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Regulated Expression of the Integrin α9β1 in the Epithelium of the Developing Human Gut and in Intestinal Cell Lines: Relation With Cell Proliferation

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Abstract The integrin α9β1 is one of the recently identified integrins whose expression is restricted to specialized tissues. Its exact function is still unknown. In the present study, we have analyzed the expression of the α9 subunit in human fetal and adult small intestinal and colonic epithelia as well as in intestinal cell lines by indirect immunofluorescence, immunoprecipitation, Western blot, and Northern blot. In intact tissues, the antigen was restricted to the basolateral domain of epithelial cells in intestinal crypts at the fetal stage and was absent in the adult. The α9β1 integrin was also detected in the intestinal cell lines HIEC-6 and Caco-2/15. The presence of α9β1 in HIEC-6 was found to be consistent with their proliferative crypt-like status. In Caco-2/15 cells, the integrin was present at high levels in proliferating cells but was downregulated when cells cease to grow and undertake their differentiation. EGF treatment, which is known to maintain Caco-2/15 cells in a proliferative state, resulted in higher levels of α9 as compared to control cells. Taken together, these observations suggest a relation between integrin α9β1 expression and proliferation in human intestinal cells. J. Cell. Biochem. 71:536–545, 1998.

Key words: intestinal epithelium; cell growth; cell differentiation; HIEC; Caco-2

Integrins are a superfamily of cell surface receptors that mediate cell-cell and cell–extracellular matrix (ECM) interactions. These αβ heterodimer glycoproteins provide a structural and functional bridge between extracellular molecules and cytoskeletal components and are involved in mediating signal transduction processes [Clark and Brugge, 1995; Rosekelly et al., 1995]. So far, 16 α and 8 β subunits have been identified, but it is mainly the integrins of the β1 family that are responsible for ECM-cell interactions. In epithelia, β1 integrins known to be widely expressed include α1β1, α2β1, α3β1, α5β1, and α6β1 [Mercurio, 1995; Sheppard, 1996; Beaulieu, 1997a]. Some of them are ligand-specific, such as α5β1 and α6β1, which bind exclusively to fibronectin and laminins, respectively, while others, such as α2β1 and α3β1 can use collagens and/or laminins depending on the cell type. The presence of multiple integrins on the cell surface allows cells to recognize and respond to a variety of different ECM molecules in the promotion and regulation of adhesion, migration, growth, apoptosis, and differentiation [Akiyama et al., 1990; Hynes, 1992; Rosekelly et al., 1995; Assoian and Zhu, 1997; Meredith and Schwartz, 1997].

The intestinal epithelium is an advantageous system for analysis of cell-matrix interactions in relation to the cell state in the intact organ [Beaulieu, 1997a]. Indeed, this epithelium, which is in constant and rapid renewal, consists of spatially confined proliferative and differentiated cell compartments located, respectively, in the crypts and on the villi. Functionally,

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the crypt-villus axis is further defined by typical morphological and functional properties displayed by the mature enterocyte, which clearly distinguish it from the crypt cell [Leblond, 1981; Louvard et al., 1992; Ménard and Beaulieu, 1994; Beaulieu, 1997b]. The regulation of intestinal cell growth and differentiation is susceptible to various influences along the crypt-villus axis [Boyle and Brenner, 1995; Podolsky and Babyatsky, 1995], including differential cell-matrix interactions [Beaulieu, 1997a,b]. Indeed, analysis of β1 integrins in the human small intestine has demonstrated peculiar patterns of expression for many of them. For instance, in the adult, the integrin α2β1 is predominantly expressed by crypt cells, and α3β1 is mainly expressed by villus cells, whereas the α6 subunit is present throughout the epithelium [MacDonald et al., 1990; Beaulieu, 1992; Beaulieu and Vachon, 1994]. Furthermore, the α7Bβ1 integrin, which has been recently identified in the human intestinal epithelium, is primarily located at the crypt-villus junction, a distribution that coincides with the onset of epithelial cell differentiation [Basora et al., 1997]. Interestingly, patterns of expression for these integrins were found to be already established by mid-gestation [Perreault et al., 1995; Basora et al., 1997; Beaulieu, 1997a].

The integrin α9β1 is one of the most recently discovered integrins [Palmer et al., 1993]. In the mouse, it has been identified in smooth and skeletal muscles as well as in a few other specialized tissues such as squamous and airway epithelia [Palmer et al., 1993; Wang et al., 1995]. A well-characterized ligand for the α9β1 integrin is tenascin-C [Yokosaki et al., 1994; Weinacker et al., 1995]. Recent observations suggest that α9β1 may also serve as a receptor for osteopontin [Smith et al., 1996]. The expression of α9β1 has not yet been studied in the small intestine, but this integrin is absent from the normal adult colonic epithelium [Palmer et al., 1993; Basora et al., 1998]. However, the α9 integrin subunit has been reported in the fetal colonic epithelium [Dieckgraefe et al., 1996] and, interestingly, was also found in adenocarcinomas of the human colon [Basora et al., 1998], suggesting that the expression of this integrin may be related to extensive remodeling/cell proliferation–related events such as those occurring in the intestinal epithelium during fetal development and tumorigenesis. This possibility prompted us to examine the expression of the α9β1 integrin in relation to intestinal cell proliferation. Using the intact fetal small intestine and colon as well as intestinal cell lines, we demonstrate in the present study that the expression of the α9β1 integrin is predominantly associated with proliferative intestinal cells both in vivo and in vitro.

MATERIALS AND METHODS

Tissues

Specimens of small intestine and colon from 15 fetuses ranging in age from 15–18 weeks (postfertilization) were obtained after legal abortion. Ten samples of adult small intestine (jejunum) and colon were obtained from nondiseased parts of resected segments (resection margins). The project was in accordance with protocols approved by the Institutional Human Research Review Committee for the use of human material. Only tissues obtained rapidly (in less than 40 min) were used in the present study.

In some experiments, fetal small intestinal and colonic epithelia were separated from mesenchymes by using a procedure derived from the method described for the isolation and production of HIEC cell lines [Perreault and Beaulieu, 1996], which allows high rates of recovery of pure intestinal epithelial cell preparations [Perreault and Beaulieu, in press].

Cell Culture

The Caco-2/15 cell line, a stable clone of the parent Caco-2 cell line [Pinto et al., 1983], has been characterized elsewhere [Beaulieu and Quaroni, 1991; Vachon and Beaulieu, 1992; Vachon et al., 1996]. These cells are unique in that upon confluence they spontaneously undergo a gradual enterocytic differentiation process, similar to that observed in the epithelium of the intact fetal small and large intestine [Ménard and Beaulieu, 1994; Zweibaum and Chantret, 1989]. Cells between passages 54 and 72 were cultured in plastic dishes as described [Vachon and Beaulieu, 1992]. In this study, Caco-2/15 cells were analyzed 2 days before confluence (50–60% confluence) and at 0–24 days postconfluence. In some experiments, epidermal growth factor (EGF) (Gibco-BRL, Life Technologies, Burlington, Ont., Canada) was added to the culture medium at a final concentration of 20 ng/ml from the day after seeding (day -5) to 4 days after confluence.

It is noteworthy that Caco-2/15 cells produce negligible amounts of endogenous transforming growth factor-α/EGF-like activity [Beaulieu and Quaroni, 1991].

The HIEC-6 cell line has been generated from the normal fetal human small intestine [Perreault and Beaulieu, 1996]. These cells express a number of crypt cell markers but no villus cell markers and are thus considered as poorly differentiated crypt-like cells [Perreault and Beaulieu, 1996; Quaroni and Beaulieu, 1997]. The HIEC cells were used between passages 5–10 and were grown as described [Perreault and Beaulieu, 1996].

Primary Antibodies

The antibodies used in this work were the monoclonal mAb13 [Akiyama et al., 1989] directed against the human β1 integrin subunit (kindly provided by Dr. S.K. Akiyama, Howard University Cancer Center, Washington, DC), HSI-14 [Beaulieu et al., 1989] directed against the human sucrase-isomaltase complex, and CY90 (Sigma Chemicals Co., St. Louis, MO) specific to the human keratin 18 [Vachon et al., 1995]. The production of the rabbit-specific antiserum directed against the α9 integrin subunit and the procedure to affinity purify the anti-α9 antibodies have been described previously [Palmer et al., 1993].

Immunofluorescence Staining

The preparation and Optimum Cutting Temperature embedding compound (OCT) (Tissue Tek; Miles Laboratories, Elkart, IN) embedding of specimens for cryosectioning was performed as previously described [Beaulieu et al., 1991]. Cryosections 2–3 μm thick were cut on a Jung Frigocut 2800N cryostat (Leica Canada Inc., Saint-Laurent, Qué., Canada), spread on silane-coated glass slides and then air-dried for 1 h at room temperature before storage at -80°C. Tissue sections were fixed in fresh 2% paraformaldehyde (60 min, 4°C) before immunostaining as described previously [Beaulieu, 1992; Beaulieu et al., 1991]. Anti-α9 subunit affinity-purified antibodies were diluted 1:200 in phosphate buffered saline (PBS) (pH 7.4) containing 10% Blotto. FITC-conjugated goat antirabbit IgG (Boehringer Mannheim Canada, Laval, Qué., Canada) were used as secondary antibodies at a working dilution of 1:25. Sections were then stained with 0.01% Evan's blue in PBS. Preparations were mounted in glycerol-PBS (9:1) containing 0.1% paraphenylene diamine and viewed with a Reichert Polyvar 2 microscope (Leica Canada) equipped for epifluorescence. In all cases, no immunofluorescent staining was observed when primary antibodies were omitted or replaced by appropriate nonimmune sera.

Western Blot Analysis

Sodium dodecyl sulfate (SDS)/12% PAGE and Western blotting were performed as described previously [Vachon and Beaulieu, 1995; Beaulieu et al., 1989]. Briefly, proteins from the various cell lines were directly solubilized in sample buffer containing 5% β-mercaptoethanol. Separated proteins (150 μg/lanes) were transferred onto nitrocellulose (ImmunoSelect; Gibco/BRL) and stained with Ponceau red to localize molecular weight markers (44–220 kD range) (BioRad, Mississauga, Ont., Canada). Membranes were blocked overnight at room temperature in PBS (pH 7.4) containing 10% Blotto and incubated with the primary antibody diluted in the blocking solution (anti-α9, 1:500; HSI-14, 1:500; mAb13, 1:1,000; CY90, 1:2,000). After washing in PBS, membranes were incubated with alkaline phosphatase–conjugated goat antimouse, -rabbit, or -rat IgG (BioRad; Cedar Lane, Hornby, Ont., Canada), further washed, and finally incubated with a chromogenic substrate for alkaline phosphatase detection according to the instructions of the manufacturer.

Immunoprecipitation

HIEC and Caco-2/15 cells at different stages of confluence were analyzed for the presence of the α9β1 complex according to a procedure described previously [Basora et al., 1998]. Briefly, the cells were washed twice in ice-cold PBS and solubilized in lysis buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 2 mM sodium orthovanadate, 2 μM PMSF, 50 μg/ml leupeptin, 50 μg/ml pepstatin, 100 μg/ml aprotinin) for 20 min on ice and then centrifuged for 15 min at 13,000*g*. mAb13 (5 ug) or a 1/200 dilution of the affinity purified anti-α9 was added to the samples for 15 h at 4°C followed by the addition of protein-G Sepharose (Gibco/BRL) for a 1 h incubation at 4°C. Immunoprecipitates were then processed for analysis by SDS-PAGE and Western blotting (see above).

Northern Blot Analysis

RNA was isolated from cell lines or tissue homogenates using Trizol reagent (Gibco/BRL). Total cellular RNA was electrophoresed on a 1%:1.8% agarose:formaldehyde gel (30–50 μg/lane) and blotted onto nylon membrane (Hybond-N; Amersham, Oakville, Ont., Canada) as described previously [Beaulieu and Quaroni, 1991]. Radiolabeled probes were synthesized from a 2.1 kb fragment of α9 cDNA [Palmer et al., 1993] and a 1.3 kb fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA used as a control using the Multiprime Labeling System (Amersham) in the presence of [α-^{32}P]dCTP. Prehybridization (1 h) and hybridization (12 h) were performed at 68°C. Kodak (New Haven, CT) Biomax MS films were exposed with intensifying screens for 2 h (GAPDH) and 42 h (α9) at -80°C.

RESULTS

Expression of the Integrin α9 Subunit in the Human Gut

The expression of the α9 subunit was first analyzed by indirect immunofluorescence in fetal and normal adult human small intestine and colon. In the fetal samples, the antigen was mainly detected at the basolateral surface of the epithelial cells located in the developing crypts in both the jejunum (Fig. 1A) and colon (Fig. 1C). The staining observed in the colon was found to be consistently stronger than in the small intestine. The antigen was also faintly detected in the cytoplasm of most epithelial cells located on both the villus and the crypt (Fig. 1A,C). Furthermore, a predominant staining in muscle layers was observed at all stages studied. The immunostaining in the epithelium was considered specific, as it was not seen with a negative control affinity-purified rabbit antibody directed against another integrin subunit not expressed in the intestinal epithelium (the α7A subunit; Basora et al., 1997). In adult specimens, the α9 subunit was not detected in the epithelium (Fig. 1B,D) but was present in the contractile network of the small intestinal mucosa (Fig. 1B) as well as in subepithelial myofibroblasts surrounding the lower two thirds of the colonic glands (Fig. 1D) and in the muscularis mucosa (Fig. 1 B,D).

Expression of the Integrin α9 Subunit in Human Intestinal Cells

The expression of the α9 subunit by fetal intestinal epithelial cells was confirmed by Northern blot analysis. The epithelium was dissociated from its underlying mesenchyme and muscle layers in both small intestine and colon with a nonenzymatic method, and the total RNA was extracted from each of these preparations. The purity of the preparations was confirmed by using different approaches including Western blot analysis for keratin 18 and vimentin contents (not shown) [Perreault and Beaulieu, in press]. As expected from immunofluorescence studies, high levels of α9 subunit transcripts were observed in the mesenchyme (Fig. 2, lanes 1,2), while a signal in the corresponding isolated epithelia was consistently detected, although much weaker in the jejunum (Fig. 2, lane 3) than in the colon (Fig. 2, lane 4).

The expression of the α9 subunit by intestinal cells was further investigated at the protein and mRNA levels in our two current in vitro models: the enterocyte-like Caco-2/15 cell line and the normal crypt-like cell line HIEC-6. Interestingly, the two forms of the α9 transcript [Palmer et al., 1993] were detected in both proliferating/poorly differentiated HIEC-6 (Fig. 3, lane 1) and Caco-2/15 cells (Fig. 3, lane 2). However, in postconfluent Caco-2/15 cells, which gradually lose their ability to proliferate as they undertake their enterocytic differentiation program, a marked reduction in the relative amounts of the α9 transcripts (both forms) was consistently observed over time (Fig. 3, lanes 3–7). Analysis of the α9 subunit at the protein level revealed similar findings. The α9 subunit was detected in both proliferating HIEC-6 and Caco-2/15 cells after immunoprecipitation with anti-α9 affinity-purified antibodies and Western blot analysis with the same antibody (Fig. 4A, lanes 1,2). As expected from the transcript levels observed above, the relative amounts of α9 decreased substantially in postconfluent differentiating Caco-2/15 cells (Fig. 4A, lanes 3–5). The analysis of α9 immunoprecipitates for the β1 subunit showed that it coprecipitates with α9 in approximately the same proportion over the culture period (Fig. 4A), confirming that intestinal cells express α9 as a typical α9β1 complex. Comparison of the relative amounts of the β1 subunit associated with α9 complex (Fig. 4A) with the total β1 population (Fig. 4B) al-

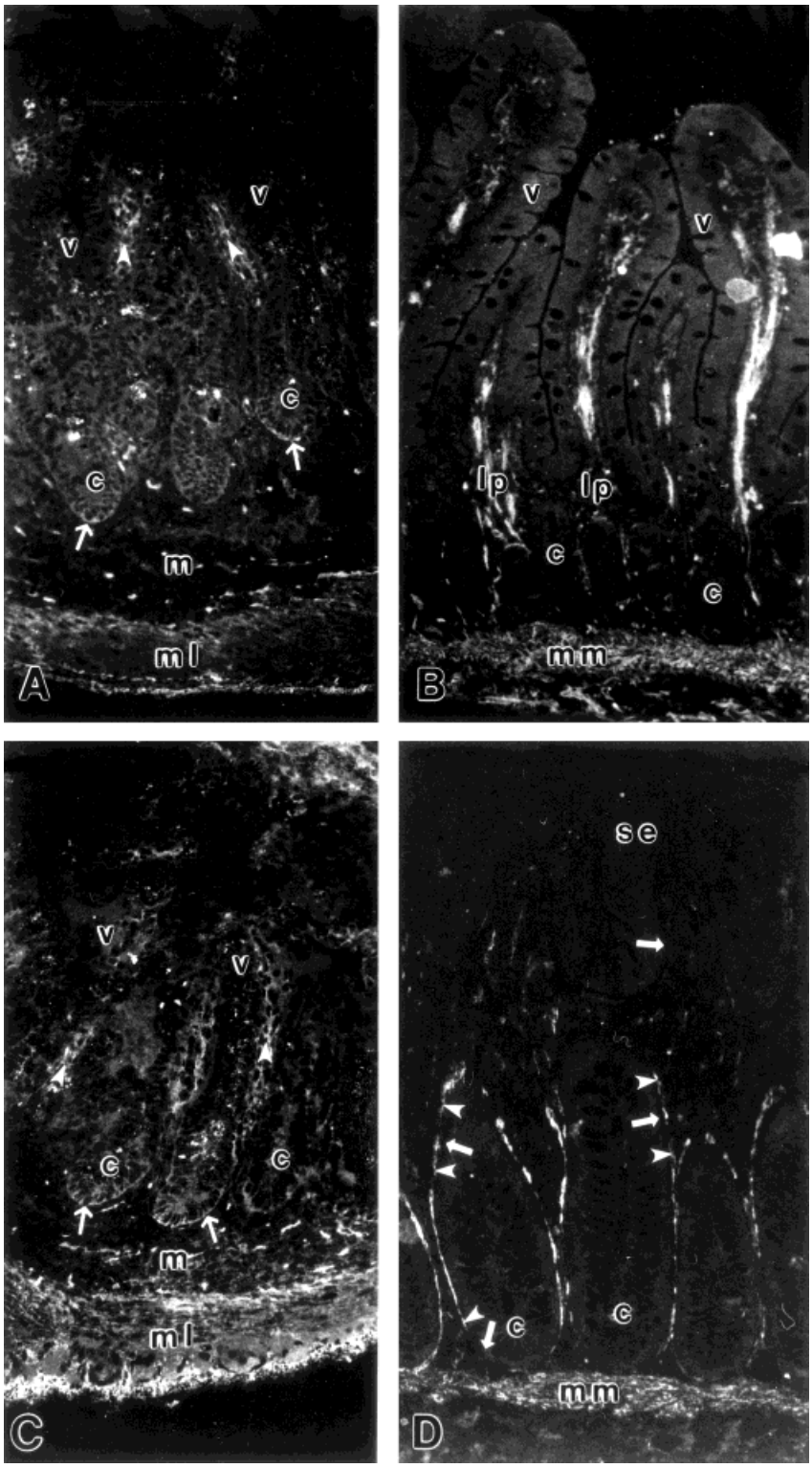

Fig. 1. Expression and distribution of the α9 integrin subunit in the developing and adult intestine. Representative immunofluorescence micrograph of human jejunum at 17 weeks of gestation (**A**) and adult (**B**) and colon at 17 weeks of gestation (**C**) and adult (**D**) stained with an affinity-purified antibody directed against the α9 integrin subunit. In both the fetal small intestine (A) and colon (C), the α9 subunit is present at the basolateral surface in the glandular epithelium (arrows) and detectable in the cytoplasm of epithelial cells in both crypt (c) and villus (v). Some staining is also apparent at the tip of the villus core (arrowheads) as well as in muscle layers (ml). In the adult small intestine (B), the α9 subunit was exclusively detected in association with smooth muscle cells of the lamina propria (lp) and muscularis mucosa (mm). The crypt (c) and villus (v) epithelium was found to be negative. In the adult colon (D), the antigen is detected in subepithelial myofibroblasts (arrowheads) surrounding the lower third of the glands and in the muscularis mucosa (mm), while the crypt (c) and surface epithelium (se) remained consistently negative both in the cytoplasm and at their basolateral surface (arrows). A–C: $\times$90. D: $\times$165.

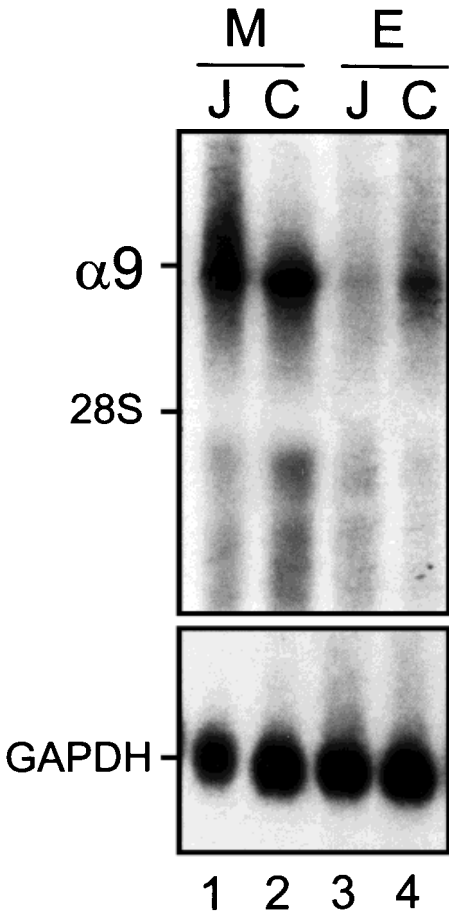

Fig. 2. Representative Northern blot analysis for the detection of the α9 mRNA in the fetal small intestinal and colonic epithelium. Epithelia (E) from both jejunum (J) and colon (C) were dissociated from their corresponding underlying mesenchyme and muscle (M) by a nonenzymatic method, and total RNA was isolated for each of these preparations and analyzed with ^{32}P-labeled DNA probes for the presence of α9 and GAPDH transcripts.

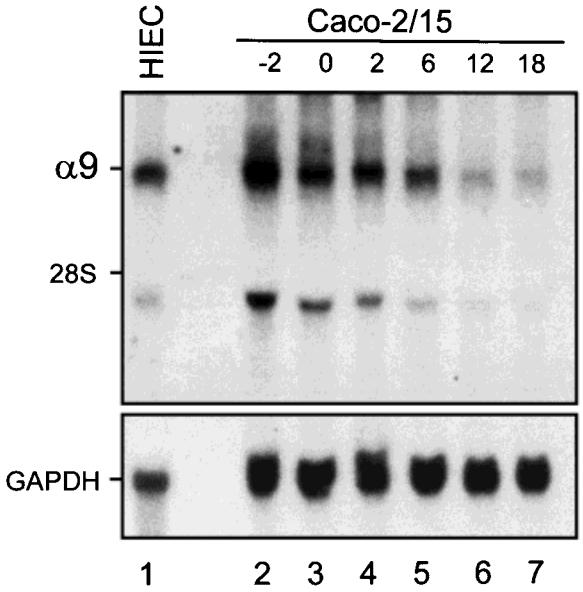

Fig. 3. Expression of the α9 subunit mRNA in intestinal cell lines. Representative Northern blot analysis. Total RNA was isolated from HIEC and Caco-2/15 cells at -2, 0, 2, 6, 12, and 18 days of confluence and analyzed with ^{32}P-labeled DNA probes for the presence of α9 and GAPDH transcripts.

lows two observations: first, that quantitatively α9β1 is not a major integrin in intestinal cells when considering that lysates used for α9 immunoprecipitation (Fig. 4A) were five times more concentrated (10 mg protein/sample) than those used for total β1 immunoprecipitation (Fig. 4B) (2 mg protein/sample); second, that in comparison with the β1 population (Fig. 4B), which is relatively stable over the culture period studied, α9β1 is indeed predominantly expressed in the proliferating subconfluent Caco-2/15 cells as opposed to postconfluent quiescent cells (Fig. 4A).

Modulation of the Integrin α9 Subunit Expression by EGF

The potential relation between expression of the α9 subunit and intestinal cell proliferation was further investigated in EGF-treated Caco-2/15 cells. EGF has been shown previously to inhibit sucrase-isomaltase expression and stimulate both DNA synthesis and proliferation of Caco-2/15 cells in postconfluent cultures (4–10 days after confluence) when added to the standard culture medium at a concentration of 20 ng/ml [Cross and Quaroni, 1991]. By using a similar experimental procedure, we found that the expression of the α9 subunit was significantly higher in lysates of EGF-treated Caco-2/15 cells than in the control cells grown under exactly the same conditions but without EGF, as determined by Western blot analysis (Fig. 5) and densitometry (relative amounts of α9/K18: -EGF, 0.351 ± 0.045; +EGF, 0.576 ± 0.014; $P < 0.01$, n = 4). As expected, a corresponding ~50% reduction in immunoreactive sucrase-isomaltase was observed (Fig. 5) (relative amounts of SI/K18: -EGF, 0.937 ± 0.025; +EGF, 0.428 ± 0.144; $P < 0.025$, n = 4), while the β1 subunit appeared to remain constant (data not shown) (β1/K18: -EGF, 0.410 ± 0.042; +EGF, 0.508 ± 0.044; n = 4).

DISCUSSION

The precise function of the α9β1 integrin still remains to be elucidated. Analysis of α9 subunit expression during murine embryogenesis has suggested that this integrin probably plays a role in the maturation and/or maintenance of

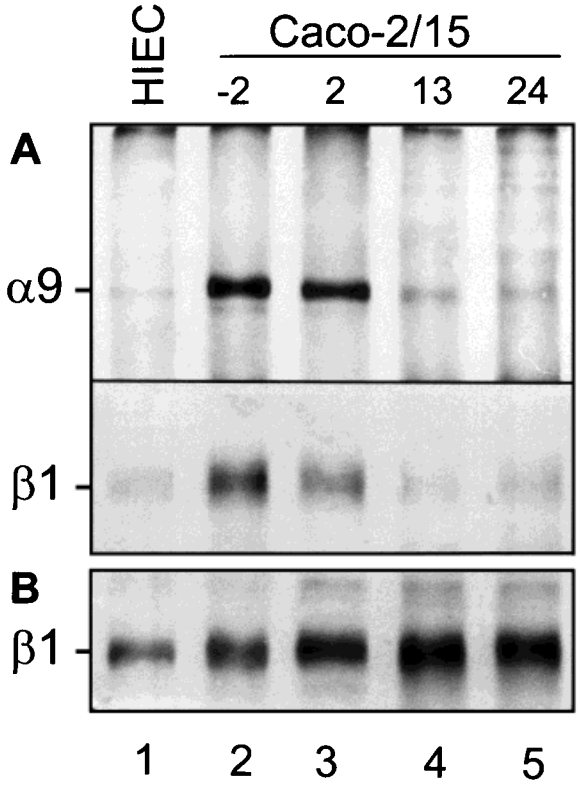

Fig. 4. Expression of the integrin α9 and β1 subunits in intestinal cell lines. Representative Western blot analysis of the α9β1 complex detected with anti-α9 and mAb13 antibodies after immunoprecipitation with an affinity-purified anti-α9 subunit (**A**) as well as corresponding total β1 subunit immunoprecipitated and detected with the mAb13 antibody (**B**) from HIEC and Caco-2/15 cells at -2, 2, 13, and 24 days of confluence. Lysates used for α9 immunoprecipitation were five times more concentrated than those used for β1.

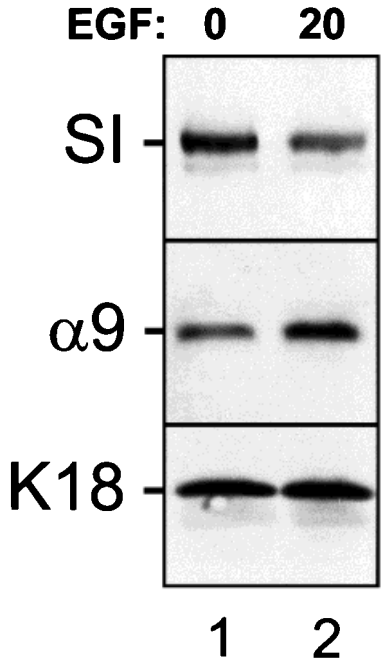

Fig. 5. Modulation of the integrin α9 subunit in Caco-2/15 cells by EGF. Representative Western blot analysis of Caco-2/15 cell lysates harvested 4 days after confluence and treated with 0 (**lane 1**) or 20 ng/ml of EGF (**lane 2**) from the day after the seeding. Sucrase-isomaltase (SI) and keratin 18 (K18) were used as control.

a variety of differentiated tissues [Wang et al., 1995], a suggestion that fits well with the constitutive expression of the α9 subunit in contractile cells of both the developing and adult intestinal mucosa [Palmer et al., 1993; Dieckgraefe et al., 1996; this work]. However, in some tissues, α9 expression is restricted to cells known to retain the capacity to proliferate, such as the basal layer of some squamous epithelia, which is indicative of another function(s) for this integrin [Palmer et al., 1993; Wang et al., 1995]. Furthermore, in the mouse cornea, the pattern of expression for the α9 integrin suggests that it may be important in helping to maintain a proliferative epithelial cell phenotype [Stepp et al., 1995]. Herein we demonstrate that the expression of the α9β1 integrin is related to proliferation in human immature intestinal epithelial cells.

In the developing fetal small intestinal and colonic epithelia, the α9 subunit was detected according to a typical basolateral staining pattern predominantly confined to cells located in the crypt, which represent the proliferative compartment [Boyle and Brenner, 1995; Podolsky and Babyatsky, 1995]. In contrast, the α9 subunit remained undetectable in the epithelium of the adult gut. These observations, which are in agreement with previous studies [Palmer et al., 1993; Wang et al., 1995], suggest that the α9 subunit is downregulated in mature small intestinal and colonic epithelia. This pattern of expression for an integrin in the gut is unusual, as most other integrins present in the fetal intestinal epithelium remain at the adult stage [Beaulieu, 1997a]. However, the lack of α9 subunit expression in the proliferative compartments of adult intestinal epithelia is of interest, as it may suggest that the presence of this integrin may not be required in these highly ordered renewing epithelia and may even not be desirable for their regulation. In support of this later possibility, we have recently demonstrated that the α9 subunit is reexpressed in human colon adenocarcinomas [Basora et al., 1998]. Such a pattern of expression for the α9 subunit in the gut appears consistent with a possible relation between the presence of the integrin α9β1 and an oncofetal cell prolifera-

tion status. In the support of this, Yokosaki et al. [1996] demonstrated recently that α9β1 expression in colon carcinoma cells and interaction with its ligand tenascin-C can promote proliferation in vitro.

To further investigate the potential relation between α9β1 and intestinal cell proliferation, we have analyzed its expression in two well-characterized human intestinal cell lines: HIEC-6 cells, which have been derived from normal human fetal small intestine [Perreault and Beaulieu, 1996], and Caco-2/15 cells, an adenocarcinoma cell line that is unique in its ability to spontaneously undertake a program of fetal intestinal cell differentiation at confluence [Beaulieu and Quaroni, 1991; Vachon and Beaulieu, 1992; Vachon et al., 1996]. Interestingly, both cell lines were found to express α9β1 as determined by immunoprecipitation and Northern blot analyses. The presence of the α9β1 integrin in HIEC-6 cells was consistent with their proliferative crypt-like status [Perreault and Beaulieu, 1996; Quaroni and Beaulieu, 1997], along with the fact that the integrin was also present in their in situ counterpart, the crypt. The pattern of α9β1 expression in differentiating Caco-2/15 cells revealed additional interesting findings. The α9β1 complex was present at relatively high levels in growing cells but declined rapidly over the first ~10 days of confluence, a culture period characterized by a substantial reduction in cell proliferation, DNA synthetic activity, and expression of the proliferation-associated nuclear antigen Ki-67 [Cross and Quaroni, 1991; Vachon et al., 1996]. The sharp reduction seen for immunoreactive α9 was closely paralleled to a substantial decrease of its corresponding transcript, suggesting that the downregulation occurs primarily at the mRNA levels. Interestingly, EGF was found to reverse the phenomenon of α9 reduction in newly confluent Caco-2/15 cells. It is noteworthy that considerable experimental evidence supports a role for EGF/transforming growth factor (TGF)-α in the control of intestinal epithelial cell proliferation [Ménard and Beaulieu, 1994; Podolsky, 1993; Drucker, 1997]. In a detailed study on the Caco-2/15 clone, which produces negligible amounts of endogenous TGF-α/EGF–like activity [Beaulieu and Quaroni. 1991], Cross and Quaroni [1991] have shown that, when added to the culture medium, even at a relatively low concentration (20 ng/ml), EGF partly abolishes the reduction of cell proliferation occurring after confluence in control cultures and inhibits the expression of the differentiation marker sucrase-isomaltase. The higher levels of the α9 subunit observed in EGF-treated Caco-2/15 cell cultures fit well with the possibility of a functional relation α9β1 expression and cell proliferation.

The transitory expression of the α9β1 integrin mostly restricted to proliferating Caco-2 cells seems unique in regard to other β1 integrins expressed in these cells. For example, the α2β1 and α7β1 integrins are expressed at significant levels in both subconfluent and postconfluent Caco-2/15 cells [Basora et al., 1997]. It is noteworthy that downregulation of gene expression in differentiating Caco-2 cells is an unusual phenomenon. For instance, most functional brush border membrane markers and extracellular matrix molecules studied in these cells have been found to be either constitutively expressed or induced after reaching confluence [Beaulieu and Quaroni, 1991; Vachon and Beaulieu, 1995; Vachon et al., 1996; Simoneau et al., 1997]. In fact, to our knowledge, cellular fibronectin is the only other molecule known to be clearly downregulated at both the protein and transcript levels in differentiating Caco-2 cells [Vachon et al., 1995], a phenomenon that, as for the α9β1 integrin, also reflects the normal in vivo situation since the molecule is predominantly associated with the proliferative epithelial cells of the crypt compartment [Beaulieu et al., 1991]. Incidentally, fibronectin levels were found significantly increased by EGF in Caco-2/15 cells (data not shown). However, a functional relevance for these two closely related patterns of expression is unlikely since α9β1 does not seem to bind to fibronectin [Yokosaki et al., 1994, 1996]. On the other hand, it has been clearly demonstrated that α9β1 can mediate functional adhesion to the third fibronectin type III repeat of tenascin-C [Yokosaki et al., 1994 1996; Weinacker et al., 1995], leading to an activation of the mitogen-activated kinase Erk-2 and stimulation of cell proliferation [Yokosaki et al., 1996]. However, the pattern of tenascin-C expression in the developing and adult human small and large intestines [Beaulieu et al., 1991, 1993; Beaulieu, 1992, 1997a; Desloges et al., 1994] differs considerably from that observed herein and elsewhere for the α9 integrin subunit [Palmer et al., 1993; Wang et al., 1995; Basora et al., 1998], suggesting that other ligands must exist for α9β1 [Wang et al.,

1995], as recently demonstrated for the N-terminal domain of osteopontin [Smith et al., 1996]. Considering the requirement of integrin-ligand interactions in the growth-promoting activity of α9β1 [Yokosaki et al., 1996], it would be of importance to identify the ligand used by this integrin in the human intestinal epithelium to further investigate its relation with intestinal cell proliferation.

In summary, these observations demonstrate that the α9 subunit is present in the fetal small intestinal and colonic epithelia as well as in their in vitro counterparts, the HIEC-6 and Caco-2/15 cell lines, and that the α9β1 integrin is predominantly associated with proliferative cells. The integrin has not been detected in the epithelium of the adult small intestine and colon, suggesting that its expression is restricted to immature proliferating intestinal cells.

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REFERENCES

Akiyama SK, Yamada SS, Chen WT, Yamada KM (1989): Analysis of fibronectin receptor function with monoclonal antibodies: Role in cell adhesion, migration, matrix assembly, and cytoskeletal organization. J Cell Biol 105:1183–1190.

Akiyama SK, Nagata K, Yamada KM (1990): Cell surface receptors for extracellular matrix components. Biochim Biophys Acta 1031:91–110.

Assoian RK, Zhu X (1997): Cell anchorage and the cytoskeleton as partners in growth factor dependent cell cycle progression. Curr Opin Cell Biol 9:93–98.

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

Basora N, Desloges N, Bouatrouss Y, Gosselin J, Chang Q, Poisson J, Sheppard D, Beaulieu J-F (1998): Expression of the α9β1 integrin in human colonic epithelial cells: Resurgence of the fetal phenotype in a subset of colon cancer and adenocarcinoma cell lines. Int J Cancer 75: 738–743.

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

Beaulieu J-F (1997a): Extracellular matrix components and integrins in relationship to human intestinal epithelial cell differentiation. Prog Histochem Cytochem 34(4): 1–76.

Beaulieu J-F (1997b): Recent work with migration/patterns of expression: Cell-matrix interactions in human intestinal cell differentiation. In Halter F, Winton D, Wright NA (eds): "The Gut as a Model in Cell and Molecular Biology." Dordretcht: Kluwer Academic Publishers, pp 165–179.

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

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

Beaulieu JF, Nichols B, Quaroni A (1989): Posttranslational regulation of sucrase-isomaltase expression in intestinal crypt and villus cells. J Biol Chem 264:20000–20011.

Beaulieu JF, Vachon PH, Chartrand S (1991): Immunolocalization of extracellular matrix components during organogenesis in the human small intestine. Anat Embryol 183:363–369.

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

Boyle WJ, Brenner DA (1995): Molecular and cellular biology of the small intestine. Curr Opin Gastroenterol 11: 121–127.

Clark EA, Brugge JS (1995): Integrin and signal transduction pathways: The road taken. Science 268:233–239.

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

Desloges N, Simoneau A, Jutras S, Beaulieu J-F (1994): Tenascin may not be required for intestinal villus development. Int J Dev Biol 38:737–739.

Dieckgraefe BK, Santoro SA, Alpers DH (1996): Immunolocalization of α-integrin subunits and extracellular matrix components during human colonic organogenesis. Gastroenterology 110:58–71.

Drucker DJ (1997): Epithelial cell growth and differentiation. I. Intestinal growth factors. Am J Physiol 273: G3–G6.

Hynes RO (1992): Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 69:11–25.

Leblond CP (1981): The life history of cells in renewing systems. Am J Anat 160:114–159.

Louvard D, Kedinger M, Hauri HP (1992): The differentiating intestinal epithelial cell: Establishment and maintenance of functions through interactions between cellular structures. Annu Rev Cell Biol 8:157–195.

MacDonald TT, Horton MA, Choy MY, Richman PI (1990): Increased expression of laminin/collagen receptor (VLA-1) on epithelium of inflamed human intestine. J Clin Pathol 43:313–315.

Ménard D, Beaulieu J-F (1994): Human intestinal brush border membrane hydrolases. In Bkaily G. (ed): "Membrane Physiopathology." Norwell: Kluwer Academic Publisher, pp 319–341.

Mercurio AM (1995): Laminin receptors: Achieving specificity through cooperation. Trends Cell Biol 5:419–423.

Meredith JE, Schwartz MA (1997): Integrins, adhesion and apoptosis. Trends Cell Biol 7:146–150.

Palmer EL, Ruegg C, Ferrando R, Pytela R, Sheppard D (1993): Sequence and tissue distribution of the integrin α9 subunit, a novel partner of β1 that is widely distributed in epithelia and muscle. J Cell Biol 123:1289–1297.

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

Perreault N, Beaulieu J-F (in press): Production of primary culture of fully differentiated and pure enterocytes. Exp Cell Res.

Perreault N, Vachon PH, Beaulieu J-F (1995): Appearance and distribution of laminin A chain isoforms and integrin α2, α3, α6, β1, and β4 subunits in the developing human small intestinal mucosa. Anat Rec 242:242–250.

Pinto M, Robine-Leon S, Appay MD, Kedinger M, Triadou N, Dussaulx E, Lacroix B, Simon-Assmann P, Haffen K, Fogh J, Zweibaum A (1983): Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Biol Cell 47:323–330.

Podolsky DK (1993): Regulation of intestinal epithelial proliferation: A few answers, many questions. Am J Physiol 264:G179–G186.

Podolsky DK, Babyatsky MW (1995): Growth and development of the gastrointestinal tract. In Yamada T (ed): "Textbook of Gastroenterology," 2nd ed. Philadelphia: JB Lippincott, pp 546–577.

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

Rosekelly CD, Desprez PY, Bissell MJ (1995): A hierarchy of ECM-mediated signaling regulates tissue-specific gene expression. Curr Opin Cell Biol 7:736–747.

Sheppard D (1996): Epithelial integrins. Bioessays 18:655–660.

Simoneau A, Herring-Gillam E, Vachon PH, Perreault N, Basora N, Bouatrouss Y, Pageot L-P, Zhou J, Beaulieu J-F (1998): Identification, distribution and tissular origin of the α5(IV) and α6(IV) collagen chains in the developing human intestine. Dev Dyn 212:437–447.

Smith LL, Cheung HK, Ling LE, Chen J, Sheppard D, Pytela R, Gianelli CM (1996): Osteopontin N-terminal domain contains a cryptic adhesive sequence recognized by α9β1 integrin. J Biol Chem 271:28485–28491.

Stepp MA, Zhu L, Sheppard D, Cranfill RL (1995): Localized distribution of α9 integrin in the cornea and changes in expression during corneal epithelial cell differentiation. J Histochem Cytochem 43:353–362.

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

Vachon PH, Beaulieu J-F (1995): Extracellular heterotrimeric laminin promotes differentiation in human enterocytes. Am J Physiol 268:G857–G867.

Vachon PH, Simoneau A, Herring-Gillam FE, Beaulieu J-F (1995): Cellular fibronectin expression is down-regulated at the mRNA level in differentiating human intestinal epithelial cells. Exp Cell Res 216:30–34.

Vachon PH, Perreault N, Magny P, Beaulieu J-F (1996): Uncoordinated, transient mosaic patterns of intestinal hydrolase expression in differentiating human enterocytes. J Cell Physiol 166:198–207.

Wang A, Patrone L, McDonald JA, Sheppard D (1995): Expression of the integrin subunit α9 in the murine embryo. Dev Dyn 204:421–431.

Weinacker A, Ferrando R, Elliott M, Hogg J, Balmes J, Sheppard D (1995): Distribution of integrins αvβ6 and α9β1 and their known ligands, fibronectin and tenascin, in human airways. Am J Respir Cell Mol Biol 12:547–556.

Yokosaki Y, Palmer EL, Prieto AL, Crossin KL, Bourdon MA, Pytela R, Sheppard D (1994): The integrin α9β1 mediates cell attachment to a non-RGD site in the third fibronectin type III repeat of tenascin. J Biol Chem 269: 26691–26696.

Yokosaki Y, Monis H, Chen J, Sheppard D (1996): Differential effects of the integrins α9β1, αvβ3 and αvβ6 on cell proliferative response to tenascin. Role of the β subunit extracellular and cytoplasmic domains. J Biol Chem 271: 24144–24150.

Zweibaum A, Chantret I (1989): Human colon carcinoma cell lines as in vitro models for the study of intestinal cell differentiation. In Smith MW, Sepulveda FV (eds): "Adaptation and Development of the Intestinal Function." Manchester: Manchester University Press, pp 103–112.