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Histological reactivity of a monoclonal antibody against rat colon cancer cells on human and rat normal gut and colonic tumours

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Summary. A monoclonal antibody, F11C, was raised against rat colon cancer cells. Its immunoreactivity on normal human and rat gut as well as human and rat colonic tumours was studied by the avidin-biotin-peroxidase complex technique. In both normal rat and human gastrointestinal tract, F11C stained surface epithelial cells from the fundus to distal colon, mainly as supranuclear vesicles. These vesicles appeared to be part of the Golgi apparatus on electron microscopy with immunogold labelling. Twenty primary rat colon tumours and 28 of 43 human colon tumours were also stained, with a heterogeneous pattern but much more strongly than the normal colonic mucosa. Biochemical purification suggested that in rat tumours F11C epitope was carried by a high molecular weight glycoprotein. Absorption experiments with synthetic oligosaccharides showed that F11C monoclonal antibody reacted with blood group A-related oligosaccharides. Nevertheless, F11C reactivity on human tissues was not related to the individual ABO or Lewis phenotype.

Key words: Monoclonal antibody – Digestive tract – Colon cancer – Rat – Golgi apparatus

Introduction

Monoclonal antibodies (mAbs) specific for oligosaccharidic epitopes of glycoproteins or glycolipids are powerful tools for studying the antigenic structure of normal and pathological gastrointestinal mucosa. Several mAbs recognize blood group ABH, Lewis and related epitopes (Clausen and Hakomori 1989). These antibodies have been used as markers of gastrointestinal cell differentiation and also as markers of tumour-associated antigens. Most of the mAbs characterized as reacting with oligosaccharide epitopes of human gastrointestinal mucosa have been obtained by immunizing mice against human material, including colon cancer cell lines. There are few reports of mAbs raised against rat colonic cancer cells (Sugiyama et al. 1988) or rat mucosal scrapings (Decaens et al. 1988). These antibodies labelled various cells in normal rat gastrointestinal mucosa and chemically induced rat colonic tumours, but their cross-reactivity with human gastrointestinal tract or its tumours has not been reported.

We report here the distribution in human and rat gastrointestinal tissues of an antigen defined by a mAb raised against a rat colon cancer cell line. This antibody, called F11C, also labels human and chemically induced rat colonic adenocarcinomas. The tissue distribution of the antigen recognized by mAb F11C seems to be different from that reported with other mAbs labelling human gastrointestinal epithelial cells.

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Materials and methods

Production and characterization of the mAb F11C has been reported elsewhere (Caignard et al. 1990). Briefly, F11C hybridoma was obtained by fusing murine myeloma cells Sp2/O-Ag-14 with spleen cells from a mouse immunized against DHD/K12/REGb cells, a tumour cell line isolated from a colonic carcinoma induced by 1,2 dimethylhydrazine (Merck, Darmstadt, FRG) in a BDIX strain rat. This hybridoma secretes an IgM kappa light chain. In this work, F11C mAb was used either as culture supernatant (2–5 μg/ml), or as ascitic fluid (2–5 mg/ml).

Rat tissues were obtained from Sprague-Dawley outbred rats and BDIX inbred rats immediately after killing by carbon dioxide inhalation. Twenty primary dimethylhydrazine-induced tumours were obtained from 14 Sprague-Dawley rats which had received a weekly subcutaneous injection of 20 mg of 1,2 dimethylhydrazine/kg body weight for 12 weeks. These rats were killed 6 months after the first injection.

Normal human gastrointestinal tissues were obtained from a panel of 68 kidney donors. Autopsies were performed immediately after death. Most of them were in the fourth decade of life. All had been free of any known neoplastic disease prior to trauma. Tissue samples measuring about 20×1 cm were taken from eight different areas of the gastrointestinal tract: oesophageal-cardiac junction; pyloric-duodenal junction; duodenal mucosa, including Wirsung's duct; jejunum; ileum; ileo-colonic junction; transverse

colon; and sigmoid. The ABO and Lewis phenotype of each donor was determined by conventional haemagglutination or by immunoperoxidase with anti-A, anti-B, anti-Le^a and anti-Le^b mAbs.

Twelve colorectal cancers with adjacent non-neoplastic mucosa were obtained as surgical resection specimens from the Dijon Anticancer Centre or from the First Department of Surgery, University of Dijon, and 31 were obtained from the Centre Médico-chirurgical de la Porte de Choisy, Paris, France.

Longitudinal bands of human or rat gastrointestinal mucosa were pinned on cork and fixed overnight in 95% ethanol (human tissues) or Gendelman's solution (rat tissues). The human colorectal tumours were fixed in Karnowski solution and rat colonic tumours were fixed in Bouin's solution or in 95% ethanol. It has been checked that the four types of fixation procedure did not modify the immunoreactivity of mAb F11C on rat colonic tissues. After fixation, normal tissues and tumours were embedded in paraffin according to the method of Sainte-Marie (1962). Human tumours were embedded in paraplast-piccolyte (Bogomoletz and Potet 1981). Sections (3 μm) were mounted in gelatin-coated slides to avoid detachment during the immunoenzymatic procedure. All the sections were stained with haematoxylin-eosin-saffron for histological study. Mucous secretion was studied by periodic acid-Schiff (PAS) staining.

Immunoenzymatic staining was performed on deparaffinized sections according to the avidin-biotin-peroxidase complex technique (Hsu et al. 1981). Undiluted F11C culture supernatant was used as the first antibody and incubated for 30 min at 4° C in a moist chamber. A non-relevant IgM antibody (anti-sheep red blood cells) and F11C culture supernatant absorbed with DHD/K12/REGb cells were used as negative controls ensuring the specificity of the reaction. Endogenous peroxidase was inhibited by incubating tissue sections in 1% hydrogen peroxide in methanol for 20 min at room temperature before addition of the first antibody. The avidin-biotin-peroxidase kit for mouse immunoglobulin (ABC Kit Vectastain) was obtained from Vector (Burlingame, Calif.). Peroxidase activity was revealed using aminoethylcarbazol (Sigma, St. Louis, Mo.) After each incubation, sections were washed three times with phosphate-buffered saline solution (PBS) containing 1% Tween 20.

For the immunogold labelling procedure tissues were fixed for 2 h in 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. After rinsing for 2 h in 0.1 M cacodylate buffer, free aldehydes were quenched with 50 mM ammonium chloride in 0.1 M cacodylate buffer for 2 h on ice. After rinsing with 0.1 M cacodylate buffer overnight, specimens were dehydrated in ethanol and embedded in epoxy medium. All immunogold staining treatments were performed at room temperature. Ultra-thin sections (75 nm) were cut, mounted on 200 mesh nickel grids with a pioloform film (Biorad, Richmond, Calif.) and treated for 30 min with 1% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS (PBSAT). Sections were treated for 1 h either with F11C supernatant or with an unrelated IgM (anti-sheep red blood cells). After rinsing 5 times in PBSAT, sections were treated with 15 nm colloidal gold-labelled anti-mouse IgM goat antibody diluted 1:5 in PBSAT. Grids were rinsed 5 times in PBSAT, 1 time in distilled water, and were post-fixed for 15 min with 2% aqueous osmium tetroxide. After rinsing in distilled water, grids were stained for 10 min with 4% aqueous uranyl acetate. Sections were examined with a Hitachi HU 12 electron microscope.

With the aim of characterizing and partially purifying the antigen recognized by F11C antibody in rat colon cancers, a transplantable tumour line, DHD/K12/PROb, growing as subcutaneous tumours or liver metastases in syngeneic BDIX rats, was used. Tumours were collected immediately before processing. Saline extracts were prepared by homogenization (Ultraturax, Janke and Kunkel, Staufen i.Br., FRG) of tissues in cooled PBS, pH 7.3, 4 ml/g tissue, followed by a 30-min centrifugation at 23,000 *g* and 4° C. The supernatant was dialysed against distillated water, lyophilized and stored at −20° C. Perchloric acid extracts were prepared according to a procedure previously reported (Martin et al. 1975). Gel filtration of saline or perchloric tumour extracts was performed on sepharose CL-6B column (Pharmacia, Uppsala, Sweden), with PBS as eluant. Fractions containing F11C antigen were detected using fluorescence inhibition as previously reported (Caignard et al. 1990). Briefly, each fraction was incubated with an equal volume of mAb F11C ascitis (1:300) for 48 h at 4° C; after centrifugation, supernatants were reacted with DHD/K12/REGb cells grown on coverslides for 2–3 days. Cells were then incubated with fluorescein-isothiocyanate (FITC)-conjugated anti-mouse Ig (Diagnostics Pasteur, Paris, France) diluted 1:50 in PBS supplemented with 1% BSA and 0.1% sodium azide (PBSAA) and washed three times in PBSAA before examination.

Table 1. F11C epitope expression in normal rat and human tissues

Tissue	Adult rat	Fetal rat (20 days)	Adult human
Oesophagus	+	ND	++
Cardia	ND	ND	+++
Forestomach	0	ND	ND
Fundus	+	ND	+++
Antrum/pylorus	++	ND	+++
Duodenum	++	+	+++
Jejunum	+++	+	+++
Ileum	++	+	+++
Proximal colon	++	ND	+
Distal colon	+	ND	+
Salivary glands	+	++	ND
Pancreas	0	ND	+
Gall bladder	ND	ND	+
Liver	0	ND	ND
Kidney	+	ND	ND
Bladder	0	ND	ND
Adrenal	0	ND	ND
Epididymis	+	ND	ND
Ovary	0	ND	ND
Mammary glands	+	ND	ND
Lung	0	ND	ND
Lymph node	0	ND	ND
Sternal bone marrow	0	ND	ND
Femoral bone marrow	0	ND	ND

Positive reactions from + to +++ represent increasing intensity of immunoperoxidase staining; 0, no staining; ND, not determined

F11C culture supernatant was absorbed on different synthetic oligosaccharides covalently bound to solid supports (Synsorb; Chembiomed, Edmonton, Canada). In a first step, the adsorption of F11C culture supernatant aliquots (100 μl) with each immunoadsorbent was performed with 5 mg Synsorb. Tubes were gently rotated for 2 h at room temperature and, after sedimentation of immunoadsorbents, supernatants were tested by immunoenzymatic staining performed on pyloric-duodenal sections from individuals showing A Le^b or O Le^b phenotype. Then the 5-mg aliquots of Synsorb able to absorb mAb F11C were retested with two different dilutions of F11C culture supernatant (1:1 and 1:5). After each test, Synsorb immunoadsorbents were recycled with glycine hydrochloride 0.1 M, pH 2.5, and ammonium hydroxide, pH 11, neutralized and stored dry.

The ability of mAb F11C to react with the Forssman antigen was tested with an agglutination test and with a fluorescence inhibition technique. For the agglutination test, serial dilutions (1:1 to 1:32000) of sheep red blood cells (SRBC; Biomérieux, Charbonnières les Bains, France) were prepared in PBSAA, mAb F11C (supernatant) was also diluted in PBSAA, from 1:1 to 1:1024 and added to the same volume (100 μl) of each SRBC dilution in the V-shaped wells of microtitration plates. Plates were sealed with an adhesive film and left at room temperature for up to 24 h. For the fluorescence inhibition test, F11C supernatant diluted 1:1 to 1:1024 in PBSAA was mixed with equal volumes of undiluted

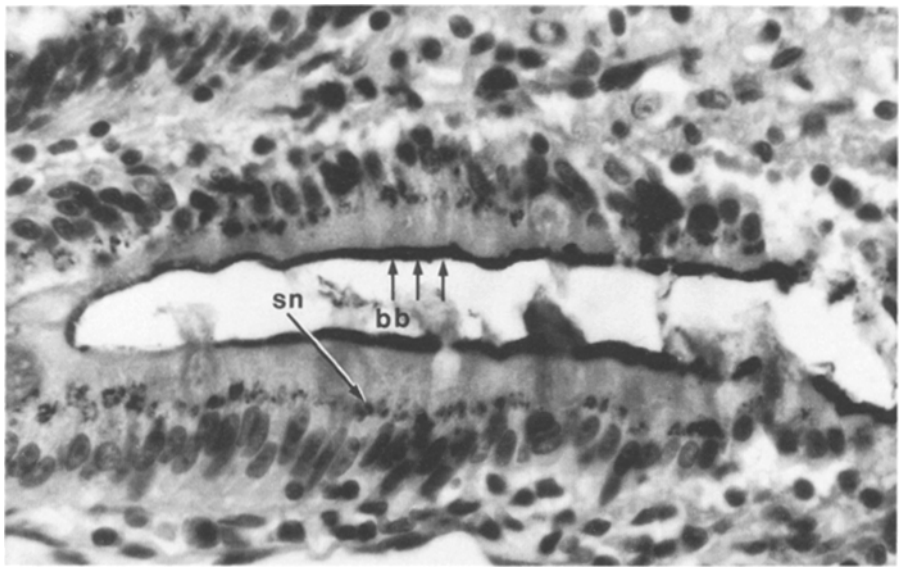

Fig. 1. Normal rat jejunum reacted with mAb F11C (counterstain: haematoxylin-eosin-saffron, HES). *Arrows* show the double localization: brush border (*bb*) and supranuclear area (*sn*). × 375

SRBC in V-shaped microtitration wells and incubated for 1 h at room temperature. The supernatants and control unabsorbed dilutions of mAb F11C (50 µl) were transferred to plates containing 5×10^5 REGb cells (50 µl/well). The plates were sealed with an adhesive film, submitted to mild agitation for 30 s, and allowed to stand for 45 min at 4° C. After being washed 3 times with PBSAA, the cells were resuspended in 50 µl FITC-conjugated anti-mouse Ig antiserum (Diagnostics Pasteur, Marnes-la-Coquette, France) and incubated as previously. Each cell suspension was washed 3 times in PBSAA, resuspended in one drop of buffered glycerine and examined on a fluorescence microscope.

Results

In the gastrointestinal tract of normal adult rat (Table 1), the F11C staining was localized in the surface epithelium from the fundus to the distal colon. In the oesophagus, there was a only a faint labelling of the intermediate layers.

In the stomach, there was a strong staining of supranuclear vesicles in the mucous cells lining the gastric pits. Cells of the fundic glands (including parietal, chief and mucous cells) were not stained.

In the jejunum (Fig. 1), most epithelial cells in the villi and Lieberkühn glands were stained with a double localization: supranuclear as in gastric cells, and brush border. Goblet cells were not labelled. A few glands, at the contact of the muscularis mucosae, were strongly stained by large, irregular granules occupying most of the cytoplasm.

In the ileum, we observed a similar pattern of F11C labelling to that in the jejunum but with a diminished intensity. The brush border of epithelial cells of the villi was not labelled.

In the caecum, there was no staining of the goblet cells. Epithelial cells of the surface and lining the tubular glands were stained by multiple small granules, irregularly sized, predominant in the supranuclear area, but also found in other parts of the cytoplasm. The brush border was not labelled.

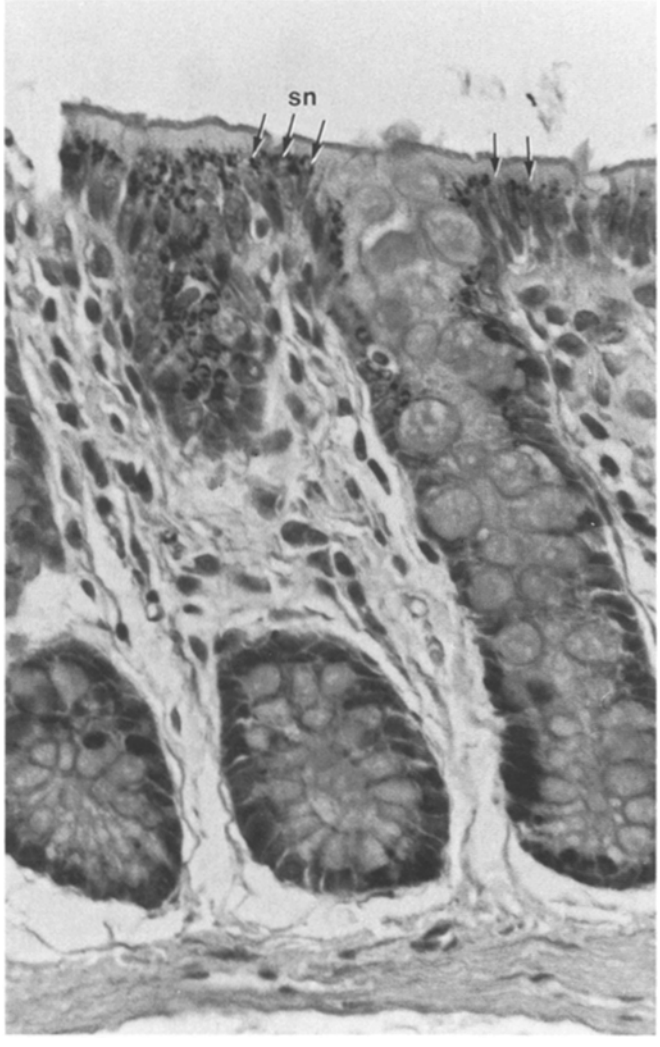

Fig. 2. Normal rat proximal colon reacted with mAb F11C (counterstain: HES). Columnar cells exhibit the same granular staining in the supranuclear area (*sn*) as in the jejunum. Glands are strongly and irregularly stained. × 375

Table 2. Expression of F11C epitope in rat primary DMH-induced colon adenocarcinomas

Tumour number	Location	Differentiation[a]	Mucin secretion (PAS)[b]	F11 staining[c]	
				Tumour	Adjacent mucosa
1	Small intestine	WD (T. PP)	+++	+	++
2	Caecum	WD (T.LP)	+++	++	0
3	Caecum	WD (T)	0	+	0
4	Proximal	WD (T.LP)	+++	+++	0
5	Proximal	WD (T)	+	+++	++
6	Proximal	WD (T+SRC)	+++	+++	0
7	Proximal	WD/PD (T+SRC)	++++	+++	0
8	Proximal	WD (T)	++	+++	0
9	Proximal	WD (T.LP)	0/+	++	++
10	Transverse	WD (T)	+++	+++	0
11	Transverse	WD (T+SRC)	0/+++	+++	++
12	Transverse	WD (T+SRC)	+++	+++	nd
13	Transverse	WD (T)	0	+++	+
14	Transverse	WD (T)	0/+	+++	+
15	Transverse	WD (T+SRC)	+++	+++	nd
16	Transverse	WD/PD (T/SRC+LP)	0/+++	+	++
17	Distal	WD (T+SRC.LP)	+++	+++	0
18	Distal	WD (T)	0/+	+++	+
19	Distal	WD (T+SRC)	+	++	0
20	Distal	WD (T+SRC)	0	++	+

[a] WD, Well-differentiated; SRC, signet-ring cells; PD, poorly differentiated; LP, tumour developed in lymphoid patches; t, tubular differentiation; PP, tumour developed in Peyer's patches

[b] PAS, Periodic acid-Schiff; 0/+ or 0/+++, simultaneous presence of weak and strongly positive and negative PAS zones on the same tissue sample

[c] Positive reactions from + to +++ represent increasing intensity of immunoperoxidase staining; 0, no staining

In proximal colon (Fig. 2), there was a very regular, thin supranuclear staining of the columnar cells lining the colonic lumen. In the glands, labelling was stronger and more irregular. In contrast to the other parts of digestive tract, many mucous goblet cells were strongly labelled. In distal colon, the supranuclear staining of the surface cells and cells lining the glands increased in intensity; the brush border and the goblet cells were not stained.

Pancreas and liver were not stained. Some sparse epithelial cells were labelled in non-digestive epithelia such as kidney, epididymis and mammary glands. F11C staining was never detected in non-epithelial cells such as fibroblasts, muscle cells or haematopoietic cells.

In 20-day-old fetuses (Table 1), the small intestine mucosa showed a weak cytoplasmic staining and salivary glands were strongly stained.

The F11C cytoplasmic expression was tested in 20 primary dimethylhydrazine-induced digestive tumours obtained from 14 Sprague-Dawley rats (Table 2). All were stained heterogeneously with an intensity varying from one area to another, according to the stage of differentiation. In the well-differentiated areas, F11C epitope was expressed as fine sparse granulations in the cytoplasm of the tumour cells surrounding the lumen of the glands (Fig. 3). In contrast, in the less well differentiated areas, F11C expression was more diffuse and was located exclusively in interstitial spaces. The expression of the F11C antigen was not correlated with mucosecretion. The normal mucosa was stained in the supranuclear area of colonic epithelial cells lining the surface and colonic glands. Goblets cells were negative, but in the proximity of the tumours, there was stronger labelling in some tubular glands, with a cytoplasmic basolateral staining surrounding the central mucus (Fig. 4).

F11C staining showed many similarities in human compared to rat normal gastrointestinal tract (Table 1). In the oesophagus, F11C stained the intermediate layers of the squamous epithelium as in rat, but also the superficial layers. There was also an irregular staining of the luminal surface, probably of luminal origin (staining by saliva stuck to the oesophageal wall).

In the stomach, there was strong F11C staining of columnar mucous cells lining the gastric lumen, the gastric pits and the superficial third of the cardiac glands. This staining had the same cellular location as in rat, with dense granules in the supranuclear area, but there were also more heterogeneous granules spread in the cytoplasm. Furthermore, the apical pole of these columnar cells was also underlined by a heavily stained, irregular coat. As in the rat, fundic and pyloric glands (including parietal and chief cells) were unlabelled.

In the duodenum, columnar epithelial cells lining duodenal villi were uniformly stained with two locations: the supranuclear area and the brush border. Epithelial cells lining the Lieberkühn glands were more heavily stained with many irregular F11C-positive granules scattered in the whole cytoplasm. The Brünner's glands were

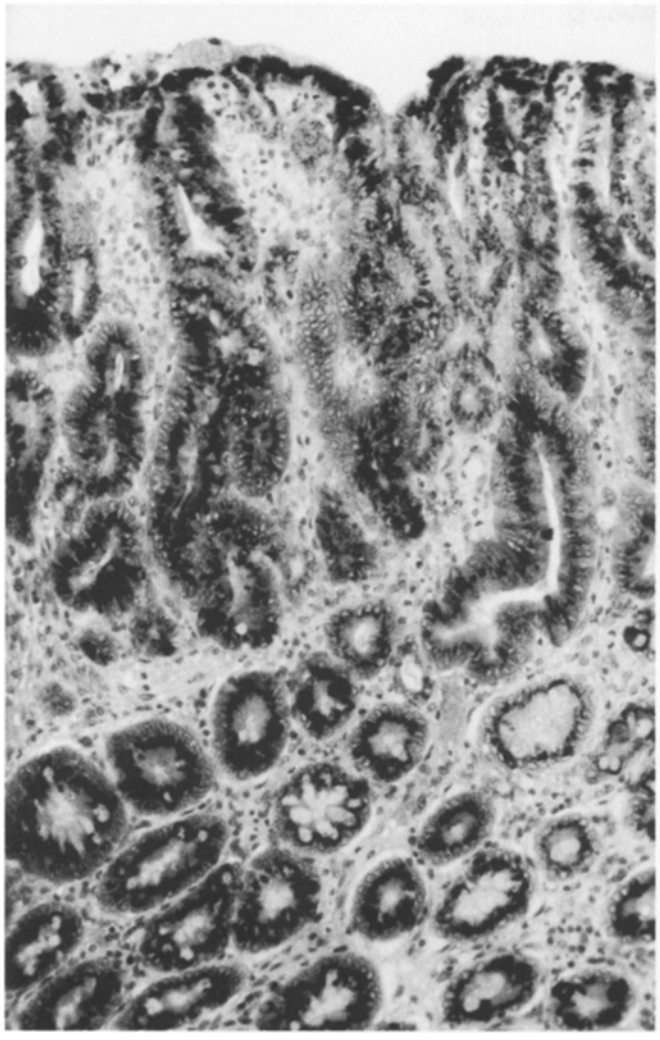

Fig. 3. Primary dimethylhydrazine-induced rat colon tumour reacted with mAb F11C (counterstain: HES). In this well-differentiated area, tumour cells exhibit a strong granular cytoplasmic staining. × 175

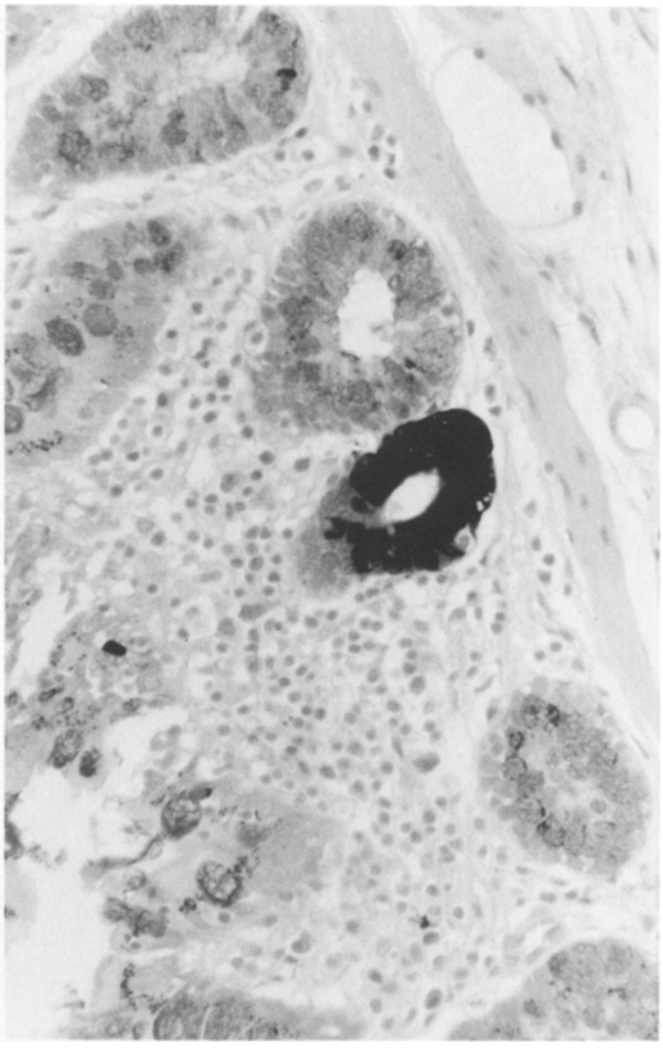

Fig. 4. Normal rat colon mucosa adjacent to tumour reacted with mAb F11C (counterstain: HES): very strong labelling of a gland (*dark tube*) contrasting with sparse cytoplasmic granulations in the surrounding glands. × 300

irregularly and weakly stained as very thin cytoplasmic granules.

In the jejunum, F11C staining increased in intensity. The enterocytes of the villi showed supranuclear staining, without staining of the brush border. The glands were negative, except for the most distal cells, which were strongly stained by multiple F11C-positive granules spread throughout the cytoplasm. There was a strong labelling of the surface epithelial cells and cells of the superficial two-thirds of Lieberkühn glands, with a double localization in the supranuclear area and in the brush border.

In the ileum (Fig. 5), the staining pattern of columnar cells lining the villi and Lieberkühn glands was about the same as that observed in the duodenum. However, this staining was more intense, particularly in the brush border, which was heavily labelled.

In the ascending colon, as in rat, mAb F11C stained the columnar cells of surface epithelium and the superficial third of the colonic glands, with a granular labelling, spread throughout the whole cytoplasm or restricted to supranuclear or subapical areas. Goblet cells were not stained.

In the transverse colon (Fig. 6), staining was restricted to thin, dusty granules found in the supranuclear area of some epithelial columnar cells lining the lumen and the upper part of the tubular colonic glands.

In the left colon and in the sigmoid, staining was quite irregular. Some colonic areas were completely negative. In other areas, columnar cells of the luminal surface and of the upper part of tubular glands were stained. They exhibited either thin and dusty, or large and heterogeneous positive granules of supranuclear location. The brush border of columnar cells remained negative.

All throughout the intestine, goblet cells did not express F11C epitope.

In the pancreas, only weak staining was found, restricted to acinar cells which contained thin heterogeneous granules concentrated at the apical part.

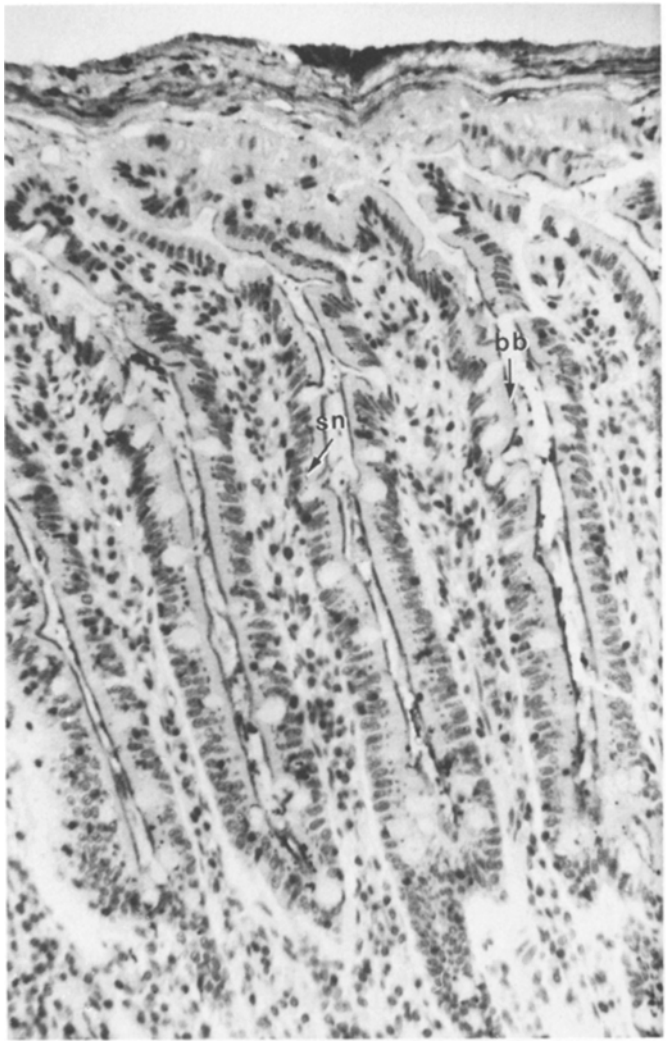

Fig. 5. Normal human ileum reacted with mAb F11C (counterstain: HES). *Arrows* show the strong labelling of the brush border (*bb*) and of the supranuclear area (*sn*) of columnar cells. ×210

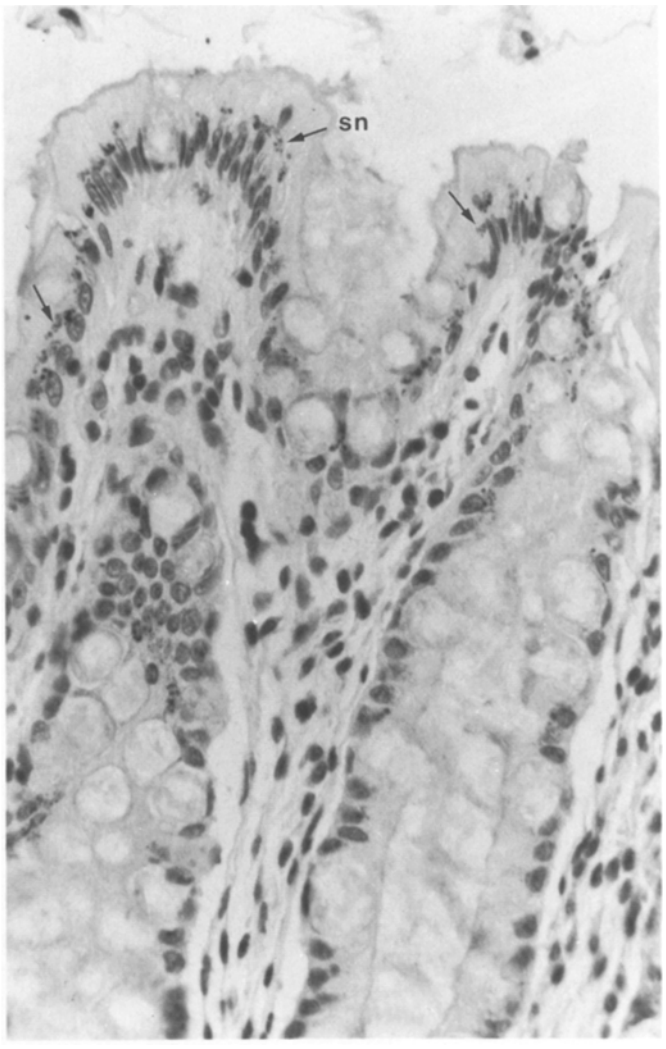

Fig. 6. Normal human transverse colon reacted with mAb F11C (counterstain: HES). Staining is restricted to a few granulations in the supranuclear area (*sn*) of cells lining the lumen. ×350

F11C immunoperoxidase labelling was not related to ABO or Lewis phenotype of the individual.

Twenty-eight (65%) of the 43 human colon tumours examined (Table 3) were stained by mAb F11C with varying intensities, unrelated to the stage of invasiveness and independently of the ABO phenotype of patients, as in normal tissues. Staining was observed in well-differentiated cancer cells (Fig. 7) and signet-ring cells. Extracellular mucus deposits and the lumen of tumour glands were also stained. As a whole, F11C staining of human colon tumours was weaker than staining of the rat tumours. Normal tubular glands adjacent to carcinomas were also strongly stained.

The F11C immunoreactivity was semi-quantified in rat tumour extracts with a fluorescence inhibition assay. Extraction was performed on PROb tumours, since large tumours are rapidly available after PROb cell inoculation to syngeneic rats. A fixed amount of F11C antibody (ascitis 1:300) was absorbed with lyophilized saline or perchloric extracts from PROb tumours, diluted 1:1 to 1:64. The protein concentration of all these undiluted extracts was 60 mg/ml. We used a saline extract from normal rat liver as a negative control to ensure the specificity of the reaction. Immunoreactivity of absorbed F11C was then tested by indirect immunofluorescence on DHD/K12/REGb cells. Results were quantified using an arbitrary scale. F11C epitope was present in saline extracts from subcutaneous, intraperitoneal and intrahepatic PROb tumours (Table 4). Perchloric extracts were 6–8 times more reactive than the corresponding saline extracts, suggesting glycoprotein nature of F11C antigen.

Perchloric extract from liver metastasis (50 mg lyophilized extract in 1 ml PBS pH 6.8) was submitted to chromatography on sepharose CL-6B and the elution fraction were tested for F11C epitope by the fluorescence inhibition assay. F11C antigen was excluded from sepharose CL-6B suggesting a molecular weight greater than 10^3 kDa.

To investigate if F11C epitope is carried by glycolipids on the surface of cultured REGb cells, we extracted total lipids from 10^8 REGb cells. REGb cells were cho-

Table 3. Expression of F11C epitope in human colon adenocarcinomas

Tumour number	Location	Differentiation[a]	ABO Phenotype	F11C staining[b]	
				Tumour	Adjacent mucosae
1	Caecum	WD	ND	++	+
2	Left colon	WD	ND	++	++
3	Left colon	WD	ND	0	+
4	Left colon	WD	A	0	+++
5	Sigmoid	WD	A	+++	+
6	Sigmoid	WD	ND	+++	ND
7	Sigmoid	WD	O	+++	+
8	Sigmoid	WD	O	++	+
9	Sigmoid	WD	O	++	+++
10	Sigmoid	WD	A	++	0
11	Sigmoid	WD	ND	++	+
12	Sigmoid	UD	ND	+	+
13	Sigmoid	WD	O	+	0
14	Sigmoid	WD	A	+	0
15	Sigmoid	WD	A	+	+
16	Sigmoid	PD	A	+	+
17	Sigmoid	MD	AB	+	0
18	Sigmoid	M	A	+	+
19	Sigmoid	WD	ND	+	0
20	Sigmoid	MD	ND	+	+
21	Sigmoid	WD	ND	+	+
22	Sigmoid	MD	A	0	+
23	Sigmoid	WD	A	0	0
24	Sigmoid	M	O	0	0
25	Recto-sigmoid	WD	A	+++	+++
26	Recto-sigmoid	WD	A	++	+
27	Recto-sigmoid	PD	A	++	0
28	Recto-sigmoid	WD	ND	+	++
29	Recto-sigmoid	WD	ND	+	0
30	Recto-sigmoid	WD	ND	+	++
31	Recto-sigmoid	MD	AB	0	+
32	Recto-sigmoid	MD	A	0	0
33	Recto-sigmoid	WD	O	0	0
34	Recto-sigmoid	WD	O	0	ND
35	Recto-sigmoid	MD	O	0	ND
36	Rectum	WD	ND	+	++
37	Rectum	WD	ND	+	ND
38	Rectum	MD	ND	+	+
39	Rectum	WD	O	0	0
40	Rectum	WD	A	0	+
41	Rectum	MD	AB	0	+++
42	ND	M	ND	0	ND
43	ND	M	ND	0	ND

[a] WD, Well differentiated; M, moderately differentiated; PD, poorly differentiated; UD, undifferentiated; M, mucinous, ND, not determined

[b] Positive reactions from + to +++ represent increasing intensity of immunoperoxidase staining; 0, no staining

Table 4. Semi-quantification of F11C epitope in tumour extracts by fluorescence inhibition

Immuno-absorbents	Dilution of tissue extracts before absorption of mAb F11C						
	1	2	4	8	16	32	64
Liver	++++	++++	++++	++++	++++	++++	++++
SE i.p.	0	0	±	±	±	+	++
SE s.c.	0	±	+	+	+	+	++
SE l.m.	0	0	0	±	+	++	++
PE l.m.	0	0	±	±	+	++	++
PBS	++++	++++	++++	++++	++++	++++	ND
LE REGb cells	++++	++++	++++	++++	++++	++++	ND

Reactivity of absorbed F11C on DHD/K12/REGb cells was tested by indirect immunofluorescence. Fluorescence intensity was ranked on an arbitrary scale: 0 and ±, no or weak fluorescence; ++++, very bright fluorescence

SE, Saline extract; i.p., intraperitoneal DHD/K12/PROb tumour; ND, not determined; PE, perchloric extract; s.c., subcutaneous DHD/K12/PROb tumour; LE, lipid extract; l.m., liver DHD/K12/PROb metastasis

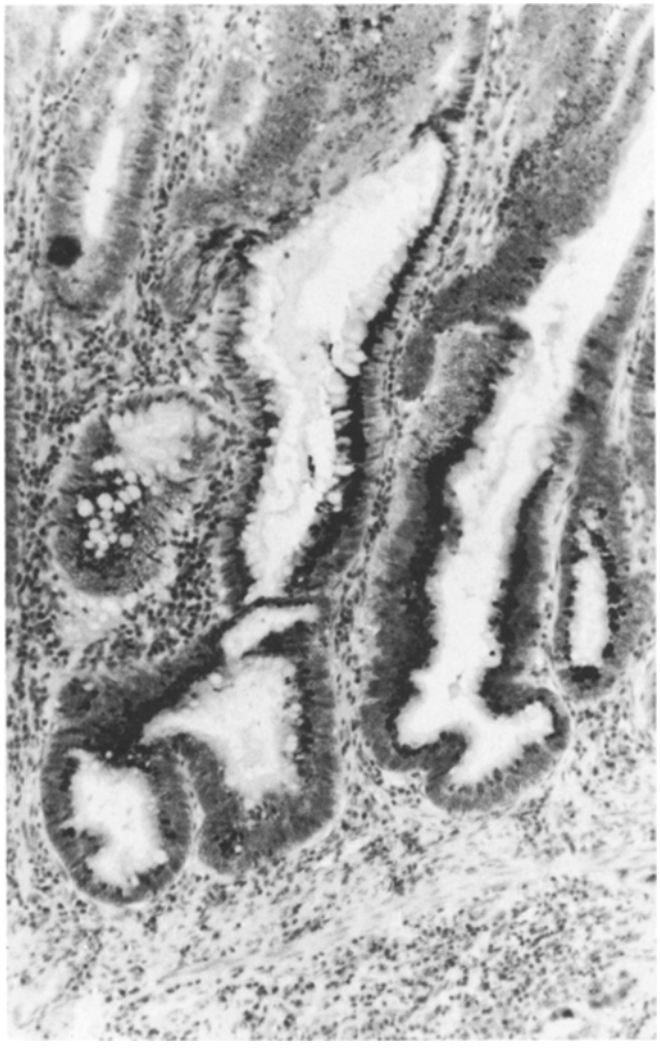

Fig. 7. Human colon tumour reacted with mAb F11C (counterstain: HES), showing a strong and heterogeneous staining pattern. ×200

sen for these experiments since they were more strongly stained by mAb F11C. This lipid extract was used to absorb mAb F11C supernatant; diluted 1:1 to 1:32 it was unable to absorb the reactivity of mAb F11C in the fluorescence inhibition assay, whereas whole cells did.

Reactivity of mAb F11C on frozen sections of human normal pylorus/duodenum was tested after absorption with oligosaccharide immunoadsorbents. Most of the A-related oligosaccharides inhibited the reactivity of F11C on these tissues. The strongest inhibition was obtained with A type 5, A linear trisaccharide and Forssman oligosaccharides (Table 5). Though reactivity of unabsorbed mAb F11C was not related to ABO phenotype of the individual when tested in direct staining technique, reactivity of absorbed mAb F11C was weaker on the O Le^b than on the A Le^b tissues.

To determine if the inhibition by Forssman Synsorb corresponds to a reactivity of mAb F11C with natural Forssman antigen, two kinds of tests were performed. In direct agglutination tests, F11C showed no reactivity with SRBC, which strongly express this antigen. In fluorescence inhibition tests, reactivity of mAb F11C with REGb cells was not inhibited by preincubation with SRBC.

Ultra-thin sections of rat normal colon were incubated with F11C and submitted to electron microscopic examination after immunogold labelling. Epithelial cells showed cytoplasmic labelling with dark supra-nuclear vesicules compatible with intermediate Golgi vesicules (Fig. 8).

Discussion

Although mAb F11C was obtained from a mouse immunized against a rat colon cancer cell line, it reacts with both human and rat gastrointestinal mucosa and colonic tumours. It was previously demonstrated that rat gastro-

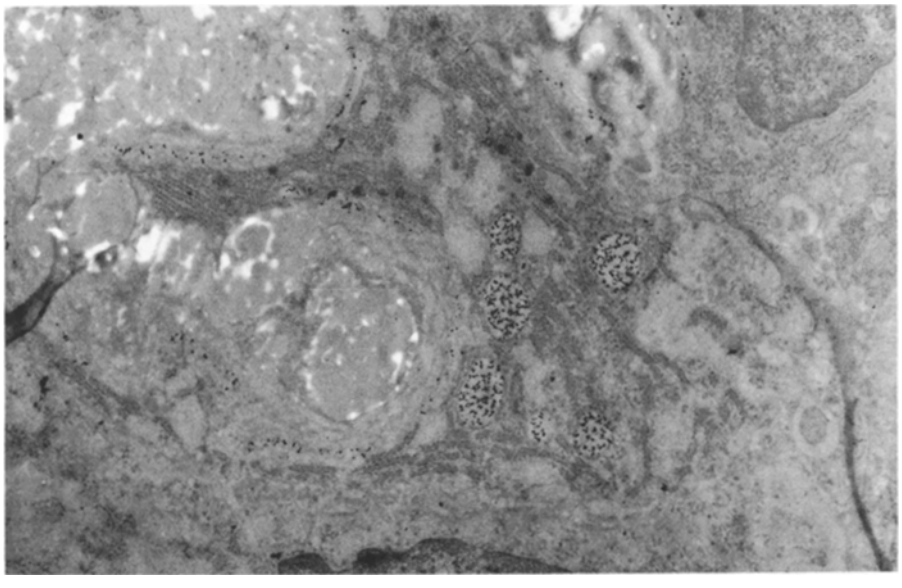

Fig. 8. Immunoelectron micrograph of a rat colon cancer tumour reacted with mAb F11C. Immunogold granules are mainly concentrated in vesicles of the Golgi region. ×12000

Table 5. Immunoreactivity of mAb F11C on human normal pylorus/duodenum sections after absorption with blood-group related oligosaccharides

Blood group related oligosaccharides		F11C staining after absorption[b]					
Trivial name	Structure[a]	A Le[b]			O Le[b]		
		M	G	B	M	G	B
A trisaccharide	αGalNac (1→3) βGal-R 2 1 αFuc	++	++	0	+	+	+
A linear type 2 trisaccharide	αGal*N*Ac(1→3)βGal(1→4)βGlc*N*Ac-R	++	+	0	0	0	0
A type 5	αGal*N*Ac(1→3) βGal(1→3) βGal-R 2 1 αFuc	0	0	0	0	0	0
A type 4	αGal*N*Ac(1→3) βGal(1→3) βGal*N*Ac-R 2 1 αFuc	+	+	0	0	0	+
A type 3	αGal*N*Ac(1→3) βGal(1→3) αGal*N*Ac-R 2 1 αFuc	++	+	0	+	0	+
A type 2	αGal*N*Ac(1→3) βGal(1→4) βGlc*N*Ac-R 2 1 αFuc	++	+	+	+	+	0
A type 6	αGal*N*Ac(1→3) βGal(1→4) βGlc-R 2 1 αFuc	+	+	0	+	0	++
Forssman trisaccharide	αGal*N*Ac(1→3) βGal*N*Ac(1→3) αGal-R	++	++	0	0	0	0
B trisaccharide	αGal(1→3) βGal-R 2 1 αFuc	++	++	0	++	+	++
H type 2 trisaccharide	αFuc(1→2) βGal(1→4) βGlc*N*Ac-R	+	+	0	+	0	+

[a] R, (CH2)8-CO-NH-Synsorb; Fuc, fucose; Gal, galactose; Glc*N*Ac, *N*-acetylgalactosamine

[b] F11C staining with the avidin-biotin peroxydase technique was measured with an arbitrary scale: 0, no staining; +, weak staining; ++, significant staining, +++, important staining. M, Staining of columnar mucous cells lining pyloric lumen; G, staining of cytoplasmic granules in the supranuclear area of columnar epithelial cells lining duodenal villi and of epithelial cells lining the Lieberkuhn glands; B, staining of Brünner glands

intestinal mucosa and colonic tumours could be labelled by antibodies raised against human antigens (Decaens et al. 1986), but this is probably the first report of the opposite situation. This could contribute to the particular reactivity of mAb F11C with human gastrointestinal tissues.

In a previous report (Caignard et al. 1990), the specificity of F11C antibody was studied extensively using a variety of oligosaccharides bound to beads which were used to absorb F11C reactivity against rat colon cancer cell surface. It was shown that F11C activity was inhibited by tetrasaccharides bearing the characteristic structure of the blood group A determinant, but only when they were monofucosylated and borne on type 3, 4 or 5 chains, but not type 1, 2 or 6 chains. Similar structures bearing blood group B or H determinants or the Forssman epitope did not modify F11C reactivity. It was concluded that mAb F11C reacted with a blood group A-related epitope, even if it did not agglutinate human blood group A erythrocytes and its activity was not inhibited by human saliva from A secretor individuals.

We have shown that F11C labels gastrointestinal tract of human donors independently of their blood group ABH or Lewis status in this study. This is at variance with other anti-A-related monoclonal antibodies which react exclusively or predominantly with the gastrointestinal mucosa from blood group A individuals (Bara et al. 1987). However, the immunohistological reactivity of mAb F11C, which selectively stains the supranuclear area (Golgi) and the brush border of enterocyte, is simi-

lar to that reported for mAb specific for the A epitope borne by type 3, 4 or 5 oligosaccharidic chains (Le Pendu et al. 1986; Vanak et al. 1989). F11C tissular reactivity differs from that of polyclonal or monoclonal antibodies reacting with blood group A epitope borne or type 1 or 2 chains, which selectively label intestinal goblet cells of jejunum, ileum and proximal colon in humans (Neutra and Leblond 1966; Szulman 1960) and in rat (Decaens et al. 1986): goblet cells were not labelled by F11C antibody. The data obtained from absorption experiments with oligosaccharides, as well as the subcellular localization of F11C reactivity, suggest that F11C could have the same reactivity as other antibodies reacting with blood group A determinant borne on type 3, 4, or 5 chains. However, unlike these antibodies, mAb F11C reacted with tissues and tumours from blood group B or O individuals. This disagreement between immunohistological data and absorption experiments suggests that mAb F11C recognizes a new epitope, cross-reacting with blood group A antigen, but independent from the genetically controlled ABH and Lewis systems. However, Forssman synthetic trisaccharides absorbed mAb F11C reactivity. Nevertheless, the epitope recognized by mAb F11C is probably also different from Forssman, since mAb F11C failed to react with SRBC, which strongly express the native Forssman antigen.

The molecules carrying the epitope recognized by mAb F11C were not precisely characterized. Glycolipids bearing blood group epitopes have been demonstrated (Clausen and Hakomori 1989). Lipid extracts from REGb cells failed to inhibit mAb F11C reactivity, suggesting that F11C epitope was not carried by glycolipids on the cell surface. In perchloric extracts from liver metastasis of PROb tumours, reactivity with mAb F11C was excluded from sepharose CL-6B, suggesting that the epitope was carried by a high molecular weight glycoprotein (more than 10^3 kDa). This could partly explain our failure to characterize F11c-reactive bands with the immunoblot technique, even with low gel densities and reducing conditions (results not shown). A similar failure has been reported by Jansson et al. (1989) with a high-molecular-weight glycoprotein identified by a rat mAb in rat colon carcinomas. This glycoprotein shares several physicochemical properties with the large molecule recognized by F11C murine mAb. However, neither its precise tissue or cellular localization, nor the nature of the recognized epitope has been reported.

In intestinal cells, F11C predominantly labels supranuclear structures, whose localization always correlated with the expected position of the Golgi apparatus. This was confirmed by electron microscopic examination, which showed dark immunogold granules scattered in supranuclear vesicles of medium size. The role of the Golgi apparatus in terminal glycosylation of glycoproteins is well known (Mollicone et al. 1986). A subsequent addition of other terminal saccharides, for instance sialic acid, could prevent the recognition of the same glycoproteins by mAb F11C when they migrate into the cytoplasm or to the cell surface. mAb F11C could be useful for studying Golgi apparatus activity in normal or pathological conditions.

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