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Expression of J1/tenascin in the crypt–villus unit of adult mouse small intestine: implications for its role in epithelial cell shedding

RAINER PROBSTMEIER*, RUDOLF MARTINI and MELITTA SCHACHNER

Department of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364, 69 Heidelberg, Fed. Rep. Germany

* For correspondence

Summary

The localization of the extracellular matrix recognition molecule J1/tenascin was investigated in the crypt–villus unit of the adult mouse ileum by immunoelectron microscopic techniques. In the villus region, J1/tenascin was detected strongly in the extracellular matrix (ECM) between fibroblasts of the lamina propria. It was generally absent in the ECM at the interface between subepithelial fibroblasts and intestinal epithelium, except for some restricted areas along the epithelial basal lamina of villi, but not of crypts. These restricted areas corresponded approximately to the basal part of one epithelial cell. In J1/tenascin-positive areas, epithelial cells contacted the basal lamina with numerous microvillus-like processes, whereas in J1/tenascin-negative areas the basal surface membranes of epithelial cells contacted their basal lamina in a smooth and continuous apposition. In order to characterize the functional role of J1/tenascin in the interaction between epithelial cells and ECM, the intestinal epithelial cell line HT-29 was tested for its ability to adhere to different ECM components. Cells adhered to substratum-immobilized fibronectin, laminin and collagen types I to IV, but not to J1/tenascin. When laminin or collagen types I to IV were mixed with J1/tenascin, cell adhesion was as effective as without J1/tenascin. However, adhesion was completely abolished when cells were offered a mixture of fibronectin and J1/tenascin as substratum. The ability of J1/tenascin to reduce the adhesion of intestinal epithelial cells to their fibronectin-containing basal lamina suggests that J1/tenascin may be involved in the process of physiological cell shedding from the villus.

Abbreviations: BSA; bovine serum albumin. DMEM; Dulbecco's modified Eagle's medium. ECM; extracellular matrix. EDTA; ethylenediaminetetra-acetic acid. HBSS; Hank's balanced salt solution. PBS; phosphate-buffered saline. TBS; Tris-(hydroxymethyl)-aminomethane buffered saline.

Key words: epithelium, HT-29 cell line, J1/tenascin, small intestine.

Introduction

In the crypt–villus unit of the small intestine of adult vertebrates, epithelial cells are continuously generated from stem cells near the crypt base, migrate towards the villus tip, and are finally extruded into the intestinal lumen (Cheng and Leblond, 1974). To our knowledge, no detailed information is available on the molecular basis of this shedding process. It has been suggested by Quaroni and Trelstad (1980) that several types of collagen, synthesized in submaximally hydroxylated form by intestinal epithelial cells, could influence and decrease the binding of these cells to their basal lamina. However, no experimental systems have been developed to test this hypothesis.

We have shown previously by immunohistology at the light microscopic level that J1/tenascin is expressed at the boundary between epithelial cells and lamina propria in an increasing gradient towards the villus tip (Thor *et al.* 1987; Probstmeier *et al.* submitted for publication). This expression pattern led us to hypothesize that J1/tenascin may be involved in the physiological process of epithelial cell shedding. Here we present data that strongly suggest that J1/tenascin plays such a functional role. To this end, we have investigated the expression of J1/tenascin in the crypt–villus unit by immunoelectron microscopy and developed a cell–substratum adhesion assay to determine the adhesion of the intestinal epithelial cell line HT-29 to several ECM components in the presence or absence of J1/tenascin.

Materials and methods

Animals

C57BL/6J and NMRI mice were maintained at the departmental animal facility.

ECM molecules

Fibronectin was prepared from human plasma according to method A described by Miekka *et al.* (1982). Laminin was isolated from the EHS mouse tumor (Timpl *et al.* 1979). Isolation of J1/tenascin from cultures of embryonic mouse fibroblasts has been described (Faissner *et al.* 1988; Probstmeier *et al.* 1990). Collagen type I was extracted from rat skin (Bornstein and Piez, 1966); type II from chick cartilage (von der Mark *et al.* 1982); type III from fetal calf skin (Timpl *et al.* 1981) and type IV from EHS mouse tumor or human placenta after mild pepsin treatment, whereby the carboxyterminal NC1 domain was destroyed (Timpl *et al.* 1981).

Antibodies

The following antibodies were used: polyclonal antibodies to laminin and fibronectin (Pesheva *et al.* 1988, 1989; a kind gift of P. Pesheva) and polyclonal antibodies to J1/tenascin from early postnatal mouse brain (Faissner *et al.* 1988; a kind gift of A. Faissner).

Cell cultures and cell–substratum adhesion assay

The epithelial cell line HT-29 derived from a human colon adenocarcinoma (Fogh and Trempe, 1975) was obtained from E. Coudrier (Unit de Biologie des Membranes, Institut Pasteur, Paris, France). In the presence of high amounts of glucose in the culture medium, HT-29 cells are in a morphologically and biochemically undifferentiated state. When glucose is replaced by galactose, they differentiate into cells with enterocytic phenotype (Pinto *et al.* 1982; Chastre *et al.* 1985; Zweibaum *et al.* 1985). HT-29 cells were grown in glucose- or galactose-containing DMEM as described (Huet *et al.* 1987). Glucose-containing DMEM was supplemented with 10% fetal calf serum (dialyzed for three days at 4°C against 0.9% NaCl), 20 mM glucose (in addition to the 5 mM already present in DMEM), 10 $\mu g\,ml^{-1}$ human transferrin, 2 mM glutamine, penicillin (100 i.u. ml^{-1}) and streptomycin (100 $\mu g\,ml^{-1}$). Galactose-containing DMEM (glucose- and pyruvate-free, from Serva, Heidelberg, FRG) was supplemented with 5 mM galactose. For the adhesion assay, monolayer cultures were treated for 5 min at room temperature with trypsin/EDTA (0.05%/0.02%; w/v) in Ca^{2+}- and Mg^{2+}-free HBSS. The cells were then collected by centrifugation, washed in Ca^{2+}- and Mg^{2+}-free HBSS and resuspended in glucose- or galactose-containing DMEM.

Fibronectin, laminin and collagen types I to IV (at a final concentration of 50 $\mu g\,ml^{-1}$ in PBS) were mixed with J1/tenascin or heat-inactivated BSA (30 to 65 $\mu g\,ml^{-1}$ in PBS), spotted (4 μl per spot) in plastic Petri dishes (5 cm in diameter, tissue culture grade, from NUNC) and incubated for 1.5 h at 37°C in a humid atmosphere. Petri dishes were washed once with PBS containing 10% heat-inactivated BSA, blocked with the same solution for 1 h at 37°C, and washed three times with glucose- or galactose-containing DMEM. Single cell suspensions of glucose- or galactose-adapted HT29 cells were applied (4×10^6 cells in 4 ml of the corresponding culture medium) and maintained for 5 h at 37°C. Dishes were washed three times with the corresponding culture medium and cells adhering to the ECM components were monitored microscopically.

Determination of coating efficiency by the immunodot binding assay

Coating efficiencies to the plastic surface of Petri dishes were measured for the ECM component by the immunodot binding assay. Petri dishes coated with ECM components as described for the cell–substratum adhesion assay were washed three times with TBS containing 0.1% BSA (TBS/BSA) and incubated overnight at 4°C with polyclonal rabbit antibodies to laminin, fibronectin or J1/tenascin diluted in TBS/BSA. Dishes were washed three times with TBS/BSA, incubated for 1 h at room temperature with horseradish peroxidase-coupled antibody to rabbit immunoglobulin, and washed three times in TBS/BSA. Bound antibodies were visualized as described (Faissner *et al.* 1984). Coating efficiencies for fibronectin or laminin were not influenced by the presence of J1/tenascin. Furthermore, coating efficiencies for J1/tenascin were the same in mixtures with fibronectin, laminin or collagen types I to IV.

Immunocytology

1. Immunofluorescence

Adult mice were anesthetized by intraperitoneal injection of an aqueous solution of chloralhydrate (3%, 0.01 $ml\,g^{-1}$ body weight) and perfused with 4% formaldehyde in PBS. Ilea were then removed, cut into pieces, 3 mm in length, and post-fixed for 2 h at 4°C. Tissue pieces were washed consecutively for 1 h in PBS, for 24 h in 10% sucrose in PBS containing 0.01% NaN_3, and for 48 h in 20% sucrose in PBS containing 0.01% NaN_3, all at 4°C. Tissue pieces were then embedded in OCT (Jung, Nussloch) and shock-frozen in 2-methylbutane. Sections, 10 μm thick, were prepared in a Frigocut 2700 (Jung, Nussloch), mounted on poly-L-lysine-coated glass coverslips, and dried for 60 min at room temperature. Sections were then incubated for 15 min at room temperature in blocking buffer (PBS containing 1% horse serum and 0.1% BSA, pH 7.4), washed once for 5 min in PBS containing 0.1% BSA, incubated for 20 min with polyclonal antibodies to J1/tenascin (diluted 1:100 in blocking buffer), washed three times for 5 min in PBS containing 0.1% BSA, incubated with FITC-conjugated antibodies to rabbit IgG (diluted 1:100 in blocking buffer), washed again three times, and embedded in glycerol/PBS (1:1, v/v).

2. Immunoelectron microscopy

Mice were perfused with fixative (4% formaldehyde, 0.25% glutaraldehyde in Palay's buffer; Palay and Chan-Palay, 1974) as described (Martini and Schachner, 1986). Ilea were removed, dissected into pieces, 3 mm in length, and post-fixed in 4% formaldehyde for 12 h at 4°C. The tissue was processed, incubated with antibodies and prepared for immunoelectron microscopy as described (Martini and Schachner, 1986).

Results

Immunohistological localization of J1/tenascin in the adult mouse ileum

1. Light microscopy

The localization of J1/tenascin was investigated in crypts and villi of cross-sections of the ileum (Fig. 1) by indirect immunofluorescence. J1/tenascin was strongly detectable in the lamina propria of villi, particularly at the interface between mesenchyme and epithelium (arrows in Fig. 2A,B). In crypts J1/tenascin was also accumulated at the epithelium–mesenchyme interface, but its expression was considerably weaker when compared to the villus region (Fig. 2C, D).

2. Electron microscopy

To investigate in more detail which structures express

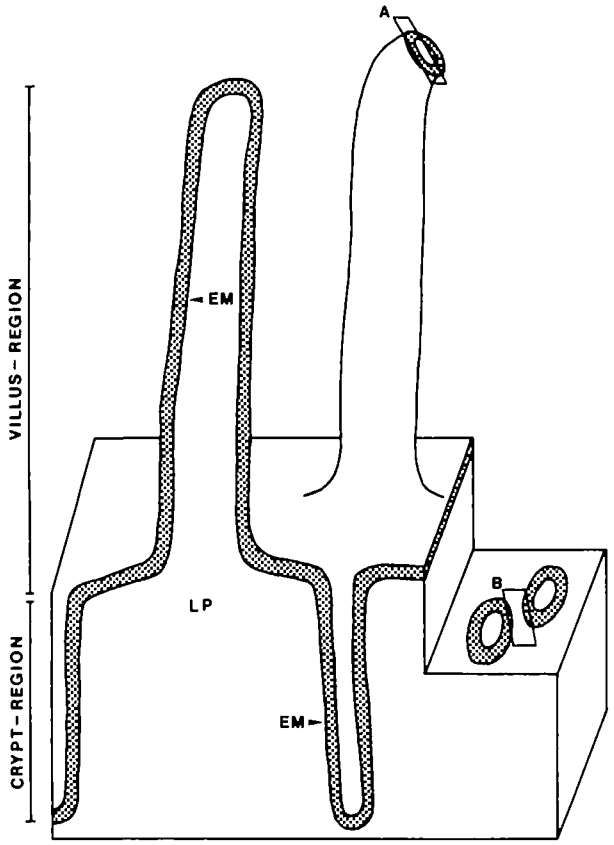

Fig. 1. Schematic diagram of the crypt–villus axis in the adult mouse small intestine. In cross-sectioned villi (A) the ring-like epithelium is surrounded by the intestinal lumen and contains in its core the lamina propria. In cross-sectioned crypts (B), the ring-like epithelium is surrounded by the lamina propria and contains in its core the intestinal lumen. The rectangles A and B indicate the areas from which immunoelectron micrographs are shown in Figs 3A and B. The intestinal epithelial sheath is marked by dots. EM, epithelium-mesenchyme interface; LP, lamina propria.

J1/tenascin in the crypt–villus axis of the adult mouse ileum, an immunoelectron microscopic characterization was carried out using pre-embedding staining procedures. Representative areas of cross-sectioned crypt- and villi-regions were cut as indicated in the diagram of Fig. 1 and are shown in Fig. 3.

J1/tenascin was scarcely detectable in the ECM of the lamina propria in the villus core. Also in the crypt region, J1/tenascin was only weakly detectable in the lamina propria. Only in the vicinity of small nerve fibers were glial cells surrounded by strongly J1/tenascin-positive ECM (Fig. 3A, inset). J1/tenascin was also strongly expressed in the villus region, in association with ECM surrounding subepithelial fibroblasts of the lamina propria (Fig. 3A). J1/tenascin was specifically localized, in that it was strongly detectable in the ECM between neighbouring fibroblasts, but was generally absent in the basal lamina at the interface between fibroblasts and epithelium. This polarized expression was most clearly seen when thin processes of subepithelial fibroblasts were oriented in parallel to the basal lamina of the epithelium, since, here, J1/tenascin-negative basal lamina was in close vicinity to J1/tenascin-positive areas separated only by slender fibroblastic processes (Fig. 3A, asterisks). However, in some confined areas (approximately 3 to 5 μm in length), J1/tenascin was detectable also in the basal lamina of the epithelial cell–fibroblast interface (Fig. 3A, large arrowheads). J1/tenascin-positive zones were separated from each other by a stretch of 5 to 50 μm of J1/tenascin-free basal lamina. In approximately 80 % of the J1/tenascin-positive stretches, the basal parts of the epithelial cells differed in their morphology from those apposed to J1/tenascin-negative basal lamina. Basal parts of epithelial cells touching J1/tenascin-negative basal lamina made smooth, uninterrupted and non-convoluted contacts with the basal lamina (Fig. 3A, epithelial cell e). In contrast, basal parts of epithelial cells apposed to J1/tenascin-positive basal lamina formed numerous microvillus-like processes (Fig. 3A; epithelial cell e').

In contrast to the villus region, no J1/tenascin-positivity was found in the crypt region associated with the ECM in the basal lamina at the basal parts of epithelial cells (Fig. 3B). Basal parts of epithelial crypt cells always engaged in smooth and uninterrupted apposition to the basal lamina, never showing microvillus-like protrusions (Fig. 3B).

Substratum adhesion of epithelial cells in the absence or presence of J1/tenascin

Since expression of J1/tenascin in the epithelial basal lamina was correlated with an apparently disturbed contact between epithelial cells and basal lamina, a functional assay system was used to investigate adhesion of intestinal epithelial cells to different ECM components in the absence or presence of J1/tenascin. In initial experiments, we used freshly isolated crypt and villus cells from adult mouse small intestine which can be separately obtained by incubation of intestinal tubes with an EDTA-containing buffer for different time periods (Weiser, 1973; Thor *et al.* 1987). Unfortunately, these cells did not adhere to any of the substrata offered in our adhesion assay (collagen types I to IV, laminin and fibronectin), probably due to the limited viability of the cells over time periods of several hours (for references, see Quaroni and May, 1980). Thus, while these cells could be successsfully used for cell aggregation assays of less than one hour (Thor *et al.* 1987), they could not be taken for cell–substratum adhesion assays with duration of five hours. Fortunately, an epithelial cell line (HT-29) is available which can be obtained in morphologically undifferentiated and differentiated states. In glucose-containing medium, this cell line exhibits predominantly the features of undifferentiated epithelial cells (HT-29 glu cells), while in galactose-containing medium it differentiates into cells with properties of villus epithelial cells (HT-29 gal cells) (Zweibaum *et al.* 1985).

When fibronectin, laminin, collagen types I to IV, or J1/tenascin were offered as substratum for cell attachment (50 $\mu g\,ml^{-1}$ in the solution used for coating), HT-

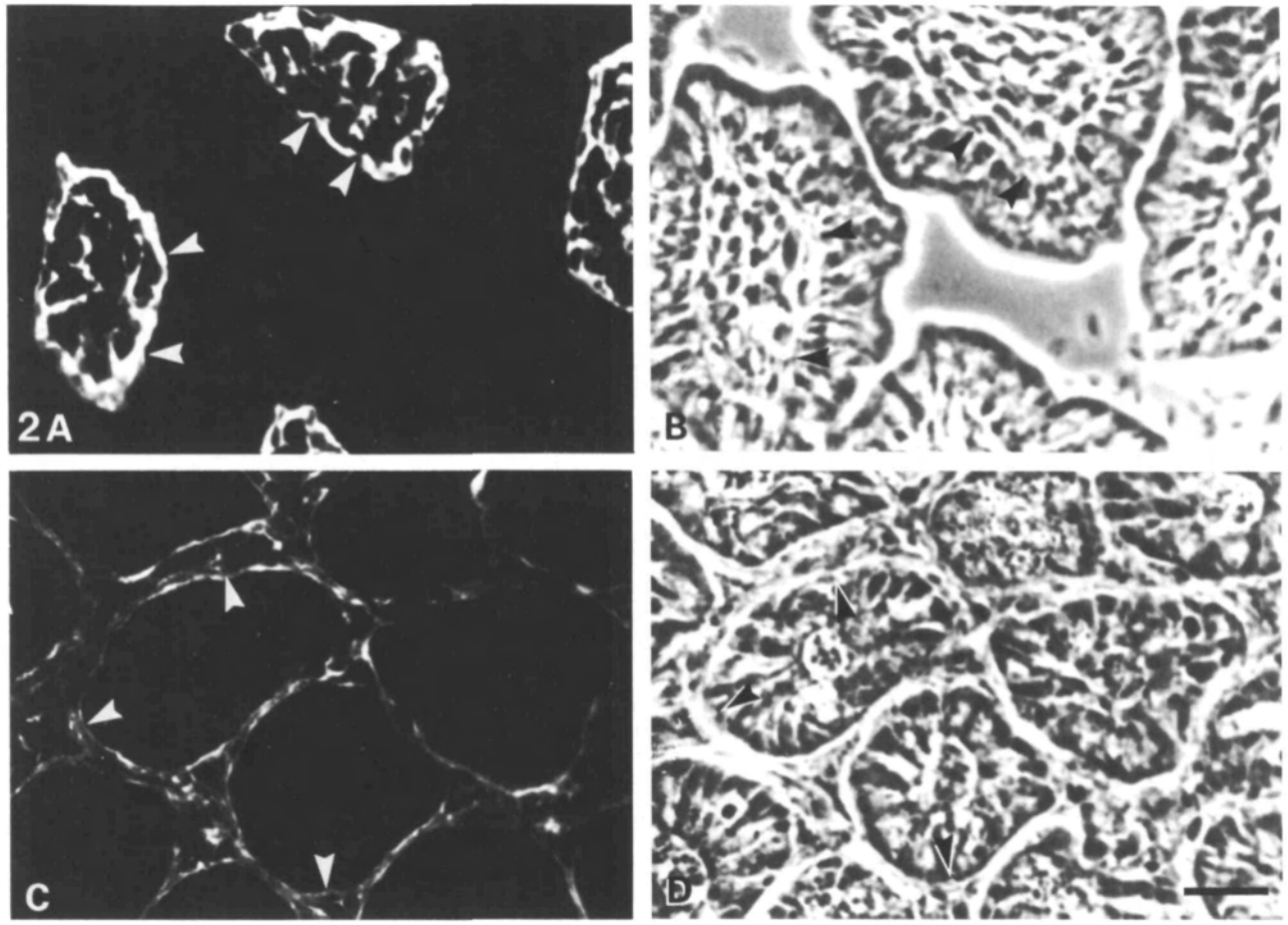

Fig. 2. Immunohistological localization of J1/tenascin in cross-sections of the villus (A, B) and crypt (C, D) regions of adult mouse ileum by indirect imunofluorescence. Arrows indicate the interface between epithelium and mesenchyme. B and D are the corresponding phase contrast micrographs to fluorescence images A and C, respectively. Bar in D represents 25 μm (A–D).

29 glu cells adhered approximately equally well to laminin, fibronectin and collagen types I to IV, but not to J1/tenascin (Fig. 4A,C,E,H and Table 1). When mixtures of J1/tenascin (coating concentration 65 $\mu g\,ml^{-1}$) with fibronectin, laminin or collagen types I to IV (coating concentration 50 $\mu g\,ml^{-1}$) were offered as substratum, adhesion of HT-29 glu cells to the mixture of fibronectin with J1/tenascin was completely abolished (Fig. 4G), while substrata containing the mixtures of J1/tenascin with laminin or collagen types I to IV did not affect cell adhesion (Figs 4B,D and Table 1). In the latter combinations, binding was comparable to that of mixtures where J1/tenascin was replaced by heat-inactivated BSA. The inhibitory effect of the mixture of J1/tenascin and fibronectin on the adhesion of HT-29 cells was J1/tenascin-concentration-dependent, in that adhesion of HT-29 cells to the mixture was increased with a lower concentration of J1/tenascin (coating concentration 30 $\mu g\,ml^{-1}$; compare Fig. 4F and G). No differences in adhesion were observed between HT-29 glu or HT-29 gal cells (Fig. 4I to L and compare to A to H), except that HT-29 gal cells bound less specifically to heat-inactivated BSA than HT-29 glu cells.

When the RGDS-peptide was added at a concentration of 100 $\mu g\,ml^{-1}$ to the culture medium prior to plating of HT-29 glu or gal cells, adhesion to fibronectin, but not to laminin or collagen types I to IV, was reduced (Table 1), indicating that HT-29 cells interact with fibronectin predominantly *via* a RGD-dependent fibronectin receptor.

Results obtained in the cell–substratum adhesion assays are summarized in Table 1.

Discussion

Proteins and glycoproteins of the ECM have been mainly investigated for their role as adhesive cell substrata. It has recently been shown, however, that J1/tenascin is a poor substratum for fibroblasts and, when mixed with fibronectin, inhibits fibronectin-dependent cell spreading, while attachment of fibroblasts is not altered (Chiquet-Ehrismann *et al.* 1988). These observations together with previous ones from our laboratory, showing, by immunohistology at the light microscopic level, an increasing gradient of J1/tenascin expression from crypt base to villus tip (Thor *et al.* 1987;

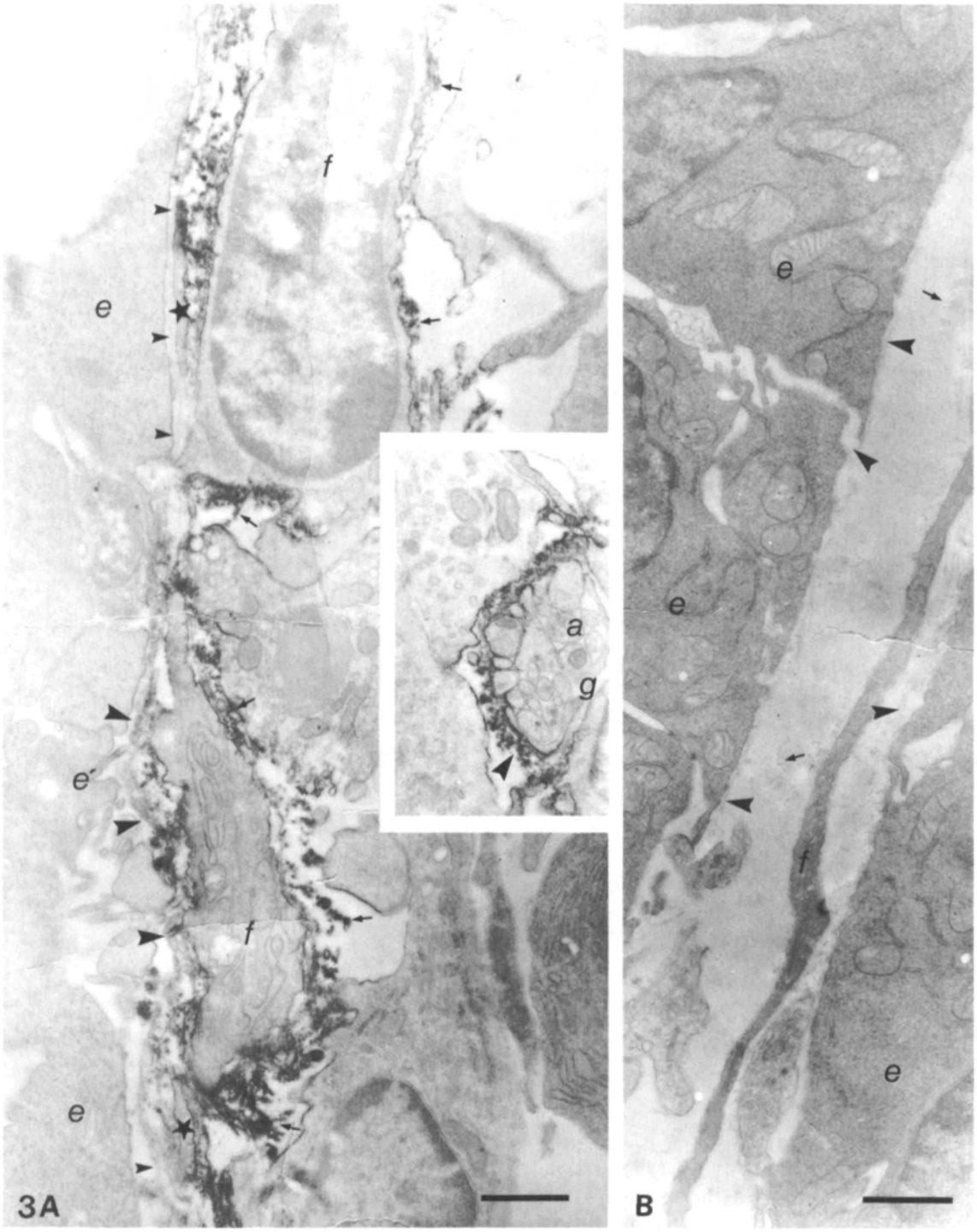

Fig. 3. Immunoelectron microscopic localization of J1/tenascin in the villus (A) and crypt (B) regions of the adult mouse ileum. Immunoelectron microscopy was carried out by pre-embedding staining procedures using polyclonal antibodies to J1/tenascin and protein A coupled to horseradish peroxidase. (A) J1/tenascin immunoreactivity is present in the ECM around fibroblast-like cells of the lamina propria of the upper villus region (arrows). Note that only a small portion of the basal lamina of the epithelium is J1/tenascin-positive (large arrowheads). In this region, the epithelial cell (e') forms microvillus-like processes touching the basal lamina. Asterisks mark slender processes of fibroblasts which are in contact with J1/tenascin-positive ECM on one side and with the J1/tenascin-negative basal lamina on the other (small arrowheads). Epithelial cell (e); subepithelial fibroblasts of the lamina propria (f). Inset: Small nerve in the lamina propria of the villus region. Note strong immunoreactivity associated with ECM around the glial cell processes of the nerve (arrowhead). Axon (a); glial cell (g). (B) In the crypt region, subepithelial basal laminae (arrowheads) and collagen fibrils (arrows) do not show detectable levels of J1/tenascin immunoreactivity. Crypts were cut in cross-section, so that the basal parts of epithelial cells (e) of neighbouring crypts are close to each other. Subepithelial fibroblasts of the lamina propria (f). Bars: 0.5 μm.

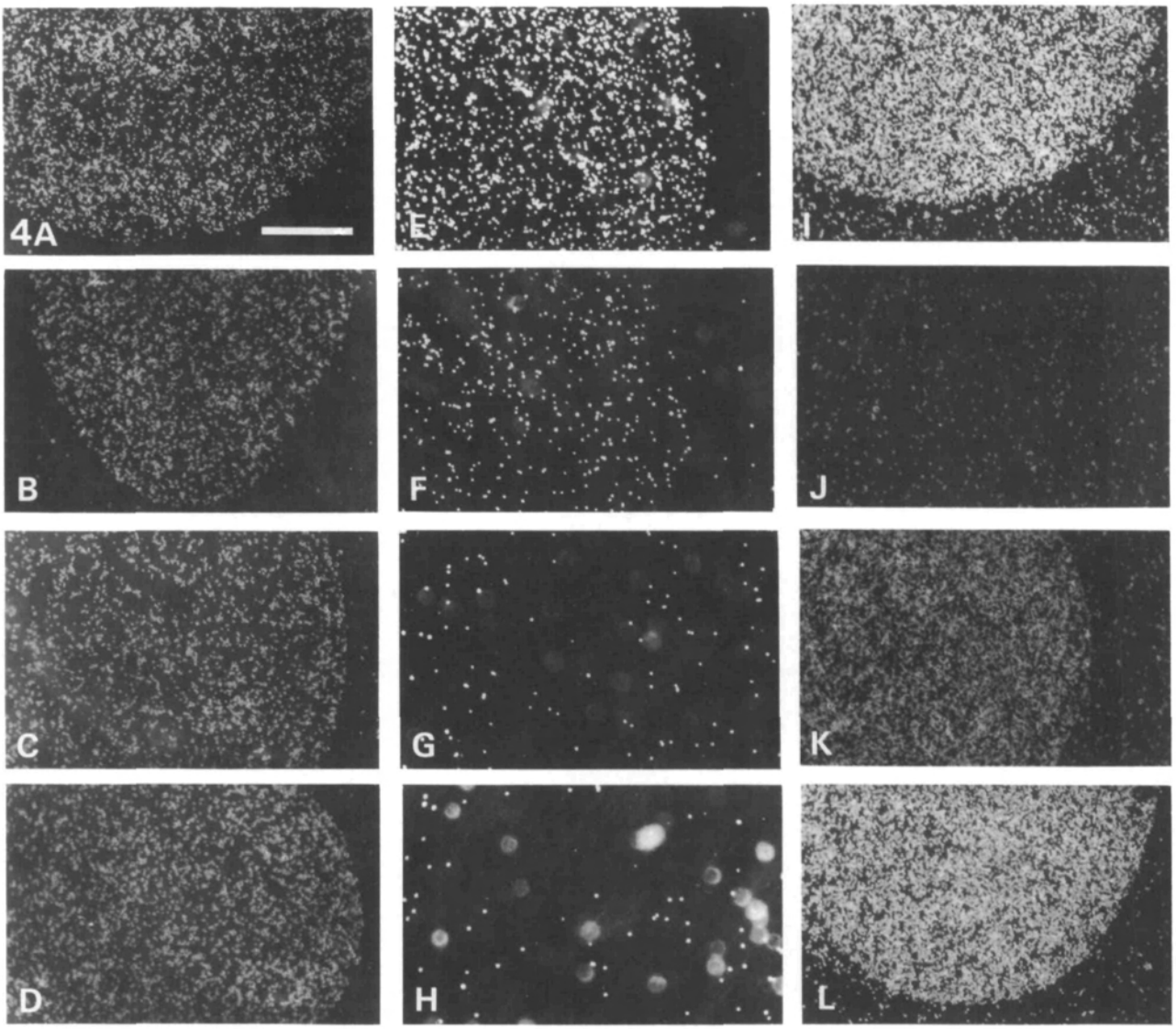

Fig. 4. Adhesion of HT-29 cells grown in glucose- (A–H) or galactose-containing medium (I–L) to different ECM components and their mixtures with J1/tenascin (J1) coated on plastic. (A) laminin plus BSA; (B,K) laminin plus J1; (C) collagen type III plus BSA; (D,L) collagen type III plus J1; (E,I) fibronectin plus BSA; (F) fibronectin plus J1 (30 $\mu g\,ml^{-1}$); (G,J) fibronectin plus J1; (H) J1. If not indicated otherwise, the concentrations used for coating were: 50 $\mu g\,ml^{-1}$ for laminin, fibronectin, and collagen type III and 65 $\mu g\,ml^{-1}$ for heat-inactivated BSA and J1/tenascin. Bar in A represents 500 μm (A–L).

Probstmeier *et al.* submitted for publication), led us to study the expression of J1/tenascin at the mesenchymal–epithelial interface at the electron microscopic level. In addition, we have analysed the substratum properties of J1/tenascin for intestinal epithelial cells.

Our study has shown that intestinal epithelial HT-29 cells adhere well to laminin, fibronectin and collagen types I to IV, but not to J1/tenascin. In mixtures with these ECM components, J1/tenascin inhibited the attachment of HT-29 cells to fibronectin, while the presence of J1/tenascin in mixtures with laminin and collagen types I to IV substrata did not influence their adhesive properties. These observations can be interpreted in several ways. On the one hand, there may be no cell surface receptor for J1/tenascin and J1/tenascin may block the RGDS-dependent or other cell binding sites for HT-29 cells on fibronectin, while it does not interfere with the cell binding site on laminin or collagen types I to IV. On the other hand, there may be a cell surface receptor that allows recognition between the cell surface and J1/tenascin, but results in repulsion of cells. In conjunction with fibronectin, J1/tenascin might thus interfere with HT-29 cell binding by allowing interaction of fibronectin with its cell surface receptor, but annihilating fibronectin-mediated adhesion. In this scheme, the J1/tenascin receptor would not annihilate laminin- or collagen-mediated adhesion. These differential responses may result from the balance of opposing intracellular consequences of recognition between cell surface and substratum. In combination with laminin or different types of collagen, the J1/tenascin receptor could trigger intracellular

Table 1. *Binding of HT-29 cells to J1/tenascin or to mixtures of J1/tenascin with other extracellular matrix components*

Protein(s) used as substratum		Adhesion of HT-29 cells +RGDS*	Adhesion of HT-29 cells −RGDS
Collagen type I	Plus BSA	464±113	503±38
	Plus J1	471±48	493±59
Collagen type II	Plus BSA	625±42	584±37
	Plus J1	610±57	580±67
Collagen type III	Plus BSA	337±50	376±78
	Plus J1	345±20	298±61
Collagen type IV	Plus BSA	603±32	717±68
	Plus J1	710±109	736±73
Laminin	Plus BSA	513±48	471±97
	Plus J1	476±114	425±165
Fibronectin	Plus BSA	78±16	329±49
	Plus J1	n.d.	18±8
J1/tenascin		n.d.	24±16
BSA		18±10	21±13

Proteins were immobilized on plastic and offered as substratum for HT-29 glu cells. Protein concentrations used for coating are indicated in the legend to Fig. 4. For estimation of the number of cells adhering to the different substrata, cells in micrographs of substratum spots were counted in microscopic fields corresponding to 500 μm^2. Values represent mean values±standard deviation of adherent cells in 4 to 6 different microscopic fields from 3 independent experiments. Comparable results were obtained with HT-29 cells grown in glucose- or galactose-containing medium. n.d., not done.

*RGDS was present in the culture medium at a concentration of 100 $\mu g\ ml^{-1}$.

signals that would not override the adhesion-promoting signals triggered by laminin or the collagens. However, when the J1/tenascin receptor would be activated in conjunction with the fibronectin receptor, their additive effects would result in cell repulsion. This repulsive action of J1/tenascin could be mediated by an integrin-independent cell surface receptor, but may possibly also act by triggering the fibronectin-dependent integrin receptor; that is J1/tenascin would attach to a domain different from the RGDS-dependent fibronectin-binding domain. That such a mechanism might exist stems from the report that the hexabrachion complex, identical to J1/tenascin, binds to an integrin-like molecule (Bourdon and Ruoslahti, 1989). However, hexabrachion binding to the integrin-like receptor could be shown to be RGDS-dependent, thus contradicting previous reports on the RGDS-independence of tenascin action (Chiquet-Ehrismann *et al.* 1988). Since tenascin has been shown to bind directly to fibronectin, but not to laminin or the RGDS peptide (Chiquet-Ehrismann *et al.* 1988), it is also conceivable that the J1/tenascin–fibronectin complex induces allosteric changes in fibronectin leading to an inability of fibronectin for cell binding or to a modification of the action on the fibronectin receptor itself. Functional connections between adhesion molecules and the cytoskeleton or intracellular second messenger systems have been described (Horwitz *et al.* 1986; Pollerberg *et al.* 1986, 1987; Schuch *et al.* 1989). The intracellular consequences of such ligand–receptor interactions could vary in different cell types, preventing the formulation of a general scheme about influences of J1/tenascin on the adhesivity of cells to different substrata. In fact, it has been shown that cells that adhere to J1/tenascin, such as epithelial, mesenchymal and glial cells (Bourdon and Ruoslahti, 1989; Chiquet-Ehrismann *et al.* 1988), can be distinguished from cells that do not, such as melanoma, F9 embryonic carcinoma (Bourdon and Ruoslahti, 1989) and HT-29 cells. The combined observations urge for a detailed analysis of the molecular mechanisms involved in recognition and adhesion on one hand and recognition and de-adhesion or repulsion on the other.

The question now is how does the de-adhesive complex of J1/tenascin with fibronectin affect the function of the crypt–villus unit? It is known that intestinal epithelial cells express fibronectin receptors (Chen *et al.* 1985) and that fibronectin and J1/tenascin display inverse gradients in immunofluorescence intensities from the villus bottom to the villus tip, with J1/tenascin being increased and fibronectin being decreased towards the villus tip (Thor *et al.* 1987; Probstmeier *et al.* submitted for publication). Furthermore, both molecules appear to be coexpressed in the basal lamina between epithelium and mesenchyme (for fibronectin, see Laurie *et al.* 1982). The immunoelectron microscopic study presented in this paper shows a locally restricted expression of J1/tenascin in the basal lamina of epithelial villus cells. There was a rough, more convoluted appearance of the surface membrane of the basal parts of some villus cells towards the J1/tenascin-positive basal lamina. It is possible that this intermittent contact between basal surface membrane and basal lamina represents de-adhesion, thus possibly implicating these J1/tenascin-associated cells in the shedding process. This would mean that epithelial cells are not shed from the villus in clusters, but as individual cells by their loss of contact with the basal lamina. Furthermore, the shedding process may be promoted by the absence of adhesion molecules at lateral cell contact sites, such as the cell adhesion molecule L1, which is expressed by the proliferating epithelial cells in the crypt region, but is no more detectable on epithelial cells in the villus region (Thor *et al.* 1987; Probstmeier *et al.* submitted for publication). Thus, an interplay between the absence of adhesive forces contributed, for instance, by L1, and the loss of adhesive forces contributed by repulsive molecules, such as the fibronectin–J1/tenascin pair, could result in the overall shedding process. It has been observed elsewhere that nidogen and laminin is another such pair in that nidogen can interfere with cell binding to laminin (Aumailley *et al.* 1987).

At present, it is not known how the gradient of J1/tenascin expression is formed along the crypt–villus axis and how only discrete patches in the basal lamina become J1/tenascin-positive. It is likely that J1/tenascin is synthesized by intestinal mesenchymal cells, since the intestinal mesenchyme has been shown to be induced by intestinal epithelium to synthesize J1/tenascin, while no J1/tenascin synthesis was detected in epithelial cells alone (Aufderheide and Ekblom, 1988).

In agreement, in intestinal explant cultures (Kondo *et al.* 1984), J1/tenascin was observed exclusively in association with mesenchymal or glial cells, but never with epithelial cells (Probstmeier, unpublished observations). The mesenchymal fibroblasts underlying the epithelial basal lamina in the small intestine form a cell layer that migrates from the crypt bottom to the villus tip (Marsh and Trier, 1974*a*). These fibroblasts proliferate in the crypt region and cease to divide in the villus region (Marsh and Trier, 1974*b*). It is thus possible that the cessation of proliferation of these cells is one of the factors that contributes to a more stochastic expression of J1/tenascin by these cells. However, Gatchalian and coworkers (1989) have shown that fibroblasts derived from perisynaptic and non-synaptic regions of denervated muscle express J1/tenascin when proliferating. Thus, it is probably not a general phenomenon that expression of high amounts of J1/tenascin is correlated with and/or implicated in cell proliferation. These observations, together with others (Martini *et al.* 1990; Steindler *et al.* 1989*a*,*b*; Stern *et al.* 1989), suggest that the regulatory events underlying the expression of J1/tenascin and its isoforms may be many fold and depend on the cell type (Probstmeier *et al.* 1990), as has been suggested for the intracellular signalling pathways that are triggered in response to cell recognition.

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