Differential expression of the VLA family of integrins along the crypt-villus axis in the human small intestine

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Summary

Regulation of epithelial cell proliferation, migration and differentiation under physiological conditions remains poorly understood. Interaction of the cells with their underlying basement membrane through integrins, a specific subset of cell surface binding proteins, is one potential mechanism. In the present work, I examined this hypothesis by investigating the distribution of a variety of epithelial basement membrane proteins and the expression of the members of the VLA family of integrins in the adult intestinal epithelium. Indeed, this rapidly renewing simple epithelium contains within its functional unit, the crypt-villus axis, essentially two distinct cell populations: the proliferative and undifferentiated crypt cells and the mature enterocytes on the villus. Although immunolocalization of basement membrane molecules revealed that laminin, type IV collagen and heparan sulfate proteoglycan are distributed homogeneously all along the crypt-villus axis, other non-exclusive basement membrane components were found differentially expressed. Tenascin was concentrated at the base of both villus and lower crypt cells while cellular fibronectin was mostly detected in association with the crypt cells. Moreover, VLA \( \beta_1 \) as well as 5 of the 6 VLA \( \alpha \) subunits tested were expressed by intestinal epithelial cells under specific patterns of staining. The \( \beta_1 \) and \( \alpha_6 \) subunits were strongly detected at the base of all enterocytes while \( \alpha_5 \), also detected all along the crypt-villus axis, was weaker and consistently appeared with a punctuated/interrupted pattern. On the other hand, the VLA \( \alpha_1 \), \( \alpha_2 \) and \( \alpha_3 \) were expressed at the basolateral domains of enterocytes under distinctive crypt-villus gradients. The \( \alpha_6 \) subunit was detected at the base of all epithelial cells but lateral staining was only observed in differentiating cells (middle and upper crypt). Finally, in most specimens, \( \alpha_2 \) and \( \alpha_3 \) displayed strictly complementary staining patterns for the lower crypt region (\( \alpha_2^+, \alpha_3^- \)) and the upper crypt-to-villus region (\( \alpha_2^-, \alpha_3^+ \)). Taken together, these data emphasize that proliferation, migration and differentiation in the normal state are susceptible to various influences including compositional changes in the basement membrane and differential expression of receptors for these components.

Key words: integrins, VLA, intestinal cell, differentiation, migration, extracellular matrix, basement membrane, human.

Introduction

Cell adhesion and migration are fundamental processes for the organization and the maintenance of tissue integrity (reviewed by Edelman, 1985, 1992; Rodriguez-Boulan and Nelson, 1989). During the past few years, major advances in our understanding of the molecular mechanisms of these cellular processes have occurred, with the identification and the characterization of numerous extracellular matrix (ECM) molecules (reviewed by Timpl, 1989; Yurchenko and Schittny, 1990) as well as several of their cell-matrix adhesion molecules (reviewed by Hynes, 1987; Juliano, 1987; Ruoslahti, 1988; Akiyama et al., 1990; Hemler et al., 1990).

The VLA family of integrins is an important class of cell surface receptors involved in cell-matrix interactions (Buck and Horwitz, 1987). As most other members of the superfamily of integrins, VLAs (for “very late activation” antigen) are transmembrane \( \alpha-\beta \) non-covalently linked heterodimers (Hynes, 1987). So far, at least 11 \( \alpha \) subunits and seven \( \beta \) subunits have been identified, which are associated to form 15 distinct heterodimers (Hemler et al., 1990; Ruoslahti, 1991). At least six of them belong to the VLA family (VLA 1 to 6) and are characterized by having distinct \( \alpha \) subunits (\( \alpha_2^-, \alpha_3^+ \)) that share a common \( \beta_1 \) subunit. Ligands for VLAs have been identified as ECM molecules; namely, laminin (VLA 1, 2, 3 and 6), collagen I and/or IV (VLA 1, 2 and 3), and fibronectin (VLA 4 and 5) (recently reviewed by Humphries, 1990; Ruoslahti, 1991). The list is however still incomplete. For example, the integrin that binds to tenascin has not been yet identified (Friedlander et al., 1988) while the ligands...
for other newly identified VLAs remain to be determined (Kajiji et al., 1989; Holzmann and Weissman, 1989). The molecular basis for integrin-mediated cellular events is not yet clearly understood but, as identified from in vitro studies, it probably involves direct links between the ECM and the cytoskeleton (Burridge et al., 1990). Moreover, the functional significance of a cytoplasmic β1 tyrosine phosphorylation site remains to be established (Tamkun et al., 1986; Hayashi et al., 1990).

Experiments with a variety of cultured normal and tumor cells have provided evidence that the members of the VLA family of integrins, in concert with their respective ECM ligands, participate in fundamental cellular processes such as adhesion, spreading, migration, proliferation and differentiation. Studies on intact tissues and organs have also provided strong indications that integrins are involved in cell-matrix interactions, acting as cell-specific receptors for basement membrane components. For example, in the human kidney the glomerular basement membrane is recognized by different integrins in different cell types (Korhonen et al., 1990). Similarly, there are differences in the integrin repertoire found on large vessels and capillary endothelia (Albelda et al., 1989). Furthermore, in the epidermis, the expression of several integrins appears closely related to the state of cell differentiation, suggesting a role for these molecules in the migration of terminally differentiating keratinocytes from the basal epidermal layer (Kajiji et al., 1989; Tamura et al., 1990; Quaranta, 1990; Nicholson and Watt, 1991; Sonnenberg et al., 1991). The aim of the present work was to obtain more basic information on VLAs in epithelial-basement membrane interactions and on their relationship to cell proliferation, differentiation, maturation and, ultimately, senescence. The adult intestinal epithelium appears to be an attractive system for such a purpose. Its continuous cell renewal consists of spatially separated stem cells, proliferative and differentiated cell compartments, located, respectively, in the lower regions of the crypts and on the villi (Bjerknes and Cheng, 1981; Leblond, 1981). The molecular basis of the dynamic process of cell production in the basal region of the crypts and of their migration along the basement membrane to the tip of the villi where they are extruded into the lumen remains to be established but, undoubtedly, requires carefully controlled modulation of cell-matrix adhesion. Until very recently, much of the effort has been concentrated on the analysis of the distribution of basement membrane components; it revealed that most of these constituents are distributed homogeneously all along the crypt-villus axis (see Discussion). Herein, to get further information on the molecular basis underlying the mechanism of migration of epithelial cells from the crypt to the villus, the patterns of expression for VLA 1 to 6 were determined by indirect immunofluorescence and analyzed in concert with the presence of various basement membrane and interstitial ECM components along the crypt-villus axis of the adult human small intestine. The data demonstrate that five of the six VLA α subunits tested are expressed by intestinal epithelial cells under specific patterns, which supports the hypothesis that these molecules are involved in the control of migration and/or proliferation and differentiation of enterocytes.

Materials and methods

Primary antibodies

The antibodies used for the immunolocalization of the VLA family of integrins were: the monoclonals mAb 13 and mAb 16 directed against the β1 and the α5 subunits, respectively (Akiyama et al., 1989; generously provided by Dr. Steven K. Akiyama); A-1A5, TS2/7 and B-3G10 directed against the β1 (Hemler et al., 1983); the α1 and the α6 subunits (Hemler et al., 1987), respectively, kindly supplied by Dr. Martin E. Hemler; GoH3 directed against the α5 subunit (Sonnenberg et al., 1988; Serotec, Cedarlane Laboratories, Hornby, Ont.); and P1E6 and P1B5 recognizing the α9 and the α6 subunits, respectively (Wayner and Carter, 1987; obtained from Oncogene Science, Uniondale, NY). Type IV collagen, laminin, basement membrane heparan sulfate proteoglycan, tenascin and cellular fibronectin were immunolocalized with mono- and polyclonal antibodies as described previously (Beaulieu et al., 1991). The mature form of sucrase-isomaltase was detected with the monoclonal antibody HSI-5 (Beaulieu et al., 1989).

Tissues

Twelve samples of small intestine (3 jejuna and 9 ileums) were studied. Only tissues obtained rapidly (in less than 30 min) from non-diseased parts of resected segments were used in the present study. The normality of the tissue was confirmed by histology. Specimens were obtained from adult patients with either inflammatory bowel disease or bowel obstruction, according to the protocol approved by the Institutional Human Research Review Committee.

Indirect immunofluorescence

The preparation and OCT (optimum cutting temperature embedding compound; Miles Tek, Elkhart, IN, USA) embedding of specimens for cryosectioning was performed as previously described (Beaulieu et al., 1990). Sections 4 μm thick were spread on gelatin-coated glass slides and air-dried for 1 hour at room temperature before storage at −70°C. Detection of the various antigens on cryosections by indirect immunofluorescence was performed as described previously (Beaulieu et al., 1991). Fluorescein-conjugated goat anti-mouse IgG (Boehringer Mannheim Canada, Laval, Québec), anti-rat IgG (Caltag, Cedarlane Laboratories, Hornby, Ont.) and anti-rabbit IgG (Boehringer Mannheim Canada, Laval, Québec) were used as secondary antibodies, at a working dilution of 1:25. After staining, preparations were mounted in glycerol/PBS (9:1, v/v) containing 0.1% paraphenylenediamine and then viewed with a Leitz Orthoplan microscope equipped for epifluorescence under immersion oil with ×10 and ×25 objectives. In all cases, no fluorescent staining was observed when the primary antibody had been omitted.

Results

Intestinal epithelial cell differentiation

Although all specimens were histologically normal, evidence that a typical differentiation gradient was
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Fig. 1. Representative indirect immunofluorescence micrographs of basement membrane (A,B) and interstitial matrix (C-F) components in transverse sections of human adult ileum. Upper half portion of villi (A,C,E) and corresponding crypt regions (B,D,F). Antibodies used were anti-human type IV collagen antiserum (A,B), anti-human tenascin antiserum (C,D), and FN-3E2, a monoclonal antibody directed to human cellular fibronectin (E,F). LP, lamina propria; mm, muscularis mucosa; arrowheads in E and F denote the epithelial basement membrane; v and c in D denote the feet of the villi and the crypts, respectively. Bar, 50 μm.

displayed along the crypt-villus axis was obtained by determining the site of expression for mature sucrase-isomaltase with the monoclonal antibody HSI-5. As shown previously (Beaulieu et al., 1989), this antibody exclusively recognizes the form of sucrase-isomaltase expressed by differentiated villus cells.

ECM component expression

The expression and distribution of various basement membrane and interstitial ECM molecules were examined in relation to epithelial cell differentiation along the crypt-villus axis of the small intestine. As summarized in Fig. 1, all the antibodies directed to basement membrane molecules such as those to type IV collagen, laminin and heparan sulfate proteoglycan produced continuous fluorescent staining at the base of intestinal epithelial cells from the bottom of the crypts to the apex of the villi. Staining for type IV collagen and laminin
was also found in fibrillar and cellular elements of the lamina propria and in the muscularis mucosa (Fig. 1A-B) while heparan sulfate proteoglycan was not found in these locations (not shown).

Staining patterns displayed for tenascin and cellular fibronectin were also analyzed in relation to the organization of the intestinal mucosal architecture. Tenascin expression was found in both the crypt and the villus regions but with distinct patterns of labeling (Fig. 1C-D). A bright staining for tenascin was detected in the connective tissue surrounding the lower part of the crypt (Fig. 1D) and a clear gradient of decreasing intensity was observed toward the neck of the gland. In the villus, strong immunofluorescent staining was observed, which was mostly confined to the base of epithelial cells from the foot to the tip of the villi (Fig. 1C). Specific, but less-intense staining, was also observed in isolated muscle cells of the entire lamina propria as well as in the muscularis mucosa but not around blood vessels (confirmed by double staining with anti-tenascin and anti-smooth-muscle α-actin antibodies; not shown). Immunolocalization of cellular fibronectin revealed some particularities in the distribution along the crypt-villus axis for another interstitial ECM molecule (Fig. 1E-F). Specific antibodies to this molecule stained all the elements of the lamina propria surrounding the crypts including muscle cells, and the interface between the connective tissue and the epithelial cells lining both the crypts and the bottom of the villi (Fig. 1F). Staining in the upper part of the villus was found to be restricted to a few isolated muscle cells and as a punctate labeling at the base of the differentiated villus cells (Fig. 1E).

VLA expression and localization
To examine the possible role of integrins in the adhesive interaction of enterocytes with their underlying basement membrane, frozen sections of normal adult intestine were stained with mAb 13 and A-1A5, which both recognize the human integrin β1 polypeptide. As shown in Fig. 2, the integrin β1 chain was primarily localized at the basolateral aspect of intestinal epithelial cells of the villus and the crypt. Staining was also evident in the lamina propria, at the level of cellular elements, in the muscularis mucosa, as well as in the outer muscle cell layers (longitudinal and circular layers of the muscularis propria; not shown).

To explore further the distribution of the β1 integrins, I then examined a set of normal small intestinal specimens with a series of subunit-specific monoclonal anti-VLA integrin antibodies. Five out of the six α subunits tested were found to be expressed under various patterns by intestinal epithelial cells. Subunit α4 was not detected at the basolateral aspect of the epithelium (Fig. 3A-B). However, because of a strong staining of pericryptal cellular elements (such as those that express tenascin (Fig. 1D) and smooth muscle α-actin (not shown)) with the anti-α4 subunit antibody, some basal expression of this molecule by the epithelial cells of the crypt can not be ruled out (Fig. 3B). Two VLA subunits (α6 and α6) were found expressed along the entire crypt-villus axis. Antibody GoH3 directed to α6 stained intensively the basolateral surface of enterocytes on the villus and in the crypt (Fig. 3C-D) while mAb 16, specific for α6, stained the basal aspect of crypt and villus epithelial cells according to a faint punctate/interrupted pattern (Fig. 3E-F). Integrin subunits α1, α2 and α3 were distributed according to a specific gradient along the crypt-villus axis (Fig. 4). Antibody TS2/7 to α2 stained evenly and with a good intensity the basolateral aspect of epithelial cells located in the middle and upper parts of the crypt (Fig. 4B) while the staining in the lower part of the crypt and on the villus was weaker and more restricted to the basal margin of the epithelium (Fig. 4A-B). Subunit α2, specifically recognized by the antibody P1E6, was found confined to the crypt epithelium. Basolateral aspects of the cells were strongly stained from the bottom to the middle part of the crypt but a clear gradient of decreasing intensity was observed from the middle to the upper parts of the crypt (Fig. 4D); the entire villus epithelium was negative (Fig. 4C). In contrast, staining for the α3 subunit with the P1B5 antibody revealed that the
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Fig. 3. Representative indirect immunofluorescence micrographs of transverse sections of human adult ileum stained for the detection of the $\alpha_4$ (A,B), $\alpha_6$ (C,D) and $\alpha_5$ (E,F) subunits of integrins. Subunit $\alpha_4$ was mainly detected in close association with muscle cells in the lamina propria of the villi (A) and around the crypts (B) as well as in the muscularis mucosa (mm; B). On the contrary, the $\alpha_6$ subunit was exclusively found at the basolateral aspect of epithelial cells lining the villus (C) and in the crypts (D). The $\alpha_5$ subunit was found at the basal aspect of both villus (E) and crypt (D) epithelial cells according to a faint punctate/interrupted pattern and in muscle cells of the lamina propria and muscularis mucosa. Arrows in A,C,E and arrowheads in B,D,F denote basal surfaces of villus epithelial cells and crypt cells, respectively. Bar, 50 $\mu$m.

molecule was only expressed by enterocytes lying in the upper part of the crypt and the entire villus (Fig. 4E-F). The complementary patterns of staining for $\alpha_2$ and $\alpha_3$ along the crypt-villus axis were striking.

Expression of integrin subunits was also observed in close association with muscle cells in the lamina propria, muscularis mucosa and the outer muscularis propria. As summarized in Table 1, smooth muscle cells from all sites express evenly $\beta_1$, $\alpha_1$ and $\alpha_5$. Staining for $\alpha_4$ was also found widespread but much weaker in the outer muscular layers. The $\alpha_2$ and $\alpha_6$ subunits were not detected in muscle cells in any location. Finally, the expression of the $\alpha_3$ subunit was found to be restricted to only particular areas of the muscularis mucosa and muscularis propria: as shown in Fig. 4F, muscle cells in the muscularis mucosa were predominantly stained on the mucosal side, the lower (submucosal) side remained only weakly stained; similarly, the staining for $\alpha_3$ in the outer muscle layers was strictly limited to the submucosal aspect of the circular layer (not shown).

Discussion

The intestine has been proven to be a valuable model for the study of cell growth and differentiation. This
**Table 1. Distribution of VLA 1 to 6 in smooth muscle cells at various locations in the human small intestine**

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++ to $-$: intensity of staining: bright (++), good (+), faint (±), or no (−) staining detected at the basal surface of intestinal epithelial cells.
$+/-$: specific regions stained while others negative.

rapidly renewing simple epithelium contains within its functional unit, the crypt-villus axis, essentially two distinct cell populations, the proliferative or undifferentiated crypt cells and the mature enterocytes of the villus. Indeed, the fully differentiated villus cell has typical morphological and functional properties that easily distinguish it from the crypt cell (Weiser, 1973; Altmann, 1976; Baylin et al., 1978; Leblond, 1981; Quaroni and Isselbacher, 1985; Raul and von der Decken, 1985). How cells move out of the crypt and
migrate onto the villus and what the molecular mechanisms are that prompt epithelial cells to stop proliferation and undertake differentiation still remains to be elucidated. In the present work we have approached these basic questions by studying the distribution of extracellular matrix proteins and their receptors along the crypt-villus axis in the adult human intestinal mucosa.

ECM components, namely those present at the basement membrane, are important macromolecules for tissue organization and maintenance (Timpl, 1989; Yurchenko and Schittny, 1990). There is also accumulating evidence that these components play a pivotal role in epithelial polarization and differentiation in the developing small intestine. Such involvement of ECM molecules was first suggested by the observation of changes in their deposition and distribution in developing rodents (Simon-Assmann et al., 1986; Auerheide and Ekblohm, 1988) and human intestine (Beaulieu et al., 1991), and is strongly supported by in vitro studies demonstrating their necessity to allow cell differentiation (Dauca et al., 1990; Hahn et al., 1990; Simon-Assmann et al., 1990). In this study, in the mature human intestine, immunolocalization of basement membrane molecules revealed that laminin, type IV collagen and heparan sulfate proteoglycan are distributed homogeneously all along the crypt-villus axis in the epithelial basement membrane. This observation, which is in accordance with previous work performed in the rodent small intestine (Laurie et al., 1982; Abrahamson and Caulfield, 1983; Probstmeier et al., 1986; Senior et al., 1988; Alho and Underhill, 1989), may suggest that basement membrane macromolecules support intestinal cell migration and differentiation but do not govern them. Several arguments plead against the latter assumption. First, the recent finding that the laminin A chain is preferentially located in the basement membrane of intestinal crypts while B chains are found all over the crypt-villus axis raises the possibility of a specific involvement for a basement membrane component in the process of cell growth and/or polarization (Simo et al., 1991). Second, as exemplified in the present work by the immunolocalization of tenascin and cellular fibronectin, some ECM components must also be considered in the crypt-villus axis gradient of basement membrane components. Indeed, both macromolecules were identified in the basal lamina of intestinal epithelial cells (Laurie et al., 1982; Probstmeier et al., 1990) and therefore may play a role in intestinal cell adhesion/shedding (Quaroni et al., 1990). It is also noteworthy that such a preferential distribution of tenascin at the base of both villus and lower crypt cells and of cellular fibronectin in association with crypt cells is already established by mid-gestation in the human fetal intestine (Beaulieu et al., 1991; Jutras and Beaulieu, unpublished). Third, some monoclonal antibodies to presumed basement membrane components are known to exhibit distinct staining patterns along the crypt-villus axis (Cleutjens et al., 1989; Milliane et al., unpublished), suggesting that other uncharacterized basement membrane components and/or unusual subunits may also participate in the establishment of the gradient. Taken together, these observations suggest that compositional changes in the basement membrane along the crypt-villus-axis play a role in the control of epithelial cell migration and/or differentiation.

As demonstrated herein by the immunolocalization study of the members of the VLA family of integrins, differential expression of cell-surface receptors for basement membrane components must also be considered among the potential regulatory mechanisms of the dynamic process of the enterocyte in the crypt-villus unit. Indeed, five out of the six VLA α subunits tested were found to be expressed by intestinal epithelial cells under specific patterns of staining. Only the α6 subunit was, as observed for the β1 subunit, present at the base of all enterocytes. This is in accordance with previous articles reporting widespread expression of this specific laminin receptor (Sonnenberg et al., 1988) in intestinal and colonic epithelial cells both in vivo (Choy et al., 1990; Koretz et al., 1991) and in vitro (Schreiner et al., 1991; Lotz et al., 1990). In contrast, the α5 subunit, which seems to be important in immunological events such as T cell activation (Hemler, 1988), was not detected in direct association with enterocytes. However, α6 being strongly expressed in cellular elements lying just beneath the epithelium, a basal expression of this subunit by enterocytes cannot be ruled out at this time. Although expressed only at a low level, the fibronectin receptor (α5β1 complex) was consistently detected at the base of enterocytes from both the villus and the crypt in a punctate pattern. In general, VLA 5 (α5β1) is poorly expressed in most tissues including epithelia (Toda et al., 1987; Wayner and Carter, 1988; Albelda et al., 1989). However, as reported recently (Guo et al., 1991), the expression of the α5β1 complex at the basal surface of migratory epithelial cells may be of functional importance. Moreover, it is worth emphasizing the close similarity in staining patterns for cellular fibronectin and the α5 subunit (compare Fig. 1E,F with Fig. 3E,F) in the context of the existence of a potential relationship between the expression of the fibronectin receptor and its ligand (reviewed by Akiyama et al., 1990).

Another interesting finding arising from the present set of observations is the apparent gradient of expression along the crypt-villus axis for the three multispecific VLAs, α1(β1), α2(β1) and α5(β1), which indeed may bind to various components present in the intestinal basement membrane such as type IV collagen, laminin or fibronectin (Humphries, 1990; Ruoslahti, 1991). In three tissue samples of the small intestine, Choy et al. (1990) reported a widespread distribution of α2 and α5 along the crypt-villus axis similar to that observed for α5, while α1 was concentrated in the crypt cells. On the other hand, Koretz et al. (1991) reported recently that the VLA-α2 chain was rather preferentially associated with the cryptic glands. In our study, we found that in most cases, α2 and α5 displayed complementary staining patterns for the lower crypt (α5+; α2−) and the upper crypt-villus
regions \((\alpha_2^-, \alpha_3^+).\) On a few occasions, staining for the \(\alpha_2^+\) subunit extended beyond the basal half of the crypt but the \(\alpha_3^+\) subunit was always poorly expressed in this latter region. Interestingly, the \(\alpha_2^+\beta_1^+\) complex, but not the \(\alpha_3^+\beta_1^+\), was recently reported to be involved in colon carcinoma cell adhesion to type I collagen and laminin (Lotz et al., 1990). The ligand for the \(\alpha_3^+\beta_1^+\) complex in the intestinal epithelium remains to be determined but, since colon carcinoma cell adhesion to fibronectin is very poor, even though these cells express significant amounts of the \(\alpha_3^+\) and \(\beta_1^+\) subunits (but not \(\alpha_5^+\); Lotz et al., 1990; Schreiner et al., 1991), fibronectin, like laminin and type-I collagen, appears to be a poor candidate. Although it was not in the main focus of the present work, it is germane that all integrin subunits expressed in the intestinal epithelium were also detected in proportional amounts in the lateral (\(\alpha_1^+, \alpha_2^+, \alpha_3^+, \alpha_5^+, \alpha_6^+, \beta_1^+\)) and even apical (\(\alpha_2^+\) and \(\alpha_4^+\)) domains of the epithelial cells (see Figs 2-4). This agrees with previous reports (De Strooper et al., 1989; Pignatelli et al., 1990; Virtanen et al., 1990; Koretz et al., 1991), which may suggest some involvement for these molecules not only in cell-matrix but also in cell-cell interactions as suggested recently (Kaufmann et al., 1989; Larjava et al., 1990). In this regard, the pattern of expression for the \(\alpha_3^+\) subunit is intriguing, since on one hand the molecule was found widelyexpressed at the base of epithelial cells all along the crypt-villus axis, while on the other hand staining of the lateral domain, which was strong in the middle and upper parts of the crypt, appeared much weaker or absent in the upper half of the villi as well as in the bottom of the crypts. The significance of such a differential pattern of staining for this putative type IV collagen (Vandenbergh et al., 1991) and/or laminin receptor (Humphries, 1990; Ruoslahti, 1991) is unknown, but could be related to the cell state, since weak lateral staining for \(\alpha_3^+\) was observed in sites where the epithelium was most fully mature (i.e. on the villus, and also at the base of the crypt where the down-migrating Paneth cells concentrate; Bjerknes and Cheng, 1981; Leblond, 1981).

Finally, diversity among integrin subunit expression in a variety of tissues (see, for example, Albelda et al., 1990; Virtanen et al., 1990) is exemplified by muscle cells of the intestinal wall that, although these are all derived from the embryonic intestinal mesenchyme, express various integrins according to their location and, therefore also, to their functions. It is pertinent to note that in the intestine, muscle cells possess a basement membrane of similar composition to that of epithelial cells (Simon-Assmann et al., 1986; Simo et al., 1991; Beaulieu, unpublished data) but, as clearly shown herein, express a quite different set of integrins.

In conclusion, these data taken together stress the potential complexity of epithelial cell-matrix interactions in a relatively simple system such as the intestinal epithelium. It now appears clear that proliferation, migration and differentiation are processes susceptible to various influences, including compositional changes in the basement membrane and differential expression of receptors for these components. What controls these changes along the crypt-villus axis? It is hoped that further in vivo as well as in vitro studies will help to answer this question, which is crucial for our understanding of the mechanisms underlying cell differentiation under normal as well as pathological conditions.

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