REPORTS

EPIDERMOLYSIS BULLOSA DYSTROPHICA–RECESSIVE: A POSSIBLE ROLE OF ANCHORING FIBRILS IN THE PATHOGENESIS

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The purpose of this study was to define the ultrastructural defects and pathogenesis of epidermolysis bullosa dystrophica–recessive (EBD–R). The only consistent ultrastructural alteration found in EBD–R was an absence of anchoring fibrils. In many specimens of nonblistered, nontraumatized EBD–R skin, absence of anchoring fibrils was the only ultrastructural abnormality observed. The possibility that lack of anchoring fibrils was a secondary change resulting from previous blistering and scarring was eliminated by our observation that anchoring fibrils were consistently absent in the never previously blistered skin of two newborns with EBD–R. In experimentally traumatized skin, the epidermis and dermis separated in the region of the epidermal–dermal junction normally occupied by anchoring fibrils. Basal lamina and dermal microfibril bundles appeared to be normal. Using recombinant grafts, we demonstrated that anchoring fibrils were not formed by EBD–R dermis when combined with EBD–R epidermis or normal epidermis. Anchoring fibrils were formed when normal dermis was combined with normal and EBD–R epidermis. These studies indicate that the defect in EBD–R resides in the dermis and that the defect may be associated with impaired formation of anchoring fibrils.

Epidermolysis bullosa dystrophica–recessive, termed epidermolyse bulleuse polydysplasique by Touraine [1], congenital, generalized, sublethal epidermolysis bullosa dystrophica by Gedde-Dahl [2] and dermolytic bullous dermatosis–recessive by Pearson [3], is an autosomal recessive disease which begins at birth or soon thereafter and pursues an unrelenting course throughout life, occasionally terminating fatally at an early age. The clinical defect is a failure of epidermal–dermal adherence resulting in blisters and subsequent erosions which involve the entire skin surface with a predilection for sites of frequent trauma, particularly the neck, shoulders, elbows, knees, buttocks, hands, and feet. Dystrophic scarring, a hallmark of the disease, is most severe in sites of recurrent blistering and leads to a variety of functional impairments including mitten-like epidermal encasements of the hands and feet, disfigurement, and joint contractures. Hair and nails are commonly lost. Mucous membranes of the oral cavity,

pharynx, esophagus, and anal canal are involved. In these sites, scarring leads to mouth and tongue deformities and frequent esophageal stenosis. Impaired growth, anemia, frequent cutaneous infection, and dental abnormalities are common.

The purpose of this paper is to define the pathologic defect in epidermolysis bullosa dystrophica–recessive (EBD-R) and to consider its pathogenesis. Pearson [4] found a consistent ultrastructural separation in the dermis just beneath the basal lamina (basement membrane). In addition, he pointed out a marked reduction of fine fibers just beneath the basal lamina (subsequently identified as anchoring fibrils) and varying degrees of collagen degradation. Subsequent reports on the ultrastructural abnormality in EBD-R have presented a more confused picture. Vogel and Schnyder [5,6] observed separation beneath the basal lamina in some patients and in the intermembranous space in others. Kobayasi [7] found blisters located in the intermembranous space in one patient with recessive epidermolysis bullosa. From the clinical descriptions in these patients, it is difficult to obtain a clear view of the diseases being studied. It is possible that several types of epidermolysis bullosa were lumped together as EBD-R. It is also possible that EBD-R is actually a group of different diseases which have a similar clinical appearance.

In the present study, we shall present evidence that the most consistent and often the only ultrastructural defect noted in EBD-R is an absence of

anchoring fibrils. Using a system in which anchoring fibrils normally re-form in recombinant grafts grown for a period of time on the chorioallantoic membrane of embryonated chicken eggs [8], we shall present evidence that formation of these structures may be impaired in EBD–R and that the defect resides in the EBD–R dermis.

MATERIALS AND METHODS

Patients

The patients comprising this study constitute a clinically homogenous group which conforms to the clinical features of EBD–R described above. All patients have a generalized bullous disorder resulting in severe dystrophic scarring. Familial cases consistent with an autosomal recessive mode of inheritance make up the bulk of the patient group.

Familial cases. Family B is of Turkish extraction with 2 of 5 siblings involved. No other family members are affected. The parents are second cousins. Patient B1 is an 18-year-old male of small stature who has mitten-like enclosures of the hands and feet and severe esophageal stenosis. Patient B2 is a 7-year-old male who has similar but milder involvement.

Family S is a Negro family in which 2 of 5 siblings are involved. No other family members are affected. Patient S1 is a 13-year-old male of small stature with severe mitten-like enclosures of the hands and feet, flexure contractures of the elbows and knees, and esophageal stenosis. Patient S2 is an 11-year-old female of small stature manifesting severe scarring about the face, scarring alopecia of the scalp, scarring of the conjunctivae with ectropion of the lower lids, and esophageal stenosis.

Family H is a Caucasian family in which 3 of 4 siblings are involved. Other members of the family are normal. Patient H1 is a 21-year-old male with very severe dystrophic scarring on his arms, legs, and face. Patient H2 is a 20-year-old female with esophageal stenosis and severe mitten-like enclosures of both hands and feet. Patient H3 is a 19-year-old male who has similar although slightly milder involvement than his siblings. He also has esophageal stenosis.

Sporadic cases. Patient JC is a 20-year-old Caucasian male with severe dystrophic scarring of the extremities, mitten-like enclosures of the hands and feet, scarring of the conjunctivae with ectropion of the lower lids, severe flexural contractures of the elbows and knees, and esophageal stenosis.

Patient IH was a 1-month-old Caucasian female with extensive blisters on the arms, legs, back, chest, and groin. Oral blisters and erosions were also present. She was observed from birth. Although extensive areas of blisters and erosions were noted, the patient also had many areas which were never involved with blisters and erosions. This patient died out of the hospital at 6 months of age of unknown causes. No autopsy was obtained.

Patient EO was a newborn Caucasian male followed since birth. An extensive area of erosion was noted at birth on the right leg. Subsequently, blisters and erosions have occurred in the oral cavity, face, arms, chest, left leg, and abdomen where subjected to minimal trauma. Large areas of normal-appearing skin which had not been previously blistered were also present. Parents are first cousins.

Source of Skin for Ultrastructural Studies in EBD–R Patients

Nontraumatized, nonblistered epidermolysis bullosa skin. An area of skin, usually on the abdomen or thigh, was chosen for biopsy. These were away from any existing blisters or obvious scarring, although it should be noted that the skin of older patients had a crinkled, atrophic appearance even in these areas. In two cases (patients IH and EO), biopsies were obtained soon after birth from completely normal-appearing skin which had never been blistered previously. Biopsies were done under local lidocaine anesthesia using a scalpel to obtain a thin split-thickness skin specimen. Special care was taken to avoid traumatizing the biopsy sites. Serial biopsies were done over a 3-year period in patient B1 and a 2-year period in patients S1, S2, H1, H2, and H3. Biopsies were also obtained from multiple sites, including thigh, abdomen, back, forearm, and upper arm to determine whether pathologic differences could be found in different anatomic sites.

Minimal frictional trauma. An area of skin free of obvious blisters and scarring was subjected to minimal trauma insufficient to produce an overt blister or epidermal–dermal separation. Five to 10 strokes with an ordinary pencil eraser with light application of pressure were used, after which the area was biopsied as previously described.

Mechanically separated epidermis and dermis. Portions of split-thickness skin specimens obtained as described above were placed in tissue culture medium (Medium 199 with 10% fetal calf serum) and immediately transported to the laboratory (lapsed time less than 5 min). Under a dissecting microscope, epidermis and dermis were easily separated using jeweler's forceps and dissecting needles to tease the components apart. These specimens will be referred to hereafter as "mechanically separated" epidermis and dermis.

Recombination Studies Using Normal and Abnormal Epidermis and Dermis

Preparation of skin components. An area of skin free of obvious blisters or scarring was chosen on patients with EBD–R. The site was prepared with tincture of iodine and alcohol and then anesthetized with lidocaine containing epinephrine. A split-thickness sheet of skin measuring approximately 1.5 by 3–6 cm was removed using a sterile Castroviejo dermatome set to cut at 0.4 mm. The graft sites were dressed with sterile petrolatum-impregnated gauze and wrapped with flexible gauze dressing. In our patients, healing was rapid and comparable to normal control subjects. No instance of secondary infection or other complication at the graft sites was noted. The split-thickness sheet of skin was placed in Medium 199 with 10% fetal calf serum added and transported to the laboratory. Isolated epidermis and dermis were prepared as described above by mechanically stripping the epidermis and dermis apart. Separated sheets of epidermis and dermis were cut into pieces approximately 8 mm in diameter for grafting.

Split-thickness sheets of skin were obtained from the thigh or buttock of normal healthy subjects in a manner identical to that described above. The skin was placed in 0.4% trypsin solution (Difco 1:250) at 4°C. for approximately 1 hr after which the skin was transferred to cold Medium 199 with 30% fetal calf serum to inactivate the enzyme, and the epidermis and dermis were separated.

Preparation and cultivation of recombinant grafts. Recombinant grafts were constructed as indicated in Figure 1 using all possible recombinations of normal and abnormal epidermis and dermis as follows: (1) normal epidermis with normal dermis, (2) EBD–R epidermis with EBD–R dermis, (3) EBD–R epidermis with normal dermis, and (4) normal epidermis with EBD–R dermis. The dermal component of each recombinant was inverted
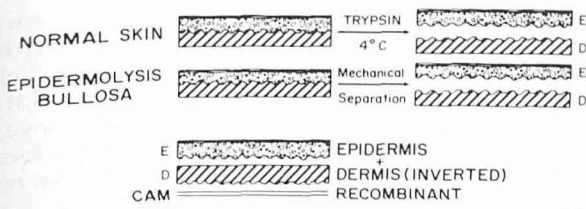
FIG. 1. Diagram showing procedures of epidermal–dermal separation and preparation of recombinants.

·from its normal position in order to present a freshly cut dermal surface at the new epidermal–dermal junction. Inversion of the dermis eliminated the possibility that dermal structures present at the former epidermal–dermal junction could be carried over into the new epidermal-dermal interface, thereby complicating interpretation of formation of new structures. The various recombinants were then grafted to the chorioallantoic membrane (CAM) of embryonated chicken eggs as described previously [8,9]. After periods of cultivation on the CAM, the recombinants were harvested and examined by light and electron microscopy. A total of 119 recombinants were done using skin components from 5 subjects with EBD–R (patients B1, S1, S2, H2, and H3). Of these, 14 were recombinants of EBD–R epidermis and EBD–R dermis. Forty-six were recombinants of EBD–R epidermis and normal dermis, 69 were recombinants of normal epidermis and EBD–R dermis, and 30 were control recombinants composed of normal epidermis and dermis. Only the recombinant grafts which were judged by light microscopic criteria [9] to be well maintained after periods of growth on the CAM were included in the study. This group of 88 recombinant grafts constituted the material for the recombination position of the study. Most recombinants were examined after 8 or 9 days' growth on the CAM. In three experiments, grafts were harvested after 3, 5, 7, and 9 days' cultivation on the CAM in order to study the sequence of formation of structures at the epidermal–dermal interface.

Electron Microscopy

Specimens for electron microscopic examination were either fixed directly in 1% phosphate-buffered osmium or prefixed in 4% phosphate-buffered paraformaldehyde and postfixed in osmium. All specimens were embedded in Epon, cut with a diamond knife, and examined in a JEM T-7 electron microscope.

RESULTS

Epidermal–Dermal Junction in Normal Human Skin

The epidermal–dermal junction in normal adult human skin before any manipulations or cultivation is shown in Figure 2 [10]. The plasma membrane of an epidermal basal cell forms the more superficial portion of the junction. Along the plasma membrane are seen focal electron-dense thickenings termed hemidesmosomes into which tonofilaments of the basal cell converge. On the dermal side of the plasma membrane is the electron-lucent intermembranous space separating the plasma membrane from the basal lamina. Fine filaments can be seen traversing the intermembranous space, particularly in the area beneath hemidesmosomes. These filaments have been called anchoring filaments [11,12]. The basal lamina is a continuous electron-opaque layer approximately 30 to 35 nanometers (nm) in thickness. In the dermis subjacent to the basal lamina, three different fibrillar components are seen: (1) collagen fibers, (2) anchoring fibrils (special fibrils of the dermis) and (3) dermal microfibril bundles (Fig. 2). Anchoring fibrils have a characteristic morphol-
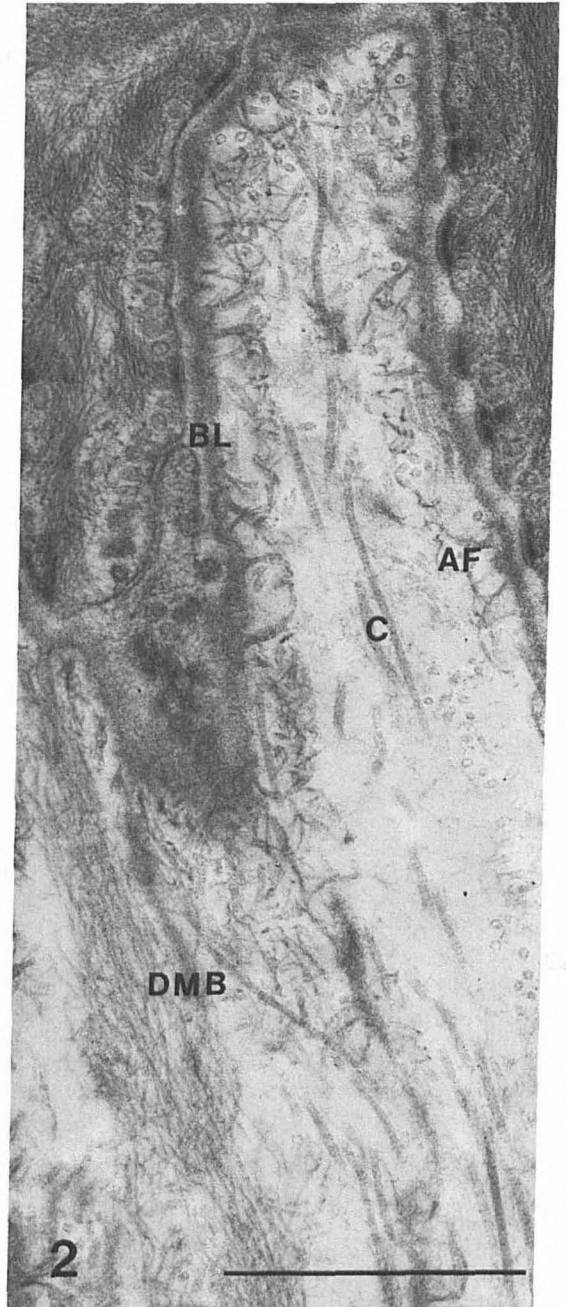

FIG. 2. Epidermal–dermal junction of normal human skin. Anchoring fibrils (*AF*) are seen beneath the basal lamina (*BL*). A dermal microfibril bundle (*DMB*) is seen extending from the basal lamina deep into the dermis. Collagen fibers (*C*) (× 39,000). Calibration bar 1.0 micrometer.

ogy consisting of an asymmetric, transverse-banded central area with filamentous or branched portions at either end extending superficially to the basal lamina and deep into the dermis [13,14].

Aggregates or parallel-arranged bundles of fine fibrils measuring approximately 10 nm in diameter (microfibrils) are attached at one end directly to the undersurface of the basal lamina. These bundles may course relatively long distances into the deeper dermis. These fibrils are similar to the microfibrils found at the periphery of elastic fibers [15]. Kobayasi [16] and Rodrigo and Pereira [17] have suggested that they are part of the elastic tissue system of skin. There is no accepted term for them. In this paper the term, dermal microfibril bundles, will be used.

Studies on the Ultrastructural Pathologic Defect in EBD–R

Nontraumatized, nonblistered EBD–R skin. The epidermis and dermis were attached in most specimens of skin from nontraumatized, nonblistered EBD–R skin (Fig. 3). The most striking and consistent abnormality noted was an absence of anchoring fibrils. Rarely an ill-defined fibril was seen in the usual position of anchoring fibrils subjacent to the basal lamina. These lacked the characteristic central cross-banding and peripheral fanlike fibrillar array. We have never seen normal anchoring fibrils in the skin of patients with EBD–R. The basal lamina and other structures comprising the epidermal–dermal junction superficial to the basal lamina appeared to be normal. Basal lamina was well preserved as an essentially continuous electron-dense lamina of fairly uniform thickness (Fig. 4). In the area of hemidesmosomes, anchoring filaments were seen traversing the electron-lucent intermembranous space. Dermal connective tissue fibers were generally intact. Collagen fibers of normal diameter and characteristic periodicity on transverse section were regularly seen in this material. Since most of the collagen fibers were cut on oblique section, the possibility that some of the fibers were abnormal could not be eliminated. However, significant areas of collagenolysis were not seen in specimens of nonblistered, nontraumatized skin. Dermal microfibril bundles were seen extending from the basal lamina deep into the dermis. Small aggregates and bundles of microfibrils and occasional individual microfibrils were noted beneath the basal lamina (Fig. 3).

In some specimens, areas of incipient epidermal–dermal separation were evident, probably resulting from unavoidable trauma sustained during the biopsy procedure (Fig. 5). Separation always occurred in the plane just below the basal lamina in the position normally occupied by anchoring fibrils. Frequently, a variable amount of dermal material remained attached to the under surface of the basal lamina, but other times, separation left the basal lamina essentially bare.

No differences were noted in biopsies of nontraumatized, nonblistered skin from patients with EBD–R who were biopsied serially over a period of 3 years (B1) and 2 years (S1, S2, H1, H2, and H3). All biopsies showed the features described above, indicating that the nature of the pathologic defect does not vary with time in a manner which can be demonstrated on ultrastructural examination.

Skin from a variety of different anatomic regions was examined to determine whether differences could be found in the pathologic defect in different regions. All regions which we examined (thigh, abdomen, back, forearm, upper arm) showed similar ultrastructural alterations conforming to the description above.

Previously unblistered EBD–R skin from newborns. Specimens of skin from 2 newborn infants with EBD–R were examined. Areas of skin which had never been blistered previously were examined to look for primary pathologic alterations. This would eliminate possible secondary changes which might have resulted from previous blistering and subsequent healing and scarring. Examination of the skin showed a picture identical to that described above. Anchoring fibrils were absent subjacent to the basal lamina. Occasionally incipient epidermal–dermal separation was seen in a plane just below the basal lamina.

Experimentally induced blistering and epidermal–dermal separation. Minimal frictional trauma: Specimens of skin purposefully exposed to mild frictional trauma insufficient to produce clinically obvious blistering in vivo commonly showed areas of frank and incipient epidermal-dermal separation. As indicated previously, separation occurs in a plane immediately subjacent to the basal lamina. Figure 6 shows separation of the epidermis and dermis with the basal lamina and attached dermal material forming the roof of the blister cavity, and intact collagen fibers, microfibril bundles, and other dermal elements forming the base.

Mechanically separated epidermis and dermis: Skin specimens removed from patients with EBD–R were separated with ease into isolated epidermal and dermal components. All patients demonstrated this finding using skin from a variety of different skin regions. Patients biopsied serially over a period of time up to 3 years persistently showed this defect. Although 5 to 10 min usually elapsed from actual biopsy to separation, this period was not required since several specimens were separated immediately after removal from the patient. One can draw an analogy between this in vitro separation and the in vivo Nikolsky sign. We believe that these observations are of importance since they demonstrate that the pathologic abnormality in EBD–R, the failure of the epidermis and dermis to adhere properly to one another, is present in apparently normal skin of these patients. We will stress this point again later. Moreover, the abnormality occurs in all skin re-
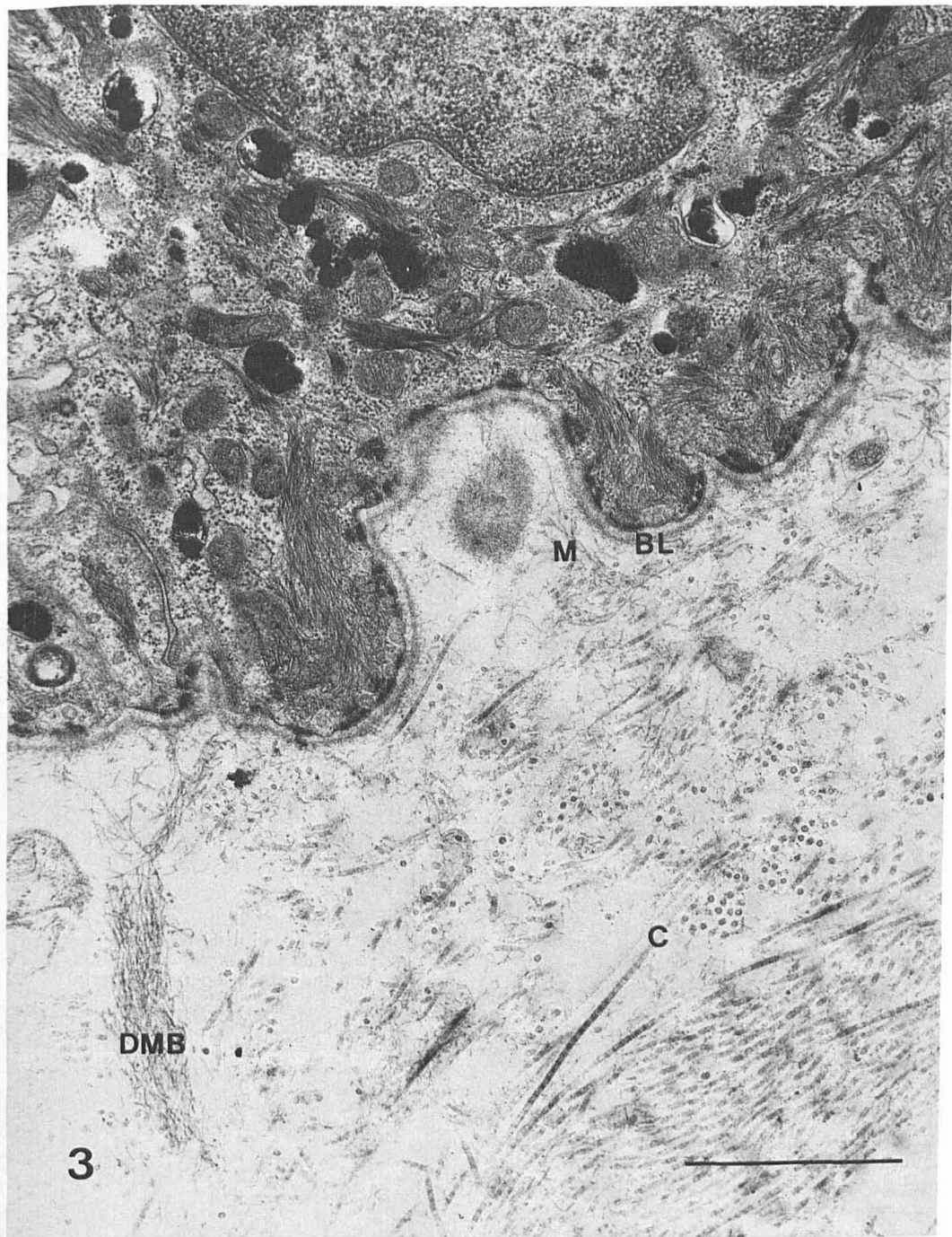
FIG. 3. Epidermolysis bullosa dystrophica. Nonblistered, nontraumatized area. Basal lamina (*BL*) is intact. Anchoring fibrils are absent. Dermal microfibril bundle (*DMB*) and individual microfibrils (*M*) are seen beneath the basal lamina. Collagen fibers (*C*) on longitudinal and cross-section appear normal (× 31,500). Calibration bar 1.0 micrometer.

gions and is persistent without significant variations over long periods of time.

Ultrastructural examination of mechanically separated epidermis and dermis offers the opportunity to determine the level at which the separation occurred. In all specimens, separation occurred consistently in a plane just under the basal lamina. As observed in Figure 7, the undersurface of mechanically separated epidermis consists of basal lamina, sometimes bare or sometimes with a thin layer of attached dermal material. The composition of dermal material could not be clearly de-
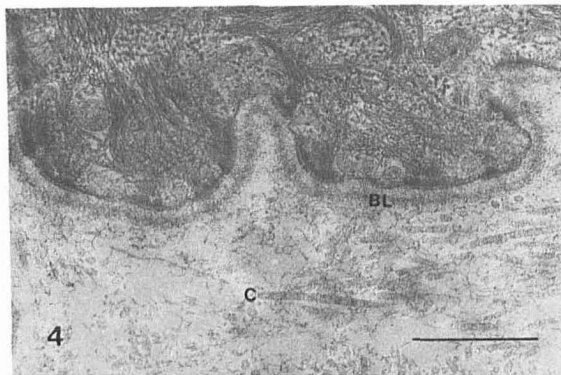
FIG. 4. Epidermolysis bullosa dystrophica. Intact epidermal–dermal junction from a nonblistered, nontraumatized area. Anchoring fibrils are absent from their normal location beneath the basal lamina (*BL*). Collagen fibers (*C*) are present (× 32,000). Calibration bar 0.5 micrometer.
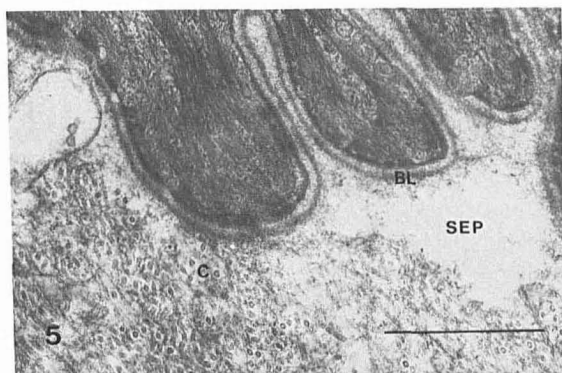

FIG. 5. Epidermolysis bullosa dystrophica. Incipient epidermal–dermal separation (*SEP*) has occurred in the plane beneath the basal lamina (*BL*) normally occupied by anchoring fibrils. Collagen fibers (*C*) (× 20,000). Calibration bar 1.0 micrometer.

fined. All structures superficial to the basal lamina appeared normal.

Recombination Studies Using Normal and EBD–R Epidermis and Dermis

Recombinants composed of normal epidermis and dermis. Basal lamina and anchoring fibrils re-formed in recombinants of normal viable epidermis and dermis as previously described [8]. Basal lamina was seen first at about day 3 of cultivation. It appeared first under hemidesmosomes, and extended from this site to form a continuous electron-dense lamina in many areas by days 7 to 9 of cultivation. Anchoring fibrils were first noted underlying basal lamina, particularly under or near hemidesmosomes at 5 to 7 days. In some areas of the recombinants, anchoring fibrils increased in number during cultivation to approach the number found in normal skin.

Recombinants composed of abnormal epidermis and abnormal dermis. In these recombinants, epidermis from patients with epidermolysis bul-

losa were combined with dermis from the same patient. At the time of grafting, the epidermal–dermal interface of these recombinants consisted of the intact basal lamina (Fig. 8) apposed to the inverted surface of the dermis. Because basal lamina was present in these recombinants prior to cultivation, no comment on basal lamina formation can be made. Anchoring fibrils were not

FIG. 6. Epidermolysis bullosa dystrophica. Minimal frictional trauma. Complete separation of epidermis and dermis has occurred forming a blister cavity (*CAV*). Basal lamina and attached dermal material (*D*) form the roof of the cavity, and intact collagen fibers (*C*), dermal microfibril bundle (*DM*), and other dermal elements form the base (× 22,500). Calibration bar 1.0 micrometer.
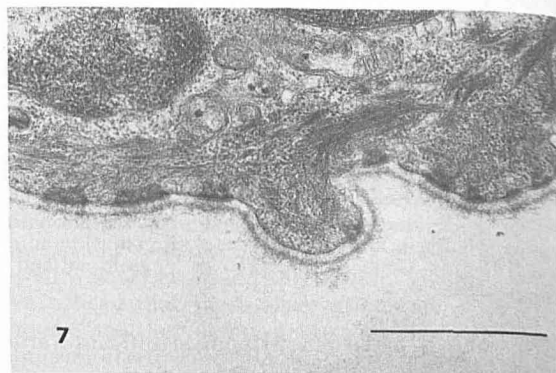

FIG. 7. Epidermolysis bullosa dystrophica. Mechanically separated epidermis. The undersurface consists of intact basal lamina. All structures superficial to the basal lamina appear normal (× 22,500). Calibration bar 1.0 micrometer.
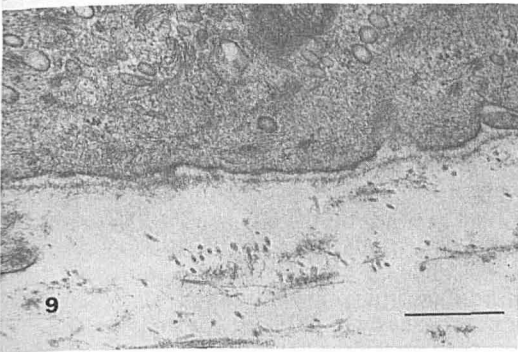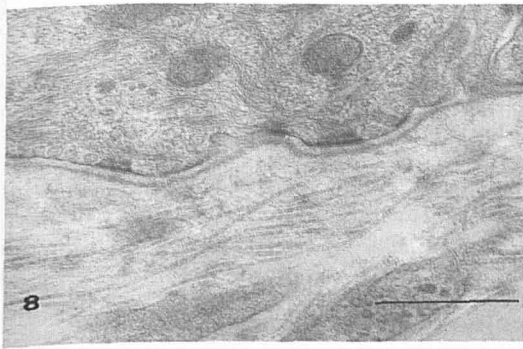
FIG. 8. Recombinant composed of epidermolysis bullosa epidermis and epidermolysis bullosa dermis. Anchoring fibrils are absent. Basal lamina and all more superficial structures of the junction appear normal (× 19,000). Calibration bar 1.0 micrometer.

FIG. 9. Recombinant composed of normal epidermis and epidermolysis bullosa dermis. Basal lamina re-formed during cultivation. Anchoring fibrils are absent from their normal position beneath the basal lamina (× 25,000). Calibration bar 0.5 micrometer.

FIG. 10. Recombinant composed of epidermolysis bullosa epidermis and normal dermis. Anchoring fibrils (AF) re-formed during cultivation on the CAM (× 35,000). Calibration bar 0.5 micrometer.

present in these recombinants before or at the time of grafting and none was noted during periods of cultivation up to 9 days. The appearance of the epidermal-dermal junction in these recombinants duplicated the appearance of the natural disease state, i.e., the anchoring fibrils were absent. Basal lamina and all more superficial structures of the junction appeared normal.

Recombinants composed of normal epidermis and EBD-R dermis. In these recombinants, nor-

mal epidermis isolated by cold trypsinization was combined with dermis from a patient with EBD-R. The plasma membrane of the epidermal basal cell was apposed to the inverted dermis. No basal lamina or anchoring fibrils were present prior to cultivation. During cultivation, basal lamina re-formed to produce continuous electron-dense lamina in many areas (Fig. 9). Anchoring fibrils were not seen in these recombinations.

Recombinants composed of EBD-R epidermis and normal dermis. In these recombinants, the new epidermal–dermal interface consisted of the basal lamina of the EBD-R epidermis apposed to the inverted surface of the normal dermis. Again, no comment can be made regarding the formation of basal lamina during cultivation since basal lamina was present prior to cultivation. Anchoring fibrils were absent in the recombinants at the time of grafting. After periods of cultivation, anchoring fibrils were found underlying the basal lamina (Fig. 10). These anchoring fibrils appeared to attach at one end to the undersurface of the basal lamina, exhibited a central cross-banded region and a deep fanlike fibrillar array extending into the dermis. They appeared first at days 5 to 7 of cultivation and increased in number during cultivation. The number of anchoring fibrils found after day 9 of cultivation in these recombinants was less than in recombinants composed of normal epidermis and dermis. Although fewer in number, there is no doubt that anchoring fibrils of characteristic morphology re-formed in recombinants of EBD-R epidermis and normal dermis. Therefore, normal dermis can correct the disease abnormality (i.e., absence of anchoring fibrils) when cultured in combination with abnormal epidermis. Ultrastructural evidence is thus provided that the site of this disease resides in the dermal component.

DISCUSSION

Role of Anchoring Fibrils in the Pathogenesis of EBD–R

We postulate that an absence of anchoring fibrils is the primary structural defect in EBD–R leading to disruption of the structural integrity of the epidermal–dermal junction (epidermal–dermal separation) and subsequent blister formation. In many specimens of nonblistered, nontraumatized EBD–R skin, absence of anchoring fibrils was the only ultrastructural abnormality observed. The possibility that lack of anchoring fibrils was a secondary change resulting from previous blistering and scarring was eliminated by our observation that anchoring fibrils were consistently absent in the newer previously blistered skin of 2 newborns with EBD–R. In experimentally traumatized skin, the epidermis and dermis separated in the region of the epidermal–dermal junction normally occupied by anchoring fibrils. Using recombinants of normal and EBD–R epidermis and dermis cultured on the CAM, we demonstrated that anchoring fibrils were

not formed by EBD–R dermis when combined with EBD–R epidermis or normal epidermis. Anchoring fibrils were formed when normal dermis was combined with normal and EBD–R epidermis. The only consistent alteration found in our experiments was an absence of anchoring fibrils. We, therefore, regard the results of this work as evidence that the defect in EBD–R is somehow associated with an abnormality of anchoring fibrils.

We agree with Pearson's finding of collagen degradation in EBD–R [3,4]. Although collagen degradation may be very extensive, particularly in or near preexisting blisters, epidermal–dermal separation can occur in EBD–R in the absence of apparent collagen degradation (Figs. 3, 4).

Dermal microfibril bundles appear normal in EBD–R (Figs. 2, 3). Although no attempt was made to quantitate their numbers, they seem to be encountered with normal frequency in EBD–R skin. The nature of these fibrils is controversial. An association of dermal microfibril bundles with dermal elastic system has been proposed [16,17] and is interesting in light of the previous suggestion that EBD–R is an abnormality of elastic fibers [18,19]. Elastic fibers were thought to be decreased on light microscopic examination of EBD–R skin [18].

Basal lamina and the more superficial structures composing the epidermal–dermal junction are remarkably unaffected in EBD–R. Normal-appearing basal lamina is consistently observed in the roof of EBD–R blisters and mechanically separated EBD–R skin (Fig. 6). The plasma membrane of epidermal basal cells and hemidesmosomes are also unaffected. The intermembranous space and the fine filaments (anchoring filaments) which cross the space, particularly subjacent to hemidesmosomes, are likewise normal.

The absence of anchoring fibrils in EBD–R yields important information about the biologic function of these fibrils. The failure of epidermal–dermal adherence and the severe blistering seen in EBD–R patients is impressive evidence of the importance of anchoring fibrils in the attachment of epidermis and dermis. The term, anchoring fibril, appears to be a very appropriate one in view of our findings.

Pathogenic Mechanisms: Destruction vs Impaired Formation

Two possible mechanisms have been proposed to explain the pathogenesis of EBD–R: (1) normally formed structures which are necessary for the attachment of epidermis and dermis are damaged or destroyed, or (2) these structures are not formed or, if formed, are defective so that separation of epidermis and dermis results from either their absence or improper function.

Evidence along several lines has been presented in support of the first possibility that normally formed structures required for attachment are destroyed by the disease process. Pearson [4]

demonstrated destruction of collagen fibers in the skin of epidermolysis bullosa dystrophica. Sometimes, collagenolysis was extensive, leaving only amorphous debris in the space under the basement membrane. Following this lead, Eisen [20] showed an elevation of collagenase activity that was highest in the blistered areas but also elevated in apparently normal, nonblistered skin. Lazarus [21] confirmed this observation of elevated collagenase activity in the blistered skin but found normal levels of activity in nonblistered skin. He suggested that the elevated collagenase activity in EBD–R might be a secondary event because of the normal activity in nonblistered skin. Collagenase activity would be expected to be elevated in nonblistered skin if collagenase played a primary role in the pathogenesis. Recently, Bauer, Gedde-Dahl, and Eisen [22] measured human skin collagenase utilizing a radioimmunoassay technique in patients with EBD–R. Tissue levels of human skin collagenase were elevated in both affected and clinically unaffected skin of these patients, indicating that the enzyme could be playing a primary role in the pathogenesis of the disease.

If human skin collagenase plays a primary role in the pathogenesis of EBD–R, the enzyme should be expected to produce damage to the epidermal–dermal junction identical to the observed ultrastructural pathology of EBD–R, namely, preferential destruction of anchoring fibrils, varying degrees of collagen degeneration, and complete sparing of the basal lamina and dermal microfibril bundles. Unfortunately, it has not been possible to investigate the effects of human skin collagenase on structures at the epidermal-dermal junction. Studies of the effect of bacterial collagenase on the epidermal–dermal junction do not correlate well with the ultrastructural pathology of EBD–R. Kahl and Pearson [23] found that bacterial collagenase, when injected into the skin of rabbits, produced collagen degeneration and loss of anchoring fibrils, but also destruction of the basal lamina which is well preserved in EBD–R, even in areas of severe collagen degeneration.

We have observed that epidermal–dermal separation may be seen in EBD–R in the absence of overt collagen degeneration with the only structural defect being a lack of anchoring fibrils. It is possible and even likely that collagenase or other proteases may be active in the dermis without overt collagenolysis seen on electron microscopy. Nevertheless, future studies in this area will have to account for the preferential destruction of anchoring fibrils compared to collagen.

An alternative pathogenic mechanism is that EBD–R may result from impaired formation of structures at the epidermal–dermal junction. Using a system in which anchoring fibrils normally re-form in recombinant grafts grown on the chick CAM, we have presented evidence that anchoring fibrils do not re-form as expected when EBD–R dermis is present as a component of the graft. We have previously demonstrated that anchoring fi-

brils are probably of dermal origin since their formation is dependent upon the dermal component of the recombinant [8]. The results of our recombination studies can be interpreted as evidence of impaired formation of anchoring fibrils by EBD–R dermis, although our studies do not exclude other possibilities. This is the first study in which the possibility of impaired formation finds experimental support.

How specific is the absence of anchoring fibrils for EBD–R? Anton-Lamprecht and Schnyder recently reported that anchoring fibrils were absent in a patient with dominantly inherited epidermolysis bullosa dystrophica (EBD–D) [24]. We, too, have found that anchoring fibrils are missing in EBD–D (unpublished observations). In 3 patients from a large kindred of EBD–D, anchoring fibrils were absent in blistered skin and in the scarred areas which resulted from previous blistering, but were present in uninvolved skin. This is different from EBD–R where anchoring fibrils are absent even in never previously blistered skin. Anchoring fibrils are present in other forms of epidermolysis bullosa, including epidermolysis bullosa simplex and junctional bullous epidermatosis (Herlitz's syndrome) [3,4].

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