Defective Expression of Plectin/HD1 in Epidermolysis Bullosa Simplex with Muscular Dystrophy

Yannick Gache,* Stéphane Chavanas,* Jean Philippe Lacour,‡ Gerhard Wiche,§ Katsushi Owaribe,‖ Guerrino Meneguzzi,* and Jean Paul Ortonne*‡

**U385 Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine, 06107 Nice Cedex 2, France; ‡Service de Dermatologie, Hôpital Pasteur, 06002 Nice Cedex 1, France; §Institute of Biochemistry and Molecular Cell Biology, University of Vienna, Austria; and ‖Department of Molecular Biology, Nagoya University, Nagoya, Japan*

Abstract

Epidermolysis bullosa simplex with muscular dystrophy (MD-EBS) is a disease characterized by generalized blistering of the skin associated with muscular involvement. We report that the skin of three MD-EBS patients is not reactive with antibodies 6C6, 10F6, or 5B3 raised against the intermediate filament-associated protein plectin. Immunofluorescence and Western analysis of explanted MD-EBS keratinocytes confirmed a deficient expression of plectin, which, in involved skin, correlated with an impaired interaction of the keratin cytoskeleton with the hemidesmosomes. Consistent with lack of reactivity of MD-EBS skin to plectin antibodies, plectin was not detected in skeletal muscles of these patients. Impaired expression of plectin in muscle correlated with an altered labeling pattern of the muscle intermediate filament protein desmin. A deficient immunoreactivity was also observed with the monoclonal antibody HD121 raised against the hemidesmosomal protein HD1. Furthermore, immunofluorescence analysis showed that HD1 is expressed in Z-lines in normal skeletal muscle; whereas this expression is deficient in patient muscle. Colocalization of HD1 and plectin in normal skin and muscle, together with their impaired expression in MD-EBS tissues, strongly suggests that plectin and HD1 are closely related proteins. Our results therefore provide strong evidence that, in MD-EBS patients, the defective expression of plectin results in an aberrant anchorage of cytoskeletal structures in keratinocytes and muscular fibers leading to cell fragility. (*J. Clin. Invest.* 1996. 97:2289–2298.) Key words: plectin • HD1 • epidermolysis bullosa simplex • muscular dystrophy • hemidesmosome

Address correspondence to Yannick Gache, INSERM U385, U.F.R. de Médecine, Avenue de Valombrose, 06107 Nice cedex 2, France. Phone: 33 93 37 77 79; FAX: 33 93 81 14 04.

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Introduction

Epidermolysis bullosa simplexes (EBS)[1] are genetic disorders characterized by separation of the epidermis from the underlying dermis, with the cleavage plane lying within the basal-cell layer of the epithelium (1). The major clinical subtypes of EBS have been associated with genetic defects in specific domains of keratins K5 and K14 that result in abnormal organization of the keratin network and cell disruption (2–4). Autosomal recessive forms of EBS associated with extracutaneous diseases, such as muscular dystrophy, have also been reported (MIM 226670) (5–8). Similar to dominant EBS, the intraepidermal cleavage localizes within the basal keratinocytes. To date, involvement of keratins in these forms of EBS has not been demonstrated. Since the keratin intermediate filaments (IF) interact with the hemidesmosomes (HD) (the complexes that anchor the basal cells of squamous and transitional epithelia to the underlying mesenchyme, reference 9), the possibility exists that the HD components contribute to the stability and organization of the keratinocyte's cytoskeleton. Mutations affecting HD proteins could therefore be implicated in the etiology of some subtypes of EBS with extracutaneous involvement.

Among the proteins that compose the HD, the cytoplasmic proteins plectin (10, 11), BP230 (12–14), HD1 (15, 16), and the integrin $\alpha 6\beta 4$ (17–19) appear to mediate the attachment of keratin IF to the plasma membrane. Plectin, a protein of 466 kD (20) found in different cell types, has been localized either throughout the cytoplasm (21) or at cellular attachment sites of cytoskeletal filaments, including the HD of epithelial cells (11), as well as the Z-lines of striated muscle (21). Based on plectin's ability to interact with IF (22), it has been proposed that one function of plectin could be the anchorage of IF to the plasma membrane. HD1 is a 500-kD protein localized in the innermost region of the HD plaque, where interaction with keratin IF is apparent (15). No structural information is yet available for HD1 and it is unclear whether HD1 is essential for the anchorage of IF to HD. In mice, disruption of the gene BPAG1, encoding the bullous pemphigoid antigen BP230 induces the loss of the inner plate of the HD and the severing of keratin IF, supporting the idea of an interaction of BP230 with keratin IF (23). Loss of HD inner plates does not seem to affect the pattern of HD1 in mutant mouse skin, suggesting an

1. *Abbreviations used in this paper:* EBS, epidermolysis bullosa simplex; HD, hemidesmosome; IF, intermediate filament; MD-EBS, EBS with muscular dystrophy.

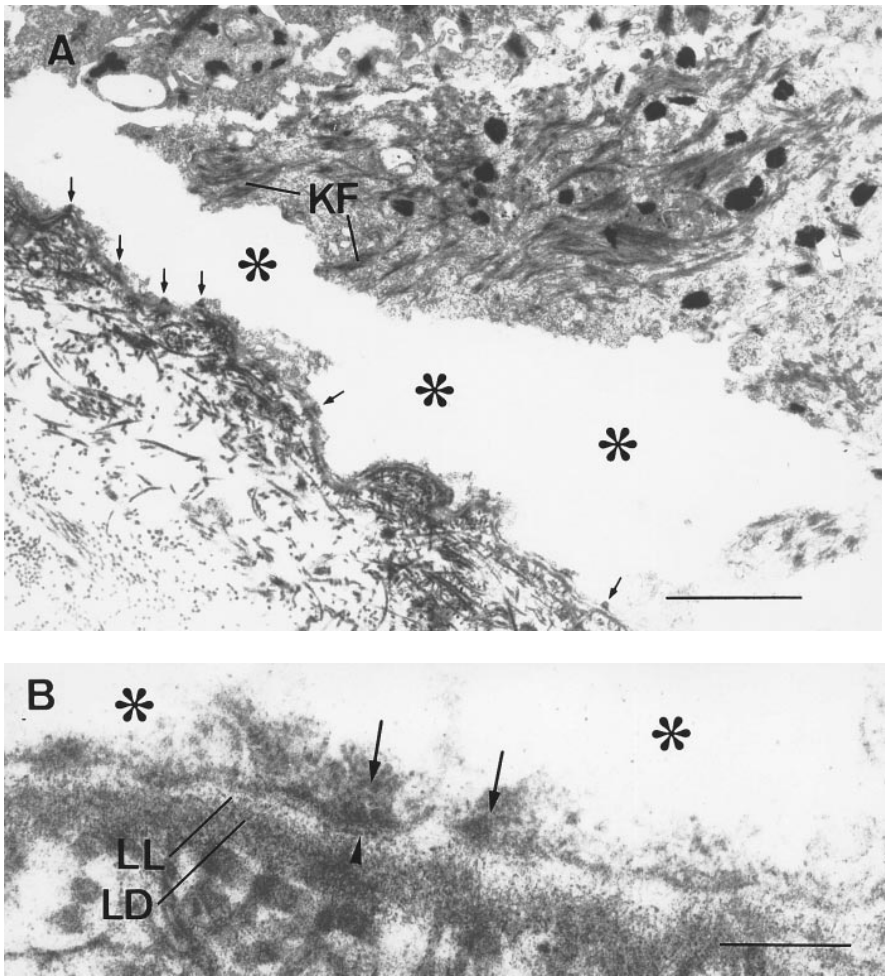


Figure 1. Electron microscopy of epidermal basal layer of skin biopsies obtained from MD-EBS patients. The separation between dermis and epidermis (*asterisks*) occurs at the level of basal cells just above the hemidesmosomes (*arrowheads*). Keratin filaments (*KF*) are dissociated from the hemidesmosomes (*arrows*). The inner plates are intact and in association with the electron-dense membrane plaque. Attachment of hemidesmosomes to the lamina densa (*LD*) of the basement membrane zone is preserved. (*B*) Higher magnification of the basal lamina. Bars: 2 μm (*A*), 200 nm (*B*).

indirect involvement of HD1 in connecting the keratin network to the HD. HD1 is also found in tissues lacking the typical HD structures, as the astrocytes of optical nerves and Schwann cells (15). Since in Schwann cells HD1 codistributes with integrin α6β4 (24), these two proteins may play a role in IF anchorage to the plasma membrane.

In this study, we analyzed the expression of HD proteins in patients displaying a form of EBS associated with muscular dystrophy (MD-EBS). We show that in these patients, plectin is immunologically defective, and that hampered expression of plectin correlates with the disorganization of IF networks. In addition, we demonstrate that this protein is not detected in the muscle of MD-EBS patients together with an abberant localization of the muscle IF protein desmin. Furthermore, we show that HD1 is expressed in normal skeletal muscle but not in skin and muscles of MD-EBS patients.

Methods

Patients. Three EBS cases with muscular dystrophy phenotype from two unrelated families were examined. Patient 1, a 29-yr-old female, was the product of a consanguineous union of unaffected parents and had 10 sisters and brothers. Two of the proband's sisters also suffered from bullous skin disease. The first died shortly after birth. The other affected sister (patient 2), now 27 yr old, also has muscular dystrophy. At birth, patient 1 presented with a generalized, recurrent bullous eruption on her elbows, knees, wrists, and hands. Healing occurred with moderate scarring without milia. The patient also displayed mild palmo-plantar keratoderma and nail dystrophy. Muscle weakness manifested in childhood and progressively led to widespread muscular atrophy and ptosis. Histological examination of muscle biopsy revealed a great variability in the diameter of muscle fibers and the presence of numerous hypertrophic and atrophic fibers. Electron microscopic analysis of MD-EBS muscle revealed that the most prominent ultrastructural feature was a striking disorganization in myofibrils and sarcomeres. Clinical description of patient 3 has been reported previously (7). Briefly, this patient, born from nonconsanguineous healthy parents, is a 29-yr-old male with epidermolysis bullosa and a severe, slowly progressive, muscle disease. Two of the proband's brothers at birth presented with a bullous skin disease which proved fatal in early infancy. A third brother was unaffected. As controls, we also examined four unrelated patients with a clini-

Table I. Immunoreactivity of MD-EBS Skin to mAbs Directed Against Components of the Dermal–Epidermal Junction

	Antibodies							
	6C6	10F6	5B3	GB3	HD121	1A8C	GoH3	FP1
Patients	*Plectin*			*Lam-5*	*HD1*	*BP180*	*α6β4*	*BP230*
Controls (n = 10)	+	+	+	+	+	+	+	+
EBS-MD (n = 3)	−	−	−	+	−	+	+	+
EBS (n = 4)	+	+	+	+	+	+	+	+

+, Strong reactivity; −, no detectable reactivity.

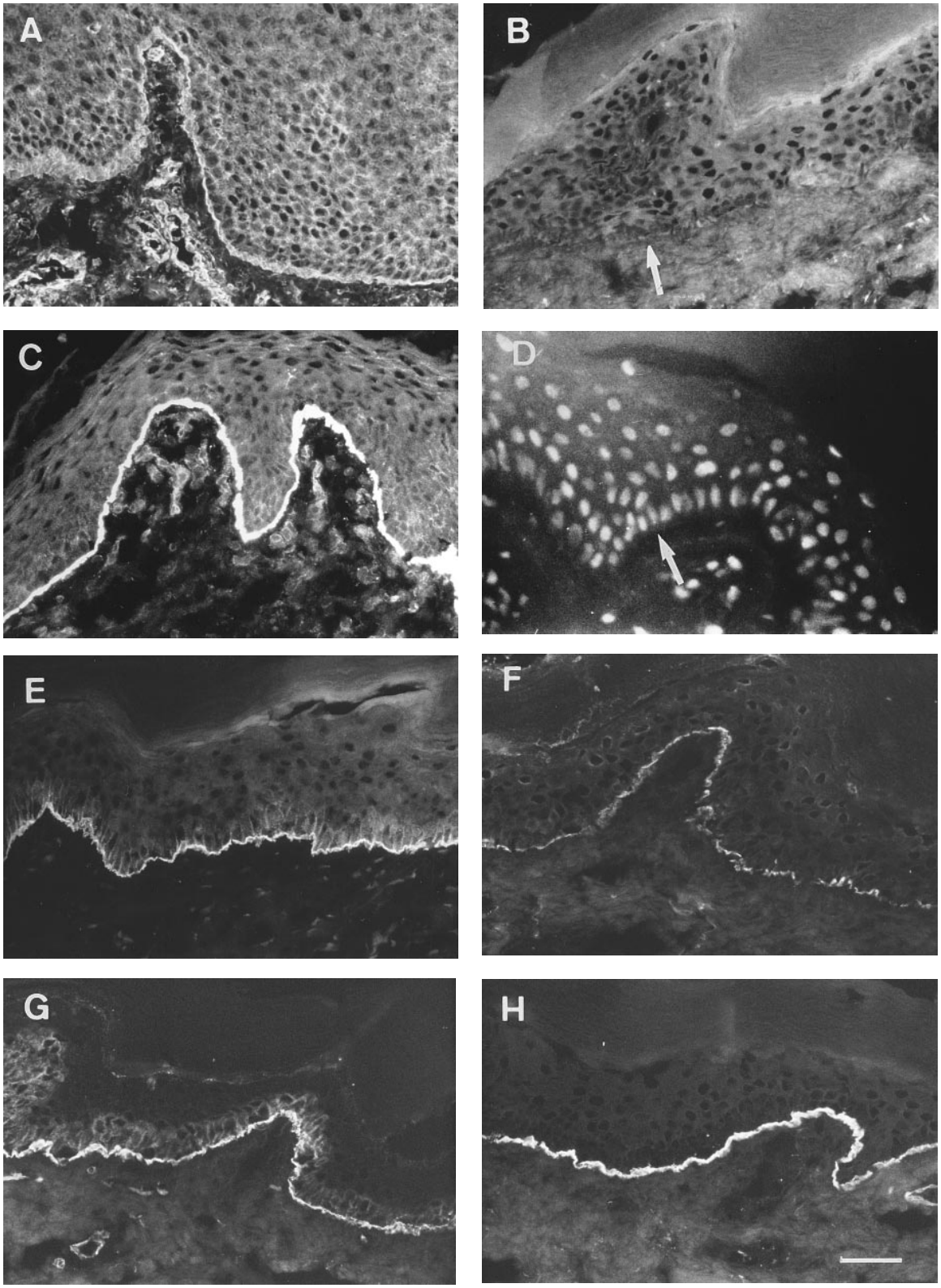


Figure 2. Immunofluorescence analysis of control skin (*A*, *C*) and nonlesional skin of MD-EBS patient 1 (*B*, *D–H*) using mAb antibody 5B3 directed against plectin (*A*, *B*), mAb HDI against HD1 (*C*, *D*), mAb 233 against BP180 (*E*), mAb FP1 against BP230 (*F*), mAb GoH3 against integrin α6 (*G*), and mAb GB3 against laminin 5 (*H*). In *D*, cells were counterstained with propidium iodide to visualize the basement membrane. *B* and *D* show the absence of staining of the epidermal basement membrane (*arrows*). Bar: 48 μm.

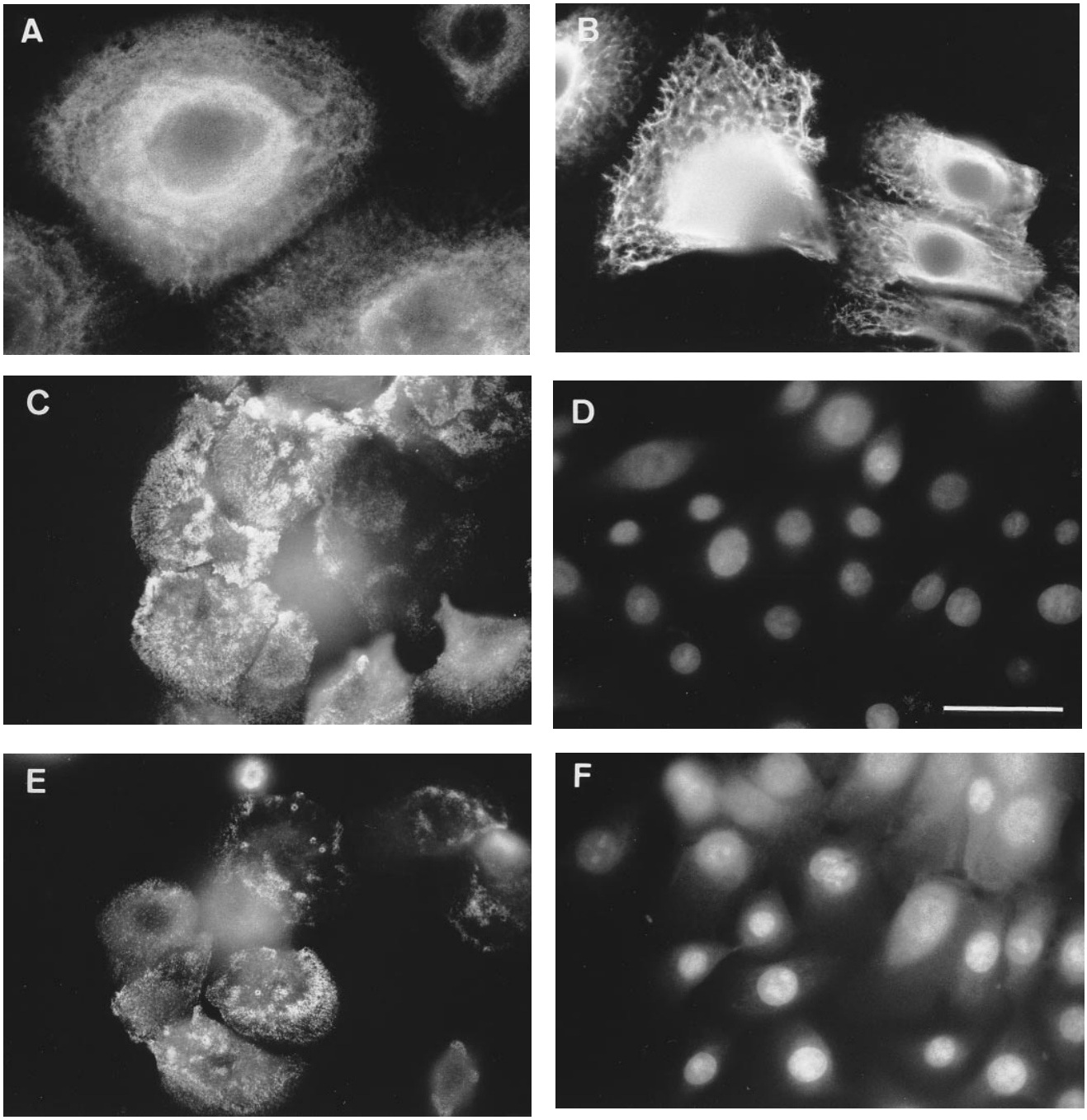


Figure 3. Distribution of keratin (*A*, *B*), HD1 (*C*, *D*), and plectin (*E*, *F*) in cultured keratinocytes from healthy control (*A*, *C*, *E*) and MD-EBS skin (*B*, *D*, *F*). Bar: 30 μm (*A*, *B*), 50 μm (*C*, *D*).

cally defined type of EBS (one affected by Dowling-Meara EBS and three others by Weber-Cockayne EBS).

Skin and muscle specimens. For electron microscopy examination, immunofluorescence, and cell cultures, skin biopsies were obtained from patients 1 and 3. Muscle biopsies were performed on the left quadriceps muscle of the two patients. Skin and muscle specimens obtained from healthy adult volunteers served as controls.

Cell culture. Epidermis from skin biopsies was separated from dermis by treatment with 0.5% dispase for 30 min at 37°C. Cells were then dissociated in 0.25% trypsin at 37°C and plated on a feeder of irradiated mouse 3T3 cells (25). Primary cultures were grown in a 1:1 mixture of DMEM and Ham's F12 medium (GIBCO BRL, Cergy Pontoise, France) containing 10% FCS (GIBCO BRL), 1 mM sodium pyruvate, 2 mM L-glutamine, 10 mg/ml penicillin and streptomycin, 10 ng/ml EGF, 100 pM choleratoxin, 400 ng/ml hydrocortisone, 5 mg/ml transferrin, 180 μM adenine, and 20 pM T3 (26). Subcultures were performed after gentle dissociation of subconfluent monolayers with PBS, pH 7.4, 0.05% trypsin, 0.02% EDTA. From passage 2, cells were cultured in serum-free keratinocyte growth medium (Promocell, Heidelberg, Germany), containing 0.15 mM $CaCl_2$, 5 mg/ml insulin, 0.1 ng/ml recombinant-EGF, 0.5 mg/ml hydrocortisone, and 30 mg/ml of bovine pituitary extract.

Antibodies. mAbs 10F6, 6C6, and 5B3, specific to the rod domain of plectin and distributed over a wide portion of this domain,

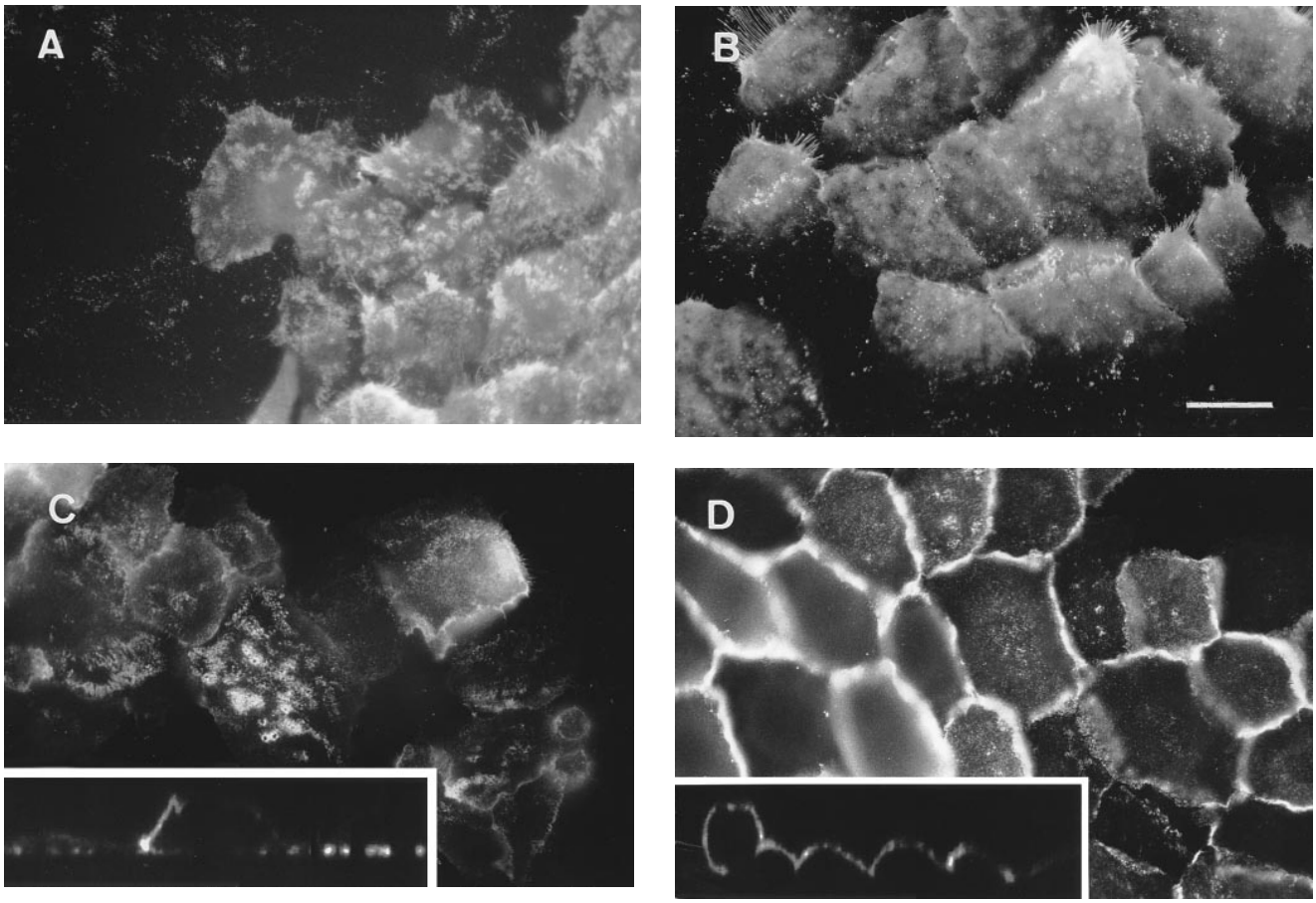


Figure 4. Immunofluorescence analysis of cultured keratinocytes from control (*A*, *C*) and MD-EBS skin (*B*, *D*) using mAb GoH3 (*A*, *B*) and mAb 233 (*C*, *D*). Bar: 30 μm. (*Insets*) confocal analysis of samples *C* and *D*.

were described previously (27). mAb HD121, specific to HD1, and mAbs 233 and 1A8C, raised against the external and cytoplasmic domains of BP180, respectively, were previously described (28). Rabbit antisera FP1 directed against BP 230 was a gift of J.R. Stanley (University of Pennsylvania Medical Center, Philadelphia, PA) (13). The antikeratin K14 mAb (clone CKB1) was purchased from Sigma Chemical Co. (Poole, UK). mAb GoH3, specific to integrin α6 was a gift of A. Sonnenberg (The Netherlands Cancer Institute, Amsterdam, Netherlands) (29). mAb GB3, which recognizes native human laminin 5, and pAb SE85, specific to the laminin α3 chain, were described previously (30, 31). mAb Mandra 1, elicited against the COOH-terminal domain of dystrophin, was from Sigma Chemical Co. pAb specific to desmin was from Euro-Diagnostics B.V., (Apeldoorn, Netherlands).

Immunofluorescence procedures. Immunohistochemical studies of skin and muscle biopsies were performed following standard methods (30). For immunofluorescence microscopy on cultured keratinocytes, cells at passage two or three were seeded on glass coverslips in serum-free, keratinocyte-defined medium and grown for 1 or 2 d. The cultures were then fixed for 20 min with PBS, 3% formaldehyde, 1 mM $CaCl_2$, 1 mM $MgCl_2$, rinsed for 15 min with PBS, 1 mM glycine, and permeabilized for 2 min at room temperature with 0.1% Triton X-100. After 30 min incubation in PBS containing 1% BSA, the primary antibody was applied for 1 h at room temperature. Cells were then washed with PBS and incubated for 30 min at room temperature in the presence of the secondary antibody. Secondary antibodies were FITC-conjugated goat anti–mouse, anti–rat IgG (Dako, SA, Glostrup, Denmark) or swine anti–rabbit IgG (Dako SA). Tissue sections and cell cultures were analyzed using a microscope (Zeiss Axiophot, Carl Zeiss Inc., Thornwood, NY). Cultured cell samples were also observed with a confocal microscope (Carl Zeiss Inc.).

Protein extracts. Cultured keratinocytes were incubated in PBS containing 10 mM EDTA for 10–30 minutes at 37°C. Detached cells were pelleted, washed with PBS, 1 mM $CaCl_2$, and 1 mM $MgCl_2$ and lysed 15 min at 4°C in RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Na deoxycholate, 1% Triton X-100, 0.1% SDS, 10 mM leupeptin, 1 mg/ml aprotinin, 0.5 mM PMSF, and 0.5 mM EDTA). Muscle biopsies were processed as previously described (32). Briefly, muscle biopsy specimens were homogenized in 7 vol (wt/vol) of extraction buffer (80 mM Tris-HCl, pH 6.8, 115 mM sucrose, 10% SDS, 1% β-mercaptoethanol, 0.5 mM PMSF, 10 mM leupeptin, 0.5 mM EDTA, and 1 mg/ml aprotinin), incubated 10 min at 50°C, and sonicated. Protein concentration was then quantified using a Bradford protein assay (Bio-Rad Laboratories, Richmond, CA).

Gel electrophoresis and immunoblotting. SDS-PAGE was performed on 3.5% acrylamide gels using the dissociating SDS-phosphate continuous buffer system described by Weber and Osborn (33). Protein samples were prepared as described by Laemmli (34). Electrotransfer to nitrocellulose membrane, immunostaining with antibodies, and stripping of the membrane for reprobing were done according to standard procedure using the ECL detection system (Amersham Intl., Little Chalfont, UK).

Results

Immunohistochemistry and ultrastructural studies of MD-EBS skin. Electron microscopic examination of the MD-EBS skin

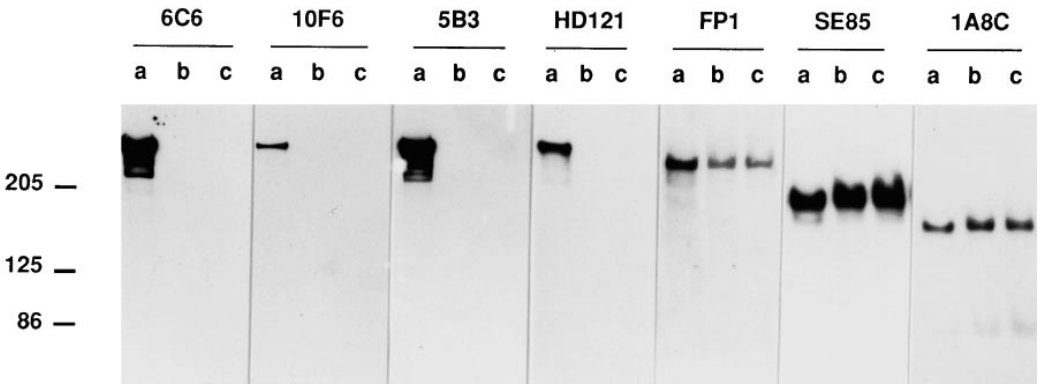


Figure 5. Western blot analysis of keratinocyte extracts obtained from the healthy control (lane *a*) and MD-EBS patients 1 (lane *b*) and 3 (lane *c*) using mAb 6C6, mAb 10F6, and mAb 5B3 directed against plectin, mAb HD121 directed against HD1, mAb FP1 directed against BP230, pAb SE85 raised against the laminin 5 α3 chain, and mAb 1A8C directed against BP180. 36 μg of cell extracts were separated on 3.5% SDS-PAGE.

revealed a cleavage within the basal keratinocytes, just above the cell basement membrane. HD adhered to the basement membrane, but its association with keratin IF was interrupted (Fig. 1 *A*). The HD electron-dense membrane plate, thought to comprise BP180 (28), α6β4 (18), and the inner plate structures where BP230 (13) and HD1 (15) have been immunolocalized were clearly identified (Fig. 1 *B*). These observations suggest that the defect responsible for skin cleavage might involve a cell component localized above the inner plate, at the anchorage site of keratin IF to HD.

To assess whether blister formation correlated with an altered immunoreactivity of HD components, immunofluorescence studies of lesional and nonlesional skin were performed using the panel of antibodies listed in Table I. In MD-EBS nonlesional skin treated with the antiplectin mAbs 6C6, 10F6, and 5B3, staining of the epidermal basement membrane zone was negative (Fig. 2 *B*). On the contrary, labeling of skin samples from healthy controls and from EBS patients without muscular dystrophy showed reactivity of plectin all along the epidermal basement membrane (Fig. 2 *A*) together with a cytoplasmic and faint staining of suprabasal keratinocytes. Absence, or strongly reduced staining, was also observed in MD-EBS skin with the monoclonal antibody HD121 directed against HD1 (Fig. 2 *D*); whereas this antibody labeled strongly the epidermal basement membrane of control skin samples (Fig. 2 *C*). Antibodies specific to BP180, BP230, integrin α6, and laminin-5 all gave a continuous labeling of the dermo–epidermal junction both in patients (Fig. 2, E–H) and control skin (not shown). In lesional zones, staining with antibodies specific to laminin-5, BP180, and integrin α6 was located at the blister floor; whereas staining of BP230 localized both at the floor and at the roof of the blister (not shown).

Expression of cytoskeletal and HD proteins in cultured keratinocytes. Previous reports demonstrated that keratinocytes cultured from patients with EBS display an abnormal keratin IF network (3, 35). To assess whether the loss of plectin and HD1 immunoreactivities affected the keratin IF organization, keratinocytes were expanded in culture from biopsies obtained from patients 1 and 3. According to the clinical diagnosis of EBS, keratinocytes obtained from both MD-EBS patients displayed an aberrant keratin IF network, as evidenced by immunofluorescence analysis performed using antibodies specific to human keratin 14 (Fig. 3 *B*). Indeed, in MD-EBS cells, thickness of the IF network (Fig. 3 *B*) contrasted with the thin and extended filamentous network of control keratinocytes (Fig. 3 *A*). The actin network appeared intact and interaction of actin stress fibers with vinculin was preserved (not shown).

MD-EBS keratinocytes cultured in medium at low calcium concentrations revealed a strongly reduced immunoreactivity to antiplectin (Fig. 3 *F*) and anti-HD1 antibody (Fig. 3 *D*). In similar culture conditions, keratinocytes from healthy controls showed a strong staining of areas of the cells in close contact with the culture substrate (Fig. 3, *C* and *E*). Localization of HD components was then performed both in normal and MD-EBS cells using mAb GoH3 and mAb 233 directed against integrin a6 and the extracellular domain of BP180, respectively. In both control and MD-EBS keratinocytes, immunoreactivity to GoH3 was comparable and had the characteristic spotlike pattern at the ventral side of the cells, in close contact with the culture substrate, which denotes the presence of cell adhesion structures at the basal plasma membrane (Fig. 4, *A* and *B*). As expected, a similar staining pattern was also observed in control keratinocytes using mAb 233. On the contrary, in MD-EBS cells, the antibody gave a peripheral staining (Fig. 4, *C* and *D*). Confocal microscope analysis confirmed the absence of the basal staining characteristic of normal control keratinocytes. Immunoreactivity localized at the edge of the cells and was prominent at the apical poles (Fig. 4, *C* and *D*, *insets*).

Western blot, performed using the antibodies 6C6, 10F6, and 5B3 directed against plectin and the mAb HD121 directed against HD1 (Fig. 5), showed that the proteins visualized in control human keratinocytes are absent in the two MD-EBS extracts analyzed. Furthermore, antiplectin and anti-HD1 antibodies each detected a band with an identical electrophoretic migration pattern. Fainter bands, presumably denoting presence of degradation products, were detected by mAbs 6C6 and 5B3. Identical bands were seen in overexposed Western blot using mAbs 10F6 and HD121 (not shown). Antibodies specific to BP230, BP180, and laminin α3 chain showed a normal expression level of these components in MD-EBS cells (Fig. 5).

Immunolocalization and expression of plectin and HD1 in normal and MD-EBS muscles. In skeletal muscle from healthy controls, indirect immunofluorescence analysis of longitudinal sections was performed using the two monoclonal antibodies, 5B3 (Fig. 6) and 10F6 (not shown), directed against plectin. In MD-EBS muscle (Fig. 6 *B*), the two antibodies detected an altered organization of the fibers. A diffuse labeling of the disor-

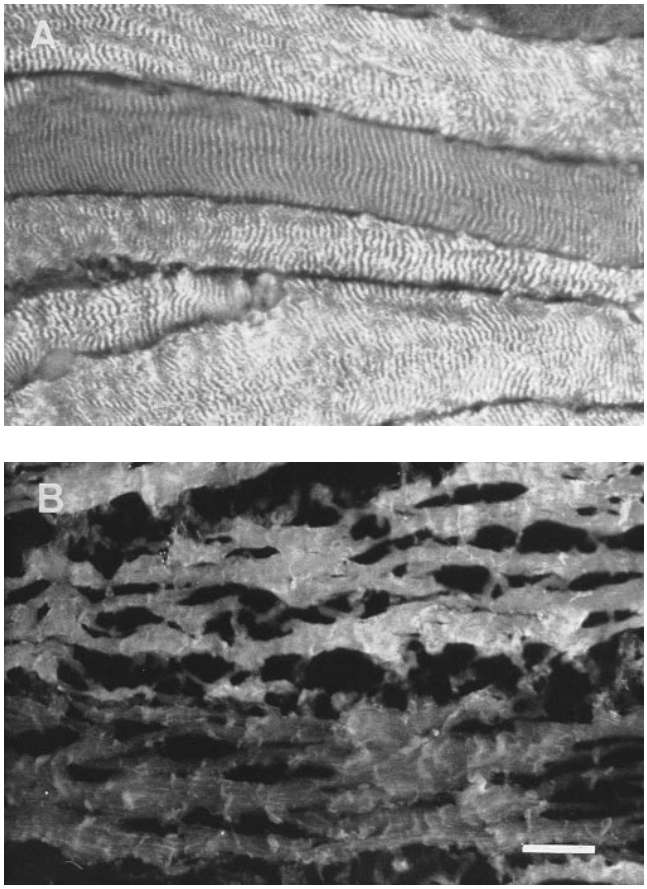


Figure 6. Immunofluorescence analysis of muscle tissues from the healthy control (*A*) and MD-EBS patient (*B*) using the antiplectin mAb 5B3. Bar: 50 μm.

ganized fibers, without cross-striations, was observed. Using the same antibodies, Z-line structures were clearly stained in all the control muscles (Fig. 6 *A*).

Immunofluorescence analysis of cross sections using mAb HD121 showed a positive immunoreactivity of the sarcolemma of the fibers in the muscle from healthy controls (Fig. 7 *A*). A strong cytoplasmic staining was also observed in ~ 40% of the muscle fibers. These immunoreactive fibers correspond to type I fibers, as demonstrated by enzymatic colorations of serial histological sections of the biopsy (not shown). In MD-EBS biopsies, staining of the fiber sarcolemma was either strongly reduced or absent (Fig. 7 *B*). A cytoplasmic labeling of type I fibers, comparable in intensity to that found in control muscle fibers was, however, observed. In longitudinal sections of control muscle, the HD121 positive type I fibers presented intracellular cross-striations (Fig. 7 *C*). Double immunofluorescence with antibodies directed against desmin showed a colocalization of HD1 and desmin labeling, suggesting that HD121 mAb reacts with a protein located in the Z-line structures (Fig. 7, *C* and *G*). In other fibers, immunoreactivity to antibody HD121 was reduced, although faintly stained Z-lines could be identified. Longitudinal sections of the MD-EBS muscle biopsies showed the disorganization of the fibers. Staining of the Z-disks was not detected and most of the fibers showed a diffuse labeling (Fig. 7 *D*). In MD-EBS patients, staining with antibodies specific for the COOH-terminal domain of dystrophin presented a usual pattern at the sarcolemmal membrane (not shown).

To determine if the abnormal plectin immunoreactivity observed in muscle by immunofluorescence correlated with a defect in the expression of the protein, muscle extracts of healthy donors and of MD-EBS patients were analyzed by Western blot. In total extracts of control muscle biopsies, the antibodies 5B3 (Fig. 8 *A*), 10F6, and 6C6 (not shown) reacted with a band corresponding to the mobility of the plectin protein expressed in keratinocytes. This band was not detected in the extract obtained from muscle of the MD-EBS patient. Furthermore, to identify the antigen detected by mAb HD121 in muscle fibers, immunoblotting was performed using mAb HD121 (Fig. 8 *B*). In extracts of control muscle, the antibody detected a band corresponding to the mobility of HD1 and plectin. As with antiplectin antibodies, this band was not detected in the MD-EBS patient extracts. In the muscle extracts, the myosin heavy chain (200 kD) cross-reacted with the HD121 antibody.

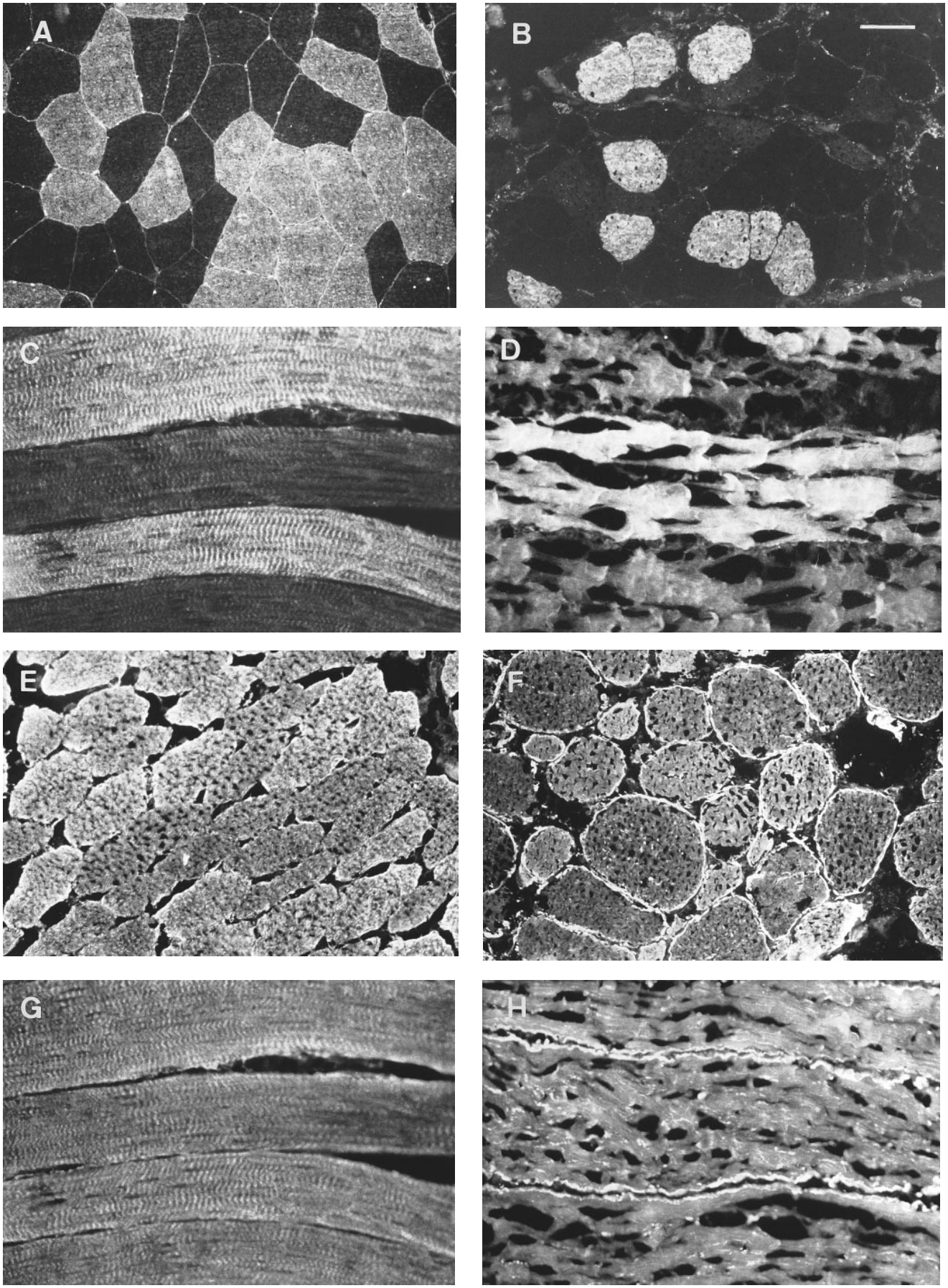
Localization of desmin in patient muscle. To assess whether the abnormal expression of plectin and HD1 in patient muscle also affected the IF network, the localization of the muscle-specific IF protein desmin was analyzed in MD-EBS muscle biopsies. Polyclonal desmin antibody presented an unusual pattern of immunoreactivity in MD-EBS muscle. Indeed, the labeling of the Z-disks typical of healthy muscle was not observed (Fig. 7 *H*) and an enhanced immunoreactivity appeared concentrated in the subsarcolemmal region (Fig. 7 *F*).

Discussion

In the present study, we report that three mAbs directed against different epitopes of the rod domain of plectin (27) and an mAb specific to the HD protein HD1 are not reactive with the basement membrane of the skin and the Z-lines of muscles of patients with MD-EBS.

Ultrastructural examination of involved MD-EBS skin clearly demonstrates that blistering arises from basal cell ripping localized in a highly defined zone, just above the basal plasma membrane which remains apposed to the lamina lucida. The keratin IF appear organized but disconnected from the HD, which suggests a defective anchorage of the IF to the plasma membrane of basal keratinocytes. The deficient immunoreactivity of plectin in MD-EBS skin and cultured keratinocytes, correlating with the impaired attachment of the keratin IF network to HD, indicates therefore that the protein may be involved in the anchorage of basal cell cytokeratins to these stable adherence junctions.

The mAb HD121 raised against HD1 also shows absence of reactivity to MD-EBS skin; therefore, an interesting question is the relationship between this protein and plectin. It has been reported that both proteins are expressed in a wide range of tissues. The two proteins are localized in the HD and display an identical apparent molecular mass. However, while plectin expression has been demonstrated in skeletal muscles, the presence of HD1 in this tissue was not reported (15). In this work, we demonstrate that HD1 is expressed in skeletal muscles and that it colocalizes with desmin, and consequently with plectin, in the Z-disks of myofibrils. We also demonstrate that HD1 is immunologically altered in plectin-deficient MD-EBS skin and muscles. Therefore, although the establishment of the cDNA sequence of HD1 is necessary to ascertain the exact degree of homology between HD1 and plectin, our results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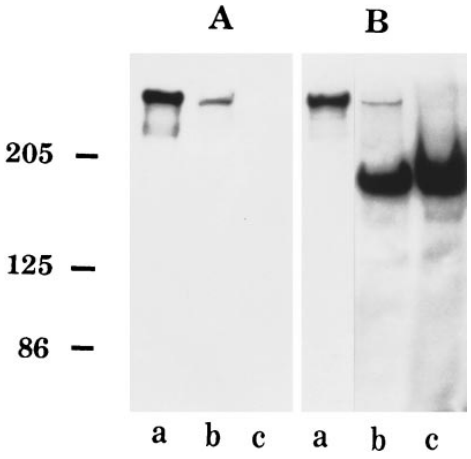

Figure 8. Western blot analysis of keratinocyte and muscle extracts using mAb 5B3 against plectin (*A*) and HD121 against HD1 (*B*). Keratinocytes from healthy donor (*a*), muscle from healthy control (*b*) and MD-EBS patient (*c*).

strongly support the hypothesis that plectin and HD1 are the same or closely related proteins.

All the other known components of HD were expressed in MD-EBS skin, suggesting that the abnormal expression of plectin (or plectin/HD1) detected in these patients is not secondary to a more generalized disorganization of the architecture of cytoskeleton and/or HD, but rather to a defect specifically affecting this protein. In cultured MD-EBS keratinocytes, however, the bullous pemphigoid antigen BP180 displayed an unusual distribution at the apical pole of the plasma membrane. This contrasts with the basal localization of integrin α6. Presence or integrity of plectin in the HD may therefore contribute to the correct polarization of BP180 in cultured keratinocytes. Alternatively, loss of BP180 polarization could be caused by propagation of cultures as it was shown for integrin α6β4 in normal cultured keratinocytes (36). However, similar abnormal distribution of BP180 was not observed in concomitant cultures of normal keratinocytes.

Examination of the MD-EBS skeletal muscle fibers shows a striking disorganization of the sarcomeres, particularly of the Z-lines, suggesting an involvement of the muscle cytoskeleton. Desmin, the muscle-specific IF protein (37), is localized in the Z-lines of the adult skeletal muscle, where it appears to form linkages between the Z-lines of adjacent myofibrils and between the peripheral myofibrils and the sarcolemma (38). In MD-EBS muscles, desmin appears delocalized at the periphery of the muscle fibers, which suggests a defective anchorage of the IF cytoskeleton. This abnormal localization of desmin, which correlates with the deficient reactivity of plectin antibodies in MD-EBS muscle, confirms that plectin is an IF-associated protein and suggests its involvement in the anchorage of cytoplasmic filaments to the cell membrane. Interestingly, a similar increased subsarcolemmal staining of desmin is observed in the muscle of the patients presenting with desmin myopathy (39). Thus far, muscular dystrophy has been associated with mutations in proteins involved in the attachment of the cytoskeleton of muscle fibers to the extracellular matrix (40). Defects in dystrophin and adhalin, two membrane-associated proteins linking the subsarcolemmal cytoskeleton to the extracellular matrix, have been associated with Duchenne's and severe childhood autosomal recessive muscular dystrophy, respectively (32, 41–43). Moreover, a defective expression of the extracellular matrix protein laminin-2 (44) was observed in patients affected by congenital muscular dystrophy (45) and in mice affected by an autosomal recessive muscular dystrophy (46). In this work, we provide an additional example of a form of muscular dystrophy associated with the impaired synthesis of a structural protein which contributes by its interaction with the cytoskeleton to the mechanical stability of muscle fibers.

The specific deficiency of plectin in the skin and muscles of two MD-EBS patients identifies a candidate gene for the disease. The deficiency was observed using three antibodies recognizing epitopes covering a widespread portion of the rod domain of the molecule. This observation suggests that in these patients, plectin either presents large deletions or is missing. The molecular analysis of the cDNA encoding plectin in these MD-EBS patients is in progress and will help to elucidate the genetic defect underlying this disabling disease.

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Figure 7. Immunofluorescence analysis of muscle tissues obtained from the healthy control (*A*, *C*, *E*, *G*) and MD-EBS patient (*B*, *D*, *F*, *H*) using mAb HD121 (*A*, *B*, *C*, *D*) and pAb antidesmin (*E*, *F*, *G*, *H*) antibodies. Crosssections (*A*, *B*, *E*, *F*) and longitudinal sections (*C*, *D*, *G*, *H*) of the two muscle biopsies were analyzed. Bar: 80 μm (*A*, *B*, *E*, *F*), 50 μm (*C*, *D*, *G*, *H*).

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