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Short Contribution

Tenascin may not be required for intestinal villus development

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ABSTRACT Tenascin, a large extracellular matrix protein, is subject to complex spatial and temporal patterns of expression in the course of various organogenetic processes including the formation of the small intestinal villus. In the present study, the presence of tenascin was analyzed in human fetal colonic villi, which are transient structures that are programmed to disappear at the time of colonic mucosal remodeling. While the labeling of muscles and peripheral mesenchyme was similar in both segments, surprisingly the colonic villus cores were mostly devoid of tenascin as opposed to those of the small intestine. Western blot analysis revealed that the 220 and 320 kDa forms of tenascin were detected in both segments. However, the 200 kDa form present in small intestinal villi was absent in colonic villi. These data suggest that tenascin under its 200 kDa form is not required for villus formation.

KEY WORDS: *tenascin, development, small intestine, colon, human*

Tenascin (Tn), a large glycoprotein of the extracellular matrix, is formed of six subunits of 190 to 320 kDa displaying both adhesive and anti-adhesive properties (Chiquet-Ehrismann *et al.*, 1991). Distinctive tissular distribution and transitory expression of Tn have been reported during embryonic development, suggesting a crucial role for this molecule in various morphogenetic processes. Tenascin gene knockout experiments by Saga *et al.* (1992) have cast a doubt on this role by reporting that homozygous mutant mice can develop normally. However, recent findings have provided evidence that tenascin is one member of a family of related proteins, encoded by related genes, and that some of these may be essential for development (Bristow *et al.*, 1993).

We have recently identified a new form of Tn of 200 kDa in the human small intestine (Beaulieu *et al.*, 1993b). Whether this form of Tn results from unusual differential mRNA splicing or protein processing of either Tn-C (the classical form of Tn also termed Cytotactin) or Tn-X (Bristow *et al.*, 1993), or whether it is encoded by an as yet unidentified gene remains to be established. However, biochemical data has shown that this 200 kDa form is predominantly expressed under a strict developmentally-regulated pattern in small intestinal villi (Beaulieu *et al.*, 1993b), suggesting that the molecule is involved in villus formation and/or maintenance. In the present study, as a first step toward investigating the potential role of the 200 kDa form of Tn, we took advantage of a well-known particularity of human colonic development: the transient presence of small intestinal-like villi (Ménard, 1989). The expression of this molecule was studied in developing jejunum and colon by indirect immunofluorescence and Western blot analysis by using a specific polyclonal antiserum.

The expression of Tn in developing jejunum and colon was first determined by indirect immunofluorescence (Fig. 1). At all stages studied (12-20 weeks of gestation), external muscle layers of both small and large intestinal segments were found strongly reactive for Tn immunodetection. Mesenchymes corresponding to the presumptive submucosa were not stained at 12 weeks (Fig. 1A,B). At 19 weeks, the muscularis mucosa which separates the submucosa from the mucosal connective tissue (the lamina propria) is present (Beaulieu *et al.*, 1993a). Submucosa and muscularis mucosa were stained for Tn in the small intestine (Fig. 1C) and the colon (Fig. 1D).

Surprisingly, in the mucosa, Tn expression was found to be strictly segment-dependent. In the small intestine, from the proximal jejunum to the distal ileum, a widespread distribution of Tn in the lamina propria from the tip of the villus to the base of the intervillus area (12-16 weeks, Fig. 1A) or of the crypts (18-20 weeks; Fig. 1C) was observed according to a typical gradient of intensity (Beaulieu *et al.*, 1991, 1993a). In contrast, the proximal and distal colonic lamina propria were mostly devoid of immunoreactive Tn. Although this extracellular matrix protein was detected near the lower crypt epithelium at 19 weeks (Fig. 1D), villus cores were either completely negative or only weakly stained for Tn immunodetection. Indeed, among all samples analyzed for Tn (12-20 weeks), 25 colons were found to bear negative villus

Abbreviations used in this paper: Tn, tenascin; SDS, sodium dodecylsulfate; SDS PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis.

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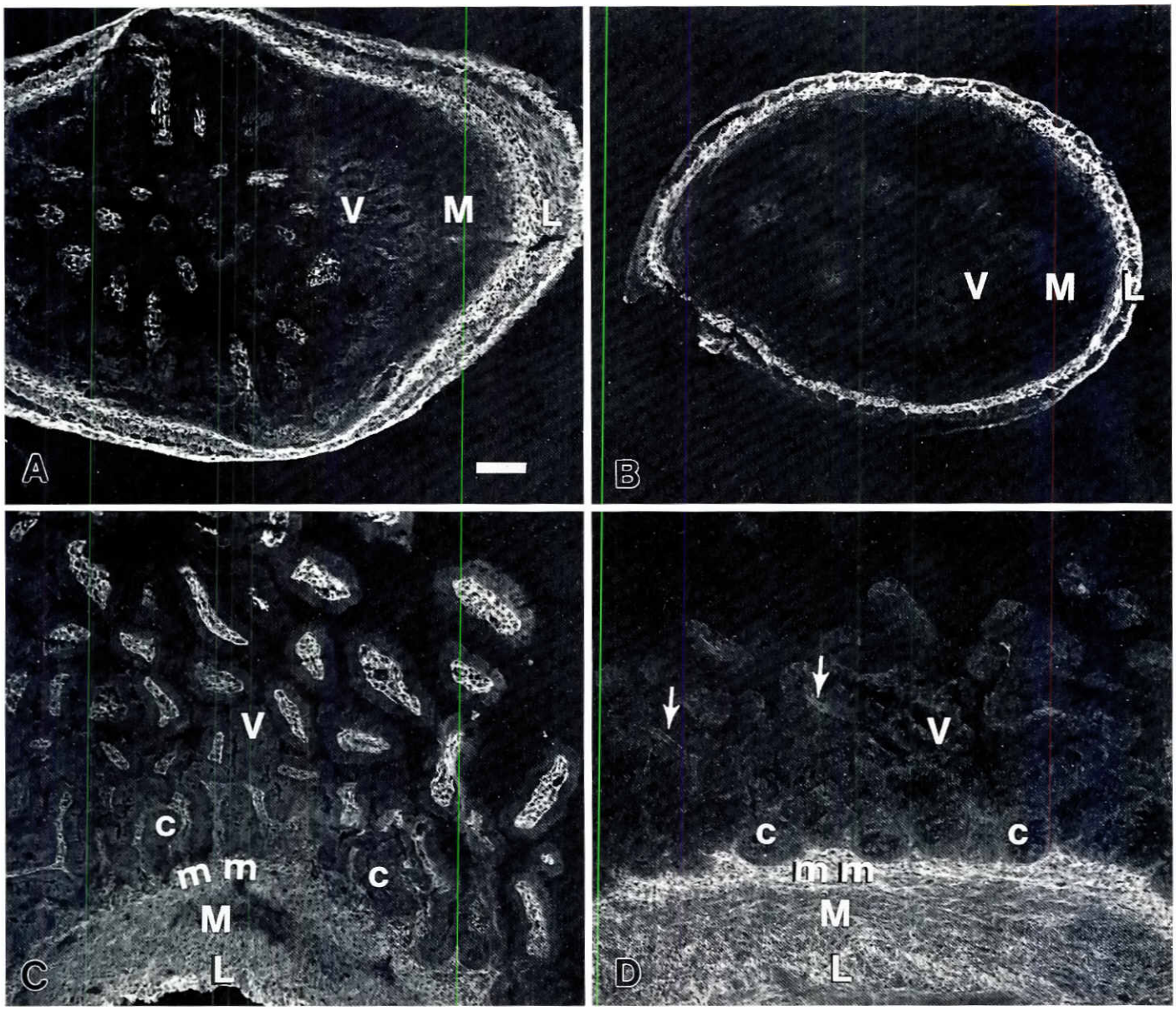

Fig. 1. Distribution of Tn in the human fetal small intestine and colon. *Representative indirect immunofluorescence micrographs of cryosections of jejunum* **(A,C)** *and proximal colon* **(B,D)** *at 12* **(A,B)** *and 19 weeks* **(C,D)** *of gestation stained for the detection of Tn. Staining in the jejunum and the colon was similar in muscle layers (L), mesenchyme (M) and muscularis mucosa (mm). In contrast, villus cores, which show intense staining in the jejunum, were mostly negative in the colon. Arrows in D denote faintly stained colonic villus cores as observed in a limited number of villi in 26% of all specimens studied. V: villi; c: crypts. Bar, 100 μm.*

cores (Fig. 1B), while a very weak staining for Tn was observed in a limited number of villus cores in the remaining 9 specimens (26%), as illustrated in Fig. 1D.

To further investigate the molecular forms of Tn in the colonic mucosa, villi were separated from the mucosa and submucosa of 18-20 week-old fetal colons as well as from the corresponding jejunum as previously described (Beaulieu *et al.*, 1993b) and these fractions were analyzed by Western blot. As shown in Fig. 2, a characteristic band of 200 kDa was identified in intestinal villus fractions prepared from the jejunum (lane 1) but was completely absent in the colonic villus fractions (lane 3), although a faint reactivity in the 180-300 kDa range, also seen in other samples (lanes 1,2 and 4), was observed. This 200 kDa form of Tn in the human small intestine has been found to be predominantly expressed in the villus under a developmentally-regulated pattern (Beaulieu *et al.*, 1993b). For comparison, villus-depleted jejunum and colon tissues were also analyzed in parallel. As expected from immunolocalization studies, the major 220 kDa form of Tn as well as the minor one (320 kDa) present in the small intestine (Beaulieu *et al.*, 1993b) were both detected in the colon.

These observations provide evidence that villi of the small and large intestine differ fundamentally in terms of Tn composition. Considering that the processes of mucosal organogenesis

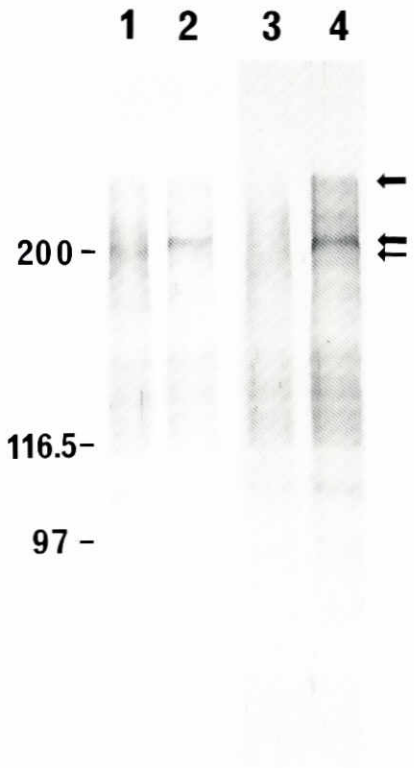

Fig. 2. Tn isoforms in the human fetal small intestine and colon. *Representative Western blot analysis of microdissected jejunum* **(lanes 1,2)** *and colon* **(lanes 3,4)** *for Tn immunodetection. Forms of Tn were analyzed in villus-enriched fractions (100 μg/well;* **lanes 1,3***) and in the corresponding villus-depleted segments (50 μg/well;* **lanes 2,4***). The 200 kDa villus isoform was detected in the jejunum* **(lane 1)** *but not in the colon* **(lane 3)**. *Molecular weight markers are indicated (in thousands) in the left margin.*

are similar, if not identical, in the two segments during the first two trimesters of human fetal development (Ménard, 1989), it is hypothesized that Tn is not required for intestinal villus formation since the molecule was absent or below the level of detection in the majority of colonic villi between 12 and 20 weeks. In support of this hypothesis, it has been established that the formation of villus rudiments precedes Tn appearance in the small intestinal mucosa (Beaulieu *et al.*, 1991, 1993a). Whether the 200 kDa form of Tn in the colon is subject to a rapid degradation or, more likely, to a differential regulation of its expression as observed for various Tn oligomeric forms in different systems (Aufderheide and Ekblom, 1988; Chiquet-Ehrismann *et al.*, 1991) remains an open question.

It is noteworthy that the human colonic villi are transient structures which disappear during the third trimester of fetal life (Ménard, 1989) while in the adult colonic glandular mucosa, Tn is strongly detected at the epithelial-stromal interface (Riedl *et al.*, 1992; Beaulieu, unpublished). The mechanism of mucosal remodeling during late development in the fetal colon is still unknown (Ménard, 1989) but in light of the present observations, it is suggested that Tn in its 200 kDa form could be important for intestinal villus structural stability.

Experimental Procedures

Specimens of intestine (jejunum, proximal and distal colon) from 34 fetuses ranging from 12-20 weeks of age (post-fertilization) without abnormalities were obtained after legal abortion. The project was in accordance with the protocol approved by the Institutional Human Research Review Committee for the use of human material. Only specimens obtained rapidly were used; the overall period required before freezing the tissue after abortion never exceeded 45 min.

The preparation and embedding of human fetal gut for cryosectioning and indirect immunofluorescence staining procedures for Tn detection were performed as previously described (Beaulieu *et al.*, 1991). The rabbit-specific antiserum directed to human Tn (used at a 1:200 dilution) has been well characterized (Beaulieu *et al.*, 1993a,b) and was obtained from Chemicon International (El Segundo, CA, USA).

Western blot analysis of 4 distinct 18-20 week fetal specimens (jejunums and colons) for the detection of Tn in villus-enriched fractions was done according to a procedure described previously (Beaulieu *et al.*, 1993b). Briefly, fractions enriched in villi were prepared from jejunums and corresponding colons by microdissection at 4°C. Total protein from villus fractions and remaining gut wall homogenates (in 20 mM Tris-HCl, pH 6.8 containing 0.1 mM phenylmethylsulfonyl fluoride, 50 μg/ml leupeptine, 50 μg/ml antipain, 100 μg/ml aprotinin and 1M ultrapure urea) were rapidly processed for solubilization in 2X sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 2% ß-mercaptoethanol, 20% glycerol and 0.08% bromophenol blue) at 90°C for 10 min. Proteins separated by SDS PAGE were transferred to nitrocellulose and Tn forms were detected with the same anti-human Tn as above at a 1:400 dilution.

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