Evaluation of Anchoring Fibrils and Other Components of the Dermal-Epidermal Junction in Dystrophic Epidermolysis Bullosa by a Quantitative Ultrastructural Technique*

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To examine the possibility that differences in the structure and population density of anchoring fibrils (AF) and other components of the dermal-epidermal junction might distinguish between genetically and clinically distinct varieties of dystrophic epidermolysis bullosa (DEB), a controlled ultrastructural morphometric study of nonseparated keratinocyte-associated dermal-epidermal junction was undertaken in a total of 17 patients with DEB. Seven patients had dominant DEB, 3 had localized recessive DEB, and 7 had severe, generalized recessive DEB. Nonlesional, unscarred skin was obtained from standard body regions. Criteria for the identification of AF were a mandatory union with the lamina densa and the presence of central banding and/or fanning of the extremities.

No AF were detected in 9 technically suitable samples from patients with severe recessive DEB. Structurally normal AF were present, but significantly reduced in number, in both dominant and localized recessive DEB, compared with site-matched samples from 12 healthy adults. There was no difference in AF characteristics between dominant and localized recessive DEB, or between sites of predilection and nonpredilection for blisters. The presence or absence of allopapuloid lesions in dominant DEB did not influence AF counts. There was no difference in numbers of hemidesmosomes, basal cell plasmalemmal vesicles, or dermal microfibril bundles in any group of DEB patients compared with controls.

Thus, although severe mutilating DEB can be distinguished by routine transmission electron microscopy, the dominant and localized recessive forms cannot be differentiated on the basis of AF structure or numbers.

Dystrophic epidermolysis bullosa (DEB) may be inherited autosomally in either a dominant or a recessive manner, each type having a wide spectrum of severity [1]. This distinction often proves difficult in sporadic cases because the clinical features of the mild localized form of recessive DEB (RDEB) may be indistinguishable from the dominant condition (DDEB) [2]. Severe RDEB, however, is a much more aggressive disease characterized by generalized fragility of the skin and widespread scarring of the skin and mucous membranes, associated with stunted growth, chronic anemia, esophageal strictures, dental decay, pseudosyndactyly, and flexion contractures [1]. This typical clinical picture allows ready distinction from the localized form.

In all variants of DEB, blisters arise immediately beneath the lamina densa of the dermal-epidermal junction (DEJ) in the most superficial region of the dermis [3], where anchoring fibrils (AF) are normally located. The biochemical nature and function of AF are still not known, but the strong union that normally exists between the lamina densa and AF [4], which are derived from epidermis and dermis respectively [5], suggests that AF serve as attachment devices. In DDEB the AF have been reported to be reduced in number [6] and abnormal in structure [7,8]. Furthermore, in the Pasini type of DDEB the reduction in AF numbers was found in both blister predilection and nonpredilection sites [7], whereas in the milder Cockayne-Touraine form, AF numbers were noted to be decreased only in predilection sites [8]. Some workers have found that AF were present, but reduced in number, in both localized and generalized forms of RDEB [9], whereas others have described a total absence of AF in severe generalized RDEB [10,11].

Quantitative data on the AF in DEB are limited, but a morphometric analysis of the components of normal DEJ has demonstrated that AF numbers vary widely among individuals, between body regions in the same individual, and even within a single skin sample, and has highlighted the potential pitfalls in evaluating AF numbers from isolated microscopic fields [12]. The aim of the present investigation was to use the same morphometric technique on skin from patients with DEB to quantify AF and other components of the DEJ, and to ascertain whether DDEB and RDEB can be distinguished using ultrastructural criteria. This would have obvious implications for both prognostication and genetic counseling, and may facilitate the prenatal diagnosis of DEB.

MATERIALS AND METHODS

Patients and Controls

Seventeen patients with DEB were studied, comprising 7 with DDEB, 3 with mild localized RDEB, and 7 with severe generalized RDEB. In each case the diagnosis was unequivocal and based on clinical and genetic features. Electron microscopy was used to establish that blistering occurred beneath the lamina densa. All the subjects with DDEB had affected family members in 2 or more generations, and each of the 3 patients with the mild localized form of RDEB had a similarly affected sibling in the absence of parental involvement. Five of the patients with severe generalized RDEB were sporadic cases and the other 2 were siblings (CH and SH).

The DDEB group included a father and daughter (DP and SG) and a grandmother and grandson (BB and KM). In 6 of the 7 patients the condition was localized, with blistering, scarring, and milia formation confined to bony prominences on the extremities, and with typical dystrophic changes of the nails. The seventh patient (KB) had a more...
widespread involvement with extensive scarring of his trunk and limbs, and active blistering continuing in these areas despite his advanced age. He occasionally suffered with blistering of the buccal mucous membranes and episodes of dysphagia had been associated with radiologic evidence of an esophageal web. However, the condition could be traced through 3 generations and there was no evidence of finger webbing. In this group the presence or absence of albo-papuloid lesions, which are thought to be a distinguishing feature of the Pasini variant of DDEB [1,13], was recorded (Table I). Albo-papuloid lesions were defined as hypopigmented macules or papules, with a wrinkled surface, usually located over the lower back. The absence of fresh, natural blisters in all 7 subjects with DDEB at the time of consultation and the inability to experimentally provoke blister formation by vigorous friction with a pencil eraser, as is often the case [14], prevented the ultrastructural confirmation of a sub-lamina densa separation, although clinically the diagnosis in each patient was beyond doubt. The 3 subjects with mild localized RDEB included 2 sisters (MD and BB). In both, skin lesions were confined to bony prominences on the extremities and consisted of old scarring as blisters had occurred only infrequently since childhood. Both had suffered episodes of dysphagia and mild iron-deficiency anemia, and an esophageal web had been confirmed radiologically in one. The third subject (IH) had extremely mild disease affecting knees and ankles without nail dystrophy or gastrointestinal involvement. The 7 patients with severe generalized RDEB all manifested the mutilating changes that typify this condition [1,2], with, in particular, extensive cutaneous and oral mucous membrane scarring, pseudosyndactyly, and flexion contractures of the digits.

The control group consisted of 7 males and 5 females, aged 20–60 years, who were the subjects in a detailed morphometric study of normal DEJ [12].

**Tissue Samples and Processing**

Nonlesional and clinically unscarred skin, with no history of having previously blistered, was obtained, after infiltration of the surrounding area with local anesthetic, from the inner aspects of the lower leg (just above the ankle) and thigh. These 2 sites represent, respectively, areas of predilection and nonpredilection for blister formation in the milder forms of DEB, and in normal skin AF counts in these 2 regions do not differ [12]. In severe RDEB there is a generalized blistering tendency, and the skin is so fragile that the trauma of the biopsy may be sufficient to cause dermal-epidermal separation. Samples were processed in a standard fashion for transmission electron microscopy as previously described [12]. Briefly, tissue was fixed in 2% formaldehyde and 2.5% glutaraldehyde in 0.4 M (final concentration) cacodylate buffer containing 0.05 M calcium chloride and 5% sucrose (pH 7.4), and then postfixed in 1.3% osmium tetroxide buffered with 0.067 M s-collidine (pH 7.4) on ice, before dehydration in ethanol and embedding in Epon 812. Ultrathin sections were cut from randomly selected blocks, stained with uranyl acetate and lead citrate, and viewed with a JEOL 100 CX electron microscope. Electron micrographs of overlapping fields of randomly selected segments of interappendageal keratinocyte-associated DEJ were taken, printed, and assembled into montages with a final magnification of between 35,000× and 43,000×, accurately determined by means of a calibration grating.

**Morphometry**

A M.O.P. semiautomatic image analysis system (Reichert-Jung, Vienna, Austria) was used to measure 40-μm lengths of lamina densa and basal plasma membrane, excluding DEJ associated with melanocytes. The numerical densities of AF and dermal microfibril bundles, with respect to lamina densa, and of hemidesmosomes and plasmalemmal vesicles, with respect to basal plasma membrane, were determined, using the criteria previously defined [12], by the same observer. An AF was counted only if it united with the lamina densa and demonstrated irregular banding of the central portion or fanning of its extremities or both these characteristics, thus excluding wisplike structures normally present beneath the lamina densa, and avoiding confusion with other fibrillar structures such as elastic microfibrils and collagen fibrils.

**RESULTS**

The clinical details and AF counts for the DDEB group are recorded in Table I. The presence of albo-papuloid lesions did not appear to bear a direct relationship to the clinical severity of the condition, and it is of interest that 1 of the subjects (IH) with the mild localized form of RDEB also had albo-papuloid lesions over the lumbar region, as did his 7-year-old brother. The possession of albo-papuloid lesions does, however, seem to be a familial trait.

In subjects with DDEB, compared with site-matched samples from healthy adults using an unpaired t-test, AF were significantly reduced in number in both the lower leg (p < 0.001) and thigh (p < 0.001), with no difference apparent between these 2 sites (p > 0.5). Compared with normal skin (Fig 1), the AF appeared morphologically normal (Fig 2) with no evidence

![Fig 1. The normal dermal-epidermal junction, with the anchoring fibrils arrowed. Calibration bar = 0.25 μm.](image-url)

**Table 1. Dominant dystrophic epidermolysis bullosa: patient details and anchoring fibril counts**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age/sex (yrs)</th>
<th>Clinical severity</th>
<th>Albo-papuloid lesions</th>
<th>Anchoring fibril count*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower leg</td>
</tr>
<tr>
<td>Controls (n = 12)</td>
<td>20-60/7M:5F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>50/M</td>
<td>Localized</td>
<td>Absent</td>
<td>28</td>
</tr>
<tr>
<td>SG</td>
<td>28/F</td>
<td>Localized</td>
<td>Absent</td>
<td>12</td>
</tr>
<tr>
<td>BB</td>
<td>73/F</td>
<td>Localized</td>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>KM</td>
<td>25/M</td>
<td>Localized</td>
<td>Present</td>
<td>0</td>
</tr>
<tr>
<td>MS</td>
<td>39/F</td>
<td>Localized</td>
<td>Present</td>
<td>25</td>
</tr>
<tr>
<td>PE</td>
<td>29/M</td>
<td>Localized</td>
<td>Not known*</td>
<td>2</td>
</tr>
<tr>
<td>KB</td>
<td>60/M</td>
<td>Generalized</td>
<td>Absent</td>
<td>0</td>
</tr>
</tbody>
</table>

* Number of anchoring fibrils per 40-μm lamina densa.

b Corrected to allow for individual differences.

c Not recorded at initial consultation and patient died shortly afterwards from a cerebrovascular accident.

d Tissue sample technically unsatisfactory.
elsewhere of damaged or rudimentary structures. Comparing patients with and without albopapuloid lesions, AF counts in thigh skin were not significantly different \( (p > 0.5) \). One patient (DP) had AF numbers in both the lower leg and thigh which fell within the lower part of the normal range, although his daughter (SG) had fewer. The 2 subjects (BB and KB) for whom no AF were recorded, both possessed sparse numbers of morphologically normal AF, which, by chance, were not located within the measured lengths of DEJ.

The AF data from the 3 patients with mild localized RDEB showed a similar pattern (Table II). All 3 subjects possessed AF with a normal morphology (Fig 3), but their numbers were significantly reduced in the thigh \( (p < 0.001) \) and also reduced in the lower leg, although the data from the latter site were too few to enable a proper statistical comparison. No difference was apparent in the AF counts in the thigh skin of patients with mild RDEB and DDEB \( (p > 0.5) \). One subject with localized RDEB (MD) had AF counts in both sites which fell within the lower part of the normal range, and although no AF were recorded in the thigh of her sister (BB), scanty AF were present outside the measured lengths of DEJ.

In 5 of the 7 subjects with the severe mutilating form of RDEB, the trauma of the biopsy procedure caused dermal-epidermal separation throughout skin samples taken from the lower leg (Table II). In the 9 remaining specimens, no AF were seen (Fig 4) along lengths representing up to 130 \( \mu m \) of intact DEJ. There was no evidence of structures that could be interpreted as damaged or abnormal AF.

The numerical data on the other major components of the DEJ are recorded in Table III. No significant differences from

**TABLE II. Recessive dystrophic epidermolysis bullosa: patient details and anchoring fibril counts**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age/sex (yrs)</th>
<th>Clinical severity</th>
<th>Sublamin a densa split</th>
<th>Anchoring fibril counta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower leg</td>
<td>Thigh</td>
</tr>
<tr>
<td>Controls (n = 12)</td>
<td>20-60/7M:5F</td>
<td></td>
<td>63.5 ± 12.5 (SEM) (range 16-100)</td>
<td>61.9 ± 12.4 (SEM) (range 19-120)</td>
</tr>
<tr>
<td>MD</td>
<td>57/F</td>
<td>Localized</td>
<td>Present</td>
<td>19</td>
</tr>
<tr>
<td>BB</td>
<td>61/F</td>
<td>Localized</td>
<td>Present</td>
<td>11</td>
</tr>
<tr>
<td>IH</td>
<td>12/M</td>
<td>Localized</td>
<td>Not done</td>
<td>NDb</td>
</tr>
<tr>
<td>CH</td>
<td>24/F</td>
<td>Generalized</td>
<td>Present</td>
<td>0c</td>
</tr>
<tr>
<td>SH</td>
<td>22/M</td>
<td>Generalized</td>
<td>Present</td>
<td>NDd</td>
</tr>
<tr>
<td>SO</td>
<td>15/M</td>
<td>Generalized</td>
<td>Not done</td>
<td>ND*</td>
</tr>
<tr>
<td>VH</td>
<td>3/M</td>
<td>Generalized</td>
<td>Present</td>
<td>ND*</td>
</tr>
<tr>
<td>ES</td>
<td>12/F</td>
<td>Generalized</td>
<td>Not done</td>
<td>ND*</td>
</tr>
<tr>
<td>JB</td>
<td>3/M</td>
<td>Generalized</td>
<td>Present</td>
<td>ND*</td>
</tr>
<tr>
<td>SB</td>
<td>32/M</td>
<td>Generalized</td>
<td>Not done</td>
<td>ND*</td>
</tr>
</tbody>
</table>

a Number of anchoring fibrils per 40-\( \mu m \) lamina densa.

b Not done because tissue sample technically unsatisfactory.

c Only 30 \( \mu m \) of unseparated skin available for evaluation.

d Only 24 \( \mu m \) of unseparated skin available for evaluation.

* Not done because dermal-epidermal separation throughout the biopsy specimen prevented evaluation.
This technique cannot distinguish between the dominant and localized recessive forms of DEB.

control values were present for hemidesmosome, plasmalemmal vesicle, and dermal microfibril bundle counts in any of the 3 variants of DEB.

**DISCUSSION**

The present study showed a total absence of AF in the severe generalized form of RDEB. This confirms previous reports based on observations using routine transmission electron microscopy [10] and a monoclonal antibody to AF [11], and suggests that the presence of AF in skin samples from cases causing diagnostic uncertainty excludes the possibility of severe mutilating RDEB. The loss of AF is unlikely to be a result of unrecognized scarring because scar tissue from healthy subjects contains normal AF (Tidman and Eady, unpublished data).

AF were present, but in reduced numbers, in all subjects with DDEB and localized RDEB, irrespective of clinical severity, although they were often very scanty, and examination of long lengths of DEJ was at times necessary to find them. There was no difference in AF counts between DDEB and localized RDEB, and in both conditions the AF appeared morphologically normal, with no evidence of degradation. These results indicate that DDEB and the localized form of RDEB cannot be distinguished by their ultrastructural features.

The distinction between the Cockayne-Touraine and Pasini variants of DDEB depends largely on the presence, in the latter, of albo-papuloid lesions [11], the etiology and significance of which are not known. The clinical features of these 2 variants are otherwise similar, despite the emphasis placed on the hyperplastic or atrophic nature of the scar tissue [2]. The abnormality of glycosaminoglycan metabolism in the Pasini variant [15], however, supports the existence of heterogeneity within the DDEB group, although the present study did not show ultrastructural differences between DDEB patients with and without albo-papuloid lesions.

In an earlier study of the Cockayne-Touraine variant of DDEB [8] up to 10 times as many AF were found compared with the numbers obtained in the present investigation. This discrepancy is probably due to the use of different criteria for counting AF, and emphasizes the difficulty in identifying AF unequivocally by their morphologic characteristics.

Thus, although all forms of DEB are associated with a numerical abnormality of AF, whether this is the primary defect is uncertain. It remains to be determined whether the increased production of collagenase by fibroblasts [16,17] is the fundamental abnormality in RDEB.

In conclusion, the examination by routine transmission electron microscopy of long lengths of DEJ, sectioned perpendicular to the skin surface, is of limited diagnostic and therefore prognostic value in dystrophic forms of epidermolysis bullosa. This technique cannot distinguish between the dominant and localized recessive forms of DEB.

We thank Mr. J. E. E. Brassey and Mr. D. B. Gunner for technical assistance, Mrs. L. J. Barducci for typing the manuscript, and our clinical colleagues, especially Dr. R. S. Wells, for allowing us to study their patients.

**REFERENCES**