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Expression and function of the keratinocyte integrins

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SUMMARY

Human keratinocytes express several adhesive receptors of the integrin family. Expression is normally confined to the basal (proliferative) layer of keratinocytes, both in mature epidermis and during development. Altered expression patterns are observed during wound healing, in psoriasis and in squamous cell carcinomas. Keratinocyte integrins are subject to both transcriptional and post-translational regulation and ligand binding ability can be modulated independently of expression. Studies with cultured keratinocytes suggest a variety of functions for the receptors: adhesion to extracellular matrix proteins, intercellular adhesion, stratification, lateral migration and the regulation of terminal differentiation. Three distinct subpopulations of basal keratinocytes, with characteristics of stem cells, transit amplifying cells and cells committed to differentiate, can be distinguished on the basis of differences in integrin expression and function.

Key words: epidermis, adhesion, differentiation

INTRODUCTION

The process of differentiation is not confined to the embryo: throughout adult life new differentiated cells are produced to replace cells lost through death or tissue damage. In stratified squamous epithelia such as the epidermis dead, terminally differentiated cells are continually shed from the outermost layers of the tissue and replaced through proliferation of a stem cell population in the basal layer. Human epidermal keratinocytes can be grown in culture under conditions in which they form stratified sheets with the same basic organisation as epidermis in vivo and these can be used to study stem cell proliferation, terminal differentiation and tissue assembly (reviewed by Watt, 1988, 1989, 1991).

Recent work has highlighted the importance of the integrin family of adhesive receptors in regulating keratinocyte behaviour. Each integrin is a heterodimer of one α and one β subunit, which are non-covalently associated (reviewed by Albelda and Buck, 1990; Hemler, 1990; Ruoslahti, 1991; Hynes, 1992). Each subunit has a large extracellular domain, a transmembrane domain and a cytoplasmic domain which is usually short and usually associates with the actin cytoskeleton. Binding of ligands, which are extracellular matrix proteins or counter-receptors of the immunoglobulin superfamily, requires both subunits; the ligand binding sites appear to be intimately associated with cation binding domains on the α subunits. So far over 7 different β subunits and 13 different α subunits have been identified and a growing number have been found to exist as two or more splice variants. An individual β subunit can potentially partner several different α subunits and vice versa: ligand binding specificity depends, to a large extent, on heterodimer composition. In addition, some integrins can bind several apparently structurally unrelated ligands and a given integrin expressed in two different cell types can show different ligand binding properties.

THE KERATINOCYTE INTEGRINS

Integrins mediate attachment of keratinocytes to the basement membrane that separates the epidermis from the dermis; this specialised extracellular matrix is rich in a number of proteins and proteoglycans, including laminin, type IV collagen and epiligrin (which may be identical to kalinin) (Yurchenco and Schittny, 1990; Rousselle et al., 1991; Watt and Hotchin, 1992). The most abundant keratinocyte integrins are $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_6\beta_4$ (see Table 1 for summary). $\alpha_2\beta_1$ is a receptor for collagen and laminin and $\alpha_3\beta_1$ is a receptor for laminin and epiligrin (Carter et al., 1990a,b, 1991; Staquet et al., 1990; Adams and Watt, 1991). $\alpha_6\beta_4$ is a component of hemidesmosomes; its ligand has yet to be established unequivocally but may be laminin (Stepp et al., 1990; De Luca et al., 1990; Lee et al., 1992).

Two other keratinocyte integrins that are well characterised are $\alpha_5\beta_1$, a fibronectin receptor (Adams and Watt, 1990, 1991; Carter et al., 1990a), and $\alpha_v\beta_5$, a vitronectin receptor (Adams and Watt, 1991; Marchisio et al., 1991). Although $\alpha_5\beta_1$ is readily detected in cultured keratinocytes it is either weakly expressed (Hertle et al., 1991, 1992) or undetectable (Peltonen et al., 1989; Nazzaro et al., 1990; Pellegrini et al., 1992) in mature epidermis. There is a low level of fibronectin in mature basement membrane (Stenman and Vaheri, 1978; Fleischmajer and Timpl, 1984) and vit-

Table 1. Integrins expressed by keratinocytes (see text for references)

Integrin	Ligand(s)	Comments
$\alpha_1\beta_1$	?	Expressed in trace amounts or undetectable
$\alpha_2\beta_1$	Collagen, laminin	
$\alpha_3\beta_1$	Laminin, epiligrin	
$\alpha_5\beta_1$	Fibronectin	
$\alpha_8\beta_1$	?	
$\alpha_6\beta_4$	Laminin?	Component of hemidesmosomes
$\alpha_v\beta_5$	Vitronectin	
β_7?		Reported expression of β_7-related mRNA

ronectin is reported to be absent (Reilly and Nash, 1988). However, in wounds in which the basement membrane is destroyed, fibronectin forms the provisional matrix over which keratinocytes migrate (Clark, 1990) and when placed in culture keratinocytes become adhesive to fibronectin (Grinnell, 1992).

Other integrin subunits reported to be expressed by keratinocytes include α_1, which forms a heterodimer with β_1 and is expressed in trace amounts (Belkin et al., 1990; Buck et al., 1990; Hertle et al., 1991; but see also De Luca et al., 1990; Nazzaro et al., 1990; Zambruno et al., 1991) and α_8, which is moderately abundant in chick embryonic epidermis and forms a heterodimer with the β_1 subunit (Bossy et al., 1991). A β_7-related mRNA has been identified in cultured mouse keratinocytes; however, its small size suggests that it is unlikely to encode full-length β_7 protein (Yuan et al., 1992). The β_6 integrin subunit forms a heterodimer with α_v and acts as a fibronectin receptor in a range of epithelial cells (Sheppard et al., 1990; Busk et al., 1992); it is not known whether keratinocytes express β_6, but antibodies to α_v do not block keratinocyte adhesion to fibronectin (Adams and Watt, 1991).

Variant forms of integrin subunits are believed to arise through alternative splicing; in each case the classical form is referred to as the A form. In addition to β_{1A}, keratinocytes express a variant known as β_{1B} in which a unique 12 amino acid sequence replaces the carboxy-terminal 21 amino acids of β_{1A} (Altruda et al., 1990; Balzac et al., 1993). α_{6A} and α_{6B} have unique cytoplasmic domains of 36 and 54 amino acids respectively; keratinocytes only express α_{6A} (Hogervorst et al., 1993); Tamura et al., 1991). Splice variants of the α_3 (Tamura et al., 1991) and β_4 (Hogervorst et al., 1990; Suzuki and Naitoh, 1990; Tamura et al., 1990) subunits have also been described. The β_4 cDNA originally cloned from keratinocytes (Hogervorst et al., 1990) contains a 53 amino acid insert in the cytoplasmic domain not found in β_4 cDNA from retinal pigment epithelial cells (Suzuki and Naitoh, 1990) or carcinoma cells (Tamura et al., 1990).

The cytoplasmic domains of integrins not only interact with cytoskeletal proteins but also play a role in signal transduction and can regulate the ligand binding ability of the extracellular domains (Hynes, 1992; Damsky and Werb, 1992; Adams and Watt, 1993; Juliano and Haskell, 1993). It therefore seems likely that the variant integrin subunits will have different cellular functions from the classical forms. Evidence for this has come from transfection experiments which demonstrate that although the ligand binding properties of β_{1A} and β_{1B} are similar, β_{1B} does not localise in focal adhesions (Balzac et al., 1993).

NORMAL AND ABNORMAL INTEGRIN EXPRESSION PATTERNS

Within the epidermis, integrin expression is largely confined to the basal layer of keratinocytes that are attached to the underlying basement membrane (see, for example, Wayner et al., 1988; De Strooper et al., 1989; Hertle et al., 1991, 1992; Fig. 1E). The integrin subunits tend to have a pericellular distribution, although the α_6 and β_4 subunits show a relative concentration at the basement membrane zone, consistent with their association with hemidesmosomes. The α_3 and β_1 subunits are expressed in embryonic epidermis prior to the initiation of stratification and do not change in abundance or distribution during subsequent development; however the other subunits (including α_2; see Fig. 1) show spatial or temporal changes in expression and it is tempting to speculate that integrins may play a role in establishing the spatial organisation of the epidermis (Hertle et al., 1991).

The distribution of integrins in stratified cultures of human keratinocytes resembles their distribution within the epidermis: expression is largely restricted to the basal layer (Nicholson and Watt, 1991; Adams and Watt, 1991). Flow cytometry can be used to analyse the relationship between integrin levels and differentiation status in individual keratinocytes, using binding of peanut lectin (PNA) as a marker of terminal differentiation (Watt and Jones, 1992). Fig. 2 shows that the α_3, α_5 and α_6 subunits are markedly downregulated in cells that bind PNA; α_2 expression is also decreased, but to a lesser extent.

Although integrin expression is normally confined to basal keratinocytes, altered expression patterns have been observed in situations in which the normal balance between proliferation and terminal differentiation is perturbed. During wound healing and in psoriatic lesions integrins are expressed suprabasally by keratinocytes that have initiated terminal differentiation (Ralfkiaer et al., 1991; Hertle et al., 1992). In squamous cell carcinomas there is considerable variation in integrin expression both within and between tumours (see, for example, Peltonen et al., 1989; Wolf et al., 1990; Jones et al., 1993), but focal loss of the α_2, α_3, α_6 and β_4 subunits is a common feature of poorly differentiated oral squamous cell carcinomas (Jones et al., 1993). Reduced expression of specific integrin subunits is observed in some keratinocyte lines derived from oral squamous cell carcinomas (Sugiyama et al., unpublished data) and experiments are underway to investigate whether 'repair' of the lines with appropriate integrin expression vectors has any effect on their proliferative potential and differentiation capacity (cf. Giancotti and Ruoslahti, 1990).

TRANSCRIPTIONAL AND POSTTRANSLATIONAL REGULATION OF INTEGRIN EXPRESSION

One technique which has been used extensively to study integrin expression in keratinocytes is suspension-induced terminal differentiation (Green, 1977; Watt et al., 1988).

Cultured human keratinocytes are disaggregated and resuspended in medium made viscous by the addition of methyl cellulose: the cells remain rounded and are prevented from adhering to one another or to the culture dish. By about 5 hours in suspension, keratinocytes have withdrawn from the cell cycle and become committed to differentiate; by 24 hours the majority of cells have initiated terminal differentiation and are expressing involucrin, a precursor of the cornified envelope that is expressed suprabasally in culture and in the upper layers of the epidermis.

When keratinocytes are placed in suspension for 24 hr there is a marked reduction in cell surface levels of integrins, although the α_2 subunit is not downregulated to the same extent as the other subunits (Fig. 3; Adams and Watt, 1990; Hotchin and Watt, 1992). There is a corresponding decline in integrin mRNA levels (Nicholson and Watt, 1991) which reflects a shut off of transcription of integrin genes (Hotchin and Watt, 1992). In situ hybridisation of sections of skin (Watt and Hertle, 1993) and of stratified keratinocyte cultures (Hodivala and Watt, unpublished data) shows that integrin mRNAs are localised to the basal cell layer. The absence of integrins from the surface of terminally differentiating cells does not simply reflect transcriptional regulation, however: when keratinocytes are suspended in methyl cellulose, N-linked glycosylation and intracellular transport of newly synthesised β_1 integrins are inhibited by a mechanism that remains to be elucidated (Hotchin and Watt, 1992).

Functional downregulation of integrins precedes loss of the receptors from the cell surface. When keratinocytes become committed to terminal differentiation, at about 5 hr in suspension, the ability of the β_1 integrins to bind extracellular matrix proteins is substantially decreased although there is no reduction in the level of integrins on the cell surface at this time (Adams and Watt, 1990). The decrease in ligand binding ability reflects functional modulation of receptors on the cell surface (Hotchin and Watt, 1992) which would be consistent with a change in receptor conformation (O'Toole et al., 1990; Faull et al., 1993; Hotchin et al., unpublished data). The decreased ability of committed cells to adhere to extracellular matrix proteins is likely to ensure that those cells are selectively expelled from the basal layer and migrate into the layer above (Adams and Watt, 1990; Fig. 4).

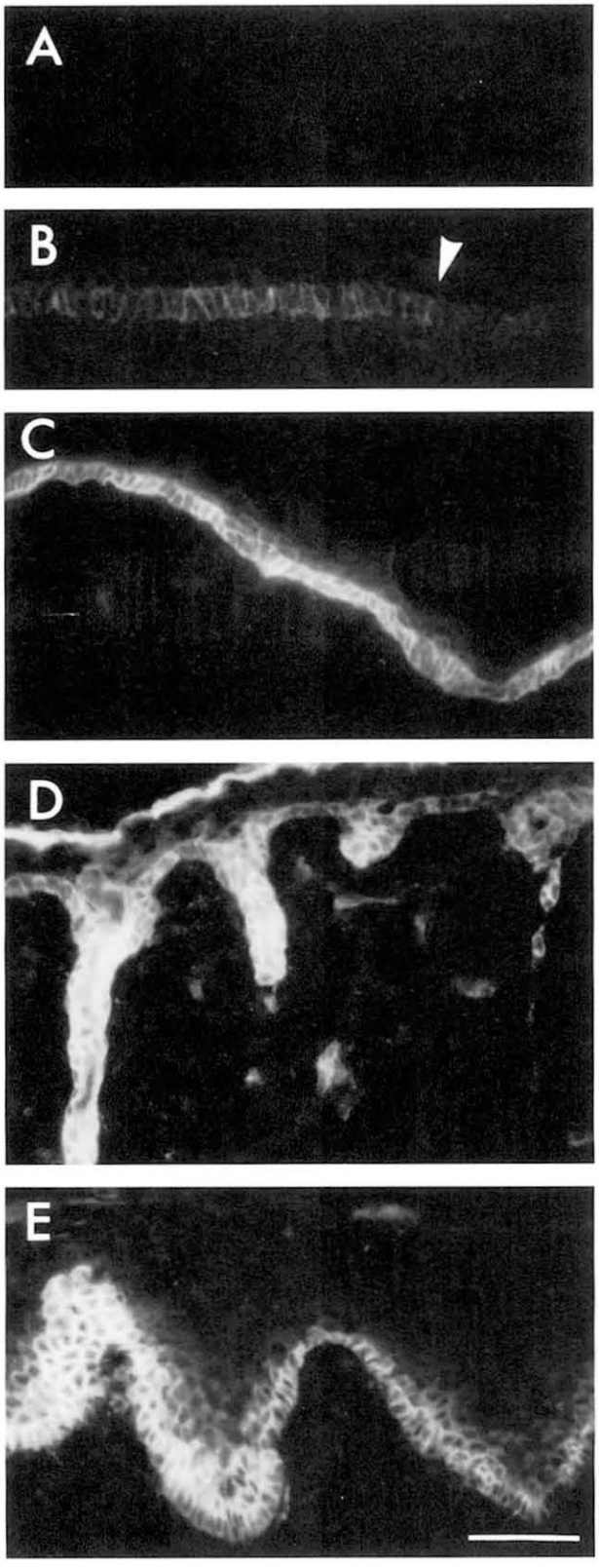

Fig. 1. Immunofluorescence staining shows expression of the α_2 integrin subunit during human epidermal development. Gestational ages are as follows. (A) 7.8 weeks; epidermis consists of one layer of keratinocytes overlaid by periderm; the α_2 subunit is undetectable. (B) 9.0 weeks; the epidermis still contains only one layer of keratinocytes, but the α_2 integrin subunit is expressed; note that α_2 is more abundant to the left of the arrow than to the right. (C) 10.7 weeks; stratification has begun and there are now two layers of keratinocytes; (D) 15.3 weeks; there are several suprabasal layers; note the developing sweat ducts which stain intensely; (E) neonatal epidermis. Scale bar, 50 μm. Reproduced from Hertle et al., 1991; copyright The Company of Biologists Ltd.

FUNCTIONS OF THE KERATINOCYTE INTEGRINS

Cell-cell and cell-extracellular matrix adhesion

The primary function of keratinocyte integrins is adhesive, but the receptors do not act simply as passive attachments to the basement membrane. As described above, functional

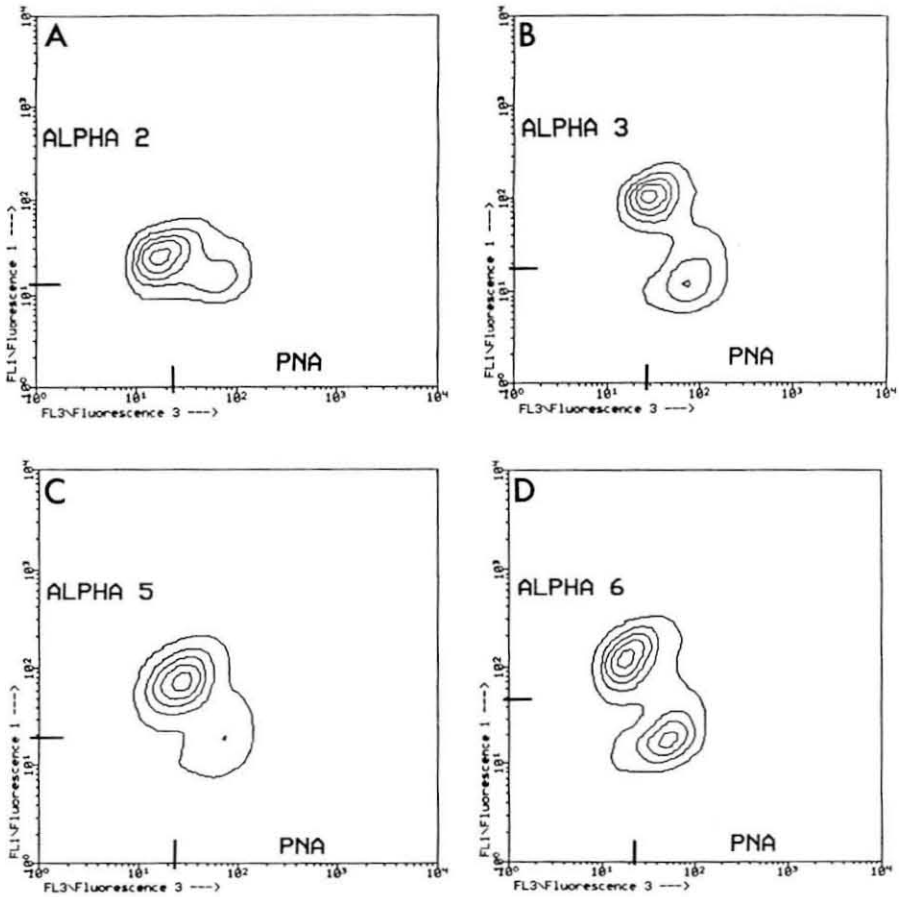

Fig. 2. Flow cytometer contour plots of single cell suspensions of confluent cultured keratinocytes labelled with antibodies to individual integrin subunits (α_2, α_3, α_5, α_6) and with peanut lectin (PNA) which binds specifically to terminally differentiating cells. Fluorescence is shown in arbitrary units on a log scale on both x and y axes and the number of contour lines reflects the number of cells with a given value of fluorescence. Anti-α_2 and α_3 antibodies (HAS6 and VM-2, respectively) were direct FITC conjugates, anti-α_5 and α_6 antibodies (mAb16 and GoH3, respectively) were detected with an FITC-conjugated anti-rat antibody. Biotinylated PNA was detected with Tricolor streptavidin (Watt and Jones, 1992). Markers on each axis show the upper limit of staining of the negative controls (CD8-FITC for α_2 and α_3, FITC-anti rat alone for α_5 and α_6 and Tricolor alone for PNA). Note that the α_3 and α_6 subunits are markedly downregulated in cells that express a higher level of PNA (approximately a 10-fold decrease in fluorescence); the α_5 subunit is similarly downregulated, but the α_2 subunit shows a much smaller decrease in fluorescence.

downregulation of the β_1 integrins ensures the migration of committed cells out of the basal layer (Adams and Watt, 1990). In addition, lateral migration of keratinocytes in vitro is mediated by integrins, implying that they play a role in wound healing in vivo (Kim et al., 1992; Grinnell, 1992).

Integrins are present on the lateral membranes of basal keratinocytes (see Fig. 1) and there is some evidence that they play a role in intercellular adhesion, perhaps through direct binding of $\alpha_2\beta_1$ to $\alpha_3\beta_1$ (Symington et al., 1993). The best characterised intercellular receptors of keratinocytes are the desmosomal and nondesmosomal cadherins (reviewed by Takeichi, 1991; Geiger and Ayalon, 1992; Buxton and Magee, 1992) which require extracellular calcium ions in order to function. In low calcium medium cell-cell contacts can be disrupted by an anti-β_1 antibody (Larjava et al., 1990); however individual anti-integrin antibodies do not block calcium-dependent aggregation of keratinocytes in suspension (Tenchini et al., 1993). Further experiments on the role of integrins in keratinocyte-keratinocyte adhesion are clearly necessary, particularly in the light of recent evidence that cadherins may play a role in the down regulation of integrin expression that occurs during terminal differentiation (Hodivala and Watt, unpublished data).

Regulation of terminal differentiation

When keratinocytes are plated on an adhesive substrate that restricts spreading, terminal differentiation is stimulated (Watt et al., 1988) and loss of contact with the extracellular matrix may be the primary differentiation stimulus in suspension (Adams and Watt, 1989). Suspension-induced terminal differentiation can be inhibited by inclusion of fibronectin or antibodies to the β_1 integrin subunit in the methyl cellulose at the time of plating (Adams and Watt, 1989; Watt et al., 1993). However, if fibronectin is added more than 2 hr after the cells have been placed in suspension differentiation is not inhibited, because of the decrease in $\alpha_5\beta_1$ ligand-binding ability that occurs on commitment to differentiation (Adams and Watt, 1989, 1990). Recent experiments have shown that laminin and type IV collagen, in combination with a low concentration of fibronectin, can participate in the inhibition of differentiation, as can a cocktail of function blocking antibodies (which presumably mimic receptor-ligand binding) to the α_2, α_3 and α_5 subunits (Watt et al., 1993). These observations suggest that, in vivo, terminal differentiation may be regulated by the total proportion of β_1 heterodimers occupied by ligand, a decrease in that proportion acting as a stimulus for differentiation (see Fig. 4). One extension of this hypothesis is that integrins on the apical and lateral membranes of basal keratinocytes may be inactive because they are not in contact with extracellular matrix proteins (see, for example, Tenchini et al., 1993).

Stem cells and transit amplifying cells

The epidermis is believed to contain two types of proliferating cell: stem cells, which retain a high capacity for self-renewal throughout adult life, and transit amplifying cells, which have a lower capacity for self-renewal and a high probability of undergoing terminal differentiation after a few

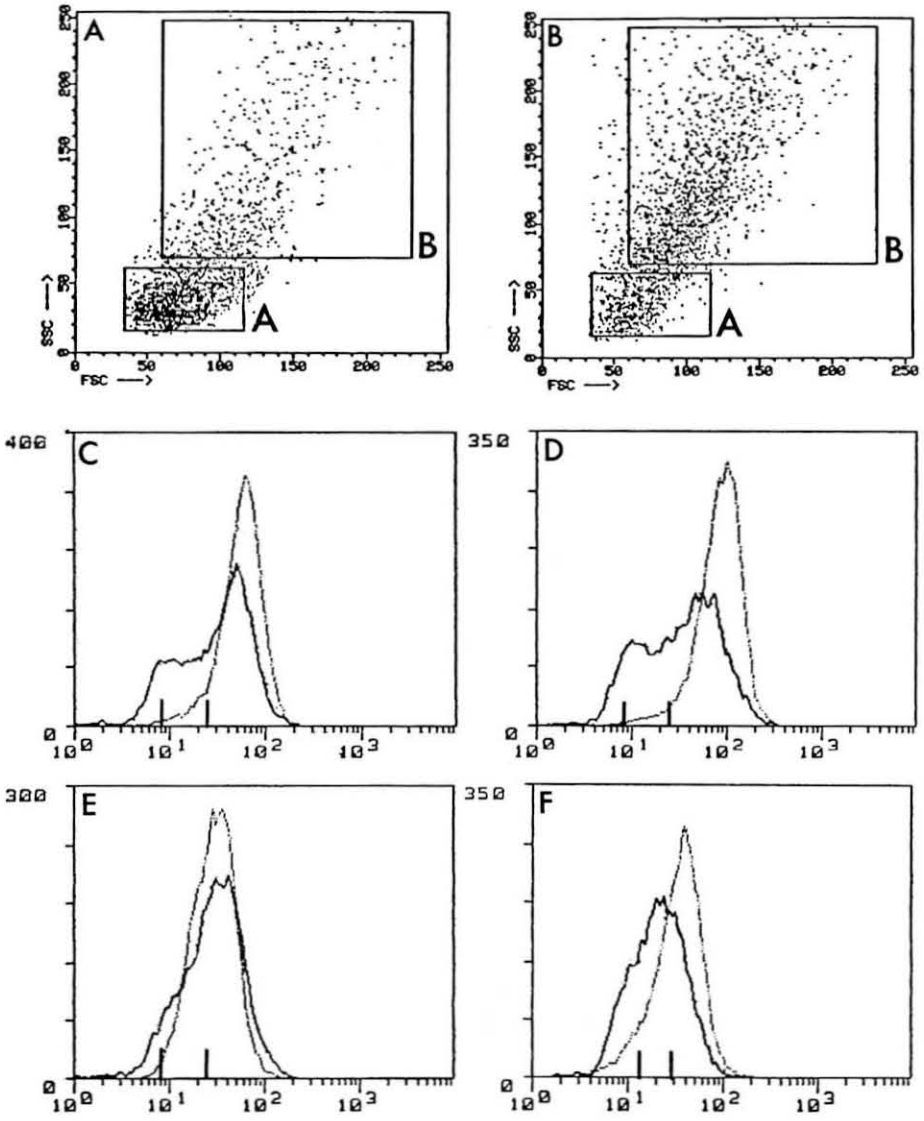

Fig. 3. Effects of suspension culture on integrin expression. (A,B) Dot plots showing the light scattering characteristics of a keratinocyte suspension before (A) and after (B) 24 hr of suspension culture in methyl cellulose. The forward and side light scatter (FSC and SSC respectively) are recorded in arbitrary units on a linear scale, each dot representing one cell. FSC is related to cell size and SSC to cytoplasmic complexity. Region A in each panel contains basal cells and region B contains terminally differentiating cells (Jones and Watt, 1993); there is a marked increase in the number of terminally differentiating cells after suspension. (C-F) Histograms showing the level of fluorescence of integrin subunits (C, β_1; D, α_3; E, α_2;F, α_5) in cells before (dotted line) and after (solid line) 24 hr of suspension. Fluorescence is in arbitrary units on a log scale; the vertical axis is cell number. The markers show the levels of control fluorescence before (left hand marker) and after (right hand marker) suspension. There is a decrease in the modal fluorescence of β_1, α_3 and α_5 integrins after 24 hr, with a second peak or a shoulder of dull cells appearing to the left of the peak seen in 0 hr cells. Cells in the integrin dull populations fall into region B on the basis of their light scatter characteristics. In contrast most α_2 expressing cells do not show a decrease in fluorescence. Anti-integrin antibodies are the same as in Fig. 2 (α_2, α_3, α_5) or CD29-FITC (β_1).

rounds of division (reviewed by Potten, 1981; Hall and Watt, 1989). Cells with characteristics of stem cells can be isolated on the basis of high surface expression of β_1 integrins and rapid adhesion to fibronectin, type IV collagen or keratinocyte extracellular matrix proteins (Jones and Watt, 1993; Fig. 4). There is a log linear relationship between β_1 receptor density, as measured by flow cytometry, and the ability of keratinocytes to form colonies of 32 or more cells by 14 days in culture. Basal cells with the highest level of β_1 integrins have about 4 times the colony forming efficiency of cells with the lowest level. There is specificity in the relationship between keratinocyte adhesiveness and proliferative capacity, since α_6 expression and rate of adhesion to laminin do not correlate with proliferative ability.

Cells with characteristics of transit amplifying cells adhere more slowly to the matrix proteins and express lower levels of β_1 integrins. These cells divide 1-5 times and then initiate involucrin expression. One implication of these results is that if a reduction in the number of β_1 integrins with bound ligand is a stimulus for terminal differentiation (Adams and Watt, 1989; Watt et al., 1993) transit cells will be more sensitive to that stimulus than stem cells because they have fewer surface β_1 integrins (see Fig. 4).

In a range of stratified epithelia there is indirect evidence that stem cells occupy a specific location within the basal layer (see, for example, Cotsarelis et al., 1989, 1990). It is difficult to determine the location of the high integrin-expressing putative stem cell population within the basal epidermal layer because in histological sections the staining of lateral membranes reflects integrin levels on adjacent cells. Nevertheless, non-uniform staining of the α_2 subunit has been reported in developing epidermis (Hertle et al., 1991; Fig. 1) and we are currently investigating whether there is variation in integrin levels in the basal layer of mature epidermis.

CONCLUSIONS AND PROSPECTS. INTEGRINS ARE NOT THE ONLY RECEPTORS. . .

In this review we have presented evidence that integrins have several important functions within the epidermis. It would be wrong, however, to give the impression that all

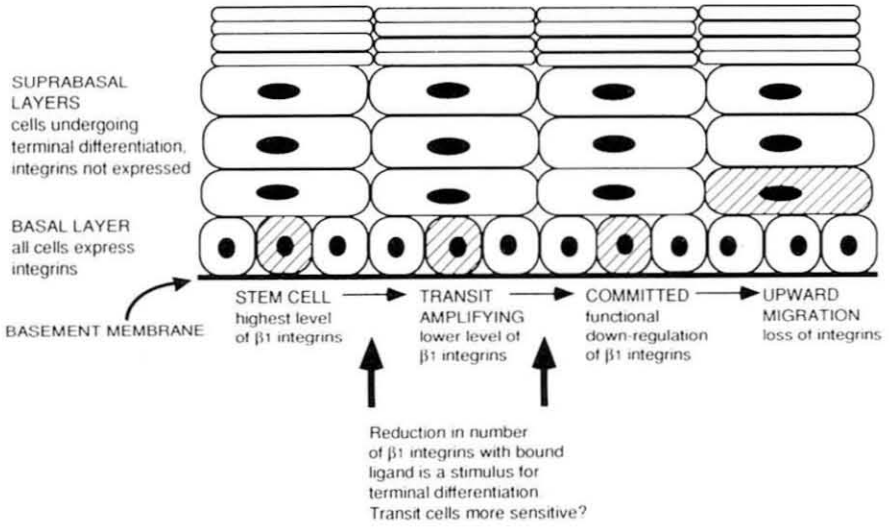

Fig. 4. Model of the relationship between keratinocyte adhesiveness, proliferative capacity and terminal differentiation potential. The proposed sequence of events within the basal layer of the epidermis by which a stem cell generates a suprabasal, terminally differentiating cell is shown. Reproduced from Jones and Watt (1993) with permission. Copyright Cell press.

aspects of keratinocyte behaviour can be explained in terms of integrins. There is no doubt that proliferation and terminal differentiation are profoundly affected by a variety of soluble agents, including growth factors and retinoids (reviewed by Fuchs, 1990; McKay and Leigh, 1991). In addition, the cells express other families of adhesive receptor, including the cadherins, which are undoubtedly important in regulating the adhesive properties of keratinocytes (Wheelock and Jensen, 1992; Hodivala and Watt, unpublished data). One priority for future research is to understand the interplay between different regulatory factors: extracellular matrix adhesiveness may determine keratinocyte responsiveness to growth factors; growth factors may regulate integrin expression (Adams and Watt, 1993); and the fate of stem cell progeny may be to some extent predetermined and to some extent regulated by the cellular environment (Hall and Watt, 1989).

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