[Frontiers in Bioscience 4, d310-321, March 15, 1999]

INTEGRINS AND HUMAN INTESTINAL CELL FUNCTIONS

Jean-François Beaulieu

MRC Group in Functional Development and Physiopathology of the Digestive Tract, Département d'anatomie et de biologie cellulaire, Faculté de médecine, Université de Sherbrooke, Sherbrooke, Qué. Canada J1H 5N4

TABLE OF CONTENTS

1. ABSTRACT

Integrins are a large family of cell-surface receptors involved in cell adhesion to the extracellular matrix. In epithelia, it is mainly the integrins belonging to the beta1 and beta4 classes that bind to basement membrane molecules such as the laminins and the type IV collagens. Beta1 and beta4 integrins regulate the assembly of adhesive junctions as well as the activation of various signaling pathways leading to the modulation of gene expression. In this review, I will discuss what is currently known about integrins in human intestinal epithelial cells. The interest in the intestinal cell model to analyze cell-matrix interactions will be delineated and the recent experimental evidence showing that these interactions can regulate cell proliferation and differentiation will be presented.

2. INTRODUCTION

The intestinal epithelium, which is in constant and rapid renewal, represents an attractive system for the study of mechanisms involved in the determination of the cell state. Within its functional unit, the crypt-villus axis, are two main distinct cell populations: the proliferating and poorly differentiated crypt cells and the mature enterocytes of the villus (1-3). The regulation of epithelial cell growth and functional differentiation is susceptible to various influences along the crypt-villus axis (4,5), including cell interaction with the extracellular matrix (6). As in many organs, the intestinal epithelium lies on a thin and continuous sheet of specialized extracellular matrix, the basement membrane, which separates parenchymal cells from the interstitial connective tissue. It is now recognized that the basement membrane composition defines the necessary microenvironment required for multiple cellular functions during development and at maturity such as adhesion, proliferation, migration and cell survival as well as tissue-specific gene expression (7-10). These functions are themselves mediated by various cell receptors, many of which are members of the integrin superfamily (11-15). Integrins are transmembrane heterodimeric glycoproteins composed of an alpha and a beta subunit. Seventeen alpha and eight beta subunits have been identified to date that associate to form at least twenty two different receptors (11-17). It is mainly the integrins belonging to the beta1 and beta4 classes that bind to basement membrane molecules such as the laminins and the type IV collagens.

In this review, I will summarize the role of integrins in epithelial cell-matrix interactions as major signaling molecules, and will discuss their involvement in the regulation of intestinal cell functions, namely proliferation and differentiation. After a brief update on the functional aspects of the crypt-villus unit in the human small intestine, I will present our current knowledge concerning basement membrane composition and differential expression of integrins in relation to the cell state in human intestinal cells (see ref. 6 for a more comprehensive review). I will also present recent work showing experimental evidence that cell-matrix interactions can regulate intestinal cell proliferation and differentiation.

3. INTEGRINS AS MEDIATORS OF CELL-MATRIX INTERACTIONS

The view of the extracellular matrix as a biologically inert support, frequently referred to as the "ground substance", has considerably changed over the past 10 years with the identification of several of its constituting

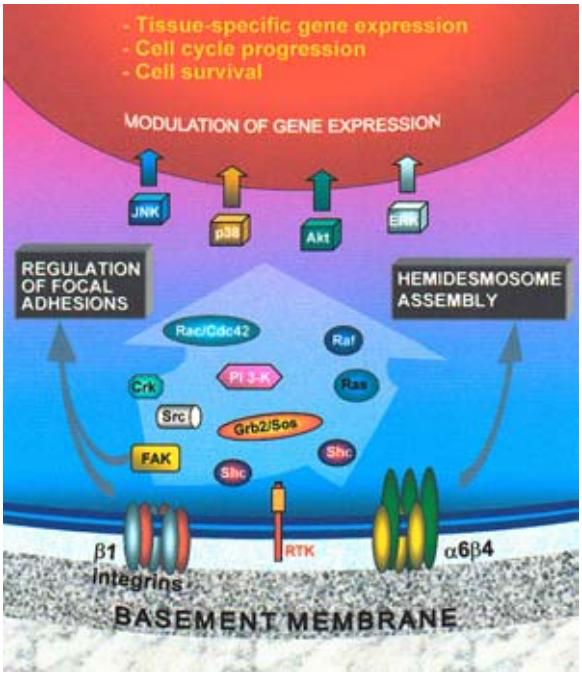

Figure 1. Integrin-mediated signaling events in epithelial cells. Adhesion of cells to basement membrane components induces integrin clustering and activation of various molecules involved in the regulation of the assembly of focal adhesion complexes (beta1) or hemidesmosomes (beta4), as well as in the activation of various signaling pathways in cooperation with receptor tyrosine kinases (RTK), leading to a modulation of the gene expression regulating cell survival, cell cycle progression and tissue-specific gene expression.

glycoproteins and, in particular, the characterization of specific cell receptors for these extracellular components. The first family of receptors for extracellular matrix molecules to be identified, and still the best characterized, is the integrin family (11,12). The classical example of cell-matrix interaction is the recognition of a particular RGD sequence in fibronectin by a specific cellular receptor, which was later found to share a common beta1 subunit with a number of independently discovered leucocyte proteins, the very late antigens (VLAs) (18-19). The nomenclature now refers to this fibronectin receptor as the alpha5beta1 integrin, which is ligand-specific, as later shown for some other beta1-integrins, such as alpha6beta1 and alpha7beta1 that bind exclusively to laminin. Other beta1 integrins can recognize more than one ligand. Indeed, alpha1beta1 and alpha2beta1 can bind various collagen types and laminin, depending on the cell type, while alpha3beta1, which has been found to respond to a broad spectrum of extracellular ligands, seems primarily to mediate adhesion to laminin-5 (20,21). For alpha9beta1, a well-characterized ligand is tenascin-C (22) but this integrin may also serve as a receptor for osteopontin (23), while alphavbeta1 appears to function as a receptor for vitronectin, fibronectin and tenascin-C (24). Beta1 integrins known to be widely expressed in epithelial cells include alpha1beta1, alpha2beta1, alpha3beta1, alpha5beta1 and alpha6beta1 (6,14,15). Alpha6beta4 is another integrin found in a number of epithelia. The only alpha subunit known to associate with beta4 is alpha6 and this particular integrin functions exclusively as a laminin-specific binding receptor.

Interestingly, the beta1 and beta4 integrins exhibit major structural differences but, as demonstrated recently, share also some of their functional characteristics (25-30). From a structural point of view, beta4, because of its unique and very large cytoplasmic domain that can associate with keratin filaments, differs fundamentally from the other beta subunits, including beta1, which possess a short cytoplasmic domain that associates with actin-based filaments. Interactions with actin or keratin to form focal adhesion complexes or hemidesmosomes, respectively, seem to depend on their unique ability to associate with intermediate proteins such as focal adhesion proteins, including alpha-actinin, talin, paxilin and the focal adhesion kinase (FAK) for beta1 (31,32), or hemidesmosomal proteins such as HD1/plectin for beta4 (33). However, as depicted in figure 1, more importantly from a functional point of view, both sets of integrins can transduce signals across the plasma membrane through their association with proteins involved in the tyrosine kinase signaling cascade and can thus, ultimately, regulate growth, apoptosis and tissue-specific gene expression. A well documented example of this is the activation of the mitogen-activated protein kinase (MAPK) pathway in response to integrin ligation (34,35). Certain integrins, which include alpha6beta4, alpha1beta1 and alpha5beta1, are linked to the Ras-MAPK signaling pathway by the adaptor protein Shc (36-38). Upon recruitment by activated integrins, Shc becomes phosphorylated on tyrosine and binds to the Grb2-mSos complex which leads to the activation of Ras-MAPK signaling. While there is evidence that Shc can bind directly to the tyrosine-phosphorylated cytoplasmic domain of the beta4 integrin subunit, the recruitment of Shc by activated beta1 integrins appears to be indirect and mediated by a transmembrane adaptor associated with this subset of alpha subunits. Integrins, upon ligation, can activate a number of additional key molecules associated with various major signaling pathways (39) . The central role of FAK and Src in both the regulation of focal adhesions and cell signaling is well documented (26,40,41). An activation of c-Jun amino-terminal kinase (JNK) has been also reported (37,42). More recently, a significant activation of the phosphoinositide 3-kinase (PI 3-K) in response to cell adhesion was demonstrated, and appears to be involved in the activation of the serine-threonine kinase Akt (43,44). An additional element to this relative multiplicity of signaling pathways is the increasing evidence that signals from matrix adhesion receptors are integrated with those originating from growth factor and cytokine receptors (29,39,45,46).

Integrin-mediated signal transduction is therefore relatively complex. To further complicate the facts, different integrins can induce different signaling pathways, and signaling through the same integrin may be different depending on the cell type, state and environment. In the light of these elements, it is obvious that a better

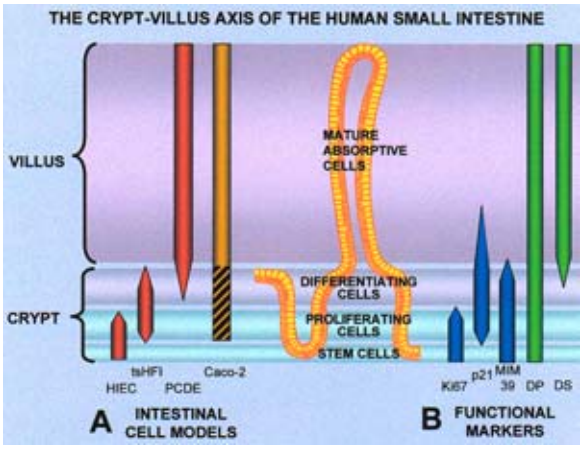

Figure 2. The crypt-villus axis is the functional unit of the small intestine. In this rapidly renewing epithelium, the proliferating cells, which arise from the stem cells located at the bottom of the crypts, loose their ability to proliferate and start to differentiate in the upper third of the crypt, then migrate toward the tip of the villus before being exfoliated into the lumen. A. Intestinal normal cell models that allow the recapitulation of the crypt-villus axis include the crypt-like proliferative cell line HIEC, the differentiating tsHFI cell line, and primary cultures of differentiated enterocytes (PCDE). The Caco-2 cell line, although of colon carcinoma origin, has been proven useful to the study of intestinal cell proliferation and differentiation. B. Functional markers of intestinal cell state include the proliferating antigen Ki67, the cyclin-dependent kinase inhibitor p21/Waf-1/Cip1 (p21), the crypt cell antigen MIM-1/39 (MIM39), the dipeptidases (DP) dipeptidylpeptidase IV and aminopeptidase N, and the disaccharisases (DS) lactase-phlorizin hydrolase and mature form of sucrase-isomaltase.

understanding of the mechanisms involving integrins as mediators of extracellular signals will require a better integration of the information gathered from relatively simple systems, such as cultured cell lines, with that of their *in vivo* normal and pathological counterparts.

4. INTESTINAL EPITHELIAL CELL-MATRIX INTERACTIONS

The human intestinal epithelium represents a particularly attractive system to investigate the regulation of integrin-related cell functions and its underlying mechanisms primarily because of the architecture of its well defined crypt-villus axis renewal unit, where proliferative, differentiating, functional and senescent cells are topologically restricted to distinct compartments, the lower half of the crypt, upper half of the crypt, villus and villus tip, respectively. Determining the pattern of expression of integrins and their corresponding ligands along the crypt-villus axis can thus provide valuable basic information relative to the potential involvement of each of these molecules, according to the cell state (6,47). Furthermore, the lack of adequate cellular models, an historical weakness of this model, has been overcome with the development of new cell lines and systems (48-50), which in conjunction with available and well characterized ones, such as the Caco-2 cell line (51-53), allow the recapitulation of the entire human intestinal crypt-villus axis (figure 2A).

4.1. The crypt-villus functional unit

The crypt-villus axis represents the functional unit in the small intestine. It can be defined by typical morphological and functional properties displayed by the mature villus enterocytes that distinguish them from crypt cells. Indeed, the villi are mainly lined by functional absorptive, goblet and enteroendocrine cells, while the crypts contain stem cells and the proliferative and poorly differentiated cells as well as a subset of differentiated secretory cells, namely Paneth, goblet and enteroendocrine cells. The differentiation of each cell type takes place as the cells move either upwards towards the villus (adsorptive, mucus and endocrine cells) or downwards to concentrate at the bottom of the crypt (Paneth cells). The compartmentalization of distinct cell populations according to their functional state is a well documented phenomenon which can be exemplified by the analysis of the localization of various markers along the crypt-villus axis (figure 2B). It is noteworthy that in all species studied, the crypt-villus junction represents a physical limit from which enterocytes acquire their final functional characteristics. For instance, immunostaining for the detection of maltase-glucoamylase, a marker of the functional enterocyte, is restricted to villus cells while MIM-1/39, a specific marker for secretory granules is expressed only by crypt cells. However, it appears more and more evident that the situation in the human varies from that observed in laboratory animals, some of the classical enterocytic markers being expressed by immature cells located below this border. For instance, aminopeptidase N and dipeptidylpeptidase IV have been found constitutively expressed by both proliferative and differentiated intestinal cells while an immature form of sucrase-isomaltase is present in crypt cells. These differences have to be considered when choosing markers to study human intestinal cell differentiation both *in situ* and *in vitro*. More importantly, they point out that the regulation of gene expression along the crypt-villus axis fundamentally differs between man and animal models (3-6,47).

4.2. Basement membrane composition

The epithelial basement membrane (BM) of the human intestine contains all the major components specific to most BMs such as type IV collagens, laminins and proteoglycans as well as BM-associated molecules (6,47,54,55). Surprisingly, it was some of the BM-associated molecules such as tenascin and fibronectin that were first identified to be differentially expressed along the crypt-villus axis in both the adult and developing small intestine (56-62). BM components such as the classical type IV collagen, heterotrimeric laminin and various proteoglycans, were detected at the base of all epithelial cells (57,60,62,63). However, the identification of genetically distinct forms of type IV collagen and laminin has incited many laboratories to re-investigate the expression of these BM molecules in the small intestine.

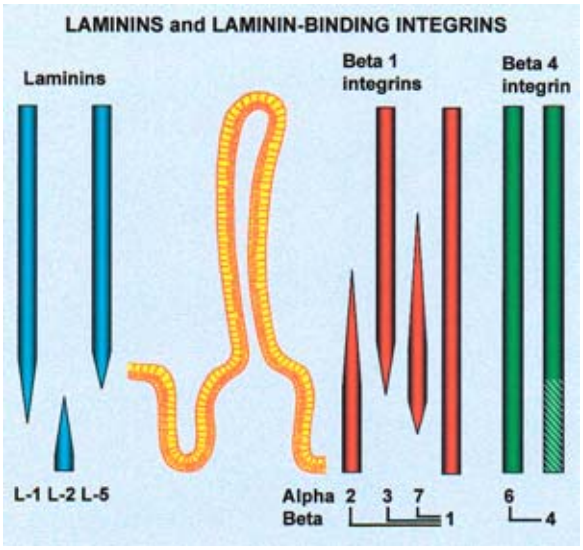

Figure 3. Distribution of laminins and their corresponding integrins along the crypt-villus axis of the adult human small intestine. Laminin-1 (alpha1beta1gamma1) and laminin-2 (alpha2beta1gamma1) show complementary locations while laminin-5 (alpha3beta3gamma2) is restricted to the villus. Among integrins, alpha6beta4, which can bind to these three laminins, is distributed uniformly, while the basal distribution of alpha3beta1 in intestinal cells coincides with the location of its specific ligand, laminin-5. In contrast, the laminin-1 binding integrin alpha7beta1 is primarily expressed in the differentiating compartment.

The analysis of the type IV collagen alpha1(IV) to alpha6(IV) chains (64) in the human small intestine with specific antibodies and probes confirmed the constitutive expression of the alpha1 and alpha2 chains, which assemble as a [alpha1(IV)]2 alpha2(IV) complex, at the epithelial BM, while the alpha3(IV) and alpha4(IV) chains were not detected (65). However, the alpha5(IV) and alpha6(IV) chains were identified (65,66). Interestingly, in contrast to the alpha1(IV) and alpha2(IV) chains which exclusively originate from the mesenchymal compartment, the alpha5(IV) and alpha6(IV) chains were found to be produced by both epithelial and mesenchymal cells (66-68). Another interesting feature pertaining to these newly identified type IV collagen chains is that, although they can presumably assemble as an [alpha5(IV)]2 alpha6(IV) complex (64) in the intestinal BM, their expression differs considerably during development, alpha6(IV) being expressed constitutively while alpha5(IV) is subject to a substantial down-regulation from the fetal to the adult stage (65,66). These observations indicate that, although the two genes may share a common bidirectional promoter (69,70), the modulation of their transcription, namely that of the COL4A5 gene, relies on additional regulatory elements in the intestine. Although its functional significance still remains to be determined, this developmentally-regulated alpha5(IV)/alpha6(IV) collagen expression in the human gut represents a good example of how complex tissues can temporally modify cell-matrix interactions.

Laminin also was initially thought to be a unique heterotrimeric molecule formed by one heavy A chain and two distinct light B chains (71,72) but has been redefined as a multigene family of related proteins (73,74). In light of this complexity, a new nomenclature has been adopted where the designated A, B1 and B2 chains have been replaced by alpha, beta and gamma respectively, and the identification of forms is distinguished by arabic numbers (75). Functionally, laminins have been shown to mediate several cellular activities, namely the promotion of adhesion, growth, polarization and differentiation depending on the cell type studied (10,76,77). Variability in spatial and temporal expression for a number of these laminin chains (78,79) suggests that different heterotrimeric forms of laminin could perform distinct functions.

The expression of laminin variants in the small intestinal BM has received major attention after a reciprocal expression of laminin-1 (alpha1beta1gamma1) and laminin-2 (alpha2beta1gamma1) along the crypt-villus axis was reported (80-82). The occurrence of laminin-1 as a villus form and laminin-2 as a crypt form (figure 3) was indeed suggesting for the first time a possible relation between laminin expression and functional intestinal cell differentiation. By further investigating this relationship in the Caco-2/15 cell model, our laboratory provided evidence that enterocytic-differentiation-related gene expression is specifically promoted by laminins, and is susceptible to a differential modulation by variant forms of this family (83). Indeed, a close relation between laminin-1 deposition and sucrase-isomaltase expression was demonstrated at the cell level suggesting that the well established potential of Caco-2/15 cells to differentiate *in vitro* (51-53) could be related to their potential to synthesize and accumulate functional laminin-1 at their basal pole. For instance, subclones of the Caco-2/15 cell line in which laminin-1 deposition was impaired were found to express considerably less sucrase-isomaltase at their apical pole (83). Furthermore, growth of Caco-2/15 cells on purified human laminin-1 and laminin-2 revealed that both substrates can promote intestinal cell marker expression but that only laminin-1 has the ability to precociously induce functional differentiation markers such as sucrase-isomaltase and lactase-phlorizin hydrolase (83). Finally, additional evidence that laminin-1 plays an important role in regulating cell differentiation was obtained by transfecting Caco-2 cells with an antisense laminin alpha1-chain cDNA fragment (84).

Laminin-5 (alpha3beta3gamma2) is the only other laminin yet identified in the human intestine (85,86; figure 3). The observations showing its presence is of interest since this laminin has previously been reported only in BMs of stratified epithelia, in association with anchoring filaments of hemidesmosomes. It is noteworthy that true hemidesmosomes are not present in the human small intestinal epithelium (86,87), consistant with the fact that type VII collagen and some of the major hemidesmosomal proteins are lacking (85,88). However, its localization according to an increasing gradient from the upper crypt to the villus tip in the human small intestine (85,86) coincides well with that of two other important

components of hemidesmosomes, HD1/plectin (85) and the beta4 integrin subunit (80; Basora et al., submitted). The presence of laminin-5, of HD1/plectin, an intermediate protein in the association of the cytoplasmic domain of the beta4 integrin subunit to cytokeratins (32) as well as of the beta4 subunit of alpha6beta4, a receptor for laminin-5 (see below), suggests that early or type II hemidesmosomes can form at the base of villus intestinal cells. Besides a potential involvement in villus cell adhesion and structural stability through the intermediate filament network, the role of laminin-5 in intestinal cells remains to be determined. Interestingly, in colonic adenocarcinoma, a specific loss of laminin-5 staining was observed at the BM of areas of invasion (89) concomitantly with a cytoplasmic accumulation of the beta3 and gamma2 chains (89,90) suggesting that the impairment of laminin-5 deposition plays a role in matrix detachment of invasive cancer cells.

4.3. Integrin expression and distribution in the human small intestinal epithelium

The expression and distribution of most beta1 and beta4 integrins has been determined along the crypt-villus axis of the human small intestine. As summarized in figure 3, the principal integrin subunits present in epithelial cells include beta1 and beta4 as well as alpha2, alpha3, alpha6 and alpha7, all of which are among the subgroup of the laminin-binding integrins (6,14). The alpha5 subunit, which associates with beta1 to act as a fibronectin receptor, was detected at the base of crypt and villus cells according to a faint and punctuated pattern of staining reminiscent of that observed for its ligand in the BM (62) while the alpha1, alpha4 and alphaV subunits were only weakly expressed or absent from the normal adult epithelium (6,62). The alpha9 subunit is another partner for beta1. This particular receptor for tenascin-C and osteopontin is absent in the normal adult intestinal epithelium but was found at the bottom of the crypts in the developing intestine (91).

The analysis of the laminin-binding integrins along the crypt-villus axis revealed interesting features. The alpha2 and alpha3 subunits were found at the basal domain of intestinal cells according to strict complementary staining patterns along the crypt-villus axis, the alpha2 subunit being predominant in the crypts and alpha3 on the villus (62,82,86). It is noteworthy that both of these subunits were also localized at the lateral domain of enterocytes, where they can serve as mediators of intercellular adhesion (92), and that the population of cells exhibiting this lateral staining extends beyond the crypt and villus compartments for the alpha2 and alpha3 subunits, respectively (6). On the other hand, the alpha6 subunit was uniformly distributed at the base of epithelial cells from the bottom of the crypt to the tip of the villus (62,80-82,93). However, difficulties in interpreting the widespread distribution of this subunit arose from the ability of alpha6 to form two distinct integrin complexes by combining with either beta1 or beta4 depending on the cell type, and from the existence of variants, referred to as alpha6A and alpha6B, which can combine with either beta1 or beta4 (94) also susceptible to being expressed as multiple variants (95-102). At the present time, the expression of alpha6, beta1 and beta4 variants and their alpha-beta association have not been directly investigated in normal human enterocytes. Finally, the expression of the alpha7 subunit has been recently analyzed. In the adult, the alpha7B isoform was detected in the epithelium and expressed under a unique pattern of expression, being restricted to the upper part of the crypt and the lower villus region (103).

Taken together, the observations showing the expression of a number of primary laminin-binding integrins in the intestinal epithelium and their differential localization along the crypt-villus axis, in concert with compositional changes in laminin-1, 2 and 5 (see above), delineates the potential complexity of epithelial cell-laminin interactions involved in the maintenance of a relatively simple system such as the human intestinal epithelium. Analyzing the distribution of these functional molecules in a spatially well organized structure such as the crypt-villus axis represents a powerful way for estimating the potential implication of individual components in a normal environment. Nevertheless, to recapitulate the mechanisms in play, several questions pertaining to the basic organization of integrin subunits, such as the exact form(s) and variant(s) expressed, their relative quantitative importance, their association with beta1 or beta4, the specificity of their ligands and, more importantly, their precise involvement in the regulation of specific cell functions such as proliferation, polarization, adhesion, migration, apoptosis and differentiation, also needs to be addressed by means of more direct approaches.

4.4. Functional relevance of integrins in the regulation of intestinal cell functions

The expression and potential roles of a number of integrins have been investigated on various intestinal experimental models, namely human cell lines. Although mostly of colon adenocarcinoma origin, these cell lines have been used advantageously to study integrin-mediated intestinal cell adhesion, migration and invasion and also cell proliferation and differentiation. In relative good agreement with the *in vivo* situation where the expression of alpha2, alpha6, beta1 and beta4 is generally maintained and that of alpha3 is reduced or absent in colorectal carcinoma cells (89,104-106), the predominant integrins expressed in colon cancer cell lines appear to be alpha2beta1 and alpha6beta4. Other integrins analyzed in intestinal cells for which a function can be proposed include alpha3beta1, alpha7beta1 and alpha9beta1.

4.4.1 Alpha2beta1

The alpha2beta1 integrin has been shown to be involved in intestinal cell adhesion to both laminin and collagen in clone A cells (107). The alpha2beta1 binding capacity for laminin-1 appears substantially greater than for laminin-2, although much lower than for collagen (108) but recent studies suggest that alpha2beta1 may act in cooperation with alpha6beta4 for attachment to laminin-1 (14). There is also evidence that this receptor may be involved in EGF-mediated Caco-2 cell migration on laminin (109). On the other hand, the promoting effect of TGFalpha on glandular differentiation of SW1222 cells grown in a 3-dimensional collagen gel was shown to be primarily mediated by the alpha2beta1 integrin (110).

Interestingly, this latter observation is reminiscent of what is observed during gland formation in the developing fetal small intestine (82). In the adult, alpha2beta1 is predominantly associated with crypt cells which exhibit a BM containing type IV collagens but which lack laminin-1 and 5 (see above). Taken together, these observations would suggest that in the intestinal epithelium, the alpha2beta1 integrin may act primarily as a collagen receptor involved in gland morphogenesis and maintenance, rather than as a laminin receptor.

4.4.2 Alpha6beta4

The alpha6beta4 integrin was first reported as a laminin receptor in intestinal cells working in cooperation with another beta1 integrin, most likely alpha2beta1 (107,111,112). In hemidesmosome-expressing cells such as those at the epidermal-mesenchymal interface, alpha6beta4 plays an essential role in the assembly and for the stability of hemidesmosomes while it mediates anchorage on laminin-5 in cooperation with alpha3beta1 (113-115). However, as mentioned in a previous section, intestinal cells do not possess true hemidesmosomes but express these receptors. Interestingly, most colon cancer cell lines tested that express alpha6beta4 (e.g., clone A, differentiated HT29, LoVo clone E2 and Caco-2) seem to adhere better to laminin than their counterparts which express lower levels of intact alpha6beta4 or alpha6 predominantly under the alpha6beta1 form (e.g., undifferentiated HT29, LoVo clone C5, and RKO) (111,112,116,117; Basora et al., submitted). Furthermore, as well illustrated with RKO cells, the low avidity for laminin-1 in cells that express alpha6beta1 can be reversed after transfection with the full-length beta4 cDNA (101) showing that alpha6beta4, but not alpha6beta1, is a high affinity receptor for laminin-1 and that in the presence of beta1 and beta4, the alpha6 subunit associates preferentially with beta4 to act as a specific laminin-1 and 5 receptor (112,118; Basora et al., submitted). Whether the same phenomenon occurs *in vivo* has not been verified but is suggested by the fact that at least some forms of the beta4 subunit are expressed in both crypt and villus cells (80,81,86; Basora et al., submitted) although alpha6 and beta1 are present in all intestinal cells.

Interestingly, the forced expression of beta4 in RKO cells induces, under a laminin-1 or laminin-5 substrate-independent manner, the expression of p21/Cip1/WAF-1, an inhibitor of cyclin-dependent kinases, and apoptosis (101). Although further work is required to verify to which extent the phenomenon can be extrapolated to the *in vivo* situation, it is pertinent to note that the intestinal epithelium of newborn mice deficient for beta4 expression was found to be susceptible to degeneration and loss of cell-substratum adhesion suggesting that alpha6beta4 interacts with laminin-5 to mediate a signal essential for cell survival in the animal (119). In this context, the recent demonstration that upon ligation, alpha6beta4 can activate PI 3-K in invasive carcinoma cells (120) is of interest. Furthermore, in mice carrying a targeted deletion of the beta4 cytoplasmic domain, which impairs both association with hemidesmosomal components and the recruitment of the signaling adaptor protein Shc, an increasing level of the cyclin-dependent kinase inhibitor p27/Kip was observed concomitantly with a reduction in the size of the proliferative compartment in the proximal small intestine (121). Finally, mice lacking the alpha6 subunit develop until birth but die soon after with severe blistering of the skin and other epithelia (122). There is also evidence that alpha6beta4 plays a key role in the modulation of adhesion properties in the human as well (113). Therefore, the suggestion that $\alpha 6\beta 4$ primarily serves as a high affinity receptor for cell adhesion to laminin-1 and laminin-5, and as a regulator for the expression of cyclin-dependent kinase inhibitors, would appear to be in good agreement with the distribution of these molecules along the crypt-villus axis in the intact intestinal epithelium (49,80,81,86,123; Basora et al., submitted).

4.4.3 Alpha9beta1

The integrin alpha9beta1 is one of the recently identified integrins whose expression is restricted to specialized tissues (124). This integrin is absent from the normal adult colonic epithelium (124,125) but has been detected in a large proportion of differentiated colon carcinomas as well as in two colon adenocarcinoma cell lines that show high potential for cell polarization, the Caco-2/15 and T84 cell lines (125). Interestingly, the alpha9 subunit was also detected in the epithelium of both human fetal small intestine and colon, predominantly confined to the cells located in the crypts (91,125), which represent the proliferative compartment. The presence of alpha9beta1 in HIEC-6 cells was thus found to be consistent with their proliferative crypt-like status (91). In Caco-2/15 cells, alpha9beta1 expression was found to be high in proliferating cells but downregulated at both protein and transcript levels in cells that cease to grow and undertake their differentiation (91,125). Epidermal growth factor treatment, which is known to maintain Caco-2/15 cells in a proliferative state (126), resulted in higher levels of alpha9 expression (91). Taken together, these data indicate that alpha9beta1 is subject to an onco-fetal pattern of expression in the human intestinal epithelium and suggest a relation between its expression and cell proliferation. In a context where none of the so-called proliferation integrins (alpha1beta1, alpha5beta1 and alphavbeta3), linked to the Ras-ERK signaling pathway through Shc (see section 3), are expressed at significant levels in the normal human intestine, the recent finding that forced expression of alpha9 in the SW480 colon carcinoma cell line stimulates, in a ligand-dependent manner, cell proliferation and concomitant phosphorylation of ERK2 (127), further emphasizes the potential role of alpha9beta1 on intestinal cell proliferation.

4.4.4 Alpha3beta1 and alpha7beta1

Much less is known about the implication of integrins as mediators of extracellular matrix-regulated cell-specific gene expression (10,28). Although it still needs to be better documented in other systems, the study of integrin implication in human intestinal cells has been further complicated by the lack of normal cell models in which the differentiation process could be initiated under *in vitro* conditions. In light of this, the finding that laminin-1 plays a key role in the establishment and maintenance of functional differentiation in the enterocytic-like Caco-2 cell

line (83,84) is of importance by providing for the first time evidence that a BM molecule, in particular laminin-1 but not laminin-2 (83), is directly involved in the modulation of the expression of enterocytic functions and, is a suitable model to analyze laminin-cell interactions in the context of intestinal differentiation. At first glance, the potential implication of integrins in this phenomenon may appear relatively complicated to investigate in light of the numerous laminin-binding integrins expressed in the small intestinal epithelium, namely alpha3beta1, alpha7beta1 and alpha6beta4 as well as alpha2beta1. However, few of them appear to be good candidates in mediating the effects of laminin-1 on intestinal functional cell differentiation (6). Indeed, alpha2beta1 is confined to the crypts which are devoid of laminin-1. Furthermore, the widespread expression of alpha6beta4 in the adult intestine as well as during fetal development and in most colon cancer cells suggests that this integrin may not be responsible for triggering terminal differentiation in the intestinal epithelium. The predominant expression of alpha3beta1 at the base of villus cells in both the developing and adult small intestine may indicate a role for this integrin. However, there is good evidence that epithelial cells, including intestinal ones, use primarily alpha3beta1 as a high affinity laminin-5 receptor (14,20,21), a laminin form also predominantly expressed in the BM of villus cells (see below). On the other hand, the integrin alpha7beta1 has only been reported recently in epithelial cells. In muscle, this integrin was first identified as a laminin-1 receptor (128) mediating laminin functions during myogenic differentiation (129-131). Further studies confirmed that alpha7beta1 can interact with laminin-1 and laminin-2 but not laminin-5 (132,133). The particular distribution of the alpha7B variant, at the upper crypts and lower villus region, in concert with its regulated expression in Caco-2 cells and its absence in HIEC cells (103), suggests that the alpha7Bbeta1 integrin plays a role in the regulation of laminin-1-mediated enterocytic differentiation.

A role for the integrin alpha3beta1 in intestinal differentiation could also be suggested considering its co-distribution, at the base of intestinal villus cells, with laminin-5, its principal ligand (62,85,86). Although neither alpha3beta1 or laminin-5 are expressed by the differentiating Caco-2 cells, one cannot exclude the possibility that a dual set of cell-matrix interactions, modulating intestinal differentiation, may occur *in situ*. For instance, the well-polarized T84 colon carcinoma cells lack laminin-1 and alpha7beta1 (103), but express both laminin-5 and alpha3beta1 (134). While this possibility remains to be explored, it has to be noted that in the epidermis, the function of alpha3beta1 appears to be in cell attachment and organization of the BM in cooperation with alpha6beta4 (115), a function also compatible with the situation described in the intestinal epithelium.

5. CONCLUSIONS AND PERSPECTIVES

Studies in the past few years have provided considerable evidence that cell-matrix interactions are central in the regulation of many fundamental cell functions and that integrins are key mediators of these interactions. In the intestinal epithelium, we have begun to appreciate the complexities of integrins and their potential roles along the crypt-villus axis.

The main challenges for the next years will obviously be to directly demonstrate cause-to-effect relationships between particular integrins and specific cell functions, and to ascertain the signaling molecules specifically involved. For instance, whether the two newly identified intestinal integrins alpha9beta1 and alpha7Bbeta1 trigger ligand-dependent cell proliferation and differentiation, respectively, in normal intestinal cells, has still to be directly demonstrated. The recent development in the establishment of normal intestinal cell models that recapitulate, *in vitro*, the entire crypt-villus axis (48-50) should be instrumental for this purpose and should help to define downstream signaling events orchestrating the regulation of functional gene expression.

6. ACKNOWLEDGEMENTS

I would like to thank the members of my laboratory for their contribution to the original research and their suggestions to the manuscript, and F.E. Herring-Gillam for reviewing the manuscript. The original work and the preparation of this review were supported by grants from the Medical Research Council of Canada (MT-11289 and GR-15186) and from the "Fonds pour la Formation des Chercheurs et l'Aide à la Recherche".

7. REFERENCES

1. Leblond C.P.: The life history of cells in renewing systems. Am J Anat 160, 114-159 (1981)

2. Louvard D., M. Kedinger & H.P. Hauri: The differentiating intestinal epithelial cell: establishment and maintenance of functions through interactions between cellular structures. Annu Rev Cell Biol 8, 157-195 (1992)

3. Ménard D. & J.-F. Beaulieu: Human intestinal brush border membrane hydrolases. In: Membrane Physiopathology, Ed: Bkaily G, Kluwer Academic Publisher, Norwell (1994)

4. Podolsky D.K. & M.W. Babyatsky: Growth and development of the gastrointestinal tract. In: Textbook of Gastroenterology. 2nd ed. Ed: Yamada T, JB Lippincott, Philadelphia (1995)

5. Boyle W.J. & D.A. Brenner: Molecular and cell biology of the small intestine. *Cur Opin Gastroenterol* 11, 121-127 (1995)

6. Beaulieu J.-F.: Extracellular matrix components and integrins in relationship to human intestinal epithelial cell differentiation. *Prog Histochem Cytochem* 31-4, 1-78 (1997)

7. Adams J.C. & F.M. Watt: Regulation of development and differentiation by the extracellular matrix. *Development* 117, 1183-1198 (1993)

8. Juliano R.L. & S. Haskill: Signal transduction from the extracellular matrix. *J Cell Biol* 120, 577-585 (1993)

9. Lin C.Q. & M.J. Bissell: Multi-faceted regulation of cell differentiation by extracellular matrix. *FASEB J* 7, 737-743 (1993)

10. Rosekelly C.D., P.Y. Desprez & M.J. Bissell: A hierarchy of ECM-mediated signaling regulates tissue-specific gene expression. *Cur Opin Cell Biol* 7, 736-747 (1995)

11. Ruoslahti E.: Integrins. *J Clin Invest* 87, 1-5 (1991)

12. Hynes R.O.: Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69, 11-25 (1992)

13. Sonnenberg A.: Laminin receptors in the integrin family. *Path Biol* 40, 773-778 (1992)

14. Mercurio A.M.: Laminin receptors: achieving specificity through cooperation. *Trends Cell Biol* 5, 419-423 (1995)

15. Sheppard D.: Epithelial integrins. *BioEssays* 18, 655-660 (1996)

16. Akiyama S.K., K. Nagata & K.M. Yamada: Cell surface receptors for extracellular matrix components. *Biochim Biophys Acta* 1031, 91-110 (1990).

17. Fernandez C., K. Clark, L. Burrows, N.R. Schofield & M.J. Humphries: Regulation of the extracellular ligand binding activity of integrins. *Front Bioscience 3,* d684-700 (1998)

18. Ruoslahti E. & M. Pierschbacher: New perspective in cell adhesion: RGD and integrins. *Science* 283, 491-497 (1987)

19. Takada Y., C. Huang & M.E. Hemler: Fibronectin receptor structure in the VLA family of heterodimers. *Nature* 336, 487-489 (1988)

20. Carter W.G., M.C. Ryan & P.J. Gahr: Epliligrin, a new cell adhesion ligand for integrin alpha3beta1 in epithelial basement membranes. *Cell* 65, 599-610 (1991)

21. Delwel G., A.A. de Melker, F. Hogervost, L.H. Jaspar, D.L.A. Fles, I. Kuikman, A. Lindblom, M. Paulsson, R. Timpl & A. Sonnenberg: Distinct and overlapping ligand specificities of the alpha3Abeta1 and alpha3Bbeta1 integrins: recognition of laminin isoforms. *Mol Biol Cell* 5, 203-215 (1994)

22. Yokosaki Y., E.L. Palmer, A.L. Prieto, K.L. Crossin, M.A. Bourdon, R. Pytela & D. Sheppard: The integrin alpha9beta1 mediates cell attachment to a non-RGD site in the third fibronectin type III repeat of tenascin. *J Biol Chem* 269, 26691-26696 (1994)

23. Smith L.L., H.K. Cheung, L.E. Ling, J. Chen, D. Sheppard, R. Pytela & C.M. Gianelli: Osteopontin N-terminal domain contains a cryptic adhesive sequence recognized by alpha9beta1 integrin. *J Biol Chem* 271, 28485-28491 (1996)

24. Vogel B.E., G. Tarone, F.G. Giancotti, J. Gailit & E. Ruoslahti: A novel fibronectin receptor with an unexpected subunit composition (alphavbeta1). *J Biol Chem* 265, 5934-5937 (1990)

25. Clark E.A. & J.S. Brugge: Integrin and signal transduction pathways: The road taken. *Science* 268, 233-239 (1995)

26. Parson J.T.: Integrin-mediated signaling: regulation by protein tyrosine kinases and small GTP-binding proteins. *Cur Opin Cell Biol* 8, 146-152 (1996)

27. Giancotti F.G.: Signal transduction by the alpha6beta4 integrin: charting the path between laminin binding and nuclear events. *J Cell Sci* 109, 1165-11272 (1996)

28. Craig S.W. & R.P. Johnson: Assembly of focal adhesions: progress, paradigms, and portents. *Cur Opin Cell Biol* 8, 74-85 (1996)

29. Ruoslahti E. & B. Obrink: Common principles in cell adhesion. *Exp Cell Res* 227, 1-11 (1996)

30. Kumar C.C.: Signaling by integrin receptors. *Oncogene* 17, 1365-1373 (1998)

31. Hemler, M.E.: Integrin associated proteins. *Cur Opin Cell Biol* 10, 578-585 (1998)

32. Yamada K.M. & B. Geiger: Molecular interactions in cell adhesion complexes. *Cur Opin Cell Biol* 9, 76-85 (1997)

33. Schaapveld R.Q.J., L. Borradino, D. Geerts, M.R. van Leusden, I. Kuikman, M.G. Nievers, C.M. Niessen, R.D.M. Steenbergen, P.J.F. Snijders & A. Sonnenberg: Hemidesmosome formation is initiated by the beta4 integrin subunit, requires complex formation of beta4 and HD1/plectin, and involves a direct interaction between beta4 and the bullous pemphigoid antigen 180. *J Cell Biol* 142, 271-284 (1998)

34. Giancotti F.G.: Integrin signaling: specificity and control of cell survival and cell cycle progression. *Cur Opin Cell Biol* 9, 691-700 (1997)

35. Howe A., A.E. Aplin, S.K. Alahari & R.L. Juliano: Integrin signaling and cell growth control. *Cur Opin Cell Biol* 10, 220-231 (1998)

36. Mainiero, F., A. Pepe, K. K. Wary, L. Spinardi, M. Mohammadi, J. Schlessinger, & F.G. Giancotti: Signal transduction by the alpha6beta4 integrin: distinct beta4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. *EMBO J* 14, 4470-4481 (1995)

37. Mainiero, F., C. Murgia, K.K. Wary, A.M. Curatola, A. Pepe, M. Blumemberg, J.K. Westwick, C.J. Der & F.G. Giancotti: The coupling of alpha6beta4 integrin to Ras-MAP kinase pathways mediated by Shc controls keratinocyte proliferation. *EMBO J* 16, 2365-2375 (1997)

38. Wary K.K., F. Maniero, S.J. Isakoff, E.E. Marcantonio & F.G. Giancotti: The adaptor protein Shc couples a class of integrins to the control of cell cycle progression. *Cell* 87, 733-743 (1996)

39. Katz B.Z. & K.M. Yamada: Integrins in morphogenesis and signaling. *Biochimie* 79, 647-476 (1997)

40. Ilic D., C.H. Damsky & T. Yamamoto: Focal adhesion kinases: at the crossroads of signal transduction. *J Cell Sci* 110, 401-407 (1997)

41. Schlaepfer D.D., K.C. Jones & T. Hunter: Multiple Grb2-mediated integrin-stimulated signal pathways to Erk2/mitogen-activated protein kinase: summation of both

c-Shc- and focal adhesion kinase-initiated tyrosine phosphorylation events. *Mol Cell Biol* 18, 2571-2585 (1998)

42. Miyamoto S., H. Teramoto, O.A. Coso, J.S. Gutkind, P.D. Burbelo, S.K. Akiyama & K.M. Yamada: Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. *J Cell Biol* 131, 791-805 (1995)

43. King W.G., M.D. Mattaliano, T.O. Chan, P.N. Tsichlis & J.S. Brugge: PI 3-kinase is required for integrin-stimulated AKT and Raf-1/MAP kinase pathway activation. *Mol Cell Biol* 17, 4406-4418 (1997)

44. Khwaja A., P. Rodriguez-Viciana, S. Wennstrom, P.H. Warne & J. Downward: Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. *EMBO J* 16, 2783-2793 (1997)

45. Juliano R.: Cooperation between soluble factors and integrin-mediated cell anchorage in the control of cell growth and differentiation. *BioEssays* 18, 911-917 (1996)

46. Sastry S.K. & A.F. Horwitz: Adhesion-growth factor interactions during differentiation: an integrated biologic response. *Dev Biol* 180, 455-467 (1996)

47. Bouatrouss Y., J. Poisson & J.-F. Beaulieu: Studying the basement membrane. In: Methods in disease: Investigating the gastrointestinal tract. Eds. Preedy V.C., Watson R.R., Greenwich Medical Media, London (1998)

48. Perreault N. & J.-F. Beaulieu: Use of the dissociating enzyme thermolysin to generate viable human normal intestinal epithelial cell cultures. *Exp Cell Res* 224, 354-364 (1996)

49. Quaroni, A. & J.-F. Beaulieu: Cell dynamics and differentiation of conditionally immortalized human intestinal epithelial cells. *Gastroenterology* 113, 1198-1213 (1997)

50. Perreault N. & J.-F. Beaulieu: Primary culture of fully differentiated and pure human intestinal epithelial cells. *Exp Cell Res* 245, 34-42 (1998)

51. Beaulieu J.-F. & A. Quaroni: Clonal analysis of sucrase-isomaltase expression in the human colon adenocarcinoma Caco-2 cells. *Biochem J* 280, 599-608 (1991)

52. Vachon P.H. & J.-F. Beaulieu: Transient mosaic patterns of morphological and functional differentiation in the Caco-2 cell line. *Gastroenterology* 103, 414-423 (1992)

53. Vachon P.H., N. Perreault, P. Magny & J.-F. Beaulieu: Uncoordinated, transient mosaic patterns of intestinal hydrolases expression in differentiating human enterocytes. *J Cell Physiol* 166, 198-207 (1996)

54. Simon-Assmann P, M. Kedinger, A. De Archangelis, V. Rousseau and P. Simo: Extracellular matrix components in intestinal development. Experientia 51, 883-900 (1995)

55. Beaulieu J.-F.: Recent work with migration/patterns of expression: cell-matrix interactions in human intestinal cell differentiation. In: The gut as a model in cell and molecular biology. Eds: Halter F., Winton D., Wright N.A., Kluwer Academic Publishers, Dordrecht (1997)

56. Quaroni A., K.J. Isselbacher & E. Ruoslahti: Fibronectin synthesis by epithelial crypt cells of rat small intestine. *Proc Natl Acad Sci USA* 75, 5548-5552 (1978)

57. Simon-Assmann P., M. Kedinger & K. Haffen: Immunocytochemical localization of extracellular matrix proteins in relation to rat intestinal morphogenesis. *Differentiation* 32, 59-66 (1986)

58. Aufderheide E. & P. Ekblom: Tenascin during gut development: appearance in the mesenchyme, shift in molecular forms, and dependence on epithelial-mesenchymal interactions. *J Cell Biol* 107, 2341-2349 (1988)

59. Probstmeier R., R. Martini & M. Schachner: Expression of J1/tenascin in the crypt-villus unit of adult mouse small intestine: implication for its role in epithelial cell shedding. *Development* 109, 313-321 (1990)

60. Beaulieu J.-F., P.H. Vachon & S. Chartrand: Immunolocalization of extracellular matrix components during organogenesis in the human small intestine. *Anat Embryol* 183, 363-369 (1991)

61. Beaulieu J.-F., S. Jutras, J. Durand, P.H. Vachon & N. Perreault: Relationship between tenascin and alpha-smooth muscle actin expression in the developing human small intestinal mucosa. *Anat Embryol* 188, 149-158 (1993)

62. Beaulieu J.-F.: Differential expression of the VLA family of integrins along the crypt-villus axis in the human small intestine. *J Cell Sci* 102, 427-436 (1992)

63. Trier J.S., C.H. Allan, D.R. Abrahamson & S.J. Hagen: Epithelial basement membrane of mouse jejunum. Evidence for laminin turnover along the entire crypt-villus axis. *J Clin Invest* 86, 87-95 (1990)

64. Hudson B.G., S.T. Reeders & K. Tryggvason: Type IV collagen: Structure, gene organization, and role in human diseases. *J Biol Chem* 268, 26033-26036 (1993)

65. Beaulieu J.F., P.H. Vachon, E. Herring-Gillam, A. Simoneau, N. Perreault, C. Asselin & J. Durand: Expression of the alpha5(IV) collagen chain in the fetal human small intestine. *Gastroenterology* 107, 957-967 (1994)

66. Simoneau A., F.E. Herring-Gillam, P.H. Vachon, N. Perreault, N. Basora, Y. Bouatrouss, L.-P. Pageot, J. Zhou & J.-F. Beaulieu: Identification, distribution, and tissular origin of the alpha5(IV) and alpha6(IV) collagen chains in the developing human intestine. *Dev Dyn* 212, 437-447 (1998)

67. Vachon P.H., J. Durand & J.-F. Beaulieu: Basement membrane formation and re-distribution of beta1 integrins in a human intestinal co-culture system. *Anat Rec* 236, 567-576 (1993)

68. Perreault N., F.E. Herring-Gillam, N. Desloges, I. Belanger, L.-P. Pageot & J.-F. Beaulieu: Epithelial vs mesenchymal contribution to the extracellular matrix in the human intestine. *Biochem Biophys Res Comm* 248, 121-126 (1998)

69. Zhou J., T. Mochizuki, H. Smeets, C. Antignac, L. Laurila, A. de Pape, K. Tryggvason & S.T. Reeders: Deletion of the paired alpha5(IV) and alpha6(IV) collagen genes in inherited smooth muscle tumors. *Science* 261, 1167-1169 (1993)

70. Sugimoto M., T. Oohashi & Y. Ninomiya: The genes COL4A5 and COL4A6, coding for basement membrane collagen chains alpha5(IV) and alpha6(IV), are located head-to-head in close proximity on human chromosome Xq22 and COL4A6 is transcribed from two alternative promoters. *Proc Natl Acad Sci USA* 91, 11679-11683 (1994)

71. Beck K., I. Hunter & J. Engel: Structure and function of laminin: anatomy of a multidomain glycoprotein. *FASEB J* 4, 148-160 (1990)

72. Engel J.: Laminins and other strange proteins. *Biochemistry* 31, 10643-10651 (1992)

73. Weiver U.M. & E. Engvall: Laminins. *Meth Enzymol* 245, 85-104 (1994)

74. Beck K. & T. Gruber: Structure and assembly of basement membrane and related extracellular matrix proteins. In: Principles of Cell Adhesion. Eds: Richardson PD, Steiner M, CRC Press, Boca Raton (1995)

75. Burgeson R.E., M. Chiquet, R. Deutzmann, P. Ekblom, J. Engel, H. Kleinman, G.R. Martin, G. Meneguzzi, M. Paulsson, J. Sanes, R. Timpl, K. Tryggvason, Y. Yamada & P.D. Yurchenco: A new nomenclature for the laminins. *Matrix Biol* 14, 209-211 (1994)

76. Paulsson M.: Basement membrane proteins: Structure, assembly, and cellular interactions. *Cr Rev Biochem Mol Biol* 27, 93-127 (1992)

77. Engvall E.: Laminin variants: why, where and when? *Kidney Int* 43, 2-6 (1993)

78. Engvall E., D. Earwicker, T. Haaparanta, E. Ruoslahti & J.R. Sanes: Distribution and isolation of four laminin variants: Tissue restricted distribution of heterotrimers assembled from five different subunits. *Cell Regul* 1, 731-740 (1990)

79. Sanes J.R., E. Engvall, R. Butkowski & D.D. Hunter: Molecular heterogeneity of basal laminae: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere. *J Cell Biol* 111,1685-1699 (1990)

80. Beaulieu J.-F. & P.H. Vachon: Reciprocal expression of laminin A-chain isoforms along the crypt-villus axis in the human small intestine. *Gastroenterology* 106, 829-839 (1994)

81. Simon-Assmann P., B. Duclos, V. Orian-Rousseau, C. Arnold, C. Mathelin, E. Engvall & M. Kedinger: Differential expression of laminin isoforms and alpha6-beta4 integrins subunits in the developing human and mouse intestine. *Dev Dyn* 201, 71-85 (1994)

82. Perreault N., P.H. Vachon & J.-F. Beaulieu: Appearance and distribution of laminin A chain isoforms and integrin alpha2, alpha3, alpha6, beta1, and beta4 subunits in the developing human small intestinal mucosa. *Anat Rec* 242, 242-250 (1995)

83. Vachon P.H. & J.-F. Beaulieu: Extracellular heterotrimeric laminin promotes differentiation in human enterocytes. *Am J Physiol* 268, G857-G867 (1995)

84. De Archangelis A., P. Neuville, R. Boukamel, O. Lefebvre, M. Kedinger & P. Simon-Assmann: Inhibition of laminin alpha1-chain expression leads to alteration of basement membrane assembly and cell differentiation. *J Cell Biol* 133: 417-430 (1996)

85. Orian-Rousseau V., D. Aberdam, L. Fontano, L. Chevalier, G. Meneguzzi, M. Kedinger & P. Simon-Assmann: Developmental expression of laminin-5 and HD1 in the intestine- Epithelial to mesenchymal shift for the laminin gamma2 chain subunit deposition. *Dev Dyn* 206, 12-23 (1996)

86. Leivo I., T. Tani, L. Laitinen, R. Bruns, E. Kivilaakso, V.P. Lehto, R.E. Burgeson & I. Virtanen: Anchoring complex components laminin-5 and type VII collagen in the intestine: Association with migration and differentiating enterocytes. *J Histochem Cytochem* 44,1267-1277 (1996)

87. Jones J.C.R., J. Asmuth, S.E. Baker, M. Langhofer, S.I. Roth & S.B. Hopkinson: Hemidesmosomes: Extracellular matrix/intermediate filament connectors. *Exp Cell Res* 213, 1-11 (1994)

88. Owaribe K., J. Kartenbeck, S. Stumpp, T.M. Magin, T. Krieg, L.A. Diaz & W.W. Franke: The hemidesmosomal plaque. I. Characterization of a major constituent protein as a differentiation marker for certain forms of epithelia. *Differentiation* 45, 207-220 (1990)

89. Sordat I., F.T. Bosman, G. Dorta, P. Rousselle, D. Aberdam, A.L. Blum & B. Sordat: Differential expression of laminin-5 subunits and integrin receptors in human colorectal neoplasia. *J Pathol* 185, 44-52 (1998)

90. Pyke C., J. Romer, P. Kallunki, L.R. Lund, E. Ralfkiaer, K. Dano & K. Tryggvason: The gamma2 chain of kalinin/laminin 5 is preferentially expressed in invading malignant cells in human cancers. *Am J Pathol* 145, 782-791 (1994)

91. Desloges N., N. Basora, N. Perreault, Y. Bouatrouss, D. Sheppard & J.-F. Beaulieu: Regulated expression of the integrin alpha9beta1 in the epithelium of the developing human gut and in intestinal cell lines: relation with cell proliferation. *J Cell Biochem* 71, 536-545 (1998)

92. Symington B.E., Y. Takada & W.G. Carter: Interaction of integrin alpha3beta1 and alpha2beta1: Potential role in keratinocyte intercellular adhesion. *J Cell Biol* 120, 523-535 (1993)

93. Choy M.Y., P.I. Richman, M.A. Horton & T.T. MacDonald: Expression of the VLA family of integrins in human intestine. *J Pathol* 160, 35-40 (1990)

94. Hogervorst F., L.G. Admiraal, C. Niessen, I. Kuikman, H. Janssen, H. Daam & A. Sonnenberg: Biochemical characterization and tissue distribution of the A and B variants of the integrin alpha6 subunit. *J Cell Biol* 121, 179-191 (1993)

95. Altruda F., P. Cervella, G. Tarone, C. Botta, F. Balzac, G. Stefanuto & L.A. Silengo: A human integrin beta1 subunit with a unique cytoplasmic domain generated by alternative mRNA processing. *Gene* 95, 261-266 (1990)

96. Languino L.R. & E. Ruoslahti: An alternative form of the integrin beta1 subunit with a variant cytoplasmic domain. *J Cell Biol* 267, 7116-7120 (1992)

97. Meredith Jr. J., Y. Takada, M. Fornaro, L.R. Languino & M.A. Schwartz: Inhibition of cell cycle progression by the alternative spliced integrin beta1C. *Science* 269, 1570-1572 (1995)

98. Zhidkova N., A.M. Belkin & R. Mayne: Novel isoform of beta1 integrin expressed in skeletal and cardiac muscle. *Biochem Biophys Res Comm* 214, 279-285 (1995)

99. Tamura RN, C. Rozzo, L. Starr, J. Chambers, L.F. Reichardt, H.M. Cooper & V. Quaranta: Epithelial integrin alpha6beta4: complete primary structure of alpha6 and variant forms of beta4. *J Cell Biol* 111, 1593-1604 (1990)

100. Hogervorst F., I. Kuikman I, A.E.G. Kr von dem Borne & A. Sonnenberg: Cloning and sequence analysis of beta4 cDNA; an integrin subunit that contains a unique 118kD cytoplasmic domain. *EMBO J* 9, 765-770 (1990)

101. Clarke A.S., M.M. Lotz & A.M. Mercurio: A novel structural variant of the human beta4 integrin cDNA. *Cell Adhesion Comm* 2, 1-6 (1994)

102. van Leusden M.R., I. Kuikman & A. Sonnenberg:The unique cytoplasmic domain of the human integrin variant beta4E is produced by partial retention of intronic sequences. Biochem Biophys Res Commun 235,826-830 (1997)

103. Basora, N., P.H. Vachon, F.E. Herring-Gillam, N. Perreault and J-F. Beaulieu: Relation between integrin α7Bβ1 expression in human intestinal cells and enterocytic differentiation. *Gastroenterology* 113,1510-1521 (1997)

104. Koretz K., P. Schlag, L. Boumsell & P. Moller: Expression of VLA-alpha2, VLA-alpha6 and VLA-beta1 chains in normal mucosa and adenomas of the colon, and in colon carcinomas and their liver metastases. *Am J Pathol* 138, 741-750 (1991)

105. Stallmach A., B. v Lampe, H. Matthes, G. Bornhoft, E.O. Riecken: Diminished expression of integrin adhesion molecules on human colonic epithelial cells during the benign to malign tumor transformation. *Gut* 33, 342-346 (1992)

106. Falcioni R., V. Turchi, P. Vitullo, G. Navarra, F. Ficari, F. Cavaliere, A. Sacchi & R. Mariani-Constantini: Integrin beta4 expression in colorectal cancer. *Int J Oncol* 5, 573-578 (1994)

107. Lotz M.M., C.A. Korzelius & A.M. Mercurio: Human colon carcinoma cells use multiple receptors to adhere to laminin: involvement of alpha6beta4 and alpha2beta1 integrins. *Cell Regul* 1, 249-257 (1990)

108. Pfaff M., W. Gohring, J.C. Brown & R. Timpl: Binding of purified collagen receptors (alpha1 beta1, alpha2 beta2) and RGD-dependent integrins to laminin and laminin fragments. *Eur J Biochem* 225, 975-984 (1994)

109. Basson M.D., I.M. Modlin & J.A. Madri: Human enterocyte (Caco-2) migration is modulated in vitro by extracellular matrix composition and epidermal growth factor. *J Clin Invest* 90, 15-23 (1992)

110. Liu D., G. Gagliardi, M.M. Nasim, M.R. Alison, T. Oates, E.N. Lalani, G.W. Stamp & M. Pignatelli: TGF-alpha can act as a morphogen and/or mitogen in a colon-cancer cell line. *Int J Cancer* 56, 603-608 (1994)

111. Schreiner C., J. Bauer, M. Margolis & R.L. Juliano: Expression and role of integrins in adhesion of human colonic carcinoma cells to extracellular matrix components. *Clin Expl Metastasis* 9, 163-178 (1991)

112. Lee E.C., M.M. Lotz, G.D. Steel Jr. & A.M. Mercurio: The integrin alpha6beta4 is a laminin receptor. *J Cell Biol* 117, 671-678 (1992)

113. Niessen C.M., L.M.H. van der Raaij-Hemler, E.H.M. Hulsman, R. van der Neut, M.F. Jonkman & A. Sonnenberg: Deficiency of the integrin beta4 subunit in junctional epidermolysis bullosa with pyloric atrasia: consequences for hemidesmosome formation and adhesion properties. *J Cell Sci* 109, 1695-1706 (1996)

114. Xia Y., S.G. Gil & W.G. Carter: Anchorage mediated by integrin alpha6beta4 to laminin 5 (epiligrin) regulates tyrosine phosphorylation of a membrane-associated 80-kD protein. *J Cell Biol* 132, 727-740 (1996)

115. DiPersio, C.M., K.M. Hodivala-Dilke, R. Jaenisch, J.A. Kreidberg & R.O. Hynes: Alpha3beta1 integrin is required for normal development of the epidermal basement membrane. *J. Cell. Biol.* 137, 729-742 (1997)

116. Simon-Assmann P., C. Leberquier, N. Molto, T. Uezato, F. Bouziges & M. Kedinger: Adhesive properties and integrin expression profiles in two colonic cancer populations differing by their spreading on laminin. *J Cell Sci* 107, 577-587 (1994)

117. Daemi N., T. Valet, N. Thomasset, M.F. Jacquier, N. Zebda, J.F. Dore, B. Sordat & L. Remy: Expression of the alpha6, beta1 and beta4 integrin subunits, basement membrane organization and proteolytic capacities in low and high metastatic human colon carcinoma xenografts. *Invas Metast* 15, 103-115 (1995)

118. Niessen C.M., F. Hogervorst, L.H. Jaspars, A.A. De Melker, G.O. Delwel, E.H.M. Hulsman, L. Kuikman & A. Sonnenberg: The integrin alpha6beta4 is a receptor for both laminin and kalinin. *Exp Cell Res* 211, 360-367 (1994)

119. Dowling J., Q.-C. Yu & E. Fuchs: Beta4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. *J Cell Biol* 134, 559-572 (1996)

120. Shaw L.M., I. Rabinovitz, H.H.-F. Wang, A. Toker & A. Mercurio: Activation of phosphoinositide 3-OH kinase by the alpha6beta4 integrin promotes carcinoma invasion. *Cell* 91, 949-960 (1997)

121. Murgia C., P. Blaikie, N. Kim, M. Dans, H.T. Petri & P.G. Giancotti: Cell cycle and adhesion defects in mice carrying a targeted deletion of the integrin beta4 cytoplasmic domain. *EMBO J* 17, 3940-3951 (1998)

122. Georges-Labouesse E., N. Messaddeq, G. Yehia, L. Cadalbert, A. Dierich & M. Le Meur: Absence of integrin alpha6 leads to epidermolysis bullosa and neonatal death in mice. *Nature Genetics* 13, 370-373 (1996)

123. Gartel A.L., M.S. Serfas, M. Gartel, E. Goufman, G.S. Wu, W.S. El-Deiry & A.L. Tyner: p21 (WAF1/CIP1) expression is induced in newly nondividing cells in diverse epithelia and during differentiation of the Caco-2 intestinal cell line. *Exp Cell Res* 227, 171-181 (1996)

124. Palmer E.L., C. Ruegg, R. Ferrando, R. Pytela & D. Sheppard: Sequence and tissue distribution of the integrin alpha9 subunit, a novel partner of beta1 that is widely distributed in epithelia and muscle. *J Cell Biol* 123, 1289-1297 (1993)

125. Basora N., N. Desloges, Q. Chang, Y. Bouatrouss, J. Gosselin, J. Poisson, D. Sheppard & J.-F. Beaulieu:

Expression of the alpha9beta1 integrin in human colonic epithelial cells: resurgence of the fetal phenotype in a subset of colon cancers and adenocarcinoma cell lines. *Int J Cancer* 75, 738-743 (1998)

126. Cross H.S. & A. Quaroni: Inhibition of sucrase-isomaltase expression by EGF in the human colon adenocarcinoma cells Caco-2. *Am J Physiol* 261, C1173-C1183 (1991)

127. Yokosaki Y., H. Monis, J. Chen & D. Sheppard: Differential effects of the integrins alpha9beta1, alphavbeta3, and alphavbeta6 on cell proliferative response to tenascin. Role of the beta subunit extracellular and cytoplasmic domains. *J Biol Chem* 271, 24144-24150 (1996)

128. Von der Mark H., J. Durr, A. Sonnenberg & K. Von der Mark: Skeletal myoblasts utilize a novel beta1-series integrin and not alpha6beta1 for binding to the E8 and T8 fragments of laminin. *J Biol Chem* 266, 23593-23601 (1991)

129. Song W.K., W. Wang, H. Sato, D.A. Bielser & S.J. Kaufman: Expression of alpha7 integrin cytoplasmic domains during skeletal muscle development: alternate forms, conformational change, and homologies with serine/threonine kinases and tyrosine phosphatases. *J Cell Sci* 106, 1139-1152 (1993)

130. Collo G., L. Starr & V. Quaranta: A new isoform of the laminin receptor integrin alpha7beta1 is developmentally regulated in skeletal muscle. *J Biol Chem* 268, 19019-19024 (1993)

131. Vachon P.H., F. Loechel, H. Xu, U.M Wever & E. Engvall: Merosin and laminin in myogenesis; specific requirement for merosin in myotube stability and survival. *J Cell Biol* 134, 1483-1497 (1996)

132. Yao C.-C., B.L. Ziober, R.M. Squillace & R.H. Kramer: Alpha7 integrin mediates cell adhesion and migration on specific laminin isoforms. *J Biol Chem* 271, 25598-25603 (1996)

133. Vachon, P.H., H. Xu, L. Liu, F. Loechel, Y. Hayashi, K. Arahata, J.C. Reed, U.M. Wewer, & E. Engvall: Integrins (alpha7beta1) in muscle function and survival; disrupted expression in merosin-deficient congenital muscular dystrophy. *J Clin Invest* 100, 1870-1881 (1997)

134. Lotz M.M., A. Nusrat, J.L. Madara, R. Ezzell, U.M. Wewer & A.M. Mercurio: Intestinal epithelial restitution. Involvement of specific laminin isoforms and integrin laminin receptors in wound closure of a transformed model epithelium. *Am J Pathol* 150, 747-760 (1997)

Key words: Cell-matrix interactions, intestine, epithelium, integrins, extracellular matrix molecules, proliferation, differentiation.

Send correspondence to: Jean-François Beaulieu, Département d'anatomie et de biologie cellulaire, Faculté de médecine, Université de Sherbrooke, Sherbrooke, Qué, Canada J1H 5N4, Tel:819-564-5269, Fax::819-564-5320, E-mail: jf.beaul@courrier.usherb.ca

Received 12/21/98 Accepted 1/20/99