The Tetraspan Molecule CD151, a Novel Constituent of Hemidesmosomes, Associates with the Integrin α6β4 and May Regulate the Spatial Organization of Hemidesmosomes

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Abstract. CD151 is a cell surface protein that belongs to the tetraspan superfamily. It associates with other tetraspan molecules and certain integrins to form large complexes at the cell surface. CD151 is expressed by a variety of epithelia and mesenchymal cells. We demonstrate here that in human skin CD151 is codistributed with α3β1 and α6β4 at the basolateral surface of basal keratinocytes. Immunoelectron microscopy showed that CD151 is concentrated in hemidesmosomes. By immunoprecipitation from transfected K562 cells, we established that CD151 associates with α3β1 and α6β4. In β4-deficient pyloric atresia associated with junctional epidermolysis bullosa (PA-JEB) keratinocytes, CD151 and α3β1 are clustered together at the basal cell surface in association with patches of laminin-5. Focal adhesions are present at the periphery of these clusters, connected with actin filaments, and they contain both CD151 and α3β1. Transient transfection studies of PA-JEB cells with β4 revealed that the integrin α6β4 becomes incorporated into the α3β1-CD151 clusters where it induces the formation of hemidesmosomes. As a result, the amount of α3β1 in the clusters diminishes and the protein becomes restricted to the peripheral focal adhesions. Furthermore, CD151 becomes predominantly associated with α6β4 in hemidesmosomes, whereas its codistribution with α3β1 in focal adhesions becomes partial. The localization of α6β4 in the pre-hemidesmosomal clusters is accompanied by a strong upregulation of CD151, which is at least partly due to increased cell surface expression. Using β4 chimeras containing the extracellular and transmembrane domain of the IL-2 receptor and the cytoplasmic domain of β4, we found that for recruitment of CD151 into hemidesmosomes, the β4 subunit must be associated with α6, confirming that integrins associate with tetraspans via their α subunits. CD151 is the only tetraspan identified in hemidesmosomal structures. Others, such as CD9 and CD81, remain diffusely distributed at the cell surface.

In conclusion, we show that CD151 is a major component of (pre)-hemidesmosomal structures and that its recruitment into hemidesmosomes is regulated by the integrin α6β4. We suggest that CD151 plays a role in the formation and stability of hemidesmosomes by providing a framework for the spatial organization of the different hemidesmosomal components.

Key words: integrin α6β4 • tetraspan CD151 • hemidesmosome • focal adhesion • cross-talk

Introduction

CD151 has recently been characterized as a member of the tetraspan superfamily. This growing family comprises >20 highly conserved molecules, which intersect the plasma membrane four times (Wright and Tomlinson, 1994; Maecker at al., 1997; Hemler, 1998). Like other tetraspan molecules, CD151 contains one small and one large extracellular loop with short cytoplasmic carboxy- and amino-terminal domains. The large extracellular loop is thought to be involved in the binding to other molecules, such as integrins. Tetraspans probably bind to the α subunit of the heterodimeric integrins (Imai et al., 1995; Mannion et al., 1996; Lagaudrière-Gesbert et al., 1997; Yauch et al., 1998). CD151 has been implicated in a wide variety of cell biological processes, including cell adhesion (Hasegawa et al., 1998; Fitter et al., 1999), cell motility (Yáñez-Mó et al.,
The human erythroleukemic cell line K 562 was maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (GIBCO BRL), 100 U/ml penicillin and 100 U/ml streptomycin. K 562 cells stably expressing α6β4 were established as described previously (D elwel et al., 1993; N isson et al., 1994). Immortalized keratinocytes derived from a PA-J E B patient were isolated as previously described (S chaapfeld et al., 1998). Keratinocytes were grown in keratinocyte serum-free medium (SFM; GIBCO BRL), supplemented with 50 μg/ml bovine pituitary extract, 5 ng/ml epidermal growth factor, 100 U/ml penicillin, and 100 μg/ml streptomycin. A ll cells were grown at 37°C in a humidified, 5% CO2 atmosphere.

**Materials and Methods**

**Cell Lines**

The human erythroleukemic cell line K 562 was maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (GIBCO BRL), 100 U/ml penicillin and 100 U/ml streptomycin. K 562 cells stably expressing α6β4 were established as described previously (D elwel et al., 1993; N isson et al., 1994). Immortalized keratinocytes derived from a PA-J E B patient were isolated as previously described (S chaapfeld et al., 1998). Keratinocytes were grown in keratinocyte serum-free medium (SFM; GIBCO BRL), supplemented with 50 μg/ml bovine pituitary extract, 5 ng/ml epidermal growth factor, 100 U/ml penicillin, and 100 μg/ml streptomycin. A ll cells were grown at 37°C in a humidified, 5% CO2 atmosphere.

**Antibodies**

Mouse mAb b J B H against the extracellular domain of the human α6 inte-


gran subunit and 29A 3 against the cytoplasmic domain of the human α3A 3 subunit have been described previously (H ogervorst et al., 1993; D e M elker et al., 1997). The mouse mAb b J 143, recognizing an extracellular epitope on the human α3 integrin subunit (K antor et al., 1987), was from the A merican T ype Culture Collection. R at mAb b B 1 E 5 against the hu-


mn α5 extracellular domain (H all et al., 1990) was a kind gift from D r. C. H. D amsky (U niversity of California San Francisco, San Francisco, C A) and the mouse mAb b 4.3E 1 (H esl e et al., 1984) and 450-11A (K ennel et al., 1996) against the extracellular and cytoplasmic domains of human β4 were donated by D r. E. E ngvall (T he B urnh urn I nstitute, L a Jolla, C A) and S. J. K ennel (O ak R idge N ational L aboratory, O ak R idge, T. N.). R abbit sera against the cytoplasmic domains of the human α6A and β4 integrin subunits were prepared as described (D elwel et al., 1993; N isson et al., 1994). T he anti-β6 polyclonal antibody, A 3, was raised in rabbits by immunizing with a GST-fused recombinant protein of the extracellular domain of α6 (amino acids [aa] 1–576). T he rabbit polyclonal antibodies against the cytoplasmic domains of α3A (D iperso et al., 1995), α5 (Delfillipi et al., 1991) and β1A were gifts from D r. S. D iperso (A lbany M edical C ollege, A lbany, N Y), G. T arone (U niversità di T orino, I taly) and U. M ayer (M ax-Planck-I nstitut f ür B iochemie, M artinsried, G ermany), respectively.

The mouse mAb b 121 and 815 directed against plectin (O kumura et al., 1993) and B P230 (N ishizawa et al., 1993) were generously provided by D r. K. O waribe (N agoya U niversity, N agoya, J apan) and the rabbit sera against vinculin (G eiger, 1979) and the carboxy-terminal domain of BP230 (T anaka et al., 1990) were gifts from D rs. B. G eiger (T he W eizmann I nstitute of S cience, R ehovot, I srael) and J. R. S tanley (U niversity of P ennsylva-



1 A bbreviations used in this paper: B P 180, bullous pemphigoid antigen 180; B P 230, bullous pemphigoid antigen 230; I L 2 R, interleukin-2A receptor; P A - J E B, pyloric atresia associated with junctional epidermolysis bullosa.
were washed three times and then incubated with goat anti–mouse IgG BSA, followed by incubation with the primary antibody for 1 h. The cells extracellular and transmembrane domain of the IL-2 receptor and the cytoplasm (Fig. 2). Cells expressing α3β1 or α6β4 were subcloned into the pRc/PA-JEB and α4, which consists of the α4 subunit, were subcloned into the pRc/A5, A6 and B4 at their surface were isolated by FACS sorting. The combined staining patterns of α3β1 and α6β4 are very similar to that of CD151 (Fig. 1 D). We next determined the exact distribution of CD151 in basal keratinocytes by immunoelectron microscopy. The results show that CD151 is concentrated in electron-dense hemidesmosomes (Fig. 2, B and C). The protein was also detected that CD151 is concentrated in electron-dense hemidesmosomes. An association between CD151 and desmosomes. Staining was conducted by mAb P48 (dilution 1:10) and the incubated with 10-nm colloidal gold–labeled protein A for 30 min. A fter washing, the cryosections were embedded in a mixture of methyl cellulose and uranyl acetate and examined with a Philips CM 10 electron microscope. For controls, the primary antibody was replaced by a nonrelevant mouse mAb.

**Results**

**Identification of CD151 as a Novel Constituent of Hemidesmosomes**

Immunohistochemical staining of frozen sections of the skin revealed that the laminin-binding integrins α6β4 (Fig. 1, A and B) and α3β1 (Fig. 1 C) are present at the basal and basolateral surfaces of the basal keratinocytes, respectively. In addition, the α3β1 integrin is present in some suprabasal cells. The combined staining patterns of α3β1 and α6β4 are very similar to that of CD151 (Fig. 1 D). We next determined the exact distribution of CD151 in basal keratinocytes by immunoelectron microscopy. The results show that CD151 is concentrated in electron-dense hemidesmosomes (Fig. 2, B and C). The protein was also detected along the lateral membranes, but not in desmosomes (Fig. 2 A). The ultrastructural location of CD151 is consistent with the results of the immunoperoxidase staining and suggests that CD151 is codistributed with α6β4 in hemidesmosomes. An association between CD151 and α3β1 is suggested by their similar patterns of staining in other parts of the cell.

**Coprojection of CD151 with α3β1 and α6β4**

Initial attempts using keratinocytes to show that CD151...
forms a complex with α6β4 all failed because α6β4, when present in hemidesmosomes, could not be extracted with a buffer containing 1% CHAPS. We therefore used K562 cells instead, which stably express the integrin α6β4, but do not contain hemidesmosomes. Control K562 cells, which only express α5β1, and K562 cells transfected with α3 were also included in the analyses. Cells were lysed in 1% CHAPS and integrins were immunoprecipitated with integrin subunit-specific antibodies. The presence of CD151 in the precipitates was assessed by immunoblotting with specific antibodies (Fig. 3). Protein bands corresponding to CD151 could be detected in the immunoprecipitates containing α3β1 and α6β4, but not in those that contain α5β1. When cells were lysed in 1% NP-40, CD151 was only detected in the immunoprecipitates containing α3β1 (not shown).

Localization of CD151 in Pre-Hemidesmosomal and Hemidesmosomal Structures in Cultured Keratinocytes

Cultured keratinocytes were used to further investigate the localization of CD151 and its possible role in hemidesmosome formation. Previously, we have shown that in PA-JEB keratinocytes, which lack β4, no hemidesmosomes are formed (Schaapveld et al., 1998). However, hemidesmosome formation is induced by transfection with β4, a process that can occur in both a ligand-dependent and -independent manner, the latter requiring the β4 cytoplasmic domain and the presence of plectin (Schaapveld et al., 1998; Nievers et al., 2000).

Staining of untransfected PA-JEB cells showed complete colocalization of CD151 and α3β1 in clusters at the basal surface of the cell (Fig. 4, A–C). The ligand for α3β1, laminin-5, was concentrated beneath these basal clusters (Fig. 4, D–F). Only CD151 is colocalized with α3. The other tetraspans CD9 and CD81 are diffusely distributed over the plasma membrane, while the distribution pattern of CD63 is granular in the cytoplasm (data not shown). The laminin-5 patches, which were left in the tracks of migrated cells, do not contain α3β1 or α6β4.

In PA-JEB/β4 cells, α6β4 was found to be concentrated in hemidesmosome-like structures at sites of cell–substrate contact. These hemidesmosomal structures appear to be much larger than the clusters formed by α3β1 in the untransfected PA-JEB cells, but, like the α3β1 clusters, they contain CD151 and are associated with laminin-5 (Fig. 4, G–L).

In transient transfection experiments, which enabled us

Figure 1. Codistribution of laminin-binding integrins and CD151 in the skin. Frozen sections of human skin are stained by immunoperoxidase reaction with (A) anti-α6 (J8H), (B) anti-β4 (4.3E1), (C) anti-α3 (J143), and (D) CD151 (P48). Anti-α6 and β4 produce strong staining of the basal region of basal keratinocytes, while anti-α3 reacts with basal and lateral surfaces of the basal cells and some suprabasal cells. The distribution of CD151 overlaps with that of both α3β1 and α6β4. Bar, 100 μm.
to visualize expression of the different components in transfected and untransfected cells in a single confocal high power field, it could be demonstrated that α6β4 becomes localized at sites, where the α3β1-CD151 clusters are present (Fig. 5). Thus, the formation of these clusters seems to precede the formation of mature hemidesmosomes, which requires expression of α6β4. Therefore, we refer to the α3β1-CD151 clusters as pre-hemidesmosomal structures. It is also shown that the amount of CD151 in the clusters increased after expression of β4 (Fig. 5, A–C and D–F). In contrast, that of α3 seems to become reduced, compared with that in pre-hemidesmosomal structures in untransfected cells (Fig. 5, G–I, see also Fig. 8). The increased reaction with anti-CD151 was not due to cross-reactivity of the antibody with epitopes unrelated to CD151, since two other antibodies against CD151, 8C3 and Sfa-1, gave similar results. Tetraspans, other than CD151, are absent from hemidesmosomes, as shown for CD81 (Fig. 5, J–L).

Recently, Yauch et al. (1998) have shown that CD151 expression is upregulated after transfection of K562 cells with α3. Similarly, we observed that CD151 surface expression increased by 35% when PA-JEB keratinocytes are transfected with β4 (Fig. 6). Thus, the clustering of CD151 in hemidesmosomes may result from the recruit-
ment of intracellular pools of CD151, although additional redistribution of surface CD151 is not excluded.

**Localization of CD151 in Type I and Type II Hemidesmosomes**

Based on their components, hemidesmosomes can be divided into two types, type I and II hemidesmosomes. Type II hemidesmosomes, which contain α6β4 and plectin, are considered to be precursors of the classical type I hemidesmosomes, which in addition contain BP180 and BP230 (Nievers et al., 1999). We investigated whether CD151 is expressed in both types. In PA-JEB/β4 cells that stably express the integrin α6β4, type II hemidesmosomes are more abundant than type I hemidesmosomes and in some cells only type II hemidesmosomes are present. As shown in Fig. 7 D, in PA-JEB/β4 cells, the staining pattern of hemidesmosomes for CD151 and α6β4 is very similar. This
is also true in those cells that only contain type II hemidesmosomes. Similar results were obtained when we compared the distribution of CD151 with that of plectin (not shown). In contrast, CD151 is more widely distributed than BP230 (Fig. 7 E). Together, these data show that CD151 is a component of both types of hemidesmosomes. The \( \alpha_3 \) subunit is expressed at the periphery of the hemidesmosomes and is not colocalized with \( \alpha_6 \beta_4 \), BP230, or plectin (Fig. 7, A–C). Importantly, while in untransfected PA-JEB cells, the expression pattern of \( \alpha_3 \) completely overlaps with that of CD151 (Fig. 4), in PA-JEB/\( \beta_4 \) cells that stably express the integrin \( \alpha_6 \beta_4 \), the integrin \( \alpha_3 \beta_1 \) and CD151 are only partially colocalized in the vicinity of hemidesmosomes (Fig. 7 F, see also A) and in some cells are not colocalized at all. This suggests that when \( \beta_4 \) is expressed in the hemidesmosomal structures the \( \alpha_3 \beta_1 \)-CD151 complexes are replaced by \( \alpha_6 \beta_4 \)-CD151.

The \( \beta_4 \) Cytoplasmic Domain Does Not Support the Recruitment of CD151 into Hemidesmosomes

The integrin \( \alpha \) and \( \beta \) subunits associate noncovalently via their extracellular domains. Using an IL-2R/\( \beta_4 \) chimera, which consists of the extracellular and transmembrane domains of the IL-2 receptor and the \( \beta_4 \) integrin cytoplasmic domain, we have shown that dimerization of \( \beta_4 \) with \( \alpha_6 \) is not required for hemidesmosome formation (Nievers et al., 1998). Because the extracellular domain of the IL-2 re-
The integrin α5β1, which is a receptor for fibronectin, is expressed by a variety of cells, such as endothelial and hematopoietic cells. In addition, immunohistochemical data indicated that the expression patterns of CD151 and the laminin-binding integrins α3β1, α6β4, and α7β1 also overlaps (Sincock et al., 1997). In this study, we analyzed the expression of CD151 in skin and show that CD151 is coexpressed with α3β1 and α6β4 at the basolateral surface of the basal keratinocytes. Furthermore, immunoprecipitation experiments using transfected K562 cells indicated that CD151 forms complexes with the α3β1 or α6β4 laminin-binding integrins, confirming previous findings (Y auch et al., 1998; Seru et al., 1999; Sincock et al., 1998). However, in contrast to earlier studies (Inoue et al., 1998; Fitter et al., 1999; Sincock et al., 1999), we could not confirm coinmunoprecipitation of CD151 with α5β1 from lysates of these cells. Finally, in line with the observation that CD151 is colocalized and associates with α6β4, the presence of CD151 in hemidesmosomes was demonstrated by immunoelectron microscopy.

**CD151 and Sequential Stages in the Formation of Hemidesmosomes**

The integrin α6β4, a receptor for laminin-5, is a major component of hemidesmosomes and crucial for initiating their formation since it forms a scaffold for the binding of the other hemidesmosomal components (Borradori et al., 1997; Rezniczek et al., 1998; Schaapveld et al., 1998; Geerts et al., 1999; Hopkinson and Jones, 2000). The formation of type II hemidesmosomes, containing α6β4 and plectin, precedes the formation of type I hemidesmosomes that additionally contain BP230 and BP180. Type II hemidesmosomes are also considered to be immature hemidesmosomes, present in the early phase of wound healing.

**Fluorescence intensity**

Figure 6. Expression of α6β4 results in an increase in the surface levels of CD151. Flow cytometry was used to analyze the surface expression of β4 (4.3E1) and different tetraspan molecules CD9 (MEM62), CD53 (MEM53), CD63 (6H1), CD81 (M38), and CD151 (P48) on PA-JEB (dotted lines) and PA-JEB/β4-transfected cells (interrupted lines). Negative control (solid lines) staining with secondary FITC-conjugated anti-mouse IgG is shown for comparison. The expression of β4 on the surface of PA-JEB cells (PA-JEB/β4) was selectively accompanied by an increase in the surface levels of CD151; the levels of CD9 and CD81 remain unaltered. Both PA-JEB and PA-JEB/β4 cells are negative for CD53 and CD63.
CD151 is a novel constituent of hemidesmosomes.

In contrast, type I hemidesmosomes are formed in the stabilizing phase of wound healing and in more stress-resistant epithelia (Uematsu et al., 1994; Goldfinger et al., 1999).

By comparing β4-deficient PA-JEB cells with such cells transfected with β4, the localization of CD151 could be studied before and after hemidesmosomes were formed. Our data demonstrate that hemidesmosomes are formed in defined, consecutive stages. At first, laminin-5 is deposited, followed by the recruitment of α3β1 and CD151 in what may be called pre-hemidesmosomal structures. At this stage, there is relatively little CD151 present. After β4 transfection in PA-JEB cells, hemidesmosomes are formed. The integrin α6β4 binds to the deposited laminin-5, and this is followed by the recruitment of plectin (Schaapveld et al., 1998). Ultimately, BP180 and BP230 become associated with the α6β4-plectin complexes (Borradori et al., 1998; Hopkinson and Jones, 2000).

The localization of α6β4 in the pre-hemidesmosomal structures is associated with an increase in the amount of CD151 and a loss of α3β1 from these structures. In addition to the clustering of CD151 in hemidesmosome-like structures, we found that the levels of CD151 at the cell surface are increased in PA-JEB/β4 cells, that stably express the integrin α6β4. It has been suggested that CD151 has a role in endocytosis and subsequent recycling of β1 integrins to the cell surface because of their similar intracellular localization in endosomal structures (Sincock et al., 1999). Recycling of the integrin α6β4 from the plasma membrane to internal pools and back to the plasma membrane has also been observed (Breitscher, 1992; Gaietta et al., 1994). Based on this knowledge and the data presented, it is tempting to speculate that CD151 is involved in the internalization of α6β4 into the cells and the sorting of it to hemidesmosomes in keratinocytes. Binding of α6β4 to an immobilized ligand may prevent the integrin from becoming internalized, thus resulting in an increased expression at the cell surface.

The mechanism responsible for the loss of α3β1 from hemidesmosomal structures is not known, but it might be explained by a higher affinity of α6β4 for laminin-5, thus preventing α3β1 from interacting with it. Both α3β1 and α6β4 bind to the same domain of their ligand (Delwel and Sonnenberg, 1997; Aumailley and Roussel, 1999). Alternatively, by inside-out signaling, the activity of α3β1 might be downregulated so that the protein can no longer inter-

Figure 7. CD151 is localized in type I and II hemidesmosomes. α6β4-expressing PA-JEB/β4 cells were fixed and double-labeled with mAb 4.3E1 against β4 (A), mAb 815 against BP230 (B), mAb 121 against plectin (C) or mAb CD151 (F), and polyclonal antibodies against α3 (A, B, C and F). Cells were also double-labeled with mAb CD151 (D, E) and polyclonal antibodies against β4 (D) or BP230 (E). Only dual-labeled images are shown. In cells that express both type I and II hemidesmosomes, the areas containing CD151 are larger than that containing BP230 (E), whereas those containing CD151 and β4 are identical (D). Focal adhesions that surround the hemidesmosomal structures contain α3β1 (A–C). CD151 is partially codistributed with α3β1 (F). Bar, 5 μm.
The affinities of \( \alpha_3 \beta_1 \) and \( \alpha_6 \beta_4 \) for laminin-5 might also become different as a result from proteolytic processing of the \( \alpha_3 \) or \( \gamma_2 \) chain of the laminin-5 molecule (Giannelli et al., 1997; Goldfinger et al., 1998, 1999). It has been suggested that unprocessed laminin-5 is the primary ligand for \( \alpha_3 \beta_1 \), while \( \alpha_6 \beta_4 \) may preferentially interact with proteolytically processed laminin-5 (Burgeson and Christiano, 1997).

Since complex formation between tetraspans is well established and since their expression patterns broadly overlap, they may compensate for each other if one of them is lacking (Berditchevski et al., 1996; Yáñez-Mó et al., 1998; Fitter et al., 1999). The localization of CD151 in pre-hemidesmosomal structures and its recruitment into hemidesmosomes in PA-JEB cells after transfection with \( \beta_4 \), however, appears to be selective as the tetraspans CD9, CD63, and CD81, which are also expressed by keratinocytes, were not detected in these structures. The distinct distribution of CD151 suggests that at least one of its functions is different from those of the other tetraspans.

**The \( \alpha_6 \) Chain Is Critical for the Recruitment of CD151 into Hemidesmosomes**

Based on experimental data, it is generally assumed that integrins bind directly or indirectly to tetraspans by the extracellular domain of their \( \alpha \)-subunit (Mannion et al., 1996; Yauch et al., 1998). This assumption is supported by our finding that the IL-2R/\( \beta_4 \) chimera cannot recruit CD151 into hemidesmosomes. The mechanism by which this chimera becomes localized into the pre-hemidesmosomal structures is not clear. We consider a direct binding of the IL2R/\( \beta_4 \) chimera to CD151 unlikely, because CD151 contains only three small intracellular domains. Previous studies have indicated that binding to plectin is required for the localization of the IL2R/\( \beta_4 \) chimera at the basal side of PA-JEB keratinocytes (Nievers et al., 2000). However, it is unlikely that plectin by itself can mediate the localization of the chimera into the \( \alpha_3 \beta_1/CD151 \) clusters, because the protein is not concentrated at these sites (Schaapveld et al., 1998). Perhaps, plectin facilitates the interaction of \( \beta_4 \) with an as yet unidentified component in the \( \alpha_3 \beta_1/CD151 \) clusters and thus contributes to the localization of the chimera at these sites.

The finding that the IL2R/\( \beta_4 \) chimera is directed to pre-hemidesmosomal structures without clustering of CD151, whereas it remains capable of recruiting BP180 and BP230 (Nievers et al., 1998), suggests that CD151 is not essential for the formation of hemidesmosomes. However, it is important to mention that although the chimera becomes lo-
calized in pre-hemidesmosomal structures, these never seem to reach the size of the hemidesmosomal clusters as seen in the PA-JEB/β4 cells that stably express the integrin α6β4 (see Fig. 4). Thus, CD151 might have a role in stabilizing the hemidesmosomal structures and in this way in determining their size. However, definition of the exact role of CD151 in hemidesmosome formation awaits further analysis.

The Role of CD151 in the Spatial Organization of Hemidesmosomes and the Surrounding Focal Adhesions

A part from their spatial proximity, a link between hemidesmosomes and focal adhesions has previously been suggested, since they share plectin (Sánchez-Aparicio et al., 1997; Nievers et al., 1998; Geerts et al., 1999). In this paper, we provide evidence that CD151 is another component shared by these structures. This protein, however, is not an essential component of focal adhesions, and its expression seems to be affected by α6β4. Only in β4-deficient PA-JEB keratinocytes did CD151 appear to be a resident protein of focal adhesions, while in β4-transfected PA-JEB cells, its distribution seems to be dynamically regulated, varying between cells and even between focal adhesions in the same cell. The absence of CD151 in focal adhesions in PA-JEB/β4 cells appeared not to affect the localization of α3β1 because this integrin is colocalized with vinculin in focal adhesions before and after β4 transfection. An explanation for the shift in the distribution of CD151 from focal adhesions to hemidesmosomes after β4 transfection could be that the protein binds more strongly

Figure 9. Differential localization of CD151 and vinculin in PA-JEB compared with PA-JEB/β4 cells. Cells were fixed and double-labeled with mAb CD151 and polyclonal antibodies against vinculin (A and D), or with polyclonal antibodies against α3, and a mAb against vinculin (B and E) or to detect F-actin with TRITC-conjugated phalloidin (C and F). Only composite images (A–F), generated by superimposition of the green and red signals are shown. In PA-JEB cells, CD151 is codistributed with α3 in focal adhesions surrounding the pre-hemidesmosomal clusters, whereas in α6β4-expressing PA-JEB/β4 cells, the codistribution with α3 is only partial. In both PA-JEB and PA-JEB/β4 cells, the α3β1 is localized in focal adhesions together with vinculin and F-actin. Note that the focal adhesions at the cell periphery do not contain CD151 and α3. Bars: (A, B, D, and E) 10 μm; (F and C) 10 μm.
to αβ6γ4 than to α3β1. However, biochemical studies do not support this conclusion because complexes of α6β4 and CD151 could not be detected in cells that have been lysed in 1% NP-40, whereas under these conditions CD151 remains complexed with α3β1. CD151 cannot only associate with the integrin α3β1 but also with α6β1 (Y auch et al., 1998; Serru et al., 1999; our own unpublished results). We established previously that expression of α6β1 is up-regulated and localized to focal adhesions in β4-deficient PA-JEB cells (Schaapveld et al., 1998). Thus, it is possible that downregulation of α6β1 after β4 is expressed contributes to the loss of CD151 from focal adhesions. However, as a result of prolonged culturing, the PA-JEB keratinocytes had lost most of their surface α6β1 and then synthesized more α5β1. At this stage, therefore, the effect of α6β1 on the localization of CD151 can only be limited.

A complete physical separation of focal adhesions and hemidesmosomes may take place after the removal of CD151 and α3β1 from these structures, respectively. Subsequently, the hemidesmosomal structures may mature and then contain BP180 and BP230. The actin cytoskeleton that is concentrically located around hemidesmosomes and associated with the focal adhesions may act as a physical barrier for hemidesmosomal components, thereby confining them to and thus maintaining hemidesmosomes.

Although the presence of α3β1 in the pre-hemidesmosomal structures suggests an important role for this integrin in the formation of hemidesmosomes, results with knockout mice revealed that hemidesmosome formation can occur in the absence of α3β1 (D'Persio et al., 1997). It is possible that in the absence of α3β1 other integrins form complexes with CD151 which, when clustered at the cell base, can serve as nucleation sites for hemidesmosome assembly by α6β4. However, there is little support for this explanation, since CD151 seems to interact specifically with laminin-binding integrins. A more likely explanation for the presence of hemidesmosomes in the α3-null mice is that for the formation of hemidesmosomes, initial α3β1-mediated clustering of CD151 is not required, but that it only facilitates the subsequent recruitment of CD151/α6β4 into hemidesmosomal structures. In that case, hemidesmosomes can still be formed in the absence of α3β1, but the kinetics of their assembly might be different from that in wild-type mice. A internally, the structure and stability of hemidesmosomes may, in fact, be compromised in the α3-null mice in a way that it is not yet evident at the ultrastructural level when the mice die at birth.

The focal adhesions found at the cell periphery neither contain α3β1 nor CD151. These focal adhesions are probably assembled on fibronectin and vitronectin derived from serum. The integrins that interact with these adhesive ligands are α5β1 and αvβ3 and both may be involved in the initial adhesion and spreading of the cells. The absence of CD151 as well as of α3β1 in these peripheral focal adhesions is consistent with the finding that this tetraspan preferentially associates with the laminin-binding integrins α3β1 and α6β4 and provides further support for its role in hemidesmosome assembly and stability.

In summary, we demonstrate that CD151 is a newly detected hemidesmosomal component. We show that CD151 plays a role in the sequence of events, which take place in hemidesmosome assembly. A n additional role for CD151 in the cross-talk with the surrounding focal adhesions is suggested.

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