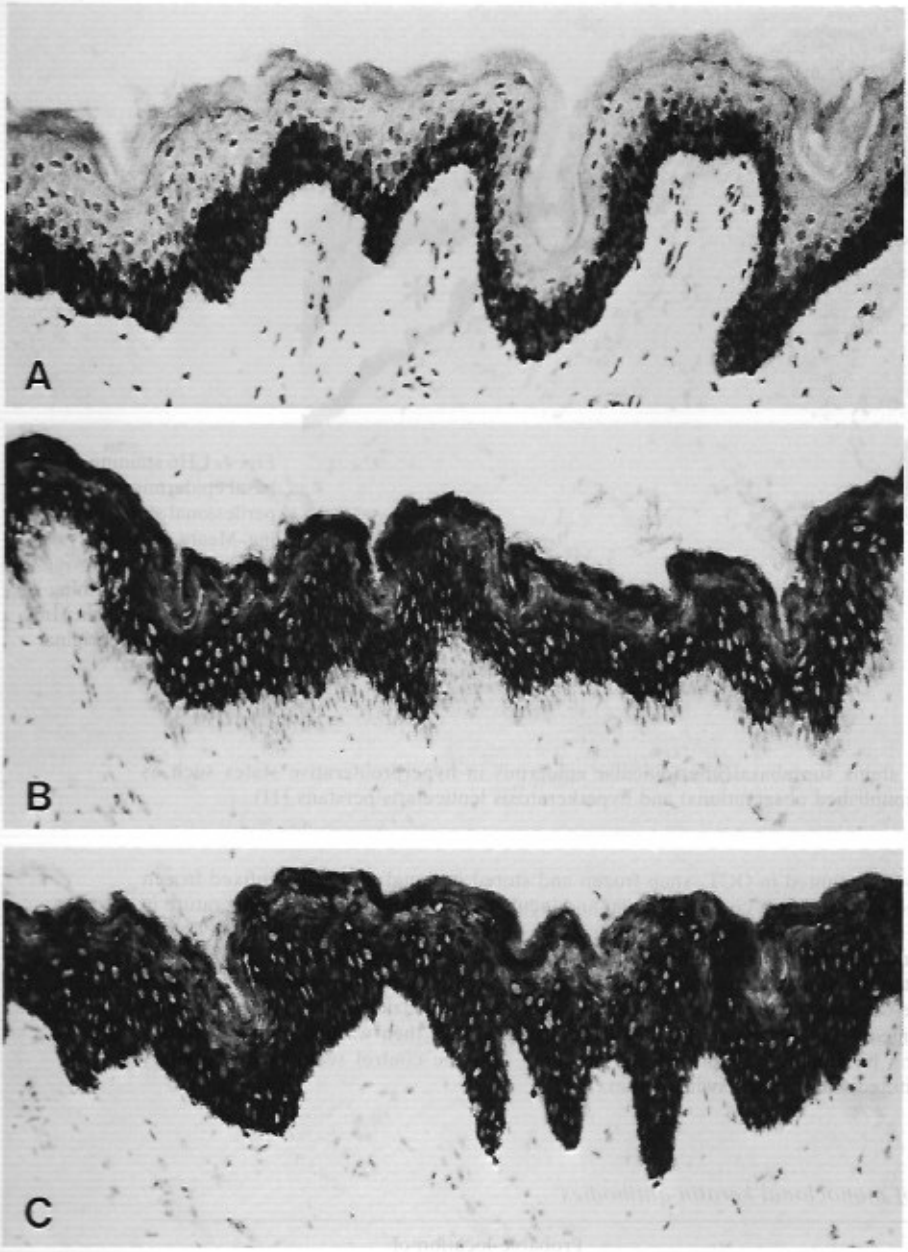

Fig. 3. Keratin antibodies in normal frozen skin. (A) LH6, localising to basal epidermis; (B) LHP3, localising to suprabasal epidermis; (C) LP34, staining entire epidermis. (Immunoperoxidase, original mag. ×35.)

the basal epidermis of LH6, made to cultured keratinocyte material, strongly suggests that it identifies keratin 14 or keratin 5 (MW 58K daltons) (Leigh, unpublished observation). LHP1, LHP2 and LHP3 identify keratin 10 (MW 56.5 K daltons), LHP1 in addition identifying keratin 1 (MW 65–67K daltons) (Leigh, unpublished observations), and LP2K is specific for keratin 19 (MW 40 K daltons) (9).

Two other antibodies were used in this study: LP34 which stains all human epithelial cells except those of the inner hair root sheath (10), and LMM3, which probably identifies keratin 16 (MW 48 K

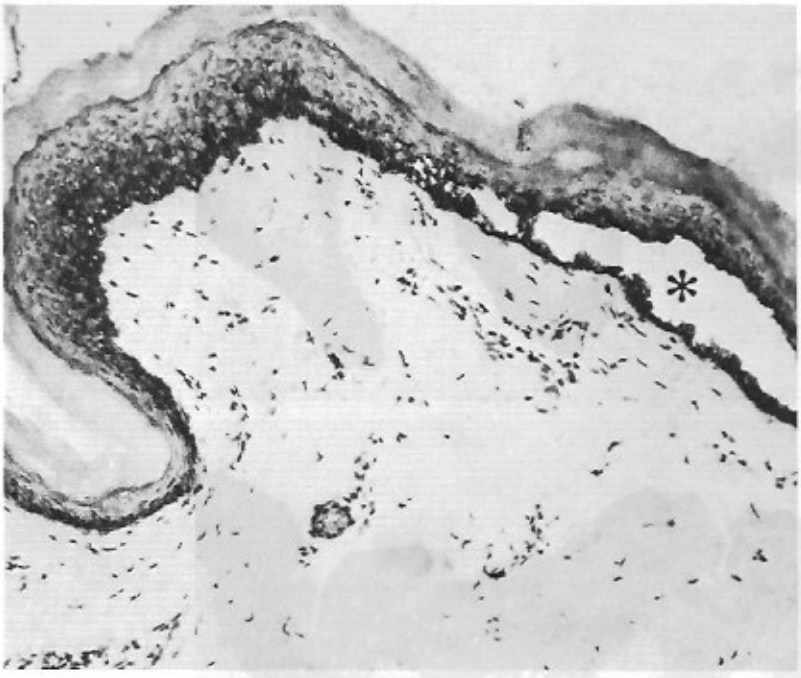

Fig. 4. LH6 staining of the basal epidermis in frozen perilesional skin from Dowling–Meara epidermolysis bullosa simplex, showing the blister cavity (*) forming within the basal layer. (Immunoperoxidase, original mag. ×35.)

daltons) and which stains suprabasal interfollicular epidermis in hyperproliferative states such as psoriasis (Leigh, unpublished observations) and hyperkeratosis lenticularis perstans (11).

Staining procedure

The skin samples were mounted in OCT, snap frozen and stored in liquid nitrogen. Unfixed frozen sections were thawed and dried in a cool air stream, and incubated for 60 min at room temperature in a moist atmosphere with the primary antibody, diluted with normal swine serum to a titre previously established as optimal on normal skin. After washing, the sections were incubated for 30 min with peroxidase-conjugated rabbit anti-mouse IgG (Dako), diluted 1:15 in normal swine serum, and, after further washing, the enzyme was localised by reaction with diaminobenzidine (Sigma), applied at a concentration of 1 mg/ml, for 10 min in the dark. The sections were then washed, lightly counterstained with Mayer's haemalum, dehydrated and mounted. For the control sections, the primary antibody was substituted with normal swine serum.

Table I. *Details of monoclonal keratin antibodies*

Antibody	Distribution of antigen	Probable location of antigenic determinant
LP34	Panepidermal	Most keratin subtypes
LH6	Basal epidermis	(Keratin 5 or 14)
LH8	Basal epidermis	Keratin 14
LHP1	Suprabasal epidermis	Keratins 1 and 10
LHP2	Suprabasal epidermis	Keratin 10
LHP3	Suprabasal epidermis	Keratin 10
LP2K	Simple epithelium	Keratin 19
LMM3	Suprabasal "hyperproliferative" epidermis	(Keratin 16)

RESULTS

Basal and suprabasal keratin expression was found to be normal in the interfollicular epidermis of all the subjects with Dowling–Meara EBS (Figs. 3 and 4). The basal compartment markers, LH6 and LH8, stained uniformly even in areas of incipient blister formation (Fig. 4), and there was no evidence of expansion of the basal layer. There was no observable tonofilament clumping with either the suprabasal markers (LHP1, LHP2 and LHP3) or the common keratin marker (LP34). Neither the simple epithelial marker (LP2K) nor the marker for hyperproliferative epidermal states (LMM3) localized to the interfollicular epidermis of Dowling–Meara EBS, although LP2K stained the eccrine apparatus normally and LMM3 stained the suprabasal epidermis in control psoriatic skin.

DISCUSSION

Keratins are considered to play a fundamental part in the differentiation process of epithelia (10), and monoclonal antibodies to antigenic determinants on tonofilaments can be considered as markers for the differentiation state of epithelial cells (12). Members of both the "keratin-pairs" (13) expressed by normal human epidermis were identified by antibodies used in this study. The normal staining pattern of interfollicular epidermis in Dowling–Meara EBS shows that at least parts of the respective keratin molecules are synthesised normally and at the correct point in the sequence of keratinocyte differentiation. The normality of staining with the 3 suprabasal antibodies, identifying different epitopes of keratin 10, provides stronger support for the normal synthesis of this particular keratin. Similarly, no cytoskeletal abnormality, based on the expression of determinants identified by AE-2 and AE-3 monoclonal antibodies to keratin, has been identified in the Weber-Cockayne and generalized forms of EBS (14).

These results are consistent, therefore, with the possibility that the aggregation of tonofilaments in basal and lower stratum spinosum cells in Dowling–Meara EBS is the result of a post-synthetic modification of keratin molecules. The confinement of tonofilament clumping to blistering skin also implies that this change is secondary to local factors. The lack of observable clumping of keratin filaments in the immunoperoxidase preparations probably reflects the relatively poor resolution of fine detail with this technique.

Blister formation in EBS may result from the action of a lytic enzyme, rather than from the presence of a defective structural protein (15). This line of evidence is also compatible with the tonofilament clumping being a secondary phenomenon, related perhaps to changes in the local physicochemical environment consequent to the release of lysosomal contents.

Whatever the cause, the effect of tonofilament aggregation on keratinocyte integrity is uncertain. However, it is of interest that antibody-induced disruption of the keratin filament network in vitro does not preclude many of the normal cellular functions (16).

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