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Specific internalization of basal membrane domains containing the integrin α6β4 in dispase-detached cultured human keratinocytes

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Keratinocytes—dispase—integrin α6β4—internalization—recycling

The integrin α6β4 is polarized towards the basal side of basal keratinocytes and helps anchor these cells to the basement membrane components. We have found that cultured human epidermal keratinocytes, when detached from their culture substratum, as for grafting, using the enzyme dispase, rapidly internalize the basal membrane domains containing the integrin α6β4, while integrins of the very late antigen subtype remain on the cell surface. Detachment and incubation at 4 °C prevent this internalization, as well as the contraction of the detached sheet area. Subsequent incubation at 37 °C initializes this contraction and allows the basal integrin α6β4 to be internalized. We took advantage of this blockage to label upon detachment using immunogold techniques, the α6 subunit present on the basal cell surface; then we studied its internalization with the electron microscope. This internalization pathway differs from classical receptor-mediated endocytosis, and intermediate filaments might possibly play a role in this process. Interestingly, 1 h after their internalization from the basal membrane, a third of the gold particles labeling the α6 subunit was found between lateral membranes of basal cells, strongly suggesting that the integrin α6β4 can be partly recycled to the cell surface in these conditions.

Introduction

Integrins are transmembrane glycoproteins that are members of a widely expressed family of cell surface receptors [18]. They are involved in cell adhesion to the extracellular matrix as well as in cell-cell interactions. These αβ heterodimeric molecules are generally divided into several subfamilies, each based on a common β subunit which can eventually associate with different α subunits. However, it is now clear that a few α subunits can also be associated with different β subunits [3, 18]. These various combinations produce different specificities for ligands, but certain integrins are able to bind several ligands, and one ligand can be bound by several integrins [17]. Two other major characteristics of integrins are their structural role in making a connection between the extracellular structures and the cytoskeleton, and their role in signalling between the intracellular milieu and the outside of the cell [3, 19].

The epidermis is a polarized tissue composed mainly of stratified layers of keratinocytes which are organized to produce an impermeable insoluble layer made up of terminally differentiated cells: the stratum corneum. The multiple integrins expressed in the epidermis are thought to determine the spatial organization of the tissue: integrins are normally expressed by the basal proliferating keratinocyte population and disappear in the suprabasal differentiating layers [2, 5, 10, 24]. The acute regulation of their function and expression [1, 19] supports the numerous suggestions that integrins play a crucial role in the behavior of cells in a wide variety of normal and pathological situations: tissue organization during development [14], wound healing [13, 15], tumor invasion [7] and psoriasis [15, 22, 31].

With the electron microscope, it can be seen that the attachment of epidermal keratinocytes to the underlying basement membrane and connective tissue is performed by highly structured adhesion complexes called hemidesmosomes, which are the basal plasma membrane anchoring sites for intermediate filaments [44]. These structures are progressively understood as their molecular content, and organization is decrypted. For example, the 230 kDa bullous pemphigoid antigen (BPA) [30], collagen VII [21, 46], kalinin [40], HD1 [16], and particularly the α6β4 complex as an integral membrane protein [20, 37, 43, 46], have all been assigned to the hemidesmosome. The localization of α6β4 suggests that this polarized integrin is of great importance in the stable adhesion of epidermal cells to the basement membrane in vivo and in vitro [5, 9, 29, 41], but this integrin could also be responsible for organizing the hemidesmosome since the presence of an antiserum directed against α6β4 impedes the reappearance of this structure in replated hemidesmosome-forming cells [20]. To date, however, there is still some discrepancy about this integrin's ligand, but laminin remains a serious candidate [27, 37].

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Recently, using dispase in order to detach the keratinocyte cultures as intact sheets [12, 45], we observed that the adhesion of the keratinocytes to an extracellular substratum controls the polarized organization of the cultured tissue into superposed proliferating and differentiating layers [36]. As clusters of basal cells were observed in the detached cultures, we suspected that integrins were responsible for this phenomenon. Here we report investigations on the fate of integrins of the very late antigen (VLA)-subtype (β1 subfamily) and integrin α6β4 when basal keratinocytes are detached from the culture substratum with dispase. We focus especially on the internalization of α6β4 in these conditions.

Materials and methods

Cell culture

Human keratinocytes were isolated from adult skin epidermis obtained after plastic surgery and cultured in the presence of a feeder layer of mitomycin C-treated 3T3 cells, in culture medium composed of one part Ham's F12 and three parts Dulbecco's modified Eagle's medium [39]. This was supplemented with 10% fetal calf serum, 5 μg/ml insulin, 0.4 μg/ml hydrocortisone, 10^{-10} M cholera toxin, 5 μg/ml transferrin, 2×10^{-9} M triiodothyronine, 1.8×10^{-4} M adenine and 10 ng/ml epidermal growth factor. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere and the medium was changed every 2 to 3 days. At confluence, the cultures were trypsinized and Multi 6 or 12-well culture plates were inoculated with 1×10^4 cells per cm^2 in the presence of the same feeder layer and cultured until confluence.

Detachment of cultured keratinocyte sheets

The cultures were first treated with 0.25% (w/v) dispase II (Boehringer Mannheim, Mannheim/Germany) in a serum-free medium, according to the method of Green and coworkers [12], for 1 h at 37 °C. Then, the incubation at 37 °C in dispase was prolonged until the cultures were completely detached; alternatively, where indicated, the incubation was prolonged at 4 °C for about 2 h before the cultured sheets were gently detached using forceps.

Storage and analysis of the detached sheets

After detachment, the keratinocyte sheets were rinsed twice in phosphate-buffered saline (PBS). Cultures detached at 37 °C were then stored in culture medium at 37 °C for different incubation times, while cultures detached at 4 °C were stored either at 4 or 37 °C. To obtain the measurements of sheet diameters, triplicate cultures were photographed and the maximum diameter was worked out on prints, using the diameter of the culture dish in order to calculate the magnification, as already described [36].

Immunofluorescent labeling of integrins

Samples of detached keratinocyte cultures were coated in O.C.T. (Tissue-Tek, Miles Inc., Elkhart, IN/USA) and immediately frozen in a methanol bath cooled with dry ice. Frozen sections perpendicular to the culture [2] were cut at 5 μm in a Microm HM 500 OM cryostat (Microm, Heidelberg/Germany) at −20 °C and stored at −80 °C until use. The fluorescent staining of integrins was slightly modified from published methods [14]. Essentially, sections were fixed for 30 min in a 4% formaldehyde PBS solution containing calcium (1 mM) and magnesium (1 mM), washed in 0.1 M glycine and blocked in 0.1% bovine serum albumin (BSA) and 0.02% Triton X-100-containing PBS. Primary antibodies, adequately diluted in the blocking solution, were performed in a humidified chamber at room temperature for 60 min; this was followed by three 15 min washes in the same solution. Fluorescein isothiocyanate (FITC)-conjugated anti-rat or anti-mouse secondary antibodies (Dakopatts, Ghent/Belgium) were also diluted in this solution and incubated for 60 min as above, with three subsequent 15 min washes. The slides were then mounted in Tris-buffered (pH 8.9) 90% glycerol containing 2.5% 1,4-diazabicyclo(2,2,2)octane (Sigma, St. Louis, MO/USA) to retard photobleaching; they were examined using a Zeiss photomicroscope equipped with RS-III epicondensor and FITC-specific filters and dichroic mirror. The primary antibodies against human integrin subunits used throughout the present study were: the rat monoclonal GoH3 against α6 subunit (donated by A. Sonnenberg, University of Amsterdam, Amsterdam/The Netherlands) [42], the rat monoclonal 439-9B against β4 subunit (donated by S. J. Kennel, Oak Ridge National Laboratory, Oak Ridge, TN/USA) [23], the mouse monoclonal DH12 against β1 subunit (donated by J.-J. Cassiman, Center for Human Genetics, University of Leuven, Leuven/Belgium) [10], and two commercially available mouse monoclonal antibodies against α2 and α5 subunits (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam/The Netherlands).

Immunogold labeling of the basal α6 subunit and analysis of gold localization

Keratinocyte cultures detached at 4 °C were used for the immunogold labeling of the basal surface α6 subunit, and the entire labeling procedure was performed at 4 °C. The cultures were first rinsed in calcium (1 mM) and magnesium (1 mM)-containing PBS. BSA (0.1%) was added to this PBS, and the cultures were washed three times for 10 min in this solution in order to block non-specific surface labeling. They were then incubated for 60 min with the primary antibody (a 1:10 dilution of culture supernatant of clone GoH3 was prepared in the blocking solution) and then for three 10 min washes in the blocking solution alone. The cultures were labeled for electron microscopic observation using 10 nm gold-labeled goat anti-rat IgG (Amersham, Ghent/Belgium) diluted at 1:20 in the BSA-containing PBS, followed by three 10 min washes in this PBS. At this moment, cultures were immediately fixed (0 min) or transferred to culture medium at 37 °C and further incubated for 5, 15 or 60 min at this temperature before fixation, processing for electron microscopy and observation as previously described [34]. This experiment was repeated 5 times with keratinocytes cultured from two unrelated donors with identical results. For the analysis of the internalization process and gold localization, during the incubation at 37 °C, of cultures labeled for the α6 subunit, random regions of immunolabeled sections were sampled; this was done by taking 80 micrographs of the basal cell layer at intervals using a uniform magnification (33000×). A blind procedure was then used: the micrographs corresponding to the different incubation times were pooled and mixed by an uninvolved person and received a code number at random before being examined by three of us. The total number of gold particles per micrograph was determined, and the particles were classed in four previously defined localizations: *1)* bound to the surface of the basal membrane, *2)* internalized in vesicles observed within 1.5 μm of the basal cell surface, *3)* internalized in more deeply located vesicles, and *4)* present in the intercellular space bound to the lateral membranes of basal cells. The code was then broken, the observations were pooled, and the number of gold particles found in each category was expressed as the percentage of the total number of particles observed for each incubation time (0, 5, 15 and 60 min) at 37 °C.

Results

Immunofluorescent localization of integrins after dispase detachment of keratinocyte cultures

Keratinocyte cultures were treated for 1 h with dispase at 37 °C and then for 2 h at 4 °C before gentle detachment with forceps. The detached sheets were then rapidly cryofixed for the preparation of frozen sections, and the fluorescent staining of integrins showed us the integrin α6β4 localized in the plasma membrane of the basal cells, but polarized particularly towards their basal membranes (Figs. 1a, b). Simultaneously, the integrins of the VLA-subtype, localized with the DH12 antibody to β1, were also observed in the basal keratinocytes only, but preferentially in sites of cell-cell contact (Fig. 1c).

On the other hand, when the cultures were detached in dispase at 37 °C, as originally described [12], the fluorescent staining showed the integrin α6β4 present over the entire margin of the basal cells, but especially its presence in the ventral cytoplasm of these cells was revealed by brightly fluorescent spots obtained with both GoH3 (Fig. 1d) and 439-9B (Fig. 1e) antibodies, suggesting the internalization of the α6β4-containing basal membrane domains in these conditions. At the same time, the staining with the DH12 antibody confirmed the preferential presence of integrins of the β1 subfamily at the margin of basal cells, concentrated on lateral and apical membranes, while no particular labeling was seen in the ventral cytoplasm of the cells (Fig. 1f). This localization was perfectly confirmed with antibodies against α2 and α5 subunits (data not shown).

The localization of α6β4 and VLA-subtype integrins, studied during the 4 h that followed dispase detachment of keratinocyte sheets, is also illustrated in Figure 1. During this storage, the reorganization of the culture consisted mainly in a vertical elongation of the cultured basal cells, producing a thickening of the sheets, while a few cells were moving progressively towards the suprabasal layers (Figs. 1g–o) [36]. The fluorescent labeling tells us that the intracellular bright staining of α6β4 was moving upwards close

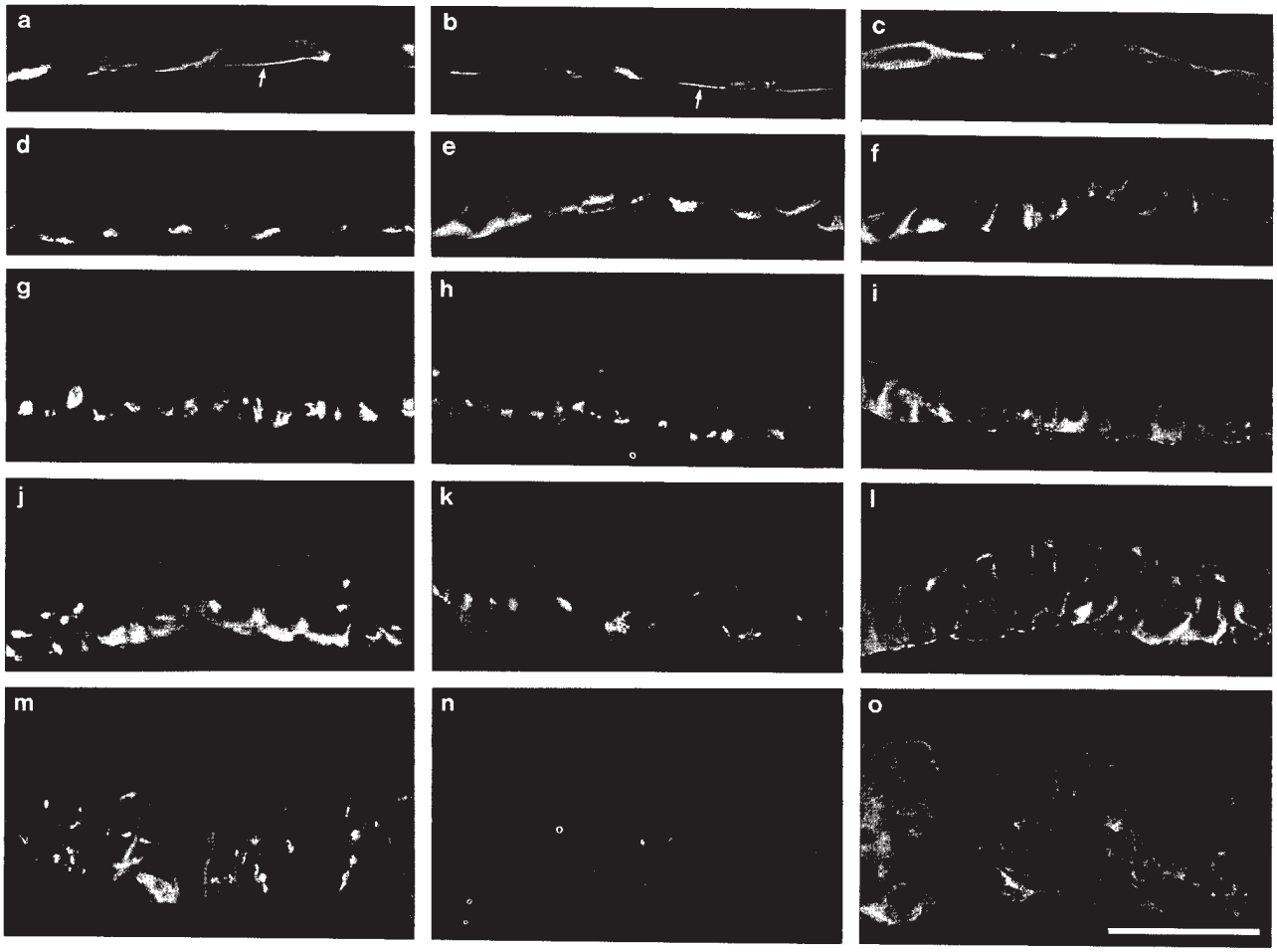

Fig. 1. Photomicrographs of cryostat sections made in cultured keratinocyte sheets dispase-detached at 4 °C (**a–c**) or 37 °C (**d–o**) and stored at this temperature for 0 h (**d–f**), 1 h (**g–i**), 2 h (**j–l**) or 4 h (**m–o**). The sections were immunolabeled for the integrin α6 subunit (**a, d, g, j, m**), β4 subunit (**b, e, h, k, n**) or β1 subunit (**c, f, i, l, o**). Note contraction and thickening of sheets during the incubation at 37 °C. *Arrows* in (**a**) and (**b**) indicate the intense basal labeling. — Bar 50 μm.

to the nucleus during the first hour following detachment (Figs. 1g, h). Subsequently, the bright spots maintained this position or sometimes, decreasing in size, moved towards the lateral membranes (Figs. 1j, k, m, n). Simultaneously, the integrins of the β1 subfamily remained on the margin of basal keratinocytes and were not incorporated in invaginating membranes (Fig. 1i, l, o).

The contraction of detached cultured keratinocyte sheets is dependent on the temperature

It has been observed by investigators, preparing dispase-detached human keratinocyte cultures for grafting, that the sheets contract rapidly to approximately 40 to 50% of their original area after detachment [6, 12, 33]. On storage, this rapid initial contraction is followed by a slower one that accompanies the spatial reorganization of keratinocytes [36]. The detachment of cultures performed at 4 °C revealed that the cells remain flattened upon detachment (Figs. 1a–c) and storage (Fig. 2) at this temperature. However, an immediate or delayed incubation at 37 °C rapidly induces their contraction and decreases the mean diameter of the sheets (Fig. 2).

The integrin α6β4 is internalized with specific basal membrane domains in detached keratinocytes

Advantage was taken of the fact that in flattened cells the polarized integrin α6β4 remains present on the surface of the basal membrane (Figs. 1a, b) and the α6 subunit was extracellularly immunogold-labeled with GoH3 for an ultrastructural study. This antibody is known to perturb cell adhesion and thought therefore to recognize an extracellular epitope on this protein [25, 43]. When the detached cultures were fixed immediately after labeling (0 min), the gold particles were found exclusively bound to the basal cell surface (Fig. 3). They were found isolated or often clustered. When clustered, the gold particles are bound over membrane domains characterized by a dense structure on the cytoplasmic side of the membrane (Fig. 3). These domains correspond to the structures that have been called 'stable anchoring contacts' in cultured keratinocytes [5] and which are immature hemidesmosomes consisting of cytoplasmic attachment plaques and sometimes subbasilar dense plaques (Figs. 3, 4) [6]. Although the cultures had been immersed during the labeling procedure, absolutely no particle was found on the apical membrane of the culture, demonstrating that the labeling is specific to an antigen present on basal layer cells only. Furthermore, when the GoH3 antibody was omitted, no labeling was found on the keratinocytes.

When detached sheets were α6-labeled on their basal membrane and then incubated for 5 min at 37 °C before fixation, numerous pits, including the gold-labeled immature hemidesmosomes, were observed forming from the basal cell surface (Fig. 4). These invaginating structures differ from classical coated pits by the absence of any clathrin coat on their cytoplasmic face. Interestingly, intermediate filaments are found between these invaginations and the nucleus while microfilaments are forming a basal cushion near the blebbing detached membrane (Fig. 4).

If the incubation period was prolonged to 15 min at 37 °C, the gold particles were found in deeply internalized vesicles of both small and large sizes, linearly arranged under the cell nucleus (Fig. 5) and corresponding to the position of the intracellular immunofluorescent staining of α6β4 in cryostat sections (Figs. 1d, e). Again, intermediate filaments were found only above the gold-labeled vesicles while microfilaments were clearly localized in close proximity to the ruffled basal membrane (Fig. 5).

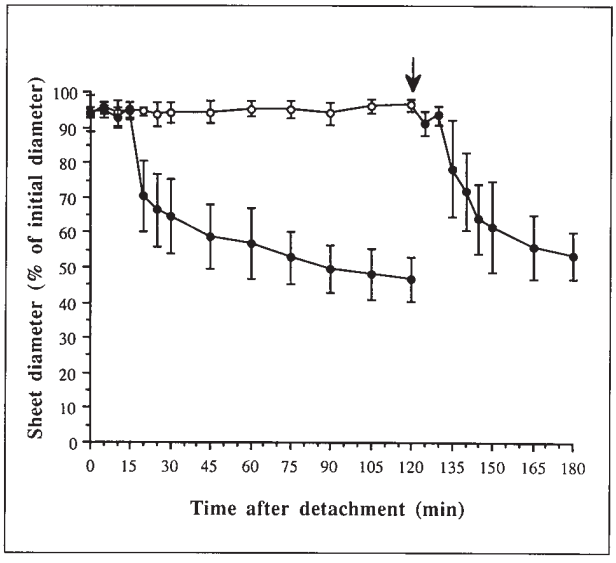

Fig. 2. Diameter of epidermal sheets dispase-detached at 4 °C and stored for different periods at 4 °C (o) or 37 °C (•). The *arrow* indicates the moment when the incubation temperature is raised from 4 to 37 °C. Each point represents the mean ± SE of diameters of triplicate sheets processed simultaneously.

The internalized integrin α6β4 is partly recycled to the plasma membrane

After 60 min of incubation at 37 °C, gold particles were still found in cytoplasmic vesicles similar to those labeled after 15 min; in addition, several were found in small vesicles located in close proximity to lateral plasma membranes, with numerous other gold particles also being observed between the basal cells, in the intercellular spaces (Fig. 6). This relatively abundant intercellular localization strongly suggests that part of the internalized α6β4 had been recycled to the lateral plasma membranes. In order to roughly quantify the pathway followed by the α6 subunit, we established a blind procedure to analyze the gold particle localizations during the process of internalization. Four localizations were defined, and the percentage of gold particles found in each of them was worked out from a large number of observations made on specially photographed cell areas (Tab. I). These results demonstrate that the incubation at 4 °C actually impedes the internalization of the α6-containing domains of the basal membrane. They also show the induction of the internalization phenomenon and the intracellular migration

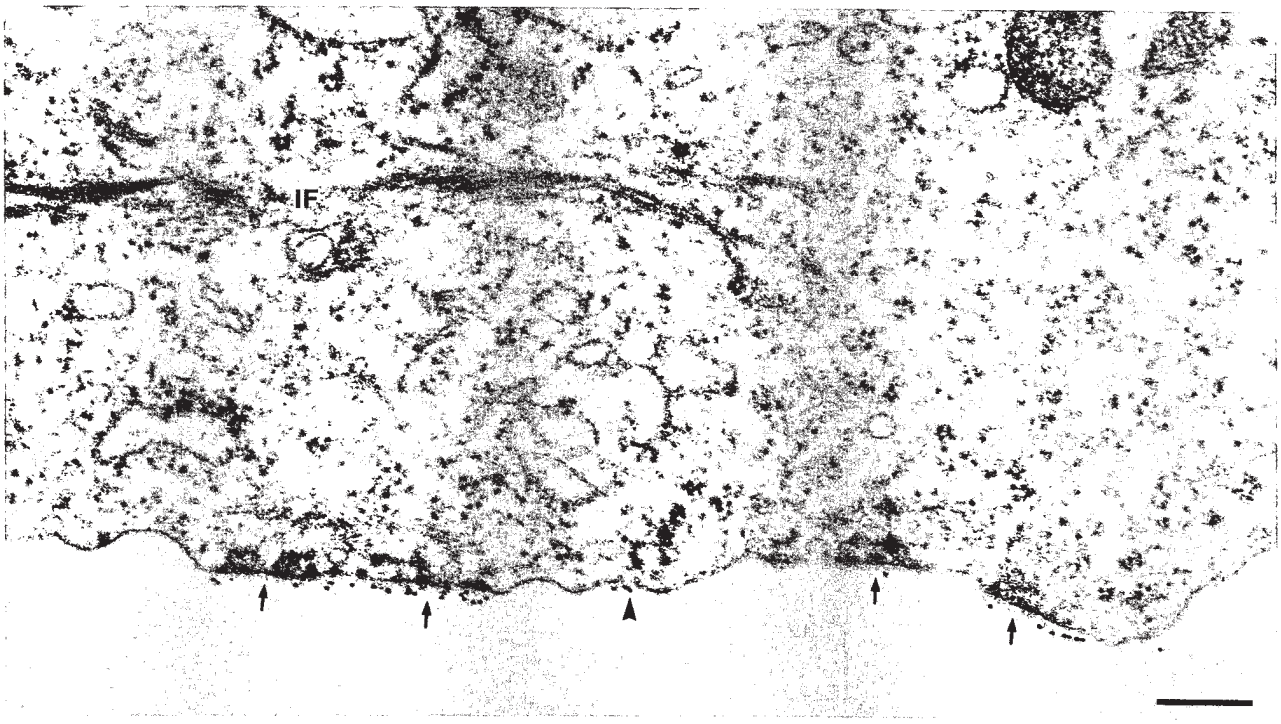

Fig. 3. Immunogold labeling of the α6 subunit on the basal surface of cultured keratinocytes detached at 4 °C and fixed immediately after the labeling procedure (0 h). Gold particles are concentrated on the basal surface of detached cells over immature hemidesmosomes *(arrows)* or over a membrane domain with no cytoplasmic plaque *(arrowhead)*. Intermediate filaments (IF) are frequently seen near the basal membrane. — Bar 0.25 μm.

of these domains when the keratinocytes are incubated at 37 °C. Very interestingly, they indicate that after 1 h at this temperature, a third of the gold label is bound to the lateral membranes, a new localization that strongly suggests a recycling phenomenon of α6β4. Indeed, pictures of gold-labeled α6 subunits secretion were very frequently observed (Fig. 6), and the quantitative results (Tab. I) suggest also that these come from intracytoplasmic localizations.

Discussion

The utilization of dispase-detached human keratinocyte cultures for clinical purposes is abundantly illustrated in the literature (for a review, see for example [32]) and only a few investigations on the consequences of this detachment of epidermal cells exist. Green and coworkers [12] demonstrated that detachment with dispase does not reduce the ability of keratinocytes to form new colonies, but we have just shown that lengthier storage at 37 °C is detrimental to these cells [35]. However, storage performed at room temperature is probably better in order to preserve the proliferative ability of the detached keratinocytes for several hours [8]. It has been shown also that blebs are present on the attachment face of detached basal cells [11] and that small foci of plasma membrane disruption are occasionally associated with the blebs [6]. Moreover, after several hours' storage, basal cells of dispase-detached keratinocyte cultures form clusters [36]. Intrigued by this clustering phenomenon, we decided to investigate the role of integrins in this process since these adhesion molecules are expressed in the basal keratinocytes only [2]. Here we report that, whereas integrins of the VLA-subtype involved mainly in cell-cell adhesion [26] remain on the cell surface, conversely the polarized integrin α6β4 is rapidly internalized from the released basal side when the keratinocytes are detached, in this case with dispase. In consequence, α6β4 is then uniformly expressed on the cell surface and the basal cells lose their characteristic polarity. Very recently, studies on suction blisters revealed in the detached epidermis (the roof of blisters) pictures suggesting also the internalization of α6β4 [15]. These observations concur exactly with what we have found in dispase-detached epidermis isolated from skin biopsies (Roland, Poumay, Leclercq-Smekens, unpublished data). Thus, in view of these immunofluorescent results, we first thought that α6β4 could be internalized by a process similar to receptor-mediated endocytosis, as has been already illustrated for other integrins [28, 38]. Keratinocyte cultures were, therefore, detached at 4 °C in order to block the internalization, and the α6 subunit was labeled with colloidal gold, in accordance with classical strategies in studies of receptor-mediated endocytosis [28, 34, 38]. Using this technique, the α6 was found to be almost exclusively present on the attachment face of basal cells: none were found in coated pits, a few were dispersed over non-specific membrane areas, while most were bound to immature

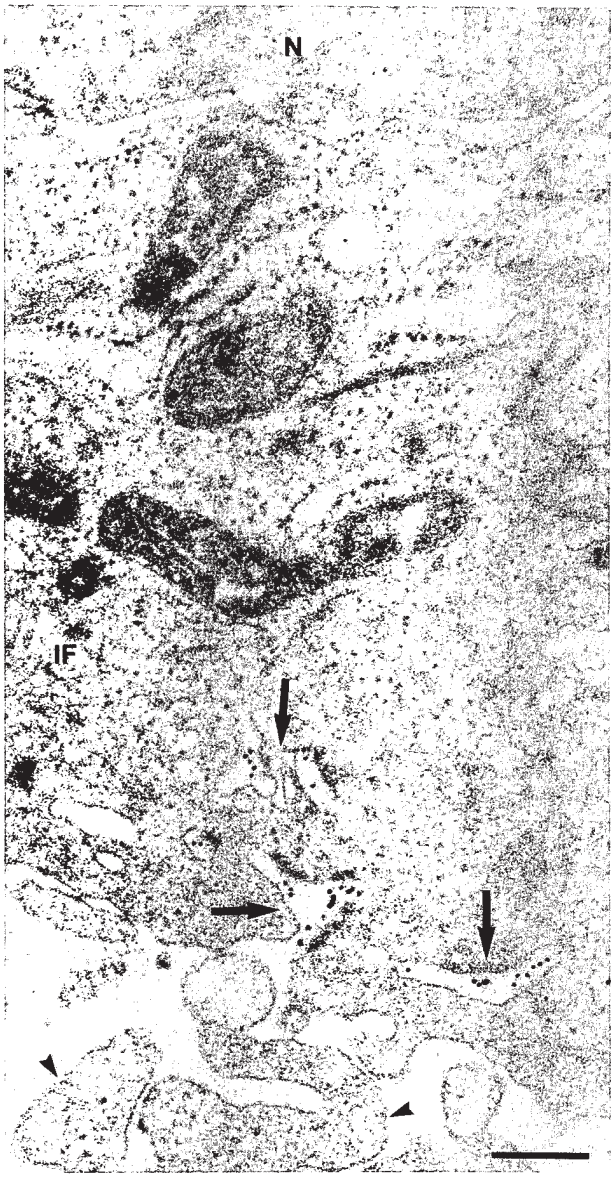

Fig. 4. Immunogold labeling performed at 4 °C of the α6 subunit on the basal surface of detached keratinocytes. The labeled cultures were then incubated for 5 min at 37 °C before tissue fixation. The electron micrograph shows a basal cell's ventral cytoplasm, between the cell nucleus (N) and the basal surface forming blebs *(arrowheads)*. The *arrows* indicate invaginating membrane domains containing α6-labeled immature hemidesmosomes. Intermediate filaments (IF) are observed in close proximity. — Bar 0.25 μm.

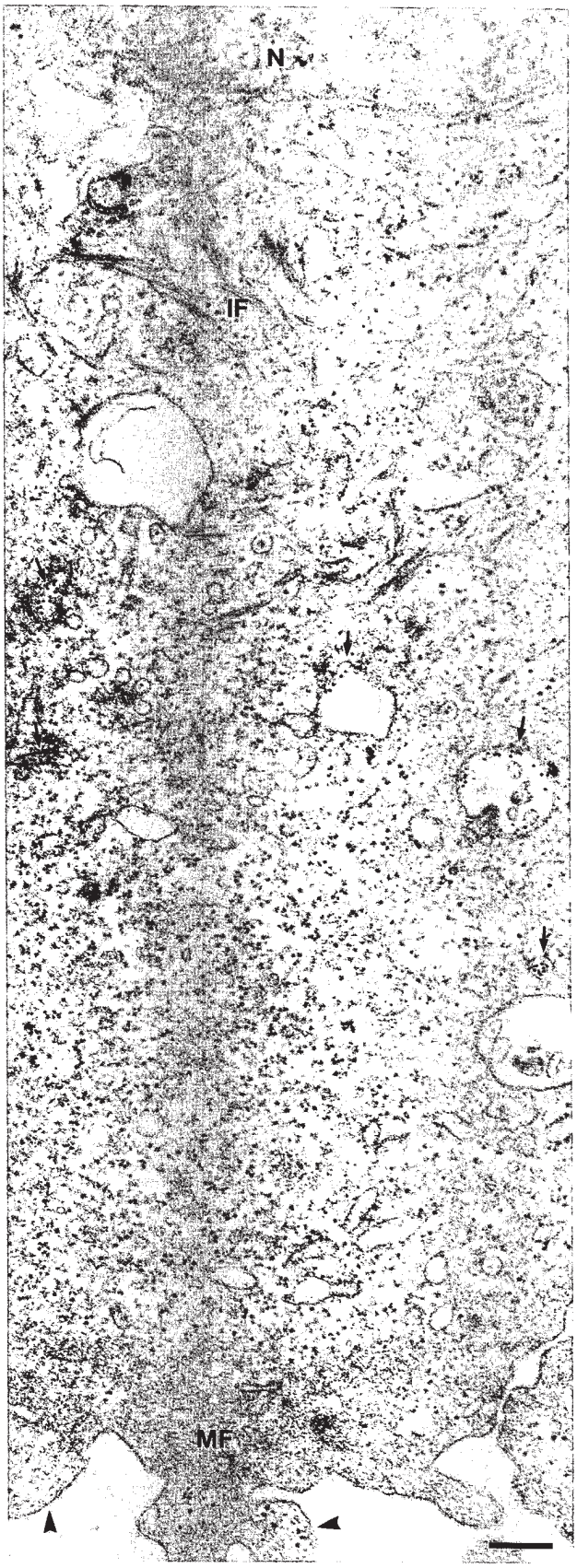

Fig. 5. Immunogold labeling performed at 4 °C of the α6 subunit on the basal surface of detached keratinocytes. The labeled cultures were then incubated for 15 min at 37 °C before tissue fixation. The electron micrograph illustrates a basal cell's ventral cytoplasm, between the cell nucleus (N) and the basal surface forming blebs *(arrowheads)*. Gold-labeled internalized vesicles *(arrows)* are located between the intermediate filaments (IF) and an "organelle-poor region" where microfilaments (MF) are observed. — Bar 0.25 μm.

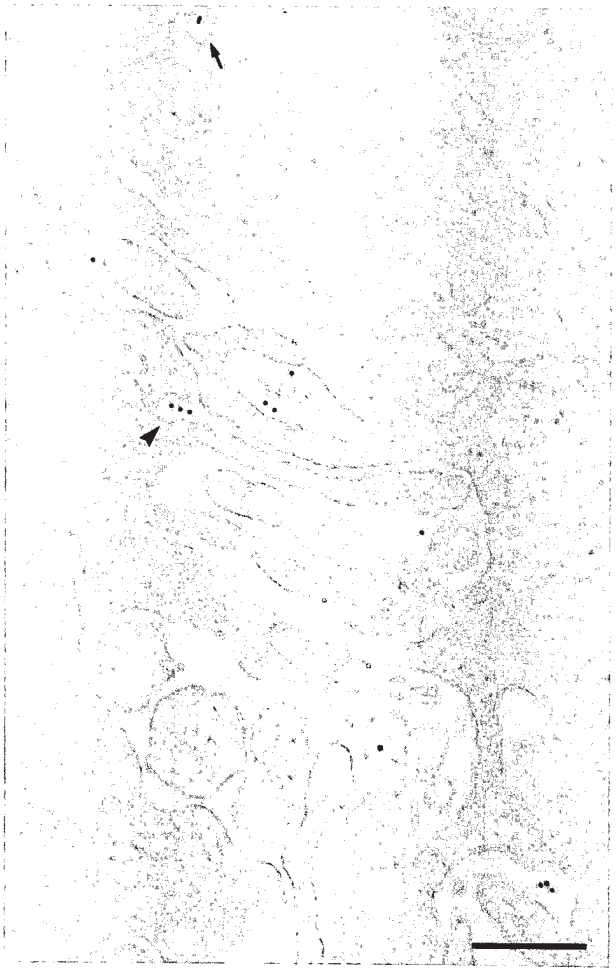

Fig. 6. Immunogold labeling performed at 4 °C of the α6 subunit on the basal surface of detached keratinocytes. The labeled cultures were then incubated for 60 min at 37 °C before tissue fixation. Detail of an intercellular space containing gold particles located between the lateral membranes of detached keratinocytes. The label is sometimes seen in small vesicles located beside the lateral membranes *(arrow)* or in vesicles that open in the intercellular space *(arrowhead)*. — Bar 0.25 μm.

Tab. I. Localization of the basal membrane integrin α6 subunit after detachment of the keratinocyte cultures with dispase and incubation at 37 °C.

Cellular localization	Incubation time (min)			
	0	5	15	60
Basal membrane	96.7%	39.7%	5.7%	6.1%
Intracellular (within 1.5 μm of the basal membrane)	1.1%	58.7%	27.8%	3.6%
Intracellular (others)	0%	1.4%	60.2%	57.2%
Lateral membranes	2.1%[a]	0.2%	6.4%	33.1%
Particles counted	615	441	753	362

[a] At this time, most gold particles in this category are located in close proximity to the basal surface. — Data are expressed as percentages of gold particles found in a particular cellular localization after the indicated times of incubation at 37 °C of the keratinocyte cultures previously detached at 4 °C in 0.25% dispase and immunogold-labeled using the anti-α6 (GoH3) antibody as primary antibody.

hemidesmosomes (Fig. 3). These structures are present along the attachment face of cultures fixed on plastic, but Compton and coworkers [6] did not find them any more after dispase detachment. Indeed, we show here that, when incubated at 37 °C, the labeled membrane domains internalize very rapidly in cultured cells. These observations confirm that hemidesmosomes are structures that epithelial cells internalize when trypsin [47] or dispase [30] are used to disrupt anchoring. This phenomenon seems to occur so rapidly in detached cultured keratinocytes, that it was not seen before in cultures detached at temperatures higher than 4 °C.

Why then are hemidesmosomes internalized upon basal cells detachment? In our results, the immature hemidesmosomes disappear within 15 min of internalization. This suggests that the internalization could take part in the dismantling of those highly structured adhesion complexes, taking the individual pieces for future utilization [11]. Consequently, the integrin α6β4 can be redistributed uniformly over the cell surface, as has been observed with migrating activated keratinocytes in a corneal wound healing model [25]. Thus, it could be that the partial recycling of the α6 subunit that was observed is complementary to the dismantling of the hemidesmosomes. The integrin recycling does in fact allow its rapid redistribution over the cell surface. Of course, further investigations, for example, with an antibody that recognizes the extracellular portion of the β4 subunit, should be necessary to assess whether at the ultrastructural level this subunit strictly follows the same pathway as its associated α6. Nevertheless, our results are in close agreement with the observation that α6β4 is a circulating integrin in A431 cells [4].

In view of our results, one may also wonder whether this internalization is a constitutive process or an emergency response to a sudden detachment of basal cells from the substrate. The massive internalization that we describe in this paper might well be scheduled in order to destroy the hemidesmosomes, because these highly structured stable adhesion complexes become unnecessary in detached cells. These cells would then be able to reorganize new hemidesmosomes upon readherence to a substrate [11], maybe around the α6β4, as has been proposed by others [20]. In other words, it is tempting to speculate that this internalization is not detrimental, but instead beneficial, to the readhesion of grafted keratinocytes on to a wound bed.

Of interest also is the difference between the internalization of α6β4 present in hemidesmosomes and classical receptor-mediated endocytosis. In the former process, cytoplasmic anchoring plaques, probably linked to intermediate filaments, characterize the majority of the pits, instead of a clathrin coat. The localization of cytoskeleton components

in detached keratinocytes is also of interest. First, it looks as if the internalization of basal membrane domains containing the integrin α6β4 could result from the retraction of intermediate filaments around the cell nucleus, exactly as has already been proposed for trypsin-isolated keratinocytes [47]. On the other hand, microfilaments remain near the blebbing basal membrane, a localization that suggests their involvement in the culture contraction. Treatment of detached cultures with cytochalasin D does in fact very much impede their contraction at 37 °C (unpublished data). However, this contraction could also result, at least in part, from the internalization phenomenon itself, since, upon detachment, numerous basal membrane domains rapidly form pits and thus reduce the basal membrane area.

The function of intermediate filaments is still an enigma, but the hypothesis of Quaranta and Jones [37], who suggested that the α6β4 could be involved in the transduction of signals from the cell surface to the nucleus via intermediate filaments, is designed to assign a possible role to these cytoskeletal elements. Interestingly, if this assumption is correct, any type of basal keratinocyte detachment could result in effects on gene expression, as it induces the internalization of α6β4-containing hemidesmosomes. The main consequences of keratinocyte detachment using trypsin [48] or dispase [36] are in fact the loss of cell proliferation and the induction of terminal differentiation.

We conclude with the reminder that in normal skin, hemidesmosomes are only observed on the basal surface of basal cells. Their normal fate, when these cells begin to differentiate and prepare to move upwards, is still unclear. Are they discretely internalized in an undetected manner or progressively dismantled when still present on the basal surface? These are challenges for future investigations.

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