Integrin Expression by Human Epidermal Keratinocytes Can Be Modulated by Interferon-γ, Transforming Growth Factor-β, Tumor Necrosis Factor-α, and Culture on a Dermal Equivalent

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Receptors of the integrin family are largely confined to the basal layer of keratinocytes, both in human epidermis and in stratified cultures of human keratinocytes. However, suprabasal integrin expression is observed during epidermal wound healing and in psoriatic lesions. We have investigated potential stimuli of suprabasal expression. Addition of transforming growth factor-β (TGF-β), interferon-γ (IFN-γ), or tumor necrosis factor-α (TNF-α) to keratinocytes cultured with a 3T3 feeder layer did not induce suprabasal expression. The cytokines caused small changes in the levels of $\alpha_2\beta_1$ or $\alpha_3\beta_1$ on the surface of basal keratinocytes but had no significant effect on the proportion of cells adhering to fibronectin, type IV collagen, and laminin, and did not cause changes in the mobility of integrin subunits on polyacrylamide gels. Injection of TNF-α or IFN-γ intradermally into healthy human volunteers induced an inflammatory response but did not induce suprabasal integrin expression. However, we did observe transient suprabasal integrin expression when keratinocytes were grown on a dermal equivalent consisting of fibroblasts in a collagen gel. One week after raising the cultures to the air-liquid interface, β_1 integrins were found in all the viable cell layers, with suprabasal cells co-expressing integrins and involucrin; 1 week later integrins were confined to the basal layer. Addition of TGF-β, IFN-γ, or TNF-α to the dermal equivalents neither induced nor inhibited suprabasal integrin expression. We conclude that suprabasal integrin expression is not induced by the inflammatory cytokines tested, and instead may reflect the proliferation/differentiation status of the epidermis.

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Integrins are α/β heterodimeric adhesive receptors that are expressed on most cell types and have a variety of functions [1]. Cultured human keratinocytes express several integrins with a common β subunit, including $\alpha_2\beta_1$, which mediates binding to collagen and laminin; $\alpha_3\beta_1$, a receptor for laminin and epiligrin; and $\alpha_5\beta_1$, the fibronectin receptor [2–6]. Keratinocytes also express $\alpha_v\beta_5$, which is a vitronectin receptor [5,7], and $\alpha_6\beta_4$, a component of hemidesmosomes, the ligand for which remains to be established unequivocally in keratinocytes [5,8–10]. Experiments with cultured keratinocytes have demonstrated that integrins have a number of functions within the epidermis. They not only mediate keratinocyte adhesion to extracellular matrix proteins, but also play a role in lateral migration, stratification, the regulation of terminal differentiation [2,11–13], and intercellular adhesion ([3,14,15], but see also [16]). Furthermore, basal keratinocytes with characteristics of stem cells can be isolated on the basis of high surface expression of the β_1 integrins [17].

Integrin expression is normally confined to the basal layer of keratinocytes, both *in vivo* and in stratified cultures [5,18–22]. However, suprabasal integrin expression has been observed during epidermal wound healing [22,23] and in certain disease states. In wound healing, suprabasal expression is transient: co-expression of integrins and specific markers of terminal differentiation is observed at the time of wound closure, when the epidermis is hyperproliferative, but is absent 1 week later [22]. Suprabasal expression also has been noted in benign hyperproliferative disorders: in psoriatic lesions [22,24–27], eczema, and lichen planus [26].

To understand the significance of suprabasal integrin expression in the epidermis, we have begun to investigate potential stimuli. Release of cytokines by the inflammatory infiltrate characteristic of psoriatic lesions and wounds has been well documented [28,29]. Cytokines regulate many aspects of keratinocyte behavior, including proliferation [30,31], migration [32,33], and communication with cells of the immune system [28]. In addition, specific cytokines have been shown to up-regulate integrin expression in a range of cell types [34–41]. We selected three cytokines for study—interferon-γ (IFN-γ), transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α)—because of their reported presence or up-regulation in inflamed skin [28,42,43], their effects on integrin

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Abbreviation: FAD + FBS + HICE, keratinocyte growth medium.

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expression [35,36,38,39], and their ability to influence keratinocyte proliferation and/or terminal differentiation [31,44–47].

We report that, in contrast to expectations, IFN-γ, TGF-β, and TNF-α did not induce suprabasal expression and had only modest effects on integrin expression by basal keratinocytes. However, if keratinocytes were cultured under standard conditions on a dermal equivalent, transient suprabasal expression was observed.

MATERIALS AND METHODS

Cell Cultures for Cytokine Treatments Human epidermal keratinocytes from neonatal foreskins (strains kc, kd, kf, kk, and z, passage numbers 3–5) were cultured in the presence of a feeder layer of mitomycin-C–treated 3T3-J2 cells, as described previously [48]. The culture medium consisted of one part Ham's F12 medium and three parts Dulbecco's modification of Eagle's medium supplemented with 1.8×10^{-4} M adenine (FAD), 10% fetal bovine serum (FBS), 0.5 μg/ml hydrocortisone, 5 μg/ml insulin, 10^{-10} M cholera toxin and 10 ng/ml epidermal growth factor (HICE) (FAD + FBS + HICE).

Preconfluent cultures were treated with cytokines in FAD + FBS + HICE for 24 or 48 h. Human recombinant IFN-γ was a generous gift of I. Kerr (Imperial Cancer Research Fund [ICRF]). Human TGF-β1 was obtained from the National Reference Standards Laboratory (South Mimms, Herts, UK) or from Sigma Chemical Co. (Poole, UK). Human recombinant TNF-α was obtained from the National Reference Standards Laboratory or was generously provided by F. Balkwill (ICRF).

Sheets of keratinocytes were harvested with 2.5 mg/ml Dispase 1–5 d post-confluence, as described previously [49], and frozen in an isopentane bath in liquid nitrogen. Six-micrometer frozen sections were prepared using a cryostat.

Immunofluorescence Staining of Sections The primary antibodies (mouse monoclonals) used were as follows: HAS4 (anti-α_2 [16]), VM-2 (anti-α_3 [50]), anti-CD29 (anti-β_1; Janssen, UK), P5D2 (anti-β_1; Developmental Studies Hybridoma Bank [51]), and 15.2 (anti-intercellular adhesion molecule-1 [ICAM-1]; gift of N. Hogg; ICRF [52]). Primary antibodies were detected with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Sigma; Zymed, South San Francisco, CA; or Jackson ImmunoResearch Laboratories Inc., West Grove, PA) or biotinylated anti-mouse IgG followed by FITC-streptavidin (Amersham International, UK).

Sections were stained essentially as described previously [21,22]. Briefly, sections were fixed for 30 min in 4% formaldehyde in phosphate-buffered saline (PBS) containing 1 mM magnesium chloride and 1 mM calcium chloride (PBS ABC), blocked in 0.1 M glycine in PBS ABC, and incubated for 30 min in PBS ABC containing 0.1% bovine serum albumin (BSA), 0.02% Triton X-100 (PBS/BSA/Triton), to which 0.02% sodium azide was added as preservative. The first antibody was applied for 1 h at room temperature, and then sections were washed three times over a period of 45–60 min in PBS/BSA/Triton. The second antibody was added, and sections were incubated and washed as before. Finally, sections were mounted in DABCO mounting medium. Controls consisted of omitting each primary antibody.

In some experiments, we performed double-label immunofluorescence for β_1 integrins and involucrin. The β_1 subunit was detected with P5D2 and involucrin with a rabbit polyclonal antiserum, DH1 [53]. Unfixed frozen sections were incubated with a mixture of P5D2 and DH1, rinsed thoroughly in PBS containing 0.1% BSA and 0.05% Tween 20, then incubated with a mixture of FITC-conjugated sheep anti-mouse IgG (Jackson ImmunoResearch Laboratories) and RITC-conjugated goat anti-rabbit IgG and washed as before.

Flow Cytometry Preconfluent keratinocyte cultures were harvested with trypsin/ethylenediamine tetraacetic acid, washed in serum-containing medium, and resuspended in cold PBS ABC (4°C) containing the appropriate antibody. Antibodies used were FITC conjugates of anti-CD29, HAS4, VM-2, and anti-CD8 (Sigma). (CD8 is involved in major histocompatibility complex class I-restricted antigen recognition and is not expressed by keratinocytes; the antibody was used as a control for nonspecific binding.) Cells were incubated with antibodies for 20–30 min on ice, washed in cold PBS ABC, and analyzed in a FACScan Flow Cytometer, acquiring 10,000 events per sample with settings determined previously for keratinocytes [17]. Propidium iodide, 50 μg/ml, was added to samples immediately before analysis so that dead cells could be gated out.

Basal keratinocytes can be distinguished from suprabasal, terminally differentiating cells on the basis of their forward and side light-scatter characteristics [17]. The fluorescence of cytokine-treated basal keratinocytes was compared with that of control basal cells by normalizing the modal fluorescence values; data are expressed as percentage differences in fluorescence.

Immunoprecipitation Preconfluent cultures of keratinocytes were labeled overnight with 50 μCi/ml ^{35}S-methionine and cysteine (TranS^{35}-Label, ICN; specific activity 1180 Ci/mmol) in FAD + FBS + HICE, then extracted and immunoprecipitated as described previously [5,12]. The lysis buffer consisted of 150 mM sodium chloride, 1% Nonidet P-40, 50 mM Tris-HCl pH 7.5, 0.1 mg/ml leupeptin, and 2 mM phenylmethylsulfonyl fluoride. Lysates containing 10^6 trichloroacetic-acid–precipitable counts were incubated with anti-CD29 for at least 90 min on ice and then mixed, end over end, at 4°C with protein A Sepharose (Pharmacia) for at least 60 min. The beads were recovered, washed, and resuspended in polyacrylamide gel electrophoresis sample buffer (non-reducing). Samples were electrophoresed on 7.5% polyacrylamide gels using the buffer system of Laemmli [54]. Gels were stained with Coomassie blue, destained, incubated in Amplify (Amersham International), dried down, and exposed to XAR-5 film (Kodak).

Adhesion Assays Bacteriologic 96-well plates (Linbro, Flow Laboratories, McLean, VA) were coated with 10 μg/ml extracellular matrix (ECM) proteins, essentially as described by Adams and Watt [5]. Laminin from the Engelbreth-Holm-Swarm murine sarcoma and human placental type IV collagen were purchased from Sigma. Human plasma fibronectin was obtained from BioProducts Laboratory (Elstree, UK). ECM proteins were diluted in PBS ABC and incubated in the 96-well plates overnight at 4°C. After coating, plates were washed three times in PBS ABC and blocked with 0.5 mg/ml heat-denatured BSA (fraction V, Ig-free, Sigma) for 1–2 h at 37°C. Keratinocytes were harvested in trypsin/ethylenediamine tetraacetic acid (after removal of 3T3-J2) and washed in 1 mg/ml soybean trypsin inhibitor (Sigma), then plated in serum-free FAD at a density of 10^3 (fibronectin, type IV collagen) or 10^4 (laminin) cells per well in triplicate wells and incubated at 37°C for 3 h. Nonadherent cells were removed, and adherent cells were fixed for 45 min in 4% formaldehyde in PBS ABC at room temperature. Plates were rinsed three times in PBS ABC and stained with 1% methylene blue in PBS for 45–60 min at room temperature. Plates were washed twice with distilled water and air-dried. Three or five fields per well were counted using a graticule, and results were expressed as a percentage of the number of cells plated per well. The number of cells that adhered to heat-denatured BSA in the absence of ECM proteins was subtracted from the results.

***In Vivo* Injection of Cytokines** Volunteers received intradermal injections to one buttock of either 10 μg IFN-γ (specific activity 1×10^7 U/mg, kind gift of Biogen Inc., Cambridge, MA) or 100 U recombinant human TNF-α (kind gift of Knoll AG, Ludwigshafen, Germany) and were biopsied at 6 h (TNF-α), 24 h (IFN-γ), or 48 h (IFN-γ). An additional group received three daily injections of IFN-γ (10 μg) or TNF-α and were biopsied 2 d after the final injection (i.e., day 5). In all volunteers, PBS was injected to the contralateral buttock in identical manner as a control. Six-millimeter punch-biopsy specimens were obtained from cytokine-injected and control sites under local anesthesia, snap frozen, and stored in liquid nitrogen before immunostaining. At least three volunteers in each group were studied. The study was carried out with the approval of Guy's Hospital Ethical Committee and with the informed consent of the volunteers.

Dermal Equivalent Cultures Dermal equivalents consisted either of a gel of 2.3 mg/ml bovine dermal collagen (Vitrogen; Collagen Corp., Freemont, CA) containing 3T3-J2 cells or human dermal fibroblasts, or a collagen-coated membrane insert (Cellagen, ICN) with 3T3-J2 cells or human dermal fibroblasts seeded on the undersurface. Cultures on collagen gels contracted during the course of the experiments, whereas cultures on collagen membranes did not. Keratinocytes were seeded immediately after the equivalent was prepared or 1 d later; the same results were obtained in each case. Keratinocytes were seeded at a density of 10^5 per 8 cm^2 of dermal equivalent and grown to confluence submerged in FAD + FBS + HICE. Thereafter, cultures were raised to the air-medium interface for 1 or 2 weeks; in the case of the collagen gels, this was achieved by placing them on a stainless-steel grid. These methods are based on those described by other workers [55,56]. Dermal equivalent cultures were frozen in an isopentane bath cooled in liquid nitrogen; 6-μm sections were prepared and stained as described above.

RESULTS

IFN-γ, TGF-β, and TNF-α Treatment Results in Small Changes in β_1 Integrin Levels in Culture Keratinocytes cultured according to the Rheinwald and Green technique [48]

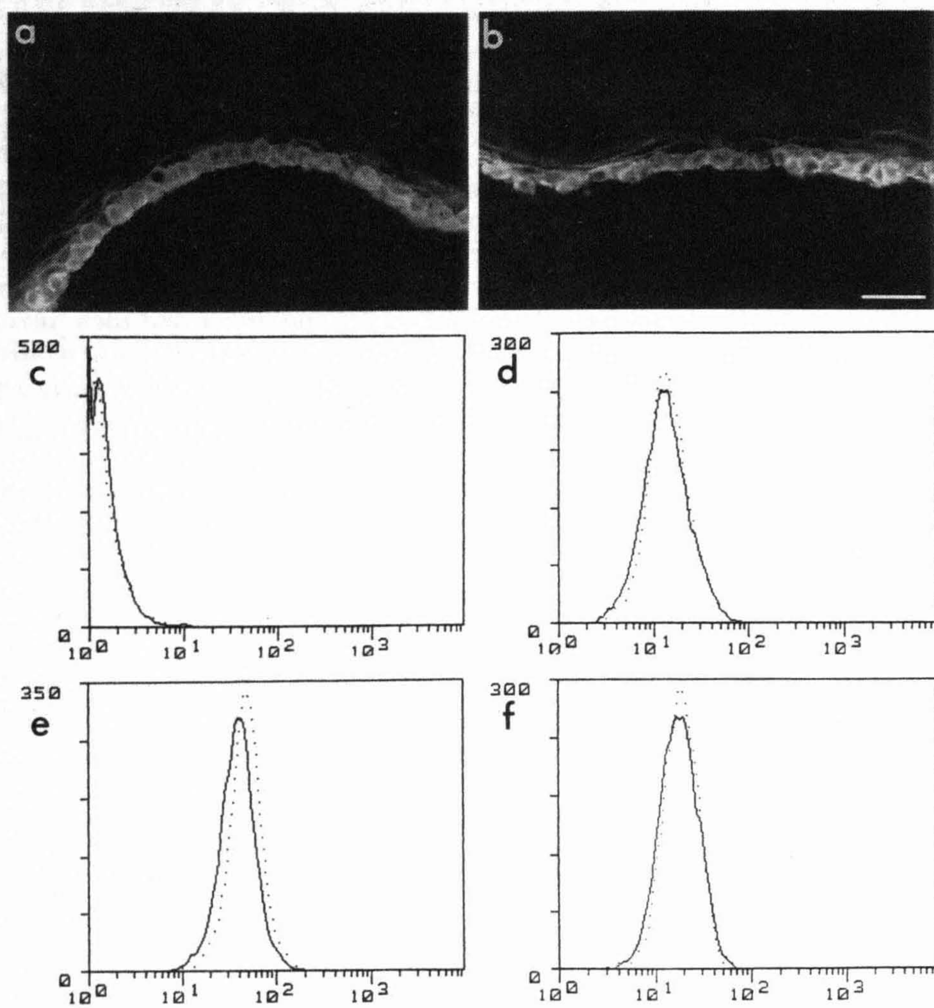

Figure 1. IFN-γ does not induce suprabasal integrin expression and has a small effect on integrin levels in basal keratinocytes. *a,b*) Sections of Rheinwald and Green cultures stained with antibody to the β_1 integrin subunit by immunofluorescence. *a*) Control; *b*) treated with 200 U/ml IFN-γ for 5 d. *Bar*, 50 μm. *c–f*) Flow cytometry profiles of keratinocytes stained with antibodies to the following antigens: *c*) CD8 (negative control); *d*) α_2; *e*) α_3; *f*) β_1 integrin subunits. *Dotted lines* show keratinocytes treated with 200 U/ml IFN-γ for 24 h. *Solid lines* show control (untreated) keratinocytes. Vertical axes, cell number; horizontal axes, fluorescence in arbitrary units on a log scale.

with a 3T3 feeder layer were treated with IFN-γ (200 or 500 U/ml), TGF-β (1 or 10 ng/ml), or TNF-α (10 or 50 ng/ml) for 24 h or 48 h, and the effects of the cytokines on integrin expression and function were determined. As reported previously [57,58], ICAM-1 was induced with both concentrations of IFN-γ (data not shown).

None of the cytokines induced suprabasal integrin expression at either time point examined. Sections through confluent cultures showed no differences in β_1 integrin distribution, with strong staining in the basal layer and weak or undetectable staining of the suprabasal cells (**Fig 1*a,b*** and results not shown).

Flow cytometric analysis revealed that the cytokines had only small effects on the level of integrin expression in basal keratinocytes (**Table I; Fig 1*c–f*** and data not shown). IFN-γ caused an increase in α_3 and β_1 fluorescence. TGF-β caused an increase in levels of the α_2, α_3, and β_1 subunits, and TNF-α induced an increase in α_2 and β_1 fluorescence and a decrease in α_3 fluorescence. Metabolic labeling and immunoprecipitation showed no differences in the mobility or relative abundance of the mature α and β_1 integrin subunits or the immature (underglycosylated) β_1 subunit (**Fig 2**), suggesting that there were no effects of the cytokines on post-translational modifications of the β_1 integrins such as glycosylation.

In keratinocytes, as in other cell types, integrin function can be regulated independently of expression levels [1,2,59]. We therefore examined whether any of the cytokines had an effect on the proportion of keratinocytes adhering to type IV collagen, laminin, or fibronectin. IFN-γ, TGF-β, and TNF-α caused no statistically significant increase or decrease in the proportion of keratinocytes adhering (**Fig 3** and results not shown). Addition of cycloheximide (20 μg/ml) at the time of plating the cells on ECM proteins inhibited protein synthesis by 95% or more and reduced the proportion of adherent cells by between 5% and 10% in both control and cytokine-treated keratinocytes (not shown). This suggests that the cytokines did not differentially affect synthesis of ECM proteins.

Suprabasal Integrin Expression is Not Induced by Intradermal Injection of IFN-γ or TNF-α Having seen little effect of the inflammatory cytokines on integrin expression in cultured keratinocytes, we investigated whether administration *in vivo* resulted in any changes in integrin expression. Healthy volunteers were injected with IFN-γ, TNF-α, or PBS (as a control) and were biopsied up to 5 d later. Sections were stained with antibodies to the α_2, α_3, and β_1 integrin subunits (**Fig 4*c–f*** and results not shown). None of the treatments resulted in any change in keratinocyte integrin expression, although ICAM-1 was induced by IFN-γ (**Fig 4*a,b***), as reported previously [60].

Suprabasal Integrin Expression Is Observed in Dermal Equivalent Cultures Having failed to induce suprabasal integrin expression by treating keratinocytes with cytokines *in vitro* or *in vivo*, we examined whether there were any other *in vitro* conditions that resulted in suprabasal integrin expression. We prepared cultures in which keratinocytes were seeded on a gel of type I collagen containing 3T3 cells and cultured at the air-medium interface, as keratinocytes grown on a dermal equivalent express a greater range of morphologic and molecular markers of terminal differentiation than do keratinocytes cultured on tissue culture plastic in the presence of a 3T3 feeder layer [61]. We found that in cultures that had been raised to the air-medium interface for 1 week, β_1 integrins could be found in all the living suprabasal layers (**Fig 5*a,b***). However, in cultures maintained for an additional week, integrin expression was confined to the basal layer (**Fig 5*c,d***). Substitution of primary human dermal fibroblasts for the 3T3 cells in the collagen gel did not alter the pattern of integrin expression (not shown), nor did using a collagen-coated culture dish insert onto which feeders had been plated.

We also tested the effects of IFN-γ (200 and 500 U/ml), TGF-β (1 and 10 ng/ml), and TNF-α (10 and 50 ng/ml). The cytokines were added to the dermal equivalent cultures on days 1, 3, 5, and 12, and the cultures were examined 48 h later. None of the cytokines either induced or suppressed suprabasal integrin expression (**Fig 5*e*** and data not shown). Double-label immunofluorescence revealed that suprabasal cells expressing β_1 integrins also expressed the differentiation marker involucrin (**Fig 5*e,f***).

DISCUSSION

The aim of the present experiments was to discover whether inflammatory cytokines might be responsible for the induction of suprabasal integrin expression that is observed in psoriasis, eczema, and wound healing [22–27]. Treatment of Rheinwald and Green cultures with TGF-β, IFN-γ, or TNF-α did not induce suprabasal expression. The cytokines caused small changes in the levels of $\alpha_2\beta_1$ or $\alpha_3\beta_1$ on the surface of basal keratinocytes but had no effect on the proportion of cells adhering to fibronectin, type IV collagen,

Table I. Cytokines Have a Small Effect on Integrin Expression Levels in Keratinocytes[a]

Cytokine	CD8	α_2	α_3	β_1
IFN-γ, 200 U/ml	0 ± 0	0 ± 0	20 ± 2	8 ± 2
IFN-γ, 500 U/ml	0 ± 0	0 ± 0	18 ± 4	16 ± 13
TGF-β	0 ± 0	31 ± 8	4 ± 2	17 ± 3
TNF-α	0 ± 0	17 ± 13	−5 ± 2	13 ± 8

[a] The values shown are mean percentage changes in modal fluorescence (representing basal cells) compared with control (no cytokine treatment) of 80% confluent cultures treated for 24 h, ± standard error of the mean. Results are based on three experiments with each cytokine. Cells were labeled with antibodies to CD8 (negative control) or to the integrin subunits indicated.

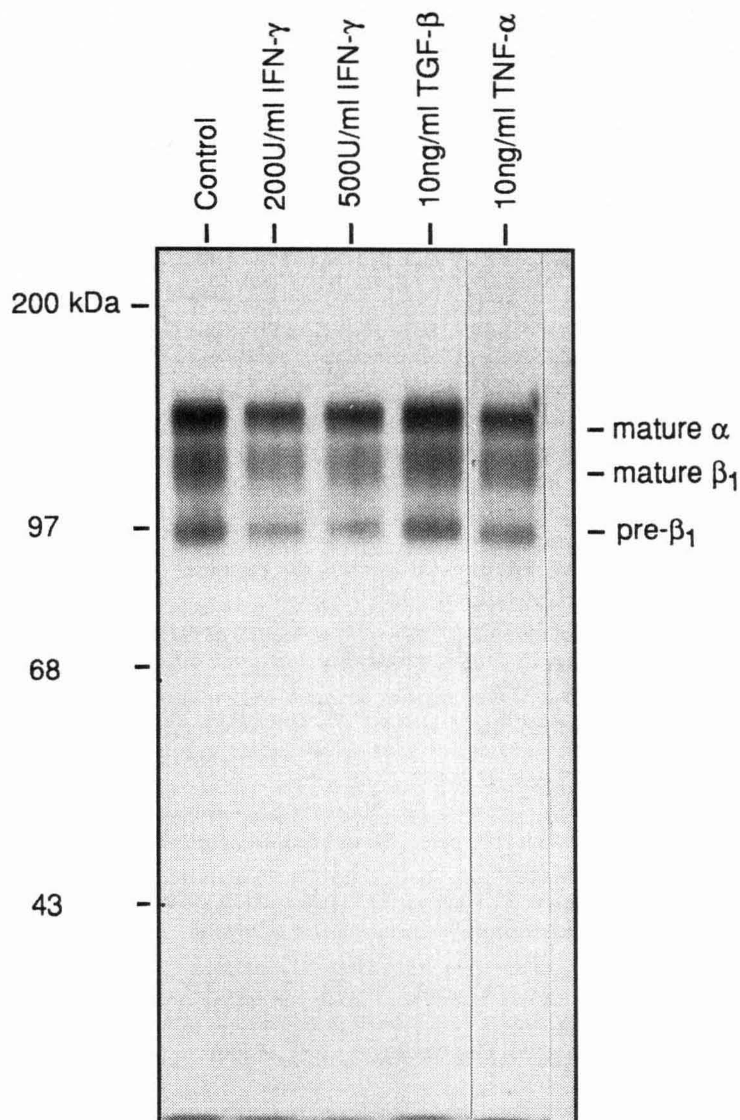

Figure 2. Cytokines do not affect the mobility or relative abundance of integrin subunits. Immunoprecipitation of β_1 integrins from metabolically labeled keratinocytes after treatment with cytokines for 24 h. Equal trichloroacetic-acid–precipitable counts were immunoprecipitated. (Note that β_1-associated α subunits are co-precipitated and migrate as a single band.) Positions of molecular-weight standards are indicated.

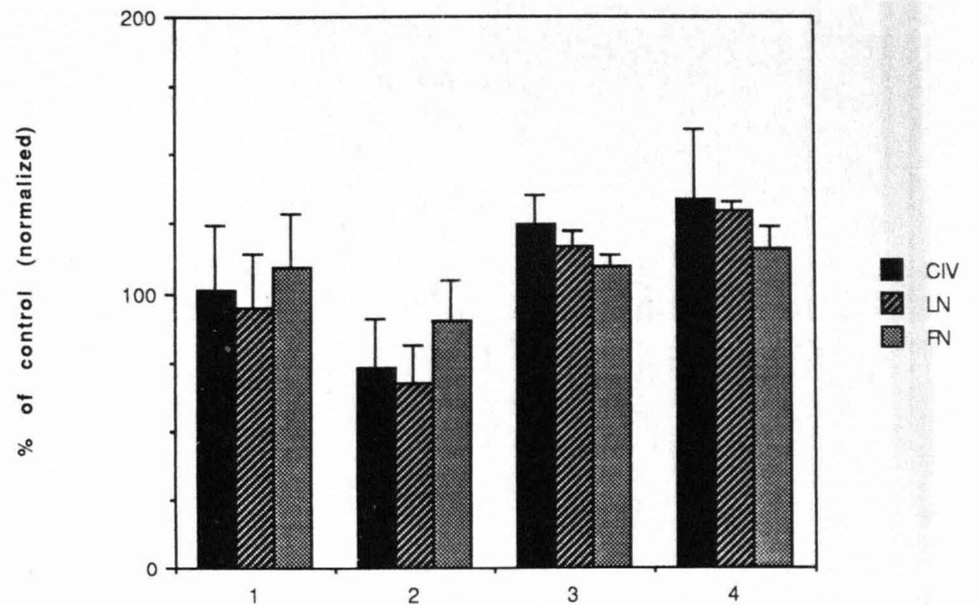

Figure 3. Cytokines do not significantly affect keratinocyte adhesion to ECM proteins. Adhesion of keratinocytes to type IV collagen (CIV), laminin (LN), and fibronectin (FN) after treatment for 24 h with (*1*) 200 U/ml IFN-γ; (*2*) 500 U/ml IFN-γ; (*3*) 1 ng/ml TGF-β; or (*4*) 10 ng/ml TGF-β. Results are expressed as a percentage of the number of control (untreated) keratinocytes adhering after subtraction of nonspecific adhesion to BSA-coated wells, ± standard error of the mean.

and laminin, and did not cause changes in the mobility of the integrin subunits on polyacrylamide gels. These results are in marked contrast to the major changes in integrin expression that are induced in other cell types by IFN-γ and TGF-β [34–36,39,40], including a 20-fold increase in transcripts originating from the β_1 proximal promoter in response to TGF-β [40].

Inflammation involves a complex cascade of events, including the interactions of multiple cell types and cytokines [62], so it is not altogether surprising that application of individual cytokines to the cultures was not sufficient to induce suprabasal integrin expression. Nevertheless, *in vivo* injections of TNF-α or IFN-γ, which induce a full inflammatory response ([63]; also Groves *et al*, submitted), did not cause suprabasal expression either.

However, we did observe suprabasal integrin expression in keratinocytes cultured on a dermal equivalent. All the viable cell layers stained positive with antibodies to the β_1 integrin subunit 1 week after the cultures were raised to the air-liquid interface. As in wound healing [22], suprabasal expression was transient, and by 2 weeks β_1 integrins were confined to the basal layer of the raft cultures, as observed by Asselineau *et al* [56] and Kaur and Carter [64]. The distribution of integrins in the rafts was not affected by substituting human dermal fibroblasts for 3T3 cells or by adding TGF-β, IFN-γ, or TNF-α to the culture medium.

We are thus left with the question of what causes suprabasal integrin expression. *In vivo*, it appears to be linked to the proliferative status of keratinocytes, being seen in situations in which the epithelium is hyperproliferative or there is a high turnover rate of the keratinocyte population [22,65]. An exception to this is Rheinwald and Green cultures, which have a high proliferative rate (average doubling time for cells in exponential growth is 24 h [66]); however, it could be argued that the truncation of the terminal-differentiation pathway in these cultures [61] prevents suprabasal expression. In this context, it is interesting that Asselineau *et al* [56] found suprabasal integrin expression in 2-week dermal equivalent cultures treated with a high concentration (10^{-6} M) of retinoic acid; in the same cultures expression of differentiation markers is delayed and uncoupled from morphogenesis. It will be worthwhile to extend our *in vivo* analysis of integrin expression to later biopsies

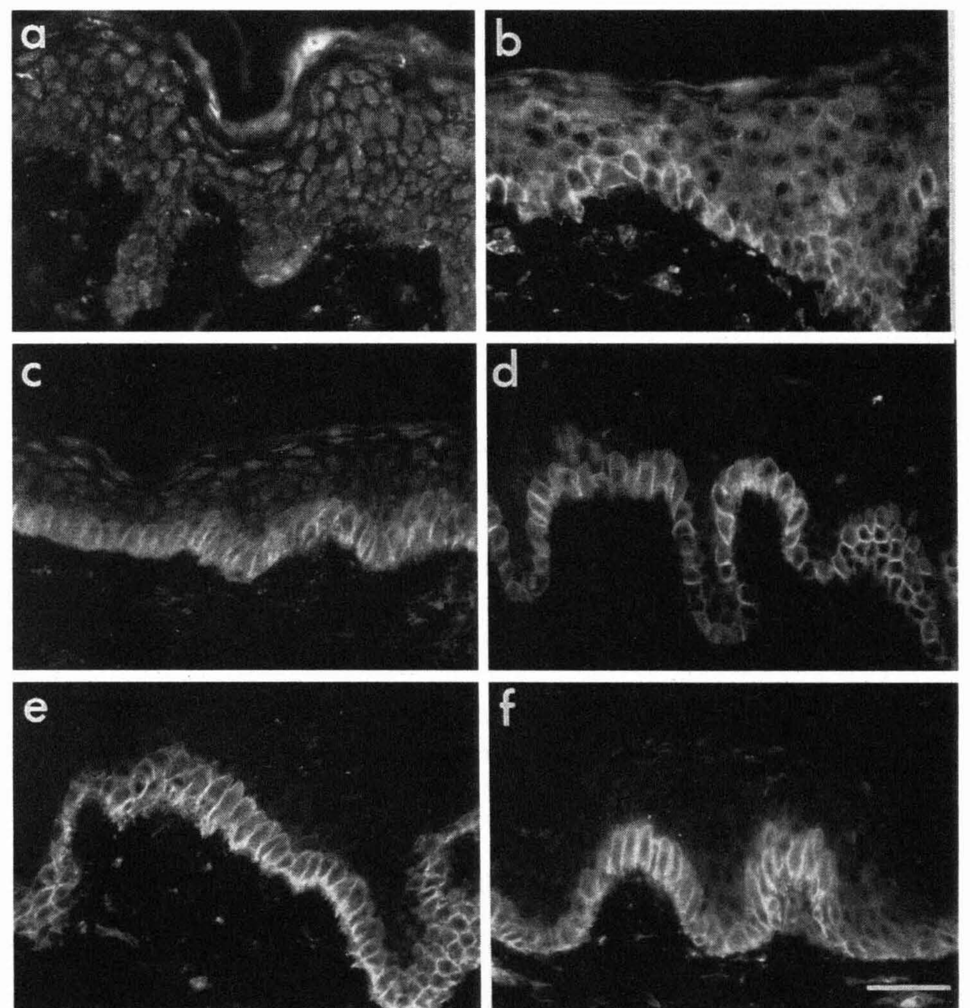

Figure 4. Cytokines do not induce suprabasal integrin expression *in vivo*. Biopsy specimens of skin of volunteers after intradermal injection of cytokines or PBS. The injected material, time of biopsy post-injection, and antibodies used for staining were as follows: *a*) PBS control, anti–ICAM-1; *b*) IFN-γ, 2 d, anti–ICAM-1; *c*) PBS control, anti-α_2; *d*) TNF-α, 1 d, anti-α_2; *e*) IFN-γ, 1 d, anti-α_3; *f*) IFN-γ, 5 d, anti-β_1.

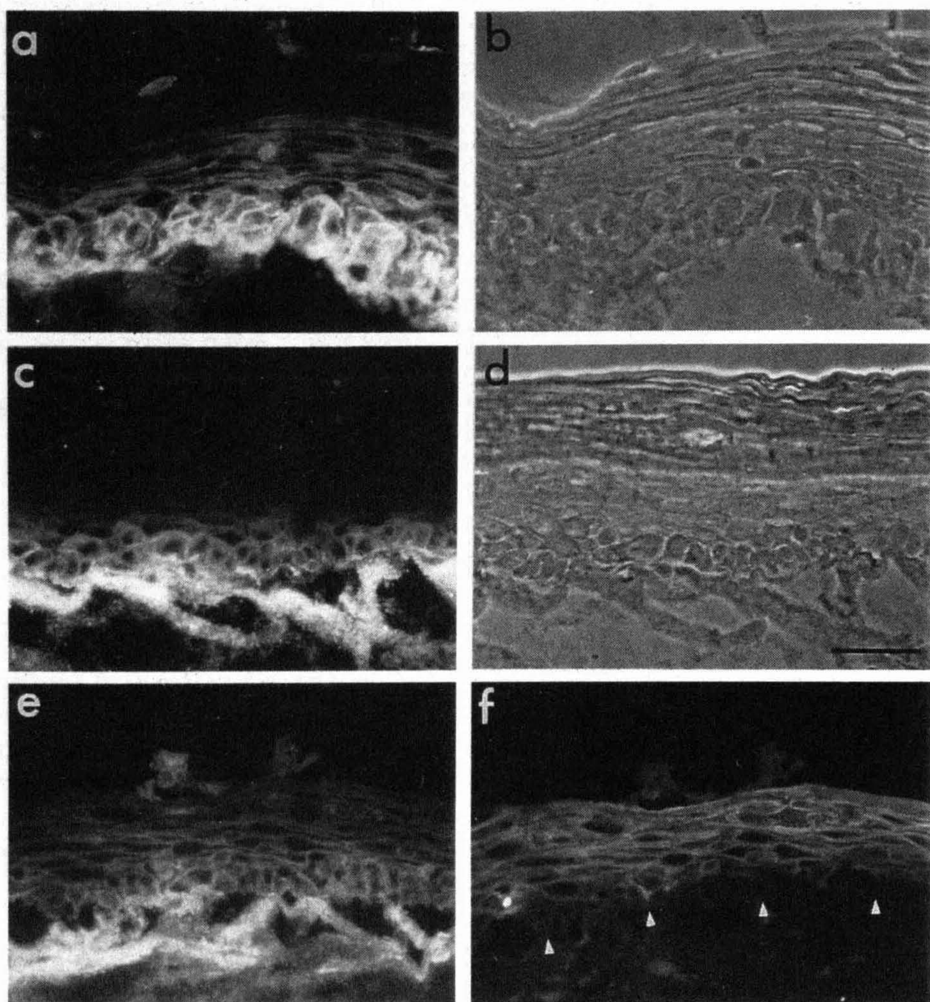

Figure 5. Integrins can be expressed suprabasally in dermal equivalent cultures. Cultures were stained with anti-β_1 antibody. *a,b,e,f*) 1 week; *c,d*) 2 weeks after raising to the air-medium interface. *a–d*) Control cultures; *e–f*) treated with 10 ng/ml TGF-β; *b,d*) Phase-contrast images of *a,c*, respectively. *e,f*) Double-label immunofluorescence: *e*) β_1; *f*) involucrin. *Arrowheads* (*f*) denote boundary between keratinocytes and underlying collagen gel. *Bar*, 50 μm.

of IFN-γ–treated skin, as increased keratinocyte proliferation is observed at day 6 post-injection [47].

In addition to the question of what stimuli lead to suprabasal integrin expression, there are questions as to the nature and function of the receptors. We need to discover whether the presence of suprabasal receptors reflects *de novo* integrin synthesis by differentiating keratinocytes or an increase in the half-life of receptors synthesized in the basal layer [12]. At least *in vivo*, there is no evidence for co-localization of the suprabasal integrins with the ECM proteins that are their ligands [22], thus opening the possibility that their primary function is in intercellular adhesion [15] or that the receptors are inactive [2,59].

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REFERENCES

1. Hynes RO: Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69:11–25, 1992
2. Adams JC, Watt FM: Changes in keratinocyte adhesion during terminal differentiation: reduction in fibronectin binding precedes $\alpha_5\beta_1$ integrin loss from the cell surface. *Cell* 63:425–435, 1990
3. Carter WG, Wayner EA, Bouchard TS, Kaur P: The role of integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$ in cell-cell and cell-substrate adhesion of human epidermal cells. *J Cell Biol* 110:1387–1404, 1990
4. Staquet MJ, Levarlet B, Dezutter-Dambuyant C, Schmitt D, Thivolet J: Identification of specific human epithelial cell integrin receptors as VLA proteins. *Exp Cell Res* 187:277–283, 1990
5. Adams JC, Watt FM: Expression of β_1, β_3, β_4, and β_5 integrins by human epidermal keratinocytes and non-differentiating keratinocytes. *J Cell Bio* 115:829–841, 1991
6. Carter WG, Ryan MC, Gahr PJ: Epiligrin, a new cell adhesion ligand for integrin $\alpha_3\beta_1$ in epithelial basement membranes. *Cell* 65:599–610, 1991
7. Marchisio PC, Bondanza S, Cremona O, Cancedda R, De Luca M: Polarized expression of integrin receptors ($\alpha_6\beta_4$, $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_v\beta_5$) and their relationship with the cytoskeleton and basement membrane matrix in cultured human keratinocytes. *J Cell Biol* 112:761–773, 1991
8. Carter WG, Kaur P, Gil SG, Gahr PJ, Wayner EA: Distinct functions for integrins $\alpha_3\beta_1$ in focal adhesions and $\alpha_6\beta_4$/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. *J Cell Biol* 111:3141–3154, 1990
9. De Luca M, Tamura RN, Kajiji S, Bondanza S, Rossino P, Cancedda R, Marchisio PC, Quaranta V: Polarized integrin mediates human keratinocyte adhesion to basal lamina. *Proc Natl Acad Sci USA* 87:6888–6892, 1990
10. Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK: $\alpha_6\beta_4$ integrin is a component of hemidesmosomes. *Proc Natl Acad Sci USA* 87:8970–8974, 1990
11. Adams JC, Watt FM: Fibronectin inhibits the terminal differentiation of human keratinocytes. *Nature* 340:307–309, 1989
12. Hotchin NA, Watt FM: Transcriptional and post-translational regulation of β_1 integrin expression during keratinocyte terminal differentiation. *J Biol Chem* 267:14852–14858, 1992
13. Kim JP, Zhang K, Chen JD, Wynn KC, Kramer RH, Woodley DT: Mechanism of human keratinocyte migration on fibronectin: unique roles of RGD site and integrins. *J Cell Physiol* 151:443–450, 1992
14. Larjava H, Peltonen J, Akiyama SK, Yamada SS, Gralnick HR, Uitto J, Yamada KM: Novel function for β_1 integrins in keratinocyte cell-cell interactions. *J Cell Biol* 110:803–815, 1990
15. Symington BE, Takada Y, Carter WG: Interaction of integrins $\alpha_3\beta_1$ and $\alpha_2\beta_1$: potential role in keratinocyte intercellular adhesion. *J Cell Biol* 120:523–535, 1993
16. Tenchini ML, Adams JC, Gilbert C, Steel J, Hudson DL, Malcovati M, Watt FM: Evidence against a major role of integrins in calcium-dependent intercellular adhesion of epidermal keratinocytes. *Cell Adhesion Communication* 1:55–66, 1993
17. Jones PH, Watt FM: Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* 73:713–724, 1993
18. Wayner EA, Carter WG, Piotrowicz RS, Kunicki TJ: The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J Cell Biol* 107:1881–1891, 1988
19. De Strooper B, Van Der Schueren B, Jaspers M, Saison M, Spaepen M, Van Leuven F, Van Den Berghe H, Cassiman J-J: Distribution of the β_1 subgroup of the integrins in human cells and tissues. *J Histochem Cytochem* 37:299–307, 1989
20. Peltonen J, Larjava H, Jaakkola S, Gralnick H, Akiyama SK, Yamada SS, Yamada KM, Uitto J: Localization of integrin receptors for fibronectin, collagen and laminin in human skin. *J Clin Invest* 84:1916–1923, 1989
21. Hertle MD, Adams JC, Watt FM: Integrin expression during human epidermal development in vivo and in vitro. *Development* 112:193–206, 1991
22. Hertle MD, Kubler M-D, Leigh IM, Watt FM: Aberrant integrin expression during epidermal wound healing and in psoriatic epidermis. *J Clin Invest* 89:1892–1901, 1992
23. Clark RAF: Wound repair. *Curr Opin Cell Biol* 1:1000–1008, 1989
24. Horrocks C, Duncan JI, Oliver AM, Thomson AW: Adhesion molecule expression in psoriatic skin lesions and the influence of cyclosporin A. *Clin Exp Immunol* 84:157–162, 1991
25. Kellner I, Konter U, Sterry W: Overexpression of extracellular matrix receptors (VLA-3,5 and 6) on psoriatic keratinocytes. *Br J Dermatol* 125:211–216, 1991
26. Ralfkiaer E, Thomsen K, Vejlsgaard GL: Expression of a cell adhesion protein (VLA β) in normal and diseased skin. *Br J Dermatol* 124:527–532, 1991
27. Pellegrini G, De Luca M, Orecchia G, Balzac F, Cremona O, Savoia P, Cancedda R, Marchisio PC: Expression, topography, and function of integrin receptors are severely altered in keratinocytes from involved and uninvolved psoriatic skin. *J Clin Invest* 89:1783–1795, 1992
28. McKenzie RC, Sauder DN: The role of keratinocyte cytokines in inflammation and immunity. *J Invest Dermatol* 95:105S–107S, 1990
29. McKay IA, Leigh IM: Epidermal cytokines and their roles in cutaneous wound healing. *Br J Dermatol* 124:513–518, 1991
30. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB: Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proc Natl Acad Sci USA* 86:6367–6371, 1989
31. Choi Y, Fuchs E: TGF-β and retinoic acid: regulators of growth and modifiers of differentiation in human epidermal cells. *Cell Reg* 1:791–809, 1990
32. Barrandon Y, Green H: Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-α and epidermal growth factor. *Cell* 50:1131–1137, 1987
33. Nickoloff BJ, Mitra RS, Riser BL, Dixit VM, Varani J: Modulation of keratinocyte motility. Correlation with production of extracellular matrix molecules in response to growth promoting and antiproliferative factors. *Am J Pathol* 132:543–551, 1988
34. Ignotz RA, Massagué J: Cell adhesion protein receptors as targets for transforming growth factor-β action. *Cell* 51:189–197, 1987

35. Heino J, Ignotz RA, Hemler ME, Crouse C, Massagué J: Regulation of cell adhesion receptors by transforming growth factor-β. Concomitant regulation of integrins that share a common β_1 subunit. *J Biol Chem* 264:380–388, 1989
36. Defilippi P, Truffa G, Stefanuto G, Altruda F, Silengo L, Tarone G: Tumor necrosis factor α and interferon γ modulate the expression of the vitronectin receptor (integrin β_3) in human endothelial cells. *J Biol Chem* 266:7638–7645, 1991
37. Mortarini R, Anichini A, Parmiani G: Heterogeneity for integrin expression and cytokine mediated VLA modulation can influence the adhesion of human melanoma cells to extracellular matrix proteins. *Int J Cancer* 47:551–559, 1991
38. Santala P, Heino J: Regulation of integrin-type cell adhesion receptors by cytokines. *J Biol Chem* 266:23505–23509, 1991
39. Bauvois B, Rouillard D, Sanceau J, Wietzerbin J: IFN-γ and transforming growth factor-β_1 differently regulate fibronectin and laminin receptors of human differentiating monocytic cells. *J Immunol* 148:3912–3919, 1992
40. Cervella P, Silengo L, Pastore C, Altruda F: Human β_1-integrin gene expression is regulated by two promoter regions. *J Biol Chem* 268:5148–5155, 1993
41. Thiery JP, Boyer B: The junction between cytokines and cell adhesion. *Curr Opin Cell Biol* 4:782–792, 1992
42. Kane CJM, Hebda PA, Mansbridge JN, Hanawalt PC: Direct evidence for spatial and temporal regulation of transforming growth factor β_1 expression during cutaneous wound healing. *J Cell Physiol* 148:157–173, 1991
43. Köck A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, Ansel JC, Luger TA: Human keratinocytes are a source for tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. *J Exp Med* 172:1609–1614, 1990
44. Nickoloff BJ, Mahrle G, Morhenn V: Ultrastructural effects of recombinant gamma-interferon on cultured human keratinocytes. *Ultrastruc Pathol* 10:17–21, 1986
45. Mansbridge JN, Hanawalt PC: Role of transforming growth factor-β in the maturation of human epidermal keratinocytes. *J Invest Dermatol* 90:336–341, 1988
46. Symington FW: Lymphotoxin, tumor necrosis factor, and gamma interferon are cytostatic for normal human keratinocytes. *J Invest Dermatol* 92:798–805, 1989
47. Barker J, Goodlad JR, Ross EL, Yu CC, Groves RW, MacDonald DM: Increased epidermal cell proliferation in normal human skin in vivo following local administration of interferon-gamma. *Am J Pathol* 142:1091–1097, 1993
48. Rheinwald JG: Methods for clonal growth and serial cultivation of normal human keratinocytes and mesothelial cells. In: Baserga R (ed.). *Cell Growth and Division. A Practical Approach.* IRL Press, Oxford, 1989, pp 81–94
49. Watt FM: Selective migration of terminally differentiating cells from the basal layer of cultured human epidermis. *J Cell Biol* 98:16–21, 1984
50. Kaufmann R, Froesch D, Westphal C, Weber L, Klein CE: Integrin VLA-3: ultrastructural localization at cell-cell contact sites of human cell cultures. *J Cell Biol* 109:1807–1815, 1989
51. Wayner EA, Gil SG, Murphy GF, Wilke MS, Carter WG: Epiligrin, a component of epithelial basement membranes, is an adhesive ligand for $\alpha_3\beta_1$ positive T lymphocytes. *J Cell Biol* 121:1141–1152, 1993
52. Dransfield I, Cabañas C, Barrett J, Hogg N: Interaction of leukocyte integrins with ligand is necessary but not sufficient for function. *J Cell Biol* 116:1527–1535, 1992
53. Dover R, Watt FM: Measurement of the rate of epidermal terminal differentiation: expression of involucrin by S-phase keratinocytes in culture and in psoriatic plaques. *J Invest Dermatol* 89:349–352, 1987
54. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–689, 1970
55. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T: Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness. *Science* 211:1052–1054, 1981
56. Asselineau D, Bernard BA, Bailly C, Darmon M: Retinoic acid improves epidermal morphogenesis. *Dev Biol* 133:322–335, 1989
57. Caughman SW, Li LJ, Degitz K: Characterization and functional analysis of interferon-γ-induced intercellular adhesion molecule-1 expression in human keratinocytes and A-431 cells. *J Invest Dermatol* 94:22S–26S, 1990
58. Dustin ML, Singer K, Tuck D, Springer TA: Adherence of T lymphoblasts to epidermal keratinocytes is regulated by IFN gamma and mediated by ICAM-1. *J Exp Med* 167:1323–1340, 1988
59. Hotchin NA, Kovach NL, Watt FM: Functional down-regulation of $\alpha_5\beta_1$ integrin in keratinocytes is reversible but commitment to terminal differentiation is not. *J Cell Sci* 106:1131–1138, 1993
60. Barker JNWN, Allen MH, MacDonald DM: The effect of in vivo interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. *J Invest Dermatol* 93:439–442, 1989
61. Fuchs E: Epidermal differentiation: the bare essentials. *J Cell Biol* 111:2807–2814, 1990
62. Sauder DN: The role of epidermal cytokines in inflammatory skin diseases. *J Invest Dermatol* 95:27S–28S, 1990
63. Barker JNWN, Allen MH, MacDonald DM: Alterations induced in normal human skin by in vivo interferon-gamma. *Br J Dermatol* 122:451–458, 1990
64. Kaur P, Carter WG: Integrin expression and differentiation in transformed human epidermal cells is regulated by fibroblasts. *J Cell Sci* 103:755–763, 1992
65. Jones J, Sugiyama M, Watt FM, Speight PM: Integrin expression in normal, hyperplastic, dysplastic, and malignant oral epithelium. *J Pathol* 169:235–243, 1993
66. Rheinwald JG, Green H: Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. *Nature* 265:421–424, 1977