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Characterization of the cell adhesion molecules L1, N-CAM and J1 in the mouse intestine

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To gain insight into the cellular and molecular mechanisms underlying epithelial cell surface interactions in the adult mouse intestine, we have characterized the cell adhesion molecules L1, N-CAM and J1 by immunocytological, biochemical and cell biological methods. Whereas N-CAM and J1 expression was found to be confined to the mesenchymal and neuroectodermally-derived parts of the intestine, L1 was localized in the proliferating epithelial progenitor cells of crypts, but not in the more differentiated epithelial cells of villi. L1 was detected in crypt cells by Western blot analysis in the molecular forms characteristic of peripheral neural cells, with apparent mol. wts of 230, 180 and 150 kd. Aggregation of single, enriched crypt, but not villus cells, was strongly inhibited in the presence of *Fab* fragments of polyclonal L1 antibodies. These observations show that L1 is not confined to the nervous system and that it may play a functional role in the histogenesis of the intestine in the adult animal.

Key words: aggregation/cell adhesion molecule/immunohistology/intestine/J1/L1/mouse/N-CAM

Introduction

Attempts to understand the cellular and molecular mechanisms underlying the development and maintenance of multicellular organisms have led to the characterization of a variety of cell surface glycoproteins that have been operationally termed cell adhesion molecules (for reviews, see Edelman and Thiery, 1985; Öbrink, 1986). Well-characterized adhesion molecules in vertebrates are uvomorulin/L-CAM/cadherin/cell-CAM120/80/Arc-1 (Bertolotti *et al.*, 1980; Kemler *et al.*, 1977; Takeichi, 1977; Damsky *et al.*, 1983; Imhof *et al.*, 1983), N-CAM/BSP-2/D2 (Edelman, 1983; Hirn *et al.*, 1981; Lyles *et al.*, 1984a,b), J1 which is probably related to cytotactin and tenascin (Kruse *et al.*, 1985; Chiquet-Ehrismann *et al.*, 1986; Grumet *et al.*, 1985), and L1/NILE/Ng-CAM (Lindner *et al.*, 1983; Rathjen and Schachner, 1984; Grumet *et al.*, 1984; Lee *et al.*, 1977; Bock *et al.*, 1985; Friedlander *et al.*, 1986). These molecules have been assumed to play distinct, yet coordinated roles not only during development and adulthood (for reviews, see Edelman and Thiery, 1985; Öbrink, 1986), but also during regeneration of the peripheral nervous system (Daniloff *et al.*, 1986; Nieke and Schachner, 1985; Sanes *et al.*, 1986). Uvomorulin and its analogues have been observed in many cell types, such as the blastomeres of preimplantation mouse embryos (Kemler *et al.*, 1977), and simple and stratified epithelium in a variety of adult organs (Edelman, 1983; Thiery *et al.*, 1984), but not in the nervous system. The neural cell adhesion molecules N-CAM and J1, although initially characterized in the central nervous system, have been detected in various other organs (Edelman, 1983; Kruse *et al.*, 1985; Sanes and Covault, 1985; Sanes *et al.*, 1986). The neural cell adhesion molecule L1 was found to be restricted to subpopulations of post-mitotic neurons in the central nervous system (Keilhauer *et al.*, 1985; Persohn and Schachner, 1987; for review, see Schachner *et al.*, 1985) and expressed not only on neurons, but also on Schwann cells in the peripheral nervous system (Faissner *et al.*, 1984; Martini and Schachner, 1986; Nieke and Schachner, 1985; Sanes *et al.*, 1986).

A particular feature of L1 and N-CAM is their involvement in the migration of granule cell neurons in the developing cerebellar cortex (Lindner *et al.*, 1983, 1986). Whereas N-CAM may well mediate migration by allowing adhesion between migrating neurons and the contact guiding Bergmann glial processes (Lindner *et al.*, 1986), L1 expression appears to be a prerequisite in neuron–neuron interaction before or during migration (Keilhauer *et al.*, 1985; Lindner *et al.*, 1986; Persohn and Schachner, 1987). The neural cell adhesion molecule J1 is apparently not involved in the migration of granule cell neurons (Antonicek, Persohn and Schachner, 1987).

Based on these observations we wondered whether the neural cell adhesion molecules L1, N-CAM and J1 could play functional roles in the intestine, where generation and migration of epithelial cells continuously take place in adulthood (Leblond and Messier, 1958; Moog, 1981). Epithelial cells proliferate as crypt cells (Cheng and Leblond, 1974), migrate into the intestinal villi (Leblond and Messier, 1958) and differentiate (Simon *et al.*, 1979; Weiser, 1973; Quaroni, 1986) to become the absorptive cells that are discharged at the tip of the villi into the lumen of the intestine. The present study was undertaken to investigate whether L1, N-CAM and J1 could play a role in the histogenetic process of epithelial cell migration and differentiation in the adult intestine. We provide evidence that L1 is present on the epithelial crypt and is involved in a Ca^{2+}-independent cell adhesion mechanism among crypt cells.

Results

Immunocytochemical localization of adhesion molecules in the adult mouse intestine

The expression of L1, N-CAM and J1 was investigated in cross-sections of the ileum of adult mice by indirect immunofluorescence. No difference in immunocytochemical staining pattern was seen in the three different parts of the small intestine, duodenum, jejunum and ileum. The localization of these adhesion molecules in the smooth muscle layer, submucosa, muscularis mucosa, lamina propria, epithelial/mesenchymal interface and epithelium formed by crypt and villus cells was distinctly different.

In the outer smooth muscle layers of the intestine L1 is readily detectable in cells of Auerbach's plexus apparently associated with both neurons and Schwann cells (Figure 1A) as is the case in the rat (Mirsky *et al.*, 1986). The mesenchymally-derived parts of the submucosa and muscularis mucosa were found to be L1-negative except for the neuronal processes which extend into the

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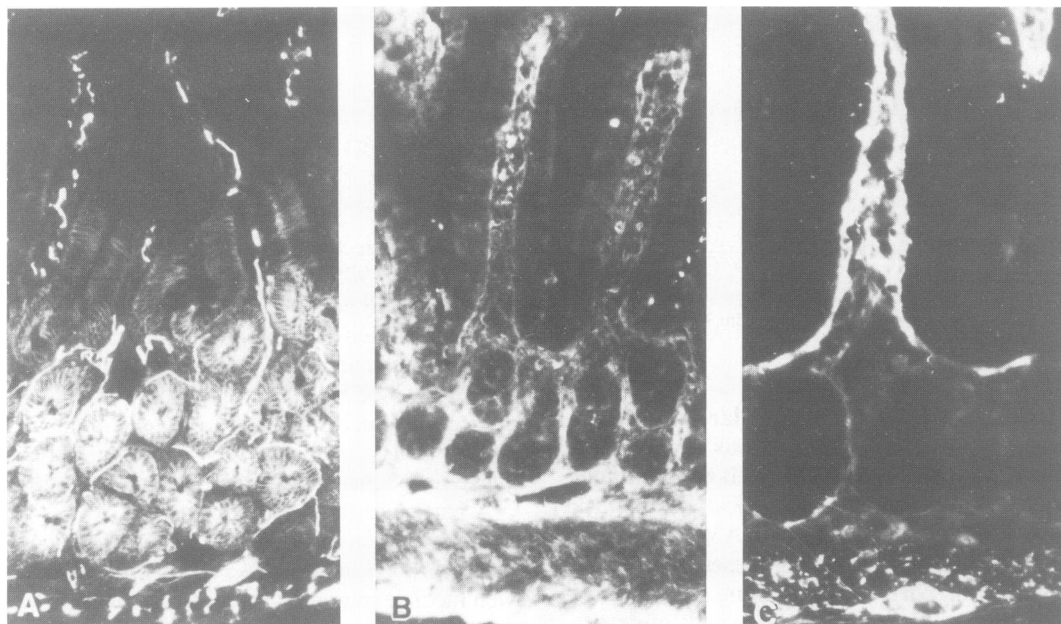

Fig. 1. Immunohistological localization of L1 (A), N-CAM (B) and J1 (C) in cross-sections of adult mouse intestine by indirect immunofluorescence. The outer muscle layers are at the bottom and the villi at the top of the micrographs. Magnification × 80.

lamina propria, which forms the mesenchymal inner part of the villi (Figure 2A). Most, if not all crypt cells expressed L1 as detected both by several monoclonal and immunoaffinity-purified polyclonal L1 antibodies (Figures 1A, 2B). The fact that three monoclonal antibodies reacting with different epitopes on the L1 molecule show the same immunostaining pattern makes it extremely unlikely that the detected molecule is not L1. L1 appeared to be present at the side contacts between crypt cells, and more towards the centre of the crypts than the periphery (Figure 2B). However, this pattern of localization was not exclusively observed and L1 was also detected at the crypt cell/lamina propria interface and at the periphery of the crypt structure (Figure 2B). L1 was more weakly detectable on epithelial cells at the villus base and was never seen in the middle and top region of a villus (Figure 1A). A more intense immunofluorescence labeling of L1 in villi was seen at the base of epithelial cells than at their apical regions (Figure 1A).

N-CAM was localized in the outer muscle layers not only in Auerbach's plexus, but possibly also in the mesenchymal parts (Figure 1B). N-CAM was also present in the submucosa, muscularis mucosa, lamina propria surrounding the crypts and in the inner part of villi (Figures 1B, 2C and D). It was never detectable on epithelial cells of crypts and villi, although a higher background fluorescence was observed for N-CAM than for J1. N-CAM was not accumulated at the interface between epithelial cells and lamina propria (Figures 1B, 2C).

J1, like N-CAM, was also detectable in the mesenchymally-derived parts of the outer muscle layers (Figure 1C). Positive structures had a spotted appearance and blood vessels were strongly surrounded by J1 reactivity (Figure 1C). The submucosa was more weakly J1-positive (Figure 1C). J1 was less strongly detectable than N-CAM around crypt cells (compare Figures 1B and C, and 2D and F). A more pronounced expression of J1 was seen in the lamina propria within villi, with a gradient of increasing immunoreactivity towards the tip of the villus and a very pronounced localization at the epithelium/lamina propria interface (Figures 1C; 2F). This epithelial/mesenchymal interface showed a much higher accumulation of J1 than N-CAM (compare Figures 2D and F).

Biochemical analysis of adhesion molecules

To study the molecular forms of each adhesion molecule expressed in the intestine, Western blot analysis was performed on total tissue of adult mouse ileum (Figure 3) and isolated populations of enriched crypt and villus cells (Figure 4). Degradation of adhesion molecules was minimized by quick dissection and immediate boiling of tissue in SDS−PAGE sample buffer containing 1% SDS. Whole brain tissue from adult mice was prepared in an identical manner for control.

L1 in brain tissue showed either a single band or the doublet of bands at 200 kd and a single strong band at 140 kd (Faissner *et al.*, 1985). In the intestine three major bands were observed at 230 kd, as it has been observed previously for peripheral nervous system neurons and Schwann cells (Seilheimer, Faissner, Keilhauer and Schachner, 1986, submitted; Sweadner, 1983a,b), at 180 and 150 kd. In some Western blots the 230-kd band was stronger than the one at 150 kd and the 180-kd component was not seen. These findings raise the possibility that the 150 kd component is a degradation product of the 230-kd band, thus paralleling the observation that the 140-kd component is a degradation product of the one at 200 kd in the brain (Faissner *et al.*, 1985). Enriched crypt cells showed the same bands at 230 and 150 kd as seen in total intestine samples (Figure 4). Enriched villus cells were L1-negative (Figure 4).

N-CAM in the adult mouse brain consists of the three components of 180, 140 and 120 kd as observed previously (Gennarini *et al.*, 1984). N-CAM in the intestine consisted of these three bands with a strong additional band at 100 kd, which is possibly a degradation product. Enriched crypt and villus cells did not show detectable levels of N-CAM.

J1 in brain showed the two and sometimes three molecular

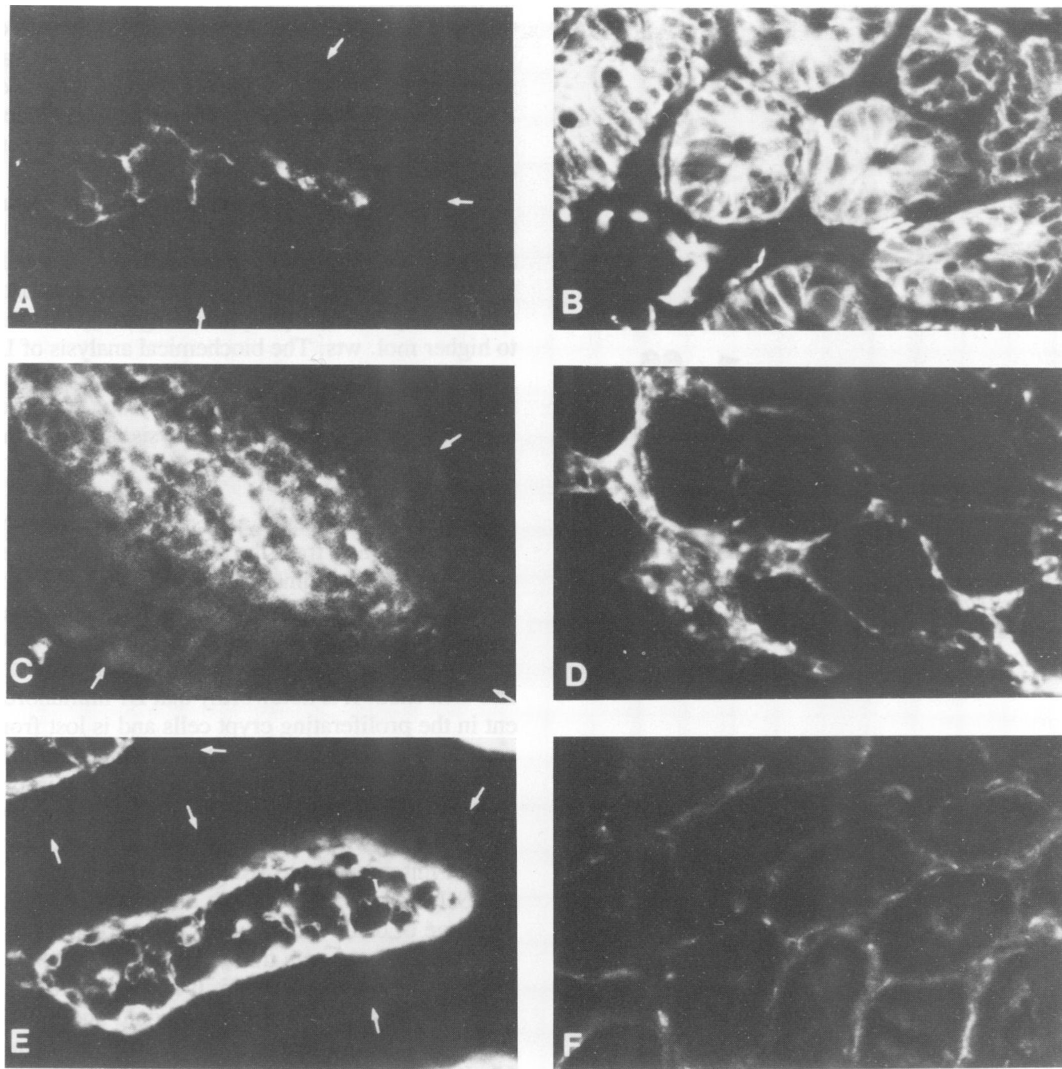

Fig. 2. Immunocytological localization of L1 **(A,B)**, N-CAM **(C,D)** and J1 **(E,F)** in cross-sections of adult mouse intestine by indirect immunofluorescence in the lamina propria of villi (A,C,D) and in the crypt region (B,D,F). The apical surface of epithelial cells of villi is outlined by arrows (A,C,E). Magnification × 160.

forms at 180 and 160 kd as described previously (Kruse *et al.*, 1985). In the intestine J1 consisted of a broad band at 170 and an additional one at ~250 kd. Enriched crypt and villus cells did not show detectable levels of J1.

Aggregation of enriched crypt and villus cells in the presence of antibodies

To investigate whether L1 on crypt cells had a role in adhesion, crypt and villus cells were enriched by a modification of differential treatment of adult intestine with EDTA-containing solutions (Weiser, 1973). Aggregation of single crypt and villus cells was measured quantitatively by Coulter counter analysis as described previously (Fischer and Schachner, 1982). Approximately 60% of all cells in the population enriched for crypt cells expressed L1 as detected by indirect immunofluorescence (Figure 5), whereas villus cells did not express detectable levels of L1. N-CAM was not detectable on crypt or villus cells. No inhibition of aggregation was seen when *Fab* fragments of an IgG fraction of non-immune rats or rabbits were used in the aggregation test with crypt cells or villus cells (Table I). Similarly, *Fab* fragments of antibodies prepared in rabbits against a crude membrane fraction of mouse liver did not inhibit aggregation of crypt cells. No significant inhibition of aggregation was seen with *Fab* fragments of antibodies to J1 and antibodies to human plasma fibronectin (for immunohistological localization, see Quaroni *et al.*, 1978; Simon-Assmann *et al.*, 1986). Polyclonal N-CAM antibodies and monoclonal L1 antibodies that have not been found to interfere with aggregation of cerebellar cells (Rathjen and Schachner, 1984) gave a small inhibitory effect on crypt cell aggregation. Since N-CAM could not be detected on isolated crypt cells by indirect immunofluorescence, this inhibition could result from low levels of N-CAM that are not detectable by indirect immunofluorescence, but could be recognized in a functional test. A strong inhibition of crypt cell aggregation by almost 70% was observed in the presence of *Fab* fragments of polyclonal L1 antibodies. Inhibition of aggregation was dependent on the concen-

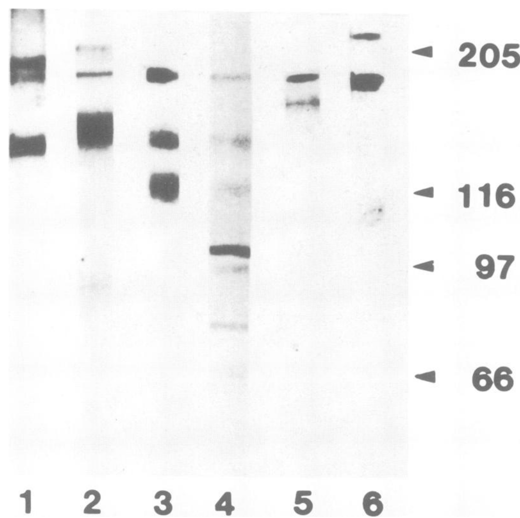

Fig. 3. Western blot analysis of molecular forms of L1 (**lanes 1** and **2**), N-CAM (**lanes 3** and **4**) and J1 (**lanes 5** and **6**) in the adult mouse brain (**lanes 1, 3** and **5**) and intestine (**lanes 2, 4** and **6**) from adult mice after SDS−PAGE on 7% slab gels.

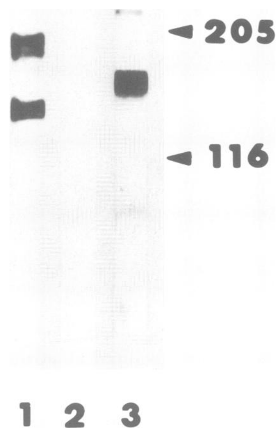

Fig. 4. Western blot analysis of molecular forms of L1 in brain (**1**), and enriched villus (**2**) and crypt (**3**) cells from adult mice after SDS−PAGE on 7% slab gels.

tation of *Fab* fragments of polyclonal L1 antibody (from 0.1 to 10 mg/ml). When per cent inhibition is plotted versus the logarithm of the concentration of *Fab* fragments a linear curve was observed with plateau values of inhibition being reached at ~ 5 mg/ml. Villus cell aggregation was not significantly inhibited by L1 antibodies. These results suggest that L1 is involved in adhesion of crypt, but not of villus cells.

Discussion

The present study has shown that the L1 cell surface glycoprotein previously thought to be involved only in adhesion among neurons (Keilhauer *et al.*, 1985) is also expressed by a non-neural cell, the epithelial cell of intestinal crypts. L1 also subserves an adhesive function on these non-neural cells, since antibodies to L1 inhibit Ca^{2+}-independent aggregation of crypt cells. Of the other adhesion molecules tested, N-CAM antibodies interfered slightly with crypt cell aggregation. This inhibitory effect could be due to vestiges of these molecules which are present at the epithelial/mesenchymal interface, but not detectable by indirect immunofluorescence. These interactions would then reflect an epithelium/mesenchyme adhesion rather than an adhesion between epithelial cells.

Expression of adhesion molecules in the intestine could indeed be seen not only by immunohistology, but also by biochemical analysis. N-CAM and J1 showed similar molecular forms as in the central nervous system. The molecular forms seen for L1 were as expected for peripheral organs in that they show a shift to higher mol. wts. The biochemical analysis of L1 in crypt cells gave a pattern similar to that of the entire intestine. The higher mol. wt of intestinal L1 is most likely due to a difference in glycosylation, since Northern blot analysis of L1 in brain and intestine showed a single band at 6 kb (R.Tacke, personal communication).

The functional roles of adhesion molecule expression on crypt cells is interesting from the point of view that intestinal epithelial cells in the adult animal form a migratory system (for reviews, see Haffen *et al.*, 1986; Potten, 1983). Epithelial cells are continuously generated in the crypt region, from where they are pushed up in a side-by-side juxtaposition to the top of the villus, and then shed. It is noteworthy that L1 immunoreactivity is present in the proliferating crypt cells and is lost from the epithelial surface with progressing migration and differentiation towards the villus tip. The fact that L1 is expressed in proliferating epithelial cells stands in contrast to observations in the central nervous system, where L1 is not present in proliferating cells, but in post-mitotic neurons (Rathjen and Schachner, 1984; Persohn and Schachner, 1987). These observations suggest that it is not the stage of differentiation *per se* that dictates the expression of L1. Rather, it appears that functional requirements in cell−cell interactions take precedence. It is tempting to speculate that one of the adhesive mechanisms that could underlie the organization of crypt cells in a single epithelium might be mediated through L1. It may be pertinent in this context that L1 appears to be more concentrated at the contact sites between neighbouring cells than at the basal surface opposing the mesenchymal interface. Also, an apparently more pronounced expression of L1 at the apical side of crypt cells facing the lumen is noteworthy and could contribute to the pronounced curvature of epithelial cells at the luminal rather than the basal side of crypt cells. It is interesting that another cell adhesion molecule, L-CAM, which is involved in Ca^{2+}-dependent aggregation, has also been localized to crypt cells (Thiery *et al.*, 1984). The co-existence of two neural cell adhesion molecules on one cell has been observed previously and was suggested to subserve cooperative effects in adhesion (Faissner *et al.*, 1984; Rathjen and Rutishauser, 1984; Keilhauer *et al.*, 1985; Thor *et al.*, 1986). Whereas L-CAM remains expressed on epithelial cells throughout their life cycle (Thiery *et al.*, 1984), L1 ceases to be expressed on villi cells as migration and differentiation proceed. It is therefore possible that the loss of L1 expression is causally related to the shedding process which would require that the cells become less adhesive to each other and the underlying extracellular matrix. Whether L1 is organized in junctional specializations between crypt cells as it has been reported for uvomorulin in the epithelial cells of villi (Boller *et al.*, 1985) and whether it is expressed by other polarized epithelia remains to be determined.

To investigate the role of L1 in the formation of crypts during early postnatal development, an immunohistological study was

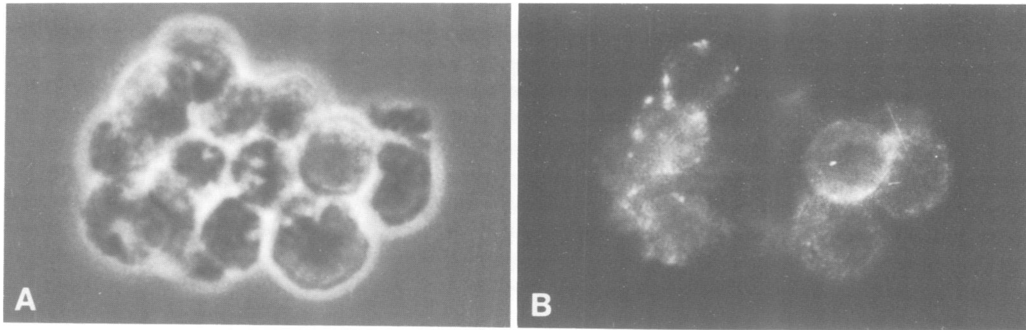

Fig. 5. Immunocytological localization of L1 on isolated crypt cells by indirect immunofluorescence (3). (A) Corresponding phase micrograph. Magnification × 1120.

Table I. Aggregation of crypt and villus cells in the presence of antibodies to adhesion molecules

Antibody	% inhibition of aggregation	
	Crypt cells	Villus cells
None	0 ± 4	0 ± 7
Non-immune from rat	7 ± 4	n.d.
Non-immune from rabbit	0 ± 6	3 ± 8
Polyclonal mouse liver membrane	2 ± 8	8 ± 9
Polyclonal fibronectin	15 ± 14	n.d.
Polyclonal N-CAM	23 ± 9	n.d.
Polyclonal J1	6 ± 4	n.d.
Monoclonal L1	26 ± 7	n.d.
Polyclonal L1	67 ± 8	17 ± 11

All antibodies were *Fab* fragments (1 mg/ml) except for polyclonal J1 antibodies which were used as IgG fraction. Numbers are mean values from five independent experiments for crypt cells and three independent experiments from villus cells carried out in quadruplicate ± SD. The experiment with polyclonal J1 antibodies was carried out once in quadruplicate.
n.d. not done.

carried out from birth onwards. Since observations from this study did not yield immediate insights into the functional role of L1 during morphogenesis of the intestine, these results will be presented elsewhere (Probstmeier *et al.*, in preparation). However, for evaluation these observations are summarized here. At birth, when crypts are still developing, L1 was not detectable. The first L1-positive crypt cells were visible at postnatal day five. The immunostaining intensity of crypt cells seen in the adult animal was reached before the end of the weaning period (~10 days). L1 was never detectable in epithelial cells of the villus region at any stage tested.

Although the functional relationships between epithelial cells, underlying basal lamina and mesenchymal cells of the lamina propria have not been assayed in this study, some inferences about these relationships can be made from the immunocytological studies. The observation that migration of epithelial cells occurs in conjunction and synchrony with the generation and migration of the pericryptal fibroblasts (Pascal *et al.*, 1968) would attribute an important role to the basal lamina and extracellular matrix between intestinal epithelial cells and underlying mesenchyme. N-CAM and, even more so, J1 are observed at the epithelial/mesenchyme interface between the epithelial cells of crypt and villus and the lamina propria. J1 shows a gradient of expression with a more pronounced expression of J1 at the tip of the villus. This gradient is in reverse to the one found previously for fibronectin (Quaroni *et al.*, 1978; Simon-Assmann *et al.*, 1986). It is difficult to conceive how the gradient in J1 expression could be related to the shedding process of epithelial cells. A decreased expression of fibronectin would appear to be more compatible with this process. The roles of N-CAM and J1, together with the extracellular matrix components laminin, fibronectin, nidogen and type IV collagen (Simon-Assmann *et al.*, 1986) in the histogenesis of the intestinal epithelium in the adult remain to be determined.

Materials and methods

Animals

NMRI mice were used for all experiments.

Antibodies

Production and specificity of polyclonal antibodies to L1, N-CAM and J1 have been described (Rathjen and Schachner, 1984; Gennarini *et al.*, 1984; Kruse *et al.*, 1985). Several monoclonal L1 antibodies have been described (Rathjen and Schachner, 1984) and produced (Asou *et al.*, in preparation). Neither poly- nor monoclonal antibodies react with the L2/HNK-1 carbohydrate epitope which is shared among several cell adhesion molecules including L1 (Rathjen and Schachner, 1984; Kruse *et al.*, 1984, 1985; Faissner *et al.*, 1984).

For indirect immunofluorescence studies polyclonal L1 and J1 antibodies were immunoaffinity-purified on L1 or J1 antigen columns as described (Martini and Schachner, 1986). The antigens for the columns were prepared by immunoaffinity chromatography purification on monoclonal L1 or L2 antibody columns as described (Rathjen and Schachner, 1984; Kruse *et al.*, 1986). For indirect immunofluorescence antibodies were used at concentrations of 4 μg/ml (L1 antibodies), 3 μg/ml (N-CAM antibodies) and 5 μg/ml (J1 antibodies). For Western blot analysis antibodies were used at concentrations of 0.4 μg/ml (L1 antibodies), 0.6 μg/ml (N-CAM antibodies) and 0.8 μg/ml (J1 antibodies). *Fab* fragments of poly- and monoclonal antibodies were prepared with mercuripapain according to Porter (1959).

Immunohistology

Indirect immunofluorescence of cross-sections of ileum and other parts of the small intestine of adult mice was carried out according to Quaroni (1985) after fixation of the tissue. In brief, the ileum was prepared and rinsed quickly with 0.15 M NaCl, pH 7.3, and immediately frozen in OCT (Jung, Nussloch). Sections, 10-μm thick, were cut, thawed onto glass coverslips and left to dry for 1 h at room temperature. They were then fixed at 4°C with 1% formaldehyde in 100 mM Na phosphate buffer, ph 7.4. Coverslips were washed three times with phosphate-buffered saline, pH 7.3 (PBS) and left for 1 h at 4°C in 100 mM glycine in PBS followed by three washes with PBS. Sections were then incubated with the first antibody in PBS containing 0.2% bovine serum albumin and 1% horse serum for 20 min followed by three washes with PBS. Sections were then incubated for indirect immunofluorescence procedures using fluorescein isothiocyanate (FITC)-conjugated antibodies as second antibodies.

Indirect immunofluorescence of single-cell suspensions of isolated crypt and villus cells was performed as described for cerebellar cells (Schachner *et al.*, 1975). During the labeling procedure aggregation of cells into small clumps could not be prevented even when they were vigorously pipetted up and down after each step. Counting was performed by focusing the cells in different planes.

Western blot analysis

Western blots were carried out as described (Faissner *et al.*, 1985). In brief, ileum or brain tissues from adult mice were dissected, cut into small pieces and immediately boiled in SDS sample buffer for electrophoresis (Laemmli, 1970). Specimens were then centrifuged, loaded onto 7% SDS gels, separated by PAGE and blotted onto nitrocellulose. Nitrocellulose filters were then incubated with polyclonal antibodies diluted for 2 h at 37°C, washed three times (15 min each) in Tris-buffered saline, pH 7.3, containing 0.2% Tween 20 and incubated with immunoaffinity-purified goat anti-rabbit IgG coupled to alkaline phosphatase (Promega-Biotech) for 0.5 h in Tris-buffered saline containing 10% bovine serum albumin and 0.2% Tween 20. After several washings with Tween 20 containing Tris-buffered saline nitrocellulose filters were developed with a mixture of 5-bromo-4-chloro-3-indolyl phosphate (50 mg/ml in dimethylformamide) and nitro blue tetrazolium (50 mg/ml in 70% dimethylformamide).

Preparation of intestinal cells

Single-cell suspensions of crypt and villus cells were prepared according to Weiser (1973) with modifications to ensure dissociation into single cells. The efficacy of dissociation was monitored by phase contrast microscopy and Coulter counter analysis. In brief, the whole intestine from adult mice was dissected out and rinsed with 10 ml of 0.15 M NaCl, 1 mM dithiothreitol, pH 7.2. One end of the intestine was closed by a clamp and the intestine filled with 1.5 mM KCl, 96.0 mM NaCl, 27.0 mM Na citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, pH 7.2 and left for 15 min at 37°C. This solution was then discarded. The intestine was then refilled with 1 mM EDTA, 0.5 mM dithiothreitol, 10 mM Hepes in Hank's balanced salt solution, pH 7.2 for 4 min at 37°C. The solution containing villus cells was then removed from the intestine, centrifuged to obtain the cells which were then washed two times with Hank's balanced salt solution. Three further incubations at 37°C, for 10 min each, generated cells from the lower villus and upper crypt regions. A final incubation at 37°C for 15 min gave the enriched crypt cell population. After two washings in Hank's balanced salt solution, crypt and villus cells were resuspended in Hank's balanced salt solution containing 10 mM Hepes and 0.004% DNase using a fire-polished Pasteur pipette. Before cell counts, cells were allowed to settle for 5 min and cell numbers in the supernatants were monitored by Coulter counter analysis.

Aggregation assay

Aggregation of single crypt and villus cells was carried out as described previously (Fischer and Schachner, 1982). In brief, 24-well Costar plates were saturated for 1 h at room temperature with basal medium Eagle's containing 10% horse serum, washed three times with distilled water and once with Hank's balanced salt solution containing 10 mM Hepes, pH 7.2. Single-cell suspensions (7.5×10^5 cells/500 μl Hank's balanced salt solution containing 10 mM Hepes and 0.004% DNase, pH 7.2) were added to each well and allowed to aggregate in a reciprocal shaker at 24°C for 20 min. Particles were counted immediately in a Coulter counter after dilution in 20 ml 0.9% NaCl containing 1.9% formaldehyde. The Coulter counter was calibrated according to the volume size of counted particles. Cell aggregation was measured as per cent decrease in particles in channels with low Coulter volumes and shift to channels with higher Coulter volumes (Fischer and Schachner, 1982).

The inhibition of aggregation in the presence of *Fab* fragments of poly- and monoclonal antibodies was measured after preincubation of single cells with 1 mg/ml *Fab* fragments for 15 min on ice before the aggregation assay. Inhibition was calculated according to the formula:

$$\text{\% inhibition of aggregation} = \frac{\text{aggregation} \begin{pmatrix}\text{in presence of}\\ \text{antibodies}\end{pmatrix} - \text{aggregation (control)}}{\text{aggregation (control)}} \times 100$$

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