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Epithelial vs Mesenchymal Contribution to the Extracellular Matrix in the Human Intestine

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The basement membrane (BM) underlying the epithelium of the intestine is generally believed to be of both epithelial and mesenchymal origin but the exact contribution of each tissue has not been directly examined in the human. In this study, we have used a newly described procedure to dissociate the human intestine into pure epithelial and corresponding mesenchymal fractions. Northern blot and RT-PCR analyses of the fractions for the presence of transcripts encoding extracellular matrix molecules revealed that the epithelium produces the formal BM molecules such as the α1, α2, and β1 chains of laminin-1 and laminin-2 and the α5(IV) and α6(IV) chains of collagen as well as fibronectin, a BM-associated molecule. Interestingly, the α1(IV) chain of collagen, which associates with the α2(IV) chain to form the main BM collagen network, as well as tenascin-C and decorin, two BM-associated molecules, was found to be exclusively of mesenchymal origin. Taken together, these data support the concept that in the human, as in experimental animals, the intestinal BM is composed of components produced from both the epithelium and the mesenchyme.

The existence of reciprocal epithelial-mesenchymal interactions during embryonic development as well as in the adult intestine is well documented (1-3). In both the developing and mature intestine, the epithelium is always closely associated with the underlying stromal cells: mesenchymal cells and their adult derivatives, the pericryptal and subepithelial myofibroblasts (4). The two tissues are separated by a specialized sheet-like extracellular matrix, the basement membrane (BM) (5). BM components have received great attention as potential key elements for the mediation of epithelial-mesenchymal interactions involved in the regulation of intestinal cell functions (6-8) based on the observation that they are in direct contact with epithelial cells and that the molecules that form them have been identified as dynamic effectors of cell adhesion, migration and tissue-specific gene expression (9-12). The human intestinal BM has been found to contain most of the major components specific to BMs, namely type IV collagens, laminins and proteoglycans as well as a number of BM-associated molecules such as fibronectin and tenascin-C. A number of specific cell receptors for these molecules, namely those of the integrin family, have also been identified (7). Furthermore, direct evidence that cell interactions with extracellular matrix molecules can control specific functions in the enterocyte has recently begun to accumulate (13-15).

Despite the importance of BM components for enterocyte regulation, little is known about the tissular origin of the molecules that compose it in the human intestine. Indeed, in contrast to other organs such as the skin, where the origin of the various BM component mRNAs has been directly determined after separation of the epithelium and mesenchyme (16), the intestine has been proven difficult to dissect into pure epithelial and mesenchymal fractions. Determining the tissular origin of BM molecules in the developing intestine appears to be of prime importance to understanding the role of these molecules in epithelial-mesenchymal interactions. For instance, in situ hybridization has revealed that the α1(IV) chain of collagen originates mainly, if not exclusively, from the mesenchymal compartment in the rodent small intestine (17) pointing out the requirement of the mesenchyme for the formation of a complete epithelial BM in this organ (7). Unfortunately, in situ hybridization has been proven difficult to apply to the detection of transcripts expressed at relatively low levels such as several of those encoding BM molecules (18, 19). Therefore, up to now, most of our knowledge about the tissular origin of extracellular matrix molecules localized at the epithelial-mesenchymal interface results from various approaches in-

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cluding co-culture systems and interspecies hybrid tissue recombination (6, 7). Although these approaches have significantly contributed to the establishment of the concept that the intestinal epithelial BM is composed of molecules produced by both cell types, the use of tissues obtained from laboratory animals and/or cancer cell lines in these studies precludes a direct extrapolation of their results to the normal human situation.

In the present study, we describe a new and simple procedure that allows the efficient dissociation of the human intestine into pure epithelial and corresponding mesenchymal fractions. Interestingly, the procedure was found particularly well suited to the fetal intestine at mid-gestation, a period at which the mucosa morphologically and functionally resembles the adult (20). Analysis of the fractions for the presence of transcripts for a number of extracellular matrix molecules revealed that the epithelium produces most of the formal BM molecules while BM-associated molecules are mostly of mesenchymal origin.

MATERIALS AND METHODS

Tissues. Specimen of small intestine and colon from 22 fetuses ranging from 15 to 20 weeks of age (post-fertilization) were obtained after legal abortion. The project was in accordance with a protocol approved by the Institutional Human Research Review Committee for the use of human material. Only specimen obtained rapidly were used in the present work.

Epithelial-mesenchymal dissociation and RNA extraction. Pools of epithelial cells and mesenchyme were obtained from small intestine (jejunum and ileum) and colon according to the following procedure. The serosa and muscularis propria were first removed and the remaining segments were opened longitudinally, washed in phosphate buffered saline (PBS) and cut into 5 mm fragments. Small intestinal and colonic fragments were transferred to ice-cold Matrisperse solution (Collaborative Biomedical Products, Becton Dickenson Labware, Mississauga, Ontario, Canada) and incubated at 4°C for 8-10h without agitation. Then, flasks were gently shaken to separate the epithelium. The remaining fragments, free of epithelial lining, were recovered and washed twice in PBS. The epithelial suspension was also washed twice in PBS (100*g*, 7 in). For each preparation, complete dissociation of the epithelium from the mesenchyme and lack of cross-contamination were confirmed at the protein level with tissue-specific markers (21). Total RNAs from intact tissues and mesenchymal and epithelial fractions from either small intestine or colon were prepared by the guanidine isothiocyanate-phenol method (22).

Northern hybridization analysis. A samples were subjected to agarose gel electrophoresis with formaldehyde and transferred for Northern blot analysis to nylon membranes (Nytran, Schleicher and Schuell). Equal RNA loading (15 μg) was confirmed by hybridization to a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene control. Prehybridization and hybridization were performed as described previously (18).

Probe preparation. Hybridizations were performed with the followed random primed ^{32}P-labeled cDNA probes (Multiprime Kit, Amersham, Oakville, Ontario). 1.3 kb Pst 1 rat GAPDH fragment (23), 1.7 kb Pst I human type IV collagen cDNA fragment (24), 1.7 kb Bgl II human sucrase-isomaltase cDNA fragment (25), 4.6 kb EcoR I murine laminin B1 chain cDNA fragment (26), 4.5 kb Hind III human fibronectin cDNA fragment (27), 1 kb Apa I and Pst I human decorin fragment (Gibco BRL) and a 2.2 kb BamH I pig SPARC fragment (kindly provided by Dr. J. Sodek). The E-cadherin probe was prepared from polymerase chain reaction (PCR) amplification of cDNA (28) prepared from total RNA extracted from a 18-week-old human fetal intestine.

Reverse-transcriptase PCR. Conditions for amplification of E-cadherin, sucrase-isomaltase and S14, used as endogenous control, have been described previously (29, 30). For laminin α1 chain, we used the sense primer 5′-aagtggcacacggtcaagac-3′ and the antisense primer 5′-gacaagagctgcatatccgc-3′ (31). For laminin α2 chain, we used the sense primer 5′-tcaaagtatctgtgtcttcagga-3′ and the antisense primer 5′-ccagtgaatgtaatcacacgtacagc-3′ (32).

For tenascin-C, we used the sense primer 5′-ctggtctgagtcttcgttcc-3′ and the antisense primer 5′-tcctgacagccgagaaaggc-3′. The sense primer hybridizes with the FnIII 5 (bases 3290 to 3309) domain, and the antisense primer hybridize with FnIII 6 (bases 5410 to 5429) (Genebank #A55618). These primers were expected to generate a major 230 bp product (Bélanger et al. unpublished data). Single stranded cDNA was amplified in PCR buffer containing 1 μM of both primers for 28 cycles of denaturation (1 min at 93°C) and annealing/extension (1 min at 61°C and 3 min at 72°C) in a thermal cycler (Perkin-Elmer DNA thermal cycler Model 480) in the presence of 250 μM dNTPs and 2.5 U of Taq polymerase (Roche, Branchburg, NJ).

RESULTS

For each preparation, complete dissociation of the epithelium from the mesenchyme was confirmed at the protein level (not shown) with tissue-specific markers including keratin 18 (epithelium), vimentin (mesenchymal cells) and α-smooth muscle actin (smooth muscle cells). However, assessing the tissular origin of extracellular matrix molecules required analysis at the transcript level. By using a combination of Northern blot and RT-PCR analyses, we have tested the origin of a number of formal BM molecules such as laminin and the type IV collagen chains as well as several BM-associated molecules. As shown in Fig. 1, Northern blot analysis revealed that the α1(IV) chain of collagen (C α1(IV)) was of exclusive mesenchymal origin (lane 2) while the β1 chain of laminin (Ln β1) and fibronectin (Fn) were both detected in the epithelial and mesenchymal fractions (lanes 2 and 3). Transcripts for decorin (Dc) and SPARC/osteonectin/BM40 (SPARC) were only detected in the mesenchyme (lane 2). RT-PCR was used for the detection of transcripts expressed at relatively low levels. As illustrated in Fig. 2, cDNAs corresponding to the α1 and α2 chains of laminin (Ln α1 and Ln α2) as well as the α5(IV) and α6(IV) chains of collagen were amplified in both epithelial and mesenchymal fractions (lanes 2 and 3). On the other hand, tenascin-C (Tn-C) was exclusively detected in the mesenchymal fraction (lane 2). Similar observations were found from epithelial and mesenchymal fractions isolated from the colon (data not shown).

It is pertinent to note that Fn and the Ln α2 chain were both consistently found at a relatively lower level in the epithelium as compared to the mesenchyme in 4 different specimen (Fn: 12% vs 88%, $p < 0.001$; Ln

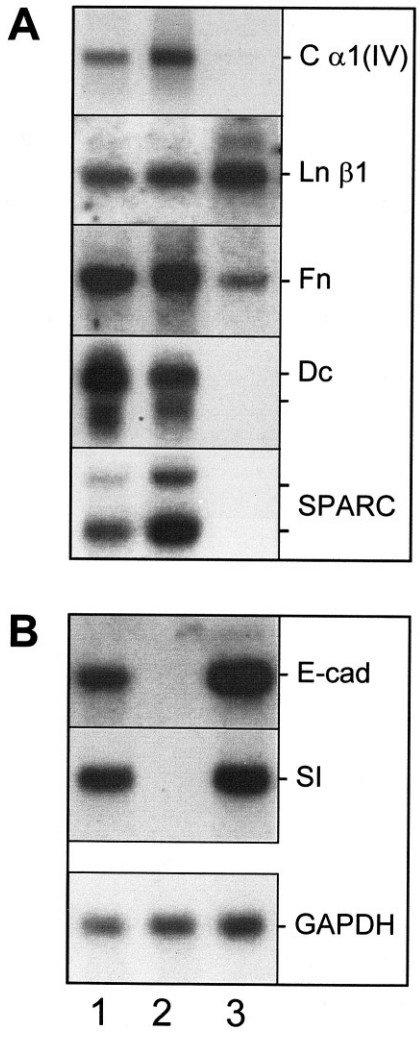

FIG. 1. Representative Northern blot analysis for the detection of transcripts encoding extracellular matrix molecules (A) and markers (B) in the intact fetal small intestine at mid-gestation (lane 1) and in corresponding isolated mesenchyme (lane 2) and epithelial fractions (lane 3). Transcripts analyzed were the α1(IV) collagen chain (C α1(IV)), laminin β1 chain (Ln β1), fibronectin (Fn), decorin (Dc), SPARC/osteonectin (SPARC), E-cadherin (E-cad), sucrase-isomaltase (SI) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

α2: 25% vs 75%, $p < 0.025$). Finally, purity of epithelial and mesenchymal fractions used for these studies was confirmed at the transcript level as determined by the exclusive detection of E-cadherin (E-cad) and sucrase-isomaltase (SI) in the epithelium (Figs. 1 and 2) and the restricted expression of Dc, SPARC and Tn-C in the mesenchyme.

DISCUSSION

This study represents the first attempt to determine the tissular origin of extracellular matrix molecules from the intact human intestine. Up to now, our knowledge of the origin of epithelial basement membrane components in the intestine comes mainly from experiments performed on laboratory animals (17, 33-36). However, it has become evident that results from experimental animals cannot always be directly extrapolated to the human. Indeed, fundamental differences in the regulation of gene expression during intestinal development and along the crypt-villus axis in the adult have been found between man and animal models (20, 37, 38). Differences in the distribution of basement membrane components have been also observed (6-8). For instance, human laminin-1 has been found to be predominantly associated with the differentiated en-

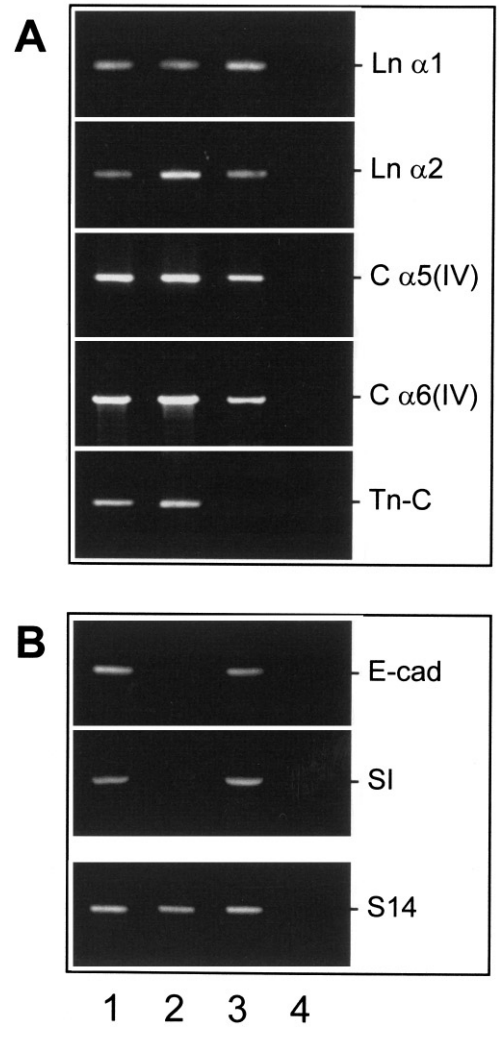

FIG. 2. Representative RT-PCR analysis of transcripts encoding extracellular matrix molecules (A) and markers (B) in the intact fetal small intestine at mid-gestation (lane 1) and in the corresponding isolated mesenchyme (lane 2) and epithelial fractions (lane 3). cDNAs were transcribed from total RNAs and amplified with primer sets specific for laminin α1 (Ln α1) and α2 (Ln α2) chains, collagen α5(IV) (C α5(IV)) and α6(IV) (C α6(IV)) chains, tenascin-C (Tn-C), E-cadherin (E-cad), sucrase-isomaltase (SI) and S14.

TABLE 1

Epithelial and Mesenchymal Contribution to the Expression of Extracellular Matrix Components in Relation to Their Distribution in the Intestinal Mucosa

	Distribution[a]						Expression[b]	
	Villus-EMI	Upper crypt-EMI	Lower crypt-EMI	Stroma	Cellular elements of the LP	Muscularis mucosa	Epithelium	Mesenchyme
C α1(IV)	+	+	+	−	+	+	−	++
C α5(IV)	+	+	+	+	+	+	++	++
C α6(IV)	+	+	+	nd	nd	nd	++	++
Ln α1	+	±	−	−	+	+	++	++
Ln α2	−	±	+	−	−	−	+	++
Ln β1	+	+	+	−	+	+	++	++
Fn	±	+	+	+	+	+	+	++
Dc	−	+	+	+	+	+	−	++
SPARC	−	−	−	−	±	+	−	++
Tn-C	+	±	±	+	+	+	−	++

Note. nd, not detected.

[a] Distribution of extracellular matrix molecules at the epithelial-mesenchymal interface (EMI) along the crypt-villus axis and in mesenchymal elements (stroma, cellular elements of the lamina propria (LP) and muscularis mucosa) summarized from Ref. 7.

[b] Expression of extracellular matrix components determined in isolated intestinal epithelium and corresponding mesenchyme as illustrated in Figs. 1 and 2.

terocytes lining the villus (39) while it has been found to be restricted to the proliferative compartment in rodents (40). Co-culture of human intestinal epithelial cells with fibroblasts has also been used by us and others to analyze epithelial-mesenchymal interactions in vitro (41-43). These systems have provided valuable information on the cellular origin of BM molecules but the use of adenocarcinoma cell lines such as Caco-2 and HT29 as the epithelial component has been, at least theoretically, their main drawback.

In this work, we have described a new procedure to separate the human intestinal epithelium from its underlying mesenchyme based on the use of Matrisperse, a dissociating solution initially designed to recover cells grown on extracellular matrix. This approach has allowed for the first time the possibility to investigate the respective epithelial and mesenchymal contributions to the BM in the normal human intestine. As summarized in Table 1, a number of molecules are localized at the epithelial-mesenchymal interface, namely formal BM components such as the laminins and type IV collagens but also BM-associated molecules such as fibronectin, tenascin-C and decorin (7). The analysis of their tissular origin has revealed interesting findings. First, we have confirmed the exclusive mesenchymal origin of the α1(IV) chain of collagen in the human as previously observed in rodents (17, 34) and in co-culture (43) while the α5(IV) and α6(IV) chains of collagen are of dual epithelial and mesenchymal origin suggesting that intestinal epithelial cells contribute to their BM-collagen network, by producing their own collagen, presumably as a $[\alpha5(IV)]_2\alpha6(IV)$ complex (19). Secondly, concerning laminins, laminin-1 chains (α1, β1 and γ1) have been shown to be of both epithelial and mesenchymal origin in the rat intestine (34, 35). An epithelial origin for laminin-1 was also suggested by its expression in intestinal Caco-2 cells (13, 14). The detection of laminin α1 and β1 chains in both compartments has confirmed the dual origin of laminin-1 in the human intestine. Laminin-2 has a very restricted pattern of expression in the human intestine, being exclusively located at the lower portion of the crypts (39, 44). Interestingly, the α2 chain of laminin-2 was also found in both the epithelial and mesenchymal fractions, suggesting that crypt epithelial cells as well as their pericryptal myofibroblasts contribute to laminin-2 deposition.

Among the BM-associated molecules tested herein, decorin and tenascin-C, which are distributed predominantly at the epithelial-mesenchymal interface in the intact human small intestine (7), were found to be exclusively of mesenchymal origin, thus confirming previous observations performed with human in vitro models (41,43). Interestingly, fibronectin was found to be of dual origin. Fibronectin is expressed at relatively high levels in the developing human small intestine, being distributed throughout the mesenchyme including the epithelial-mesenchymal interface (45; see Table 1). The possibility that intestinal epithelial cells can synthesize fibronectin was first suggested by its identification in the basal lamina of the intact tissue (46). Its expression by undifferentiated intestinal epithelial cells in vitro (47, 48) supported this possibility. Herein, we have shown that the fibronectin transcript is present in epithelial fractions. However, its relatively low amounts as compared to other molecules exhibiting a

dual origin, such as the laminin β1 chain, suggests that the epithelial contribution of fibronectin at the epithelial-mesenchymal interface is rather minor in the intact tissue. Finally, SPARC was exclusively detected in the mesenchyme. SPARC/osteonectin/BM40 has been found to be a BM component in some tissues (49, 50). However, in the human intestine, it is exclusively distributed in the smooth musculature as determined by immunofluorescence (see Table 1) and the expression of its transcript has not been detected in the epithelium (51). SPARC thus served as a mesenchymal marker in conjunction with the epithelial markers SI and E-cad to validate the lack of cross-contamination of the epithelial and mesenchymal preparations.

Taken together, these data support the concept that the intestinal BM is constituted from components produced by both the epithelium and mesenchyme. Based on their origins in the normal intact human intestine as determined herein and their well established distribution at the epithelial-mesenchymal interface (7), it is proposed that epithelial BM α5(IV)/α6(IV) collagen and laminin-1 mainly originate from the epithelium while laminin-2 and Fn are of dual epithelial and mesenchymal origin in contrast to α1(IV)/α2(IV) collagen, Tn-C and Dc which are produced exclusively by the mesenchyme.

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