Transforming Growth Factor-β1 Modulates β1 and β5 Integrin Receptors and Induces the de novo Expression of the αvβ6 Heterodimer in Normal Human Keratinocytes: Implications for Wound Healing

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Abstract. The molecular mechanism underlying the promotion of wound healing by TGF-β1 is incompletely understood. We report that TGF-β1 regulates the regenerative/migratory phenotype of normal human keratinocytes by modulating their integrin receptor repertoire. In growing keratinocyte colonies but not in fully stratified cultured epidermis, TGF-β1: (*a*) strongly upregulates the expression of the fibronectin receptor α5β1, the vitronectin receptor αvβ5, and the collagen receptor α2β1 by differentially modulating the synthesis of their α and β subunits; (*b*) downregulates the multifunctional α3β1 heterodimer; (*c*) induces the de novo expression and surface exposure of the αvβ6 fibronectin receptor; (*d*) stimulates keratinocyte migration toward fibronectin and vitronectin; (*e*) induces a marked perturbation of the general mechanism of polarized domain sorting of both β1 and β4 dimers; and (*f*) causes a pericellular redistribution of αvβ5. These data suggest that α5β1, αvβ6, and αvβ5, not routinely used by keratinocytes resting on an intact basement membrane, act as "emergency" receptors, and uncover at least one of the molecular mechanisms responsible for the peculiar integrin expression in healing human wounds. Indeed, TGF-β1 reproduces the integrin expression pattern of keratinocytes located at the injury site, particularly of cells in the migrating epithelial tongue at the leading edge of the wound. Since these keratinocytes are inhibited in their proliferative capacity, these data might account for the apparent paradox of a TGF-β1-dependent stimulation of epidermal wound healing associated with a growth inhibitory effect on epithelial cells.

HUMAN epidermis, the outermost layer of skin, is a stratified squamous epithelium mainly composed of a single cell type, the keratinocyte. The epidermis survives through a self-renewal process (Green, 1980). Small progenitor keratinocytes (Barrandon and Green, 1987*b*), forming the innermost epidermal basal layer, regularly undergo mitosis, differentiation, and upward migration to replace terminally differentiated cornified cells that are continuously shed into the environment (Green, 1980; Watt, 1989; Fuchs, 1990). Basal epidermal keratinocytes rest on a basement membrane composed of a specific subset of extracellular matrix proteins such as laminin, type IV collagen, kalinin, nidogen, and heparan sulfate proteoglycan. The firm adhesion of basal keratinocytes, hence of the whole epidermis, to the basal lamina is mediated by hemidesmosomes. These structures link the epithelial intermediate filament network to the dermal anchoring fibrils, which are mainly composed of type VII collagen and extend from the basement membrane to anchoring plaques in the papillary dermis (Jones et al., 1994). The keratinocyte behavior changes dramatically when a wound occurs and the epidermis undergoes regeneration. Indeed, wound healing is a complex phenomenon that occurs through a sequence of controlled events including: (*a*) an inflammatory stage involving aggregation of platelets and recruitment of macrophages, fibroblasts, and lymphocytes at the injury stie; (*b*) the formation of a provisional extracellular matrix, mainly composed of fibrinogen, fibrin, collagens, fibronectin, tenascin, and vitronectin; (*c*) the recruitment of epidermal stem cells at the injury boundary, as well as the formation of an epithelial tongue of migrating keratinocytes at the very edge of the wound; and (*d*) the local synthesis and secretion, operated by the cell types mentioned above, of a wide variety of growth factors and cytokines regulating the formation of the granulating tissue, the migration and proliferation of keratinocytes, and the final remodeling of the scar, through a

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network of autocrine and paracrine loops (Barrandon and Green, 1987*a*,*b*; Pierce et al., 1989, 1994; Mustoe et al., 1991; Wenczak et al., 1992; Staiano-Coico et al., 1993; Weidner et al., 1993; Di Marco et al., 1993*b*; Juhasz et al., 1993; Bennet and Schultz, 1993; Rochat et al., 1994; Boyce, 1994; Katz and Taichman, 1994).

Thus, migrating and proliferating keratinocytes at the injury site lose contact with the basal lamina and are exposed to components of the provisional extracellular matrix. Accordingly, keratinocytes modify the expression, topography, and cytoskeleton association of integrin receptors, a class of transmembrane noncovalently associated glycoprotein heterodimers composed of α and β chains that mediate cell–cell interacitons and the adhesion, spreading, and migration of cells on several components of the extracellular matrix (for reviews see Hynes, 1992; Sonnenberg, 1993). We have previously shown that normal human basal keratinocytes express $\alpha6\beta4$, $\alpha2\beta1$, $\alpha3\beta1$, and $\alpha v\beta5$ integrin receptors, exposed on discrete plasma membrane regions in a polarized fashion (De Luca et al., 1990*b*; Marchisio et al., 1991). The $\alpha6\beta4$ heterodimer is sharply localized on the basal aspect of the basal cell, is a component of hemidesmosomes, and mediates keratinocyte adhesion to the basement membrane by binding to both laminin and kalinin (De Luca et al., 1990*b*; Stepp et al., 1990; Sonnenberg et al., 1991; Zambruno et al., 1991; Niessen et al., 1994; Rousselle and Aumailley, 1994); in vitro, this integrin maintains its polar distribution and is organized in typical patches showing a "leopard skin" pattern in which spots correspond to microfilament-free areas (Marchisio et al., 1991; 1993), as expected from its association with hemidesmosomes and hence intermediate filaments (Stepp et al., 1990; Sonnenberg et al., 1991; Jones et al., 1991). Instead, the $\alpha2\beta1$ and $\alpha3\beta1$ integrins are enriched laterally, roughly at cell–cell boundaries where cadherins, vinculin, and desmoplakins are also detected, and cooperate in regulating cell–cell interactions (Kaufman et al., 1989; De Luca et al., 1990*b*, Carter et al., 1990*b*; Larjava et al., 1990; Marchisio et al., 1991, but see Carter et al., 1991), possibly by forming homotypic or hybrid bonds (Symington et al., 1993; Sriramarao et al., 1993). We propose that this polarized topography of adhesion molecules may precede and be instrumental in building up the polarized organization of epithelia (Marchisio and De Luca, 1994). During the healing of acute wounds (Cavani et al., 1993; Larjava et al., 1993; Juhasz et al., 1993), in hyperproliferative skin diseases (Pellegrini et al., 1992; Savoia et al., 1993; Giannelli et al., 1994), or in keratinocytes forced into more frequent cell cycles (De Luca et al., 1992*b*), integrin polarization is lost. Moreover, in these conditions, the $\alpha5\beta1$ fibronectin receptor, normally undetectable in healthy adult epidermal cells (Peltonen et al., 1989; Klein et al. 1990; Carter et al., 1990*a*; Hertle et al., 1991; Pellegrini et al., 1992; Cavani et al., 1993; Savoia et al., 1993; Larjava et al., 1993), becomes clearly evident on the keratinocyte plasma membrane.

TGF-β belongs to a multifunctional cytokine family composed of three highly homologous genes, TGF-$\beta1$, -$\beta2$, and -$\beta3$, that encode polypeptides with similar biological functions in many biological systems (for review see Sporn and Roberts, 1992). In mammals, the TGF-β superfamily, which includes activin, Müllerian inhibitory substance, and bone morphogenetic proteins, plays crucial roles in regulating both embryonic development and tissue repair and regeneration after injury (Sporn and Roberts, 1992). TGF-$\beta1$ is abundantly released by platelets and macrophages at the injury site (where it is present in its active form) of human skin wounds, where it initiates a complex series of events, including chemoattraction of monocytes and leukocytes, production of cytokines and inflammatory mediators, regulation of fibroblast functions, induction of angiogenesis, and modulation of the synthesis of proteases and extracellular matrix proteins (for review see Border and Ruoslahti, 1992). Moreover, TGF-$\beta1$ is synthesized and secreted by keratinocytes at the reepithelialization front of the wound, and many animal studies have shown that exogeneously applied TGF-$\beta1$ enhances and accelerates epidermal wound healing (Mustoe et al., 1991; Quaglino et al., 1991; Levine et al., 1993; Schmid et al., 1993). TGF-$\beta1$ switches keratin production from keratin 1, associated with normal epidermal maturation, to keratins 6 and 16, associated with hyperproliferative epidermis (Mansbridge and Hanawalt, 1988; Cho and Fuchs, 1990).

Yet, TGF-$\beta1$ protently inhibits the growth of primary human keratinocytes and other epithelial cell types (see Moses et al., 1990). Therefore, we decided to investigate whether TGF-$\beta1$ might regulate the regenerative/migratory phenotype of wounded keratinocytes by modulating the expression and topography of their integrin receptors and hence the properties of their entire adhesive machinery. For a model system, we used normal human epidermal keratinocytes cultured in conditions allowing full epidermal differentiation (Rheinwald and Green, 1975). This system has many advantages, since keratinocytes form epithelial colonies and cohesive sheets closely resembling normal human epidermis and maintain the differentiation pattern of their in vivo counterpart, such as to be used for autologous and permanent grafting onto patients (Green et al., 1979; Green, 1980; Gallico et al., 1984; De Luca et al., 1988, 1989, 1990*a*; Romagnoli et al., 1990).

Here we show that TGF-$\beta1$ has a profound effect on the expression and topography of preexisting integrins and induces the de novo synthesis of a new heterodimer endowed with fibronectin-binding properties, that is, the $\alpha v\beta6$ integrin (Sheppard et al., 1990). These data uncover at least one of the molecular mechanisms responsible for the peculiar expression of integrins in healing human wounds, inasmuch as TGF-$\beta1$ reproduces the integrin expression pattern of in vivo keratinocytes localized at the injury site, particularly in the epithelial tongue at the very edge of the wound (Cavani et al., 1993; Larjava et al., 1993; Juhasz et al., 1993). Since these keratinocytes are highly migratory but do not proliferate (Wenczak et al., 1992; Schmid et al., 1993), these data might account for the apparent paradox of a TGF-$\beta1$–dependent stimulation of epidermal wound healing associated with a growth inhibitory effect on epithelial cells.

Materials and Methods

Antibodies

The rabbit polyclonal antiserum to $\beta3$ and the goat antiserum to $\beta1$ have been described (Marchisio et al., 1991). The rabbit polyclonal antiserum to $\beta6$ (Sheppard et al., 1990) was a generous gift from V. Quaranta (Scripps Research Institute, La Jolla, CA); the rabbit polyclonal antiserum to $\beta5$, the murine mAb B5-IA9 to $\beta5$, and the mAb TS2/7 to $\alpha1$ were gifts from M. Hemler, Dana Farber Cancer Institute, Boston, MA). Other mAbs, and the

investigators who kindly provided them, are as follows: Gi14 to $\alpha 2$ from S. Santoso (Institute of Clinical Immunology and Transfusion Medicine, Gieffen, Germany); J 143 to $\alpha 3$ from L. J. Old (Memorial Sloan Kettering Cancer Center, New York, NY); HP1/7 to $\alpha 4$ from F. Sanchez Madrid (Hospital de la Princesa, Madrid, Spain); GoH3 to $\alpha 6$ from A. Sonnenberg (The Netherlands Cancer Institute, Amsterdam, The Netherlands); 13C2 to αv from M. Horton (ICRF, London, UK); LM609 to $\alpha v \beta 3$ from D. Cheresh (Research Institute of Scripps Clinic, La Jolla, CA); and R6G9 to $\beta 6$ from D. Sheppard (Lung Biology Center, University of California at San Francisco, San Francisco, CA). Other mAbs were commercially obtained: K20 to $\beta 1$ and SAM1 to $\alpha 5$ (Immunotech, Marseille, France); 3E1 to $\beta 4$ (Telios Pharmaceuticals, Inc., San Diego, CA).

Cell Culture

3T3-J2 cells were a gift from Dr. Howard Green (Harvard Medical School, Boston, MA) and were cultured in Dulbecco-Vogt Eagle's medium (DMEM) containing calf serum (10%), glutamine (4 mM), and penicillin-streptomycin (50 IU/ml). Human recombinant TGF-$\beta 1$ was from Boehringer-Mannheim GmbH (Mannheim, Germany). Human platelet-derived purified TGF-$\beta 1$ was from R&D Systems, Inc. (Minneapolis, MN).

Human epidermal keratinocytes were obtained from skin biopsies of healthy volunteers and cultivated on a feeder layer of lethally irradiated 3T3-J2 cells as described (Green et al., 1979). In brief, skin biopsies were minced and trypsinized (0.05% trypsin/0.01% EDTA) at 37°C for 3 h. Cells were collected every 30 min, plated ($2.5 \times 10^4/cm^2$) on lethally irradiated 3T3-J2 cells ($2.4 \times 10^4/cm^2$), and cultured in 5% CO_2 and humidified atmosphere in keratinocyte growth medium: DMEM and Ham's F12 media (3:1 mixture) containing FCS (10%), insulin (5 μg/ml), transferrin (5 μg/ml), adenine (0.18 mM), hydrocortisone (0.4 μg/ml), cholera toxin (0.1 nM), triiodothyronine (2 nM), EGF (10 ng/ml), glutamine (4 mM), and penicillin-streptomycin (50 IU/ml). Subconfluent primary cultures were passaged in secondary cultures as described (De Luca et al., 1988). Experiments were carried out on secondary cultures. Keratinocytes, either in their exponential phase of growth or after reconstitution of a confluent sheet of stratified squamous epithelium, were incubated in complete medium or in serum-free medium containing 0.1% BSA for 4–36 h (see Results) in the presence or absence of 30 ng/ml of either human recombinant or platelet-derived TGF-$\beta 1$. Cells were then processed for immunofluorescence, immunoprecipitations, Northern blot analysis, and migration assays.

Immunoprecipitation

Immunoprecipitations were carried out on metabolically and surface-radiolabeled keratinocytes as previously described (Pellegrini et al., 1992; Zambruno et al., 1993). For metabolic labeling, cells were incubated for the last 12 h of the TGF-$\beta 1$ treatment (except for the time course experiment, see Fig. 2) in methionine-cysteine-free medium alone or supplemented with 30 ng/ml of TGF-$\beta 1$, in the presence of 100 μCi/ml of [^{35}S]methionine and 100 μCi/ml of [^{35}S]cysteine (Amersham International, Amersham, UK). After labeling, cells were detached with 10 mM EDTA in PBS, pH 7.4, and washed twice in PBS containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$. Cell surface labeling was performed on keratinocytes in suspension (20×10^6 cells/ml, in PBS), detached and washed as above. Iodination was carried out for 15 min at room temperature in the presence of 1 mCi/ml of [^{125}I]iodine (Amersham International), 0.25 mg of lactoperoxidase, and 0.001% H_2O_2. Cells were then washed four times in PBS containing 5 mM KI. Metabolically and surface-radiolabeled keratinocytes were lysed for 30 min on ice in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% deoxycolate, 1% Triton X-100, 0.1% SDS, 0.2% sodium azide), pH 8.5, containing PMSF (4 mM), aprotinin (0.2 TIU/ml), and leupeptin (10 μg/ml). Immunoprecipitations were carried out by overnight incubation at 4°C of the immunoadsorbents (antibodies adsorbed onto protein A-Sepharose [Pharmacia, Uppsala, Sweden) with samples of cell lysates, followed by extensive washing and elution by boiling in Laemmli sample buffer. Samples were then analyzed by SDS-PAGE under nonreducing conditions on 6% polyacrylamide gels, followed by autoradiography. Protein-bound radioactivity in cell lysates was counted, and equivalent amounts of radioactivity were immunoprecipitated for TGF-$\beta 1$-treated and control lysates. Relative intensities of bands on autoradiograms were quantified by scanning laser densitometry using a densitometer (UltroScan XL; Pharmacia LKB, Uppsala, Sweden).

RNA Blotting

The human cDNA integrin subunit-specific probes, and the investigators who kindly provided them, are as follows: $\alpha 2$ and $\beta 5$ probes from M. Hemler (Dana Farber Cancer Institute); $\alpha 5$,αv and $\beta 1$ from G. Tarone (University of Torino, Italy); $\beta 6$ from D. Cheresh (Research Institute of Scripps Clinic).

Northern blots were performed as previously described (Di Marco et al., 1991; Zambruno et al., 1993). Briefly, total cellular RNA was isolated by lysing keratinocytes with 4.2 M guanidine thiocyanate followed by cesium chloride gradient centrifugation, as described (Di Marco et al., 1991). Polyadenylated RNA was prepared from total RNA using Dynabeads (Oligo-$(dT)_{25}$; Dynal, Oslo, Norway), according to manufacturer's instructions. 30 μg of total RNA or 10 μg of poly(A^+) RNA were separated on 1% agarose gels containing formaldehyde and transferred to nylon membranes (GeneScreen Plus; Du Pont-New England Nuclear, Bad Homburg, Germany). After immobilization by short-wave UV exposure, blots were prehybridized at 42°C for 3 h in 50% deionized formamide, 0.75 M sodium chloride, 25 mM sodium phosphate, 5 mM EDTA, 0.2 mg/ml salmon sperm DNA, and 0.5% SDS. Hybridization buffer was identical to the above buffer with the addition of the indicated ^{32}P-labeled probes (2×10^6 cpm/ml) and 10% dextran sulfate. A final wash was done at 65°C for 30 min in 15 mM sodium chloride, 1 mM sodium phosphate, 1 mM EDTA, and 0.1% SDS. All filters were autoradiographed on x-ray films (Hyperfilm-MP; Amersham International) with intensifying screens at −70°C. Equal amounts of RNA were loaded, as assessed by ethidium bromide staining. Values were normalized for the density of the band obtained by probing the same filter with a specific rRNA probe (pXCR7; F. Amaldi, Rome, Italy). Relative intensities of bands on autoradiograms were quantified by scanning laser densitometry as described above.

Immunofluorescence

Keratinocytes from subconfluent primary cultures (10^4 cells/cm^2) were plated onto 24-well plates (Costar Corp., Cambridge, MA) containing 1.4 cm^2 round glass coverslips previously coated with feeder layer and cultured as described above. When keratinocyte colonies were evident in phase contrast microscopy (3–5 d after plating), colonies were treated for 24–36 h with TGF-$\beta 1$ as described above and processed for imunofluorescence. Coverslip-attached keratinocyte colonies were fixed in 3% formaldehyde (from paraformaldehyde) in PBS, pH 7.6, containing 2% sucrose for 5 min at room temperature. After rinsing in PBS, cells were permeabilized by soaking coverslips for 3–5 min at 0°C in Hepes-Triton X-100 buffer (20 mM Hepes, pH 7.4, 300 mM sucrose, 50 mM NaCl, 3 mM $MgCl_2$, and 0.5% Triton X-100). Indirect immunofluorescence was performed as previously reported (De Luca et al., 1990*b*; Marchisio et al., 1991). Briefly, the primary antibody (10 μg/ml) was layered on fixed and permeabilized cells and incubated in a humid chamber for 30 min. After rinsing in PBS–0.2% BSA, coverslips were incubated in the appropriate rhodamine-tagged secondary antibody (Dakopatts, Copenhagen, Denmark) for 30 min at 37°C in the presence of 2 μg/ml of fluorescein-labeled phalloidin (F-PHD; Sigma Chemical Co., St. Louis, MO). Coverslips were mounted in Mowiol (Hoechst AG, Frankfurt/Main, Germany) and observed in a photomicroscope (Axiophot; Zeiss) equipped with epifluorescence lamp and usually with planapochromatic oil immersion lenses. Fluorescence images were recorded on photographic films (T-Max 400; Eastman Kodak Co., Rochester, NY) exposed at 1,000 ISO and developed in T-Max developer for 10 min at 20°C.

Migration Assays

Cell migration assays were carried out in Boyden chambers as previously described (Zambruno et al., 1993). The two Boyden chamber compartments were separated by a polycarbonate filter (8-mm pore size; Nucleopore, Concorezzo, Italy) coated with gelatin (5 μg/ml). Briefly, 30 ng/ml of TGF-$\beta 1$ was added to growing keratinocyte colonies. Cells were detached with EDTA (10 mM) 24 h later, washed thoroughly, and resuspended in DMEM containing glutamine (4 mM) and penicillin-streptomycin (50 IU/ml). Migration assays were performed in the presence or absence of 15 ng/ml of TGF-$\beta 1$. Cells (3×10^5 cells/ml) were added to the upper compartment of the Boyden chamber, and the lower compartment was filled with medium containing fibronectin (10 μg/ml) or vitronectin (10 μg/ml) as chemoattractants. Migration assays started right after the addition of keratinocytes in suspension. Keratinocytes were allowed to migrate for 16 h at 37°C in a humidified atmosphere containing 5% CO_2. Cells on the lower surface of the filter were fixed in ethanol, stained with toluidine blue, and 10 random fields per filter were counted at a magnification of 160 with a microscope. Each assay was carried out in triplicate and repeated at least four times.

Results

Expression of β1 and β5 Integrins

In preliminary experiments we noticed that, due to the presence of feeder layer, inhibition of keratinocyte proliferation, assessed by [^{3}H]thymidine incorporation (performed as in Di Marco et al., 1993*b*) and cell count, was attained with TGF-β1 concentration not lower than 25 ng/ml (not shown; see Rollins et al., 1989).

To investigate the TGF-β effects on the electrophoretic mobility and relative amounts of integrin receptors, keratinocyte colonies were metabolically labeled in serum-free medium and in the presence of 30 ng/ml of TGF-β1. Immunoprecipitations of cell lysates, performed using mAbs to α subunits (Fig. 1, lanes *α2*, *α5*, and *αv*), showed that TGF-β1 strongly increased the synthesis of the collagen(s) receptor α2β1, the fibronectin receptor α5β1, and the vitronectin receptor αvβ5. Instead, the synthesis of the multifunctional α3β1 heterodimer was reduced (lane *α3*). Immunoprecipitations with anti-β(s) mAbs (lanes *β5* and *β1*) showed that TGF-β1 strongly upregulated the expression of β5 (associated with αv). Albeit much less pronounced, there was also an increase of integrins immunoprecipitated by anti-β1 mAbs (lane *β1*). The expression of α6β4 was unchanged after TGF-β1 treatment (lane *β4*). This was confirmed by mAbs to the β6 subunit (see Figs. 3 and 4). The presence of two α3 and αv bands is probably due to immunoprecipitation of α3 and αv precursors (see also De Luca et al., 1990*b*; Marchisio et al., 1991; Koivisto et al., 1994), although we cannot exclude proteolytic degradation. The multiple β4 bands are due to proteolytic degradation (Giancotti et al., 1992). Time course experiments (Fig. 2) revealed that the stimulation of α2, α5, and αv synthesis is detactable 8 h after TGF-β1 addition and peaks after 12–24 h. The exposure of integrins on the keratinocyte plasma membrane was investigated by immunoprecipitations of cell lysates prepared from surface-radio iodinated cells. As shown in Fig. 3, TGF-β1 greatly raised the amount of α2β1, α5β1, and αvβ5 exposed on the cell membrane, whereas the α6β4 integrin was unchanged and the α3β1 complex was reduced. These data match data from metabolically labeled cells and confirm that, in the presence of the β4 subunit, the α6 monomer does not associate with β1 (De Luca et al., 1990*b*; Pellegrini

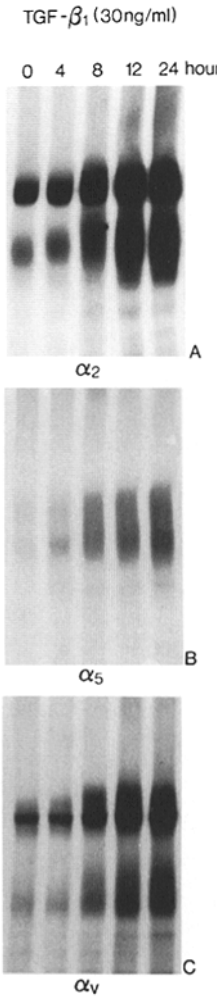

Figure 2. Time course. Growing keratinocyte colonies were incubated for 0, 4, 8, 12, and 24 h in serum-free medium supplemented with TGF-β1 (30 ng/ml), metabolically labeled during the last 4 h of incubation, and immunoprecipitated (as in Fig. 1) with mAbs to α2 (*A*), α5 (*B*), and αv (*C*): the TGF-β1–dependent upregulation of α2, α5, and αv becomes evident at 8 h and peaks at 12–24 h.

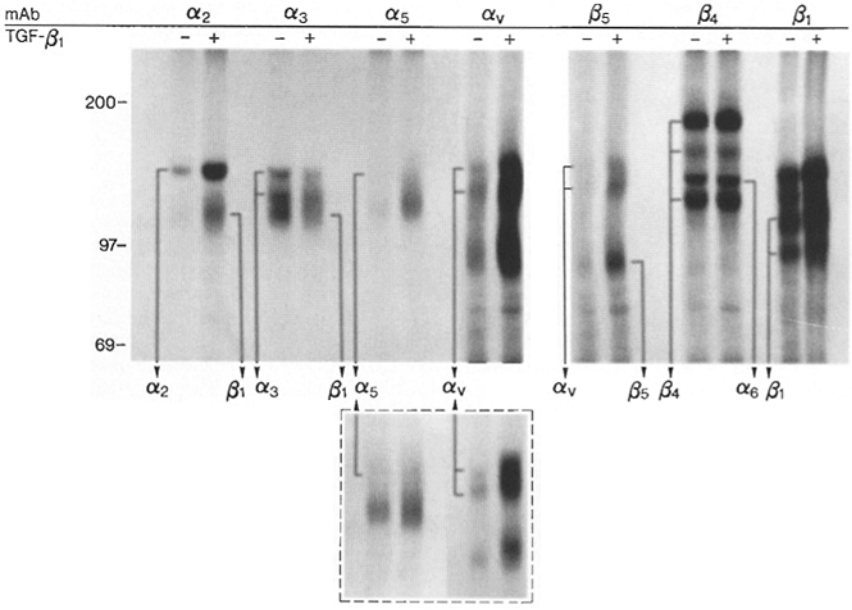

Figure 1. Immunoprecipitation of integrins. Growting keratinocyte colonies were incubated for 24 h in serum-free (0.1% BSA) medium in the presence or absence of 30 ng/ml of human recombinant TGF-β1. Cells were metabolically labeled and detergent lysates were immunoprecipitated with mAbs to different α and β subunits (*lanes*), as described in Materials and Methods. The eluates were then analyzed by SDS-PAGE under nonreducing conditions. Immunoprecipitation with anti-α5 and -αv were also performed on cells incubated in the presence of 10% FCS (*inset*).

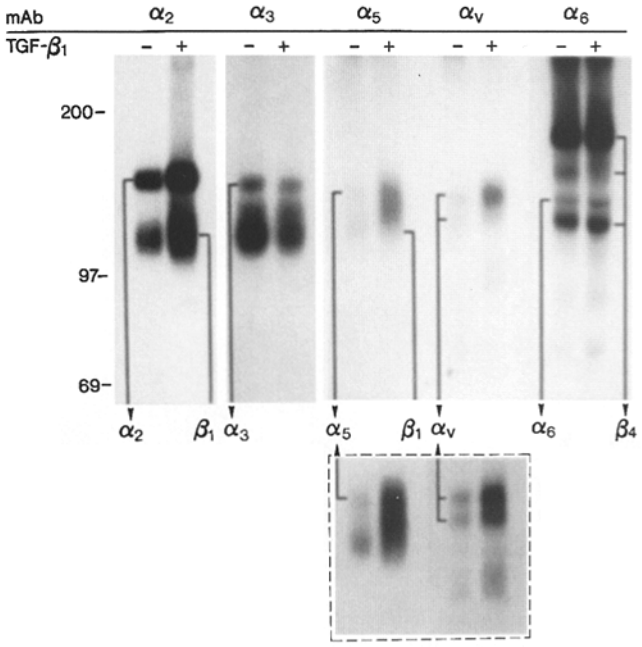

Figure 3. Immunoprecipitation of integrins. Growing keratinocyte colonies were incubated for 36 h in serum-free (0.1% BSA) medium in the presence or absence of 30 ng/ml of human recombinant TGF-β1. Cells were surface radioiodinated and detergent lysates were immunoprecipitated with mAbs to different α subunits (*lanes*) as described in Materials and Methods. The eluates were then analyzed by SDS-PAGE under nonreducing conditions. Immunoprecipitation with anti-α5 and -αv were also performed on cells incubated in the presence of 10% FCS (*inset*).

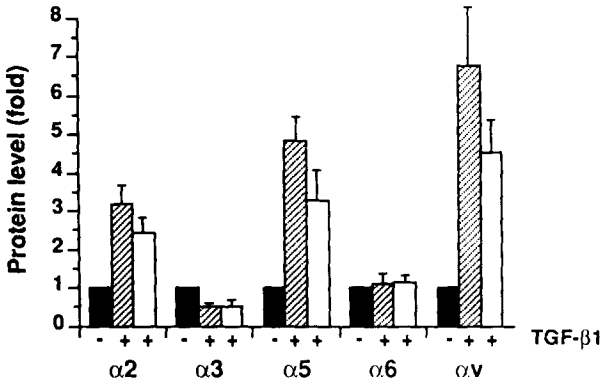

Figure 4. Densitometric analysis. Growing keratinocyte colonies from five different keratinocyte strains were incubated for 24 h in serum-free (0.1% BSA) medium in the presence (*hatched* and *open bars*) or in the absence (*solid bars*) of 30 ng/ml of human TGF-β1. Cells were both metabolically labeled (*hatched bars*) and surface radioiodinated (*open bars*). Detergent lysates were immunoprecipitated with mAbs to different α subunits (*lanes*), and eluates were analyzed by SDS-PAGE under nonreducing conditions. Relative intensities of bands on autoradiograms were then quantified by scanning laser densitometry. Values are expressed as fold variation compared with control cells (i.e., in the absence of TGF-β1, considered as 1).

et al., 1992), also after TGF-β1 stimulation. The TGF-β1-dependent regulation of both the synthesis and the surface exposure of these integrins was unaffected by the presence of 10% FCS (Figs. 1 and 3, *insets*). The lower α3, αv, and β4 bands displayed a variable intensity in different experiments, and their detection can be explained by proteolytic degradation (Giancotti et al., 1992). Densitometric analysis of five experiments performed on different keratinocyte strains showed that, after TGF-β1 stimulation, the expression of the heterodimers immunoprecipitated by anti-α2, -α5, and -αv mAbs increased approximately three-, five-, and sevenfold respectively, whereas the α3β1 complex was reduced to about one half (Fig. 4, *hatched bars*). Comparable values were obtained from densitometric analysis of parallel immunoprecipitations of cell surface-radioiodinated keratinocytes (Fig. 4, *open bars*). These data were generated from growing keratinocyte colonies, that is, from a cell culture enriched in colony-forming cells with high proliferative and migratory capacities (Green, 1980; Barrandon and Green, 1987*a*,*b*). Keratinocyte colonies will eventually fuse, giving rise to a stratified squamous epithelium mimicking the slow renewal process and the differentiated phenotype of an in situ uninjured epidermis (Green and Thomas, 1978; Green, 1980). Interestingly, when a fully stratified epidermal sheet was incubated in the presence of TGF-β1, no variation was noticed whatsoever in the expression of α2β1, α5β1, and αvβ5 integrins (Fig. 5, lanes α2, α5, and αv). It is worth noting, however, that, under these conditions, α5β1 was almost undetectable (Fig. 5, lane α5), and the lessening of α3β1 expression was still present, albeit at a lower extent (Fig. 5, lane α3). Immunoprecipitations and immunofluorescence performed with mAbs to α1, α4, β3, and αvβ3 did not show detectable levels of these integrins, both in growing keratinocyte colonies and in confluent epithelial sheets, either in the presence or absence of TGF-β1 (not shown).

Because the maximal increase of integrin expression required 12–24 h (see Fig. 2), and the level of expression of a specific heterodimer could be regulated by the synthesis

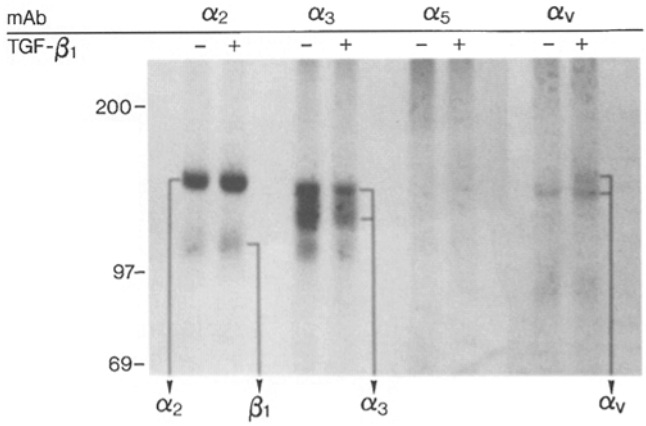

Figure 5. Immunoprecipitation of integrins. Confluent sheets of in vitro reconstituted stratified epithelium were incubated for 24 h in serum-free medium in the presence or absence of 30 ng/ml of human recombinant TGF-β1. Cells were mtabilically labeled and detergent lysates were immunoprecipitated with mAbs to different α subunits (*lanes*) as described in Materials and Methods. The eluates were then analyzed by SDS-PAGE under nonreducing conditions.

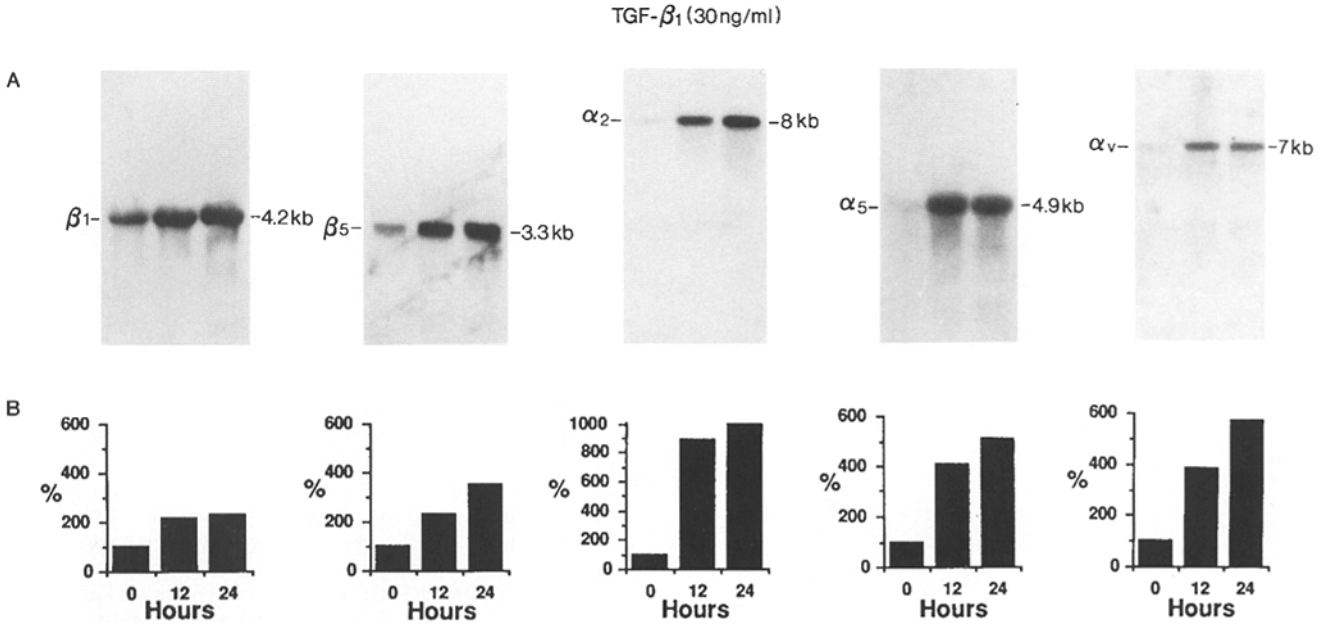

Figure 6. Northern blot analysis. 30 μg of total RNA obtained from growing keratinocyte colonies, incubated for 0, 12, and 24 h in serum-free medium supplemented with TGF-β1 (30 ng/ml), were separated by electrophoresis, transferred to nylon filters, and hybridized to ^{32}P-labeled probes specific for each integrin subunit (*A*). Equal amounts of RNA were loaded as assessed by ethidium bromide staining. *B* plots the readings obtained by laser densitometry of the autoradiograms shown in *A*, expressed as percentages of the values obtained in the absence of TGF-β1 (0 hours). Values were normalized for the density of the band obtained by probing the same filter for rRNA. Comparable results were obtained in three experiments performed on three different keratinocyte strains.

of one or both monomers, it was important to determine whether TGF-β1 would also increase the concentration of mRNAs encoding specific integrin subunits over this period of time. Thus, equal amounts of total RNA obtained from growing keratinocyte colonies exposed to TGF-β1 for 0, 12, or 24 h were separated by electrophoresis, transferred to nylon filters, and hybridized to ^{32}P-labeled probes specific for each integrin subunit (Fig. 6 *A*). Data were quantified by densitometric analysis (Fig. 6 *B*). TGF-β1 increased the α2, α5, and αv mRNA concenterations ~10-, 5-, and 6-fold, respectively. The level of β5 mRNA was increased ~4-fold, whereas there was only a very modest increase of the β1 mRNA concentration. Fig. 6 represents a single experiment. Comparable results were obtained in three experiments performed on three different keratinocyte strains.

Expression of αvβ6

It is worth noting that the relative amount of the complex immunoprecipitated by anti-αv mAbs was much higher than the amount of the heterodimer immunoprecipitated by anti-β5 mAbs (compare lanes *αv* and *β5*); moreover, the αvβ3 vitronectin receptor was never detected in human keratinocytes (Marchisio et al., 1991), also after TGF-β1 stimulation (not shown). This suggested the TGF-β1–dependent association of αv with additional β partners and prompted us to investigate if the newly described αvβ6 integrin, which has been isolated from airway epithelial cells, is expressed in several immortalized epithelial cell lines but is absent in normal human epidermis, and functions as an Arg-Gly-Asp–dependent fibronectin receptor (Sheppard et al., 1990; Busk et al., 1992; Breuss et al., 1993). 10 μg of polyadenylated mRNA, obtained from growing keratinocyte colonies cultivated in the presence or absence of TGF-β1, were thus hybridized to a ^{32}P-labeled human probe specific for the β6 subunit. Blots were exposed for up to 10 days. As shown in Fig. 7 *A* (*left lane*), the β6 mRNA was absent in control keratinocytes, whereas the 5-kb β6 transcript was readily detected in keratinocytes exposed for 24 h to 30 ng/ml of TGF-β1 (Fig. 7 *A*, *right lane*). The β6 mRNA was translated and αvβ6 was immunoprecipitated (by mAbs to β6) only from keratinocytes previously exposed to TGF-β1 (Fig. 7 *B*).

The αvβ6 association was further demonstrated by sequential immunoprecipitations with mAbs to αv and β6. Subconfluent keratinocytes were incubated for 24 h in the presence of TGF-β1, metabolically labeled, lysed, and αv immunodepleted through four sequential cycles of immunoprecipitation using mAb 13C2. The immunodepleted supernatant was then immunoprecipitated with mAbs to β6, αv, and β1. As shown in Fig. 8, the αvβ6 complex was immunoprecipitated by both anti-αv and anti-β6 mAbs before αv depletion. Note that the relative amount of αv was much higher in the αv compared with the β6 immunoprecipitate, in agreement with the presence of the αvβ5 heterodimer. In addition, in the β6 immunoprecipitate, the relative amount of the β6 subunit was higher compared with αv. After αv immunodepletion, β6 was still present, albeit at a lower level, but no association of β6 with αv, or any other α subunit, could be detected. All together, these data demonstrate that β6 associates only with αv in TGF-β1–treated keratinocytes. Moreover, these data strongly suggest that: (*a*) the β6 subunit is synthesized in excess as compared to αv; (*b*) there

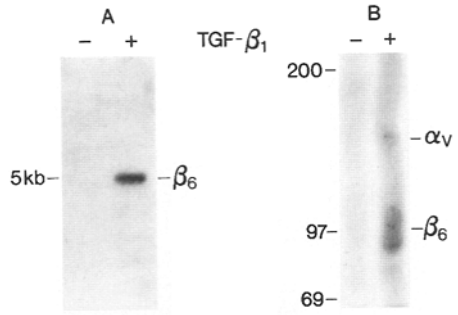

Figure 7. αvβ6 expression. (*A*) 10 μg of poly(A⁺) RNA obtained from keratinocyte colonies exposed for 0 (*left lane*) or 24 h (*right lane*) to TGF-β1 were hybridized to a ^{32}P-labeled probe specific for human β6, as described in Materials and Methods. Blots were exposed for 10 d. (*B*) Parallel cultures (as in *A*) were metabolically labeled, and detergent lysates were immunoprecipitated with the R6G9 mAb to β6 and analyzed by SDS-PAGE under nonreducing conditions. Comparable results were obtained in three experiments performed on three different keratinocyte strains.

is a cytoplasmic pool of free β6 subunit (see also immunofluorescence); and (*c*) the formation of the αvβ6 heterodimer is mainly controlled by the amount of αv available.

Topography

TGF-β1 induced a rearrangement of integrin exposure, as detected by immunofluorescence microscopy (Fig. 9). First, integrins that are responsible for adhesion-dependent polarity in most epithelial cells, that is, the laterally exposed α2β1 and α3β1 and the basal heterodimer α6β4, lost their normal distribution and became pericellular. This phenomenon was dramatically evident for α6β4 (Fig. 9, *a* and *b*), which is organized in the characteristic "leopard skin" pattern in untreated keratinocytes (Fig. 9 *a*; see also Marchisio et al., 1991, 1993). In fact, on treatment with TGF-β1, α6β4 lost its basal topography (Fig. 9 *b*), became pericellular, and was then also exposed laterally, as visualized by focusing through the section. This immunofluorescence pattern was detected with mAbs to both α6 and β4, highlighted the displacement of α6β4 to cell–cell boundaries of individual keratinocytes (Fig. 9 *b*), and indirectly indicated that the potential of forming hemidesmosomes, as well as links with the keratin cytoskeleton, was prevented or at least altered on exposure to TGF-β1. A pericellular distribution, including the basal aspect, also occurred for β1 heterodimers (not shown), indicating that TGF-β1 induced a marked perturbation of the still poorly known mechanism of polarized domain sorting of either β1 and β4 integrin receptors.

A second and even more marked event involved the de novo surface exposure of the integrin β6 chain. In untreated keratinocytes (Fig. 9 *c*), no immunofluorescent signal was detected with R6G9 mAb to β6. On treatment with TGF-β1, a specific signal for β6 was detected either in the form of tiny dots or as continuous strands at intercellular boundaries (Fig. 9 *d*, *arrowheads*). The signal was displayed at higher intensity in those cells located at the periphery of individual colonies and was lower in the cells located in the colony core (Fig. 9 *d*, *asterisk*). Since β6 binds to αv to form the αvβ6 fibronectin receptor, we checked whether αv topography was also changed on TGF-β1 exposure. Indeed, αv, which is exclusively associated with β5 in tiny focal contacts at the periphery of individual colonies in untreated cells (Fig. 9 *e*; see also Marchisio et al., 1991), also moved to lateral boundaries when it was overexpressed on TGF-β1 treatment (Fig. 9 *f*). This distribution of αv was almost identical to that of β5 (not shown). The lateral enrichment of αv was consistent with that of the newly expressed β6 chain as well as with that of β5, such as we cannot state the relative proportion of the two αv heterodimers.

We also tested the position of αv, β5, and β6 by focusing on the very edge of peripheral cells where they are in tight contact with the substratum and found that the numerous and tiny focal contacts found in this region at the end of stress fibers also contain β6 (Fig. 10 *b*), in addition to αv and β5 (not shown). Again, due to the unavailability of specific reagents, we cannot state whether individual focal contacts contain one single or both heterodimers. Finally, we observed a strong signal for β6 in the perinuclear region of peripheral cells, presumably representing a pool of internal presecretory β6 stored in endoplasmic reticulum vesicles.

In summary, our topographical studies show that TGF-β1 induces a general loss of integrin polarity, indicating weakening of basal and intercellular bonds and increased appearance of vitronectin and fibronectin receptors in peripheral focal adhesion organelles that are involved in cell migration.

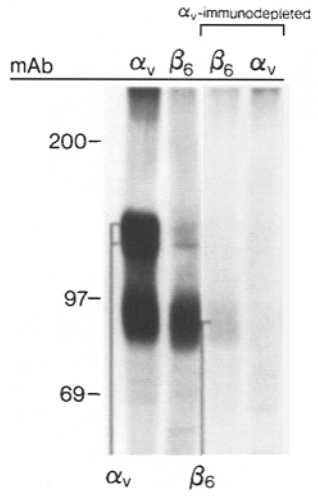

Figure 8. Sequential immunoprecipitation. Growing keratinocyte colonies were incubated for 24 h in serum-free (0.1% BSA) medium in the presence of 30 ng/ml of human recombinant TGF-β1. Cells were metabolically labeled in the presence of TGF-β1 and lysed. For sequential immunoprecipitation, two aliquots were immunoprecipitated with mAbs to αv and β6, respectively. The remaining lysate was first αv immunodepleted through four cycles of immunoprecipitation with 13C2 anti-αv mAb; aliquots of the immunodepleted supernatant were then immunoprecipitated with mAbs to β6, αv, and β1. The eluates were then analyzed by SDS-PAGE under nonreducing conditions.

Migration

These data indicate that TGF-β1 modulates the integrin receptor repertoire of epidermal cells and reproduces the integrin patterns of regenerative/migratory keratinocytes localized at the injury site of human wounds (Cavani et al., 1993; Gailit et al., 1994). The TGF-β1-dependent increase in the expression of integrins endowed with vitronectin (αvβ5) and fibronectin (α5β1 and αvβ6) binding properties prompted us to investigate whether this phenomenon was associated with an increased migration of TGF-β1–treated keratinocytes on these extracellular matrix proteins. Keratinocyte migration was assayed in Boyden chamber experiments as described in Materials and Methods. As shown in Fig. 11

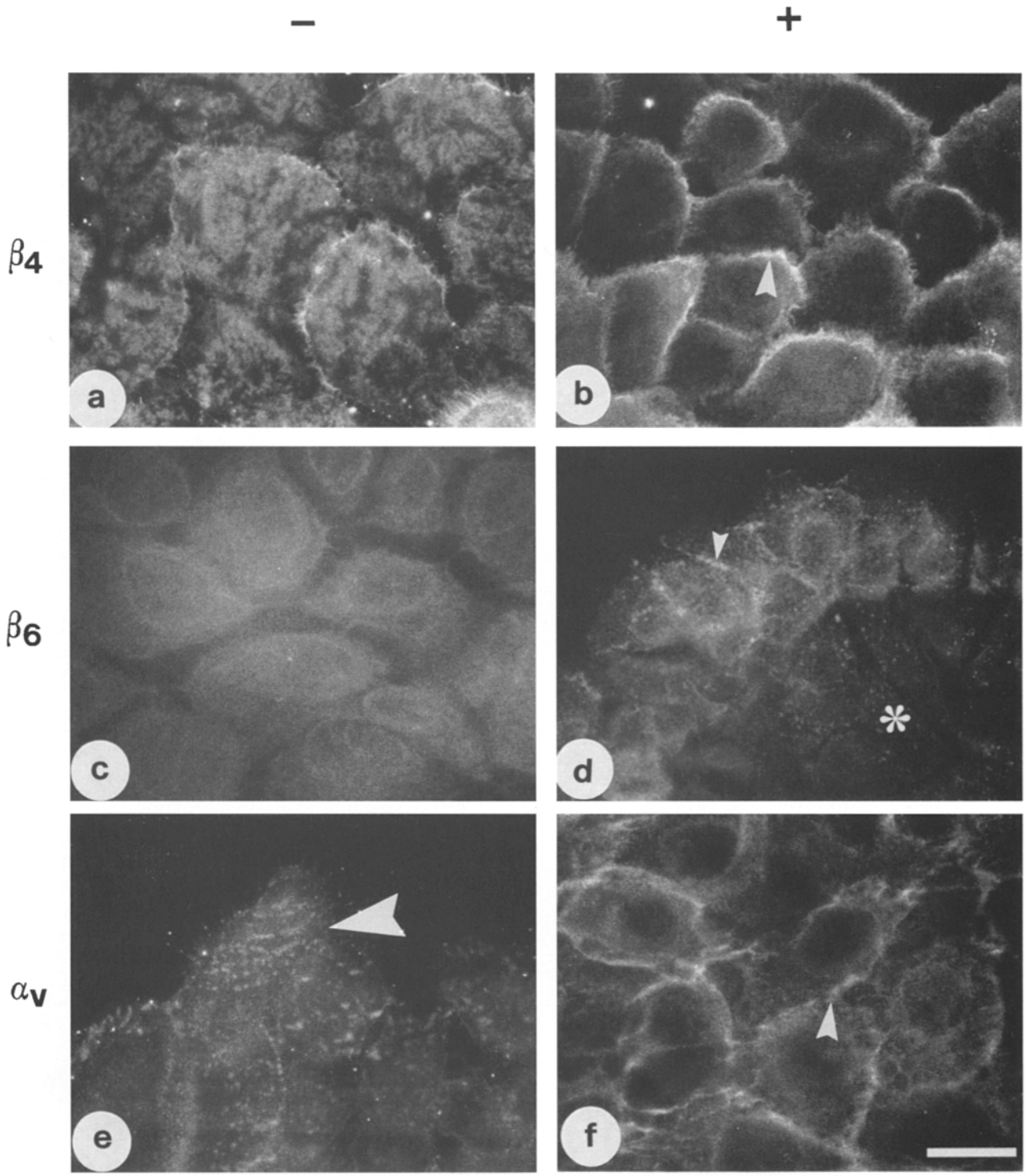

Figure 9. Immunofluorescence staining of integrin chains β4 (*a* and *b*), β6 (*c* and *d*), and αv (*e* and *f*) with (*b*, *d*, and *f*) and without (*a*, *c*, and *e*) 24-h treatment with TGF-β1. The typical basal pattern of α6β4 in cultured keratinocytes resembling a "leopard skin" (*a*) mostly disappeared on exposure to TGF-β1, and the integrin became diffuse pericellularly (e.g., *b*, *arrowhead*). β6 was not expressed in control keratinocytes, even in intentionally underexposed prints (*c*), but appeared in numerous dots or patches mostly detectable along intercellular boundaries on treatment (*d*). A significantly stronger signal was displayed by cells located at the periphery of the colony, whereas the cells forming the core generally expressed less β6 (*d*, *asterisk*). Also, αv, exclusively found in the tiny focal contacts observed in peripheral cells of control colonies (e.g., *arrowhead*) moved to give a diffuse pericellular pattern (*f*). Note that the plane of focus in *a* and *e* is closer to the attachment substratum than *b* and *f*, respectively. Bar, 10 μm.

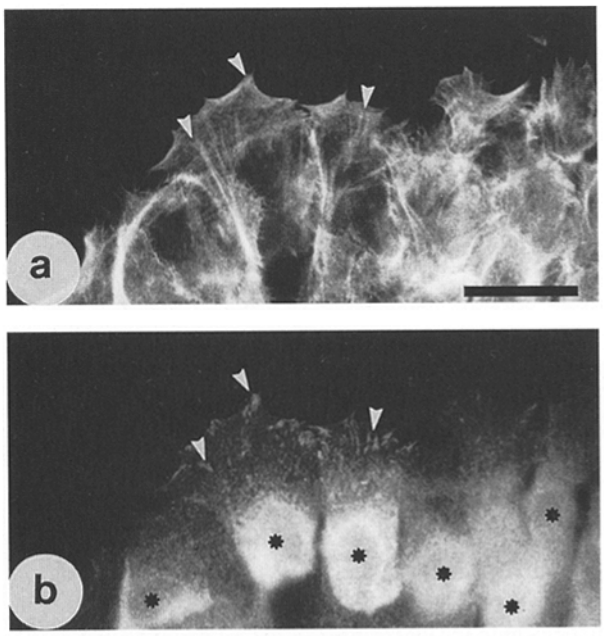

Figure 10. Double-label immunofluorescence staining for F-actin (*a*) and integrin β6 (*b*) of a group of cells located at the periphery of a keratinocyte colony on treatment with TGF-β1. The plane of focus was set close to the substratum as in Fig. 9 *e*. The arrowheads indicate that β6 is enriched at tiny focal contacts (*b*) that correspond to the terminal portion of stress fibers (*a*). At this plane of focus, a strong β6 signal appears around individual nuclei (*asterisks*) and presumably indicates that a large pool of β6 is stored in vesicular compartments in these peripheral cells. Bar, 10 μm.

(*black bars*), untreated keratinocytes migrated preferentially toward fibronectin compared with vitronectin. Keratinocyte colonies were exposed to TGF-β1 for 24 h, trypsinized, and migration assays were performed either in the presence (*white bars*) or absence (*gray bars*) of TGF-β1. In both cases, and as expected from the integrin expression data, TGF-β1 strongly enhanced keratinocyte migration towards both fibronectin and vitronectin. Keratinocytes did not migrate toward control medium lacking fibronectin or vitronectin, and TGF-β1 did not significantly increase migration toward control medium (not shown).

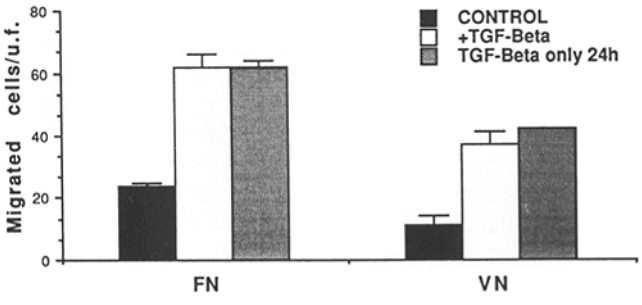

Figure 11. Migration assay. Keratinocyte migration toward fibronectin (*FN*) and vitronectin (*VN*) was assayed in Boyden chamber experiments as described in Materials and Methods. Keratinocyte colonies, untreated (*black bars*) or exposed to TGF-β1 for 24 h (*white* and *gray bars*), were detached with EDTA, and migration assays were performed either in the presence (*white bars*) or absence (*gray bars*) of TGF-β1. Each point was averaged from triplicates. The experiment was repeated four times with four different keratinocyte strains.

Discussion

When an acute wound, such as an excision or a burn, occurs, the interruption of the basement membrane causes a sequence of events leading to the reconstitution of skin integrity. One of the key events is the recruitment of proliferating and migrating keratinocytes at the injury site. Elegant studies performed on burn wounds in vivo (Wenczak et al., 1992) and on organotypic cultures (Garlick and Taichman, 1994), have demonstrated that these cells are phenotypically different and are located in quite distinct regions of the repairing wound margin (Fig. 12, *arrow*). Proliferating keratinocytes, probably enriched in stem cells (Fig. 12, *mitosis*), are confined to epithelial margins and hypertrophic epithelium adjacent to the lesion, whereas migrating keratinocytes (Fig. 12, *migration*) are mostly bordered on the marginal epithelial tongue moving on the provisional matrix underneath the eschar (Fig. 12, *C*). Ultimately, epidermal healing is achieved by a well-orchestrated balance between keratinocyte proliferation and migration. The initial cell migration is followed by the proliferative response, and these two processes envisage a sequential activation of different cell populations (Garlick and Taichman, 1994).

When keratinocytes lose contact with the basement membrane and are exposed to components of the provisional matrix at the wound margin, several modifications of their integrins occur, including a loss of polarization of β1 and β4, a strong increase of αvβ5, and the appearance of α5β1, detectable strictly in the migratory region of the wound (Cavani et al., 1993; Larjava et al., 1993; Juhasz et al., 1993). The molecular mechanism underlying these modifications has been elusive so far.

Here we show that TGF-β1, by itself, can account for these observations since: (*a*) it strongly upregulates the expression

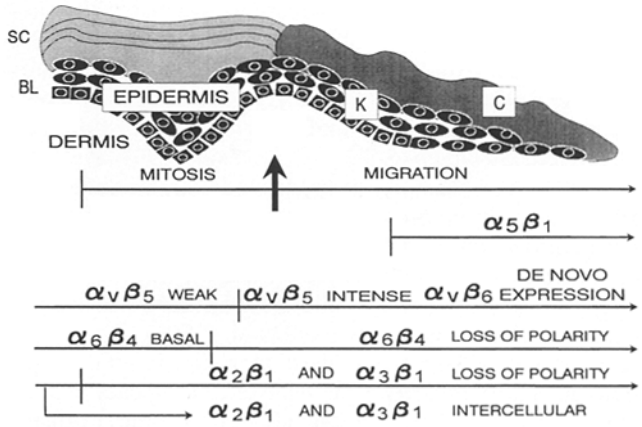

Figure 12. Schematic description of a wound-healing model. Proliferating (*motisis*) and migrating (*migration*) keratinocytes (*K*) are separated by the black arrow indicating the wound edge. The migrating epithelial tongue is located under the crust (*C*). The modulation of the expression of integrin receptors (see text) is depicted. *BL*, epidermal basal layer. *SC*, stratum corneum.

of the fibronectin receptor $\alpha5\beta1$, the vitronectin receptor $\alpha v\beta5$, and the collagen(s) receptor $\alpha2\beta1$; (*b*) it elicits its activity on growing keratinocyte colonies, but not on resting stratified cultured epidermis; (*c*) it induces the de novo expression of the $\alpha v\beta6$ fibronectin receptor; (*d*) it stimulates keratinocyte migration toward fibronectin and vitronectin; and (*e*) it causes a complete loss of the polarized expression of all integrins (See Fig. 12 for schematic description).

These data fit well with the distribution of TGF-$\beta1$ and its receptor in vivo. In fact, each isoform of TGF-β is present in a distinct temporal and spatial pattern in both normal human skin and excisional wounds (Schmid et al., 1993). In particular, the differential expression of TGF-$\beta1$ amd -$\beta3$ does suggest that the $\beta3$ isoform may be important for the epidermal maintenance, whereas TGF-$\beta1$ may play a role in epidermal regeneration. Basement membrane interruption, per se, induces TGF-$\beta1$ gene expression (Streuli et al., 1993) but not TGF-$\beta2$ or TGF-$\beta3$ in vivo, suggesting a feedback loop that results in a balance between TGF-$\beta1$ synthesis and basement membrane formation. Migrating keratinocytes in vivo synthesize and secrete biologically active TGF-$\beta1$ (Kane et al., 1991, Schmid et al., 1993) and expose TGF-β type II receptors on their plasma membrane (Schmid et al., 1993), suggesting the existence of an autocrine loop. Indeed, in human keratinocytes, TGF-$\beta1$ modulates plasminogen activator and plasminogen activator inhibitor type 1 (Wikner et al., 1990), stimulates fibronectin expression (Wikner et al., 1988) as well as other extracellular matrix proteins (see Border and Ruoslahti, 1992), and upregulates type IV collagenase (Salo et al., 1991) and collagen type VII expression (Köng and Bruckner-Tuderman, 1992).

Our data indicate that this autocrine loop may be involved in the modification of integrins as well, and suggest that the wound-healing promotion of TGF-$\beta1$ depends not only on the modulation of the extracellular matrix composition of the wound bed (Border and Ruoslahti, 1992), but also on the ensuing modification of the adhesive machinery of keratinocytes localized at the wound margin. TGF-$\beta1$ acts mainly on a specific subset of wounded keratinocytes by both inhibiting their proliferation and hence stimulating their migration (Fig. 12, *migration*). This accounts for by the apparent paradox of a TGF-$\beta1$-dependent stimulation of wound healing associated with a growth inhibitory effect on epithelial cells. Keratinocyte multiplication is then limited to a different subset of keratinocytes (Fig. 12, *mitosis*) responding to different autocrine and paracrine stimuli (Barreca et al., 1992; Di Marco et al., 1993*a*,*b*; Staiano-Coico et al., 1993; Pierce et al., 1994). For example, in the wound margin, epidermal growth factor receptors are highly expressed by proliferating keratinocytes, but undetectable on migrating keratinocytes in the epithelial tongue (Wenczak et al., 1992).

It is worth noting that TGF-$\beta1$ mRNA expression is not found in chronic wounds (Schmid et al., 1993). This might be associated with protracted healing tendencies of these lesions and might partially explain the clinical efficacy of allogenic cultured epidermal sheets (allografts) in the treatment of chronic leg ulcers (De Luca et al., 1992*b*). The enhanced migration of keratinocytes from the edge of these lesions (De Luca et al., 1992*b*) could indeed be ascribed to TGF-$\beta1$ eventually released from allografts. Thus, keratinocyte-derived TGF-$\beta1$ can cooperate with the wide variety of other keratinocyte-derived growth factors and cytokines (see Luger and Schwarz, 1990; De Luca and Cancedda, 1992; Boyce, 1994 for reviews; Di Marco et al., 1993*b*) in promoting epidermal wound healing. Conversely, TGF-$\beta1$ overproduction, or its prolonged synthesis during the healing process, has been correlated with pathological scarring such as hypertrophic skin scarring, lung and cardiac fibrosis, and liver cirrhosis (for a review see Border and Ruoslahti, 1992). The absence of any TGF-$\beta1$ effect on fully stratified epidermis (see Fig. 5) fits well with the connective origin of those lesions.

TGF-$\beta1$ potently enhances the vitronectin- and fibronectin-binding properties of keratinocytes by increasing the expression of the $\alpha v\beta5$ and $\alpha5\beta1$ integrins and by inducing the de novo expression of the newly described (Sheppard et al., 1990; Busk et al., 1992; Weinacker et al., 1994) fibronectin receptor $\alpha v\beta6$. The de novo synthesis of $\alpha v\beta6$ integrin is a unique effect of TGF-$\beta1$. Indeed, the $\alpha v\beta6$ integrin, identified in primary cultures of airway epithelium (for a review see Sheppard, 1993) is absent from intact skin, and its expression appears to be restricted to selected populations of mucosal epithelial cells, suggesting different roles of the same integrin in normal lining epithelia. We show that TGF-$\beta1$, in situations mimicking a wound-healing process, induces both the $\beta6$ mRNA and the expression of the $\alpha v\beta6$ heterodimer in keratinocytes. To our knowledge, this is the first demonstration of a novel fibronectin receptor on normal human keratinocytes. Since it has been reported that TGF-$\beta1$ regulates the levels of different fibronectin isoforms in normal human cells (Balza et al., 1988), it is tantalizing to speculate that TGF-$\beta1$ regulates keratinocyte migration on different isoforms of fibronectin by inducing the synthesis of both the ligands and their receptors. Experiments are in progress to test this hypothesis and to demonstrate the expression of $\alpha v\beta6$ in healing wounds in vivo. Several authors have reported that $\alpha5\beta1$ is not expressed in normal adult human epidermis, whereas in vitro, $\alpha5\beta1$ is weakly and diffusely distributed on the keratinocyte plasma membrane and is not organized in defined adhesive structures (see Pellegrini et al., 1992; Cavani et al., 1993; Larjava et al., 1993; Juhasz et al., 1993), suggest that $\alpha5\beta1$, $\alpha v\beta6$, and $\alpha v\beta5$ integrins, not routinely utilized by basal keratinocytes resting on an intact basement membrane, act as "emergency" receptors, and that their synthesis is strongly stimulated by TGF-$\beta1$ to allow keratinocyte migration over the vitronectin- and fibronectin-rich environment of a wound (see also Gailit et al., 1994). This fits well with previous data showing that availability and accumulation of the ligand are critical to relocate integrin receptors to defined adhesive structures (Singer et al., 1988), and that $\alpha5\beta1$ plays a cooperative role with vitronectin receptors in regulating cell motility (Bauer et al., 1992).

The modulation of integrin receptors by TGF-$\beta1$ has been observed in other cell types (Roberts et al., 1988; Heino et al., 1989; Heino and Massagué, 1989; Sheppard et al., 1992). However, the regulation of both the heterodimers and the single subunit differs in different cell types, even of the same origin. For example, in guinea pig airway epithelial cells (Sheppard et al., 1992), TGF-$\beta1$ enhances the expression of the $\alpha3\beta1$ heterodimer and increases the expression of the $\alpha v\beta3$ and $\alpha v\beta5$ integrins by only raising the transcriptino of the β subunits. Instead, in human keratinocytes, TGF-$\beta1$ downregulates the expression of the $\alpha3\beta1$ integrin and increases the expression of the $\alpha v\beta5$ heterodimer by raising

the synthesis of both the αv and the β5 subunits. This further suggests different regulation and function of the same integrin receptor in different lining epithelia. In epidermis, the downregulation of the α3β1 heterodimer might be correlated with the expected role of this elusive multifunctional integrin in intercellular adhesion (Symington et al., 1993; Sriramarao et al., 1993), but may also have a role in the adhesion to the epidermal basement membrane (Carter et al., 1990*a*, 1991). Such a relative decrease may loosen keratinocytes both from the basement membrane and from neighbor cells and trigger their motility during the process of wound healing. Larjava and colleagues (1993) have recently shown that, during human mucosal wound healing, migrating keratinocytes continuously express kalinin but not the other components of the basement membrane, which appear gradually when the epithelial sheets confront each other. This suggests that kalinin is probably the first basement membrane protein synthesized during the regeneration of the basal lamina and might explain why migrating keratinocytes keep the expression of the α6β4 kalinin receptor unaltered.

The observation that TGF-β1 potently increased the synthesis and surface exposure of β1-containing heterodimers without significantly increasing the concentration of the β1 mRNA could be explained either by the presence of an excess pool of β1 or β1 precursor in unstimulated cells or an increased rate of translation of β1 mRNA in cells stimulated by TGF-β1. Together, the comparable levels of the β1 precursor, the dramatic increase in the α(s) mRNA levels, and the very modest increase in the β1 mRNA favor the former hypothesis. This suggests that, as in WI-38 lung fibroblasts (Heino et al., 1989), the regulation of synthesis and surface exposure of β1 integrins in human keratinocytes is mainly controlled by the regulation of the synthesis of the α subunit partners. Instead, the increased expression of the αvβ5 integrin is associated with increased concentrations of both their mRNAs. Further experiments are required to establish whether the strong rise in the α2, β5, αv, and β5 mRNA levels is determined by a true increase in their transcription rate and/or by other regulatory mechanisms, such as mRNA stability.

A similar mechanism as for β1 integrins appears to be involved in the regulation of αvβ6 expression (see Figs. 8 and 10). Indeed, our data strongly suggest that, after TGF-β1 treatment, the β6 subunit is synthesized in excess, the β6 pool stays in the endoplasmic reticulum, and the availability of the αv subunit is the rate-limiting step in the formation of the αvβ6 heterodimer.

Finally, it is worth noting that recent work (for reviews see Zachary and Rozengurt, 1992; Schwartz, 1993) has shown that integrins located in adhesive structures, such as focal contacts, can be phosphorylated and are associated with several tyrosine kinases (such as $pl25^{FAK}$) and retroviral oncogenes (such as $pp60^{src}$), which can be stimulated on integrin activation. Thus, integrins can have a second functional role as signaling receptors. It will be of great interest to investigate whether TGF-β1 can influence the keratinocyte-signaling pathways through the modulation of its integrin repertoire.

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