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Fetal characteristics of small intestinal crypt cells

(fetal antigens/small intestine/crypt cells/monoclonal antibodies)

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ABSTRACT **Nine monoclonal antibodies were prepared against luminal membranes purified from rat intestinal cells at day 19 of gestation, and seven of them were found to define antigens common to adult crypt cells and fetal or embryonic intestinal epithelial cells. The FBB 2/29 antigen was first detected over the entire intestinal epithelial population at days 14–15 of gestation, a period of development characterized by formation of a stratified intestinal epithelium and differentiation of the surrounding mesenchyme. This antigen, identified as a set of high molecular mass proteins, became restricted to the crypt and lower villus cells after birth and was exclusively expressed by the crypt cells in adult intestine. It also was found to be expressed by the epithelial cells of the distal tubuli in the kidney of adult rats and by cultured human tumor colonic cells. The FBB 1/54/1, FBB 3/46, and FBB 3/78/9 antibodies stained only the epithelial cells present at the base of the villi in fetal intestine, starting at days 20–21 of gestation (about 1–2 days before birth), and stained the crypt and lower villus cells in newborn and adult intestine; these antigens may be regarded as specific markers for the developing crypt cells in fetal intestine shortly before birth. The FBB 1/20 and FBB 4/2 antigens were first detected on the fetal intestinal cells at day 18 of gestation; they were located over the entire epithelium in newborn rats and became restricted to the crypts after weaning. The FBB 2/28 antigen was expressed by the entire intestinal epithelium at all stages of development, starting from days 18–19 of gestation in the fetus. Two antibodies, FBB 3/4 and FBB 3/24, were found to be specific for lactase. These results have demonstrated the expression of cell- and tissue-specific components in rat intestine during early embryonic development and revealed a marked similarity in surface membrane antigens between fetal intestinal epithelial cells and adult crypt cells.**

In adult animals, the small intestinal epithelium is characterized by continuous cell renewal (1): undifferentiated crypt cells proliferate rapidly, move towards the top of the crypts where they undergo terminal differentiation, and then migrate along the villi until they are sloughed off into the lumen. Evidence has been presented for the existence of a common precursor, the crypt stem cell, for all differentiated cell types present in the intestinal epithelium (2). The morphological and structural characteristics (3) and the growth kinetics of the crypt cells (4) have been well documented, but their biological and functional properties are still largely unknown. In mice and rats, crypts are known to develop at the time of birth (5) from the epithelium present at the base of the villi, and experiments using mouse aggregation chimeras have demonstrated that the epithelium of each intestinal crypt is derived from a single progenitor cell (6). In the small intestine of fetal rats, DNA synthesis and cell proliferation occur in epithelial cells along the entire length of the villi (7), and the restriction of the proliferative zone to the base of the villi is first detectable at about day 21 of gestation, one day before birth. Earlier stages of development of the small and large intestine in fetal rats are characterized by the presence of an undifferentiated stratified epithelium (8), for which no specific markers have been described to date.

Previous studies with monoclonal antibodies prepared against cell membrane fractions obtained from intestinal cells of newborn rats and crypt cells of adult rats have demonstrated distinct patterns of antigen expression in crypt cells during postnatal development of the small and large intestines (9–12). These and other (13) studies have suggested also that adult crypt cells and fetal intestinal cells are not only ultrastructurally but also phenotypically similar. To further investigate the expression of fetal characteristics by adult crypt cells, I prepared and characterized a panel of monoclonal antibodies to luminal membrane components of fetal small intestinal cells. These antibodies were shown to define antigens expressed at specific stages of fetal intestinal development and retained by crypt cells at all times. Their limited distribution in other rat tissues or exclusive localization in the intestinal epithelium suggest that these newly identified cell surface-associated components represent specific products of at least some subpopulations of adult crypt cells.

MATERIALS AND METHODS

Membrane Purification. The luminal (brush border) membrane of intestinal epithelial cells was purified by the method of Kessler *et al.* (14), starting with homogenization of (*i*) the entire small intestines obtained from fetal and newborn (until day 30 after birth) rats and (*ii*) mucosal scrapings from adult rat small intestine. A mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, containing 50 μg of leupeptin, 50 μg of antipain, and 0.1 mg of aprotinin per ml) was added to all buffers and solutions used for homogenization and membrane purification.

Immunofluorescence Staining. Portions of small and large intestine from rats of different ages were rinsed with 0.155 M NaCl, cut into small fragments (0.5–1 cm long), embedded in OCT compound, and quickly frozen in liquid nitrogen. Sections 4–6 μm thick were spread on glass slides and allowed to dry at room temperature for at least 1 hr. Staining was by the double-antibody fluorescence technique as described (10).

Preparation and Characterization of Monoclonal Antibodies. BALB/c mice were immunized s.c. or i.p. with luminal membrane fractions purified from fetal rat intestine at day 19 of gestation (200 μg of protein per injection). Spleen cells were obtained from the immunized mice 3 days after administration of the last booster, and monoclonal antibodies were prepared as described (10, 15). Four independent fusions yielded ≈360 hybridoma cultures, which were tested for antibody production by an enzyme-linked immunoabsorbent assay (10) using fetal intestinal brush border membranes as target antigen and by immunofluorescence staining of frozen sections of small intestine from fetal, suckling, and adult rats.

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Nine of the selected hybridoma cultures were successfully cloned twice by dilution plating (10), and double-cloned hybridomas were used for immunoglobulin subtype determination (Table 1) and large-scale antibody production as ascites fluid. The monoclonal antibodies were purified from the ascites fluids by affinity chromatography on protein A-Sepharose 4B (IgG-type antibodies) (16) or rabbit anti-mouse Ig-Sepharose 4B (IgM-type antibodies) (10, 15) columns.

Antigen Identification and Characterization. The purified monoclonal antibodies were bound to CNBr-activated Sepharose 4B and tested for their ability to bind Triton X-100-solubilized intestinal brush border enzymes as described (10, 15).

Proteins of brush border membranes purified from fetal (day 20 of gestation), suckling (age 9 days), and adult (age 45 days) rat small intestine were labeled by reductive alkylation with [^{14}C]formaldehyde and cyanoborohydride (10, 15), solubilized with Triton X-100, and incubated with monoclonal antibodies bound to Sepharose 4B beads. The specifically bound antigens were separated by $NaDodSO_4$ slab gel electrophoresis under reducing conditions (50 mM dithiothreitol) and were detected on the dried gels by fluorography as described elsewhere (10).

RESULTS

Characterization of Antigens. On $NaDodSO_4$/polyacrylamide gels, many of the antigens appeared as multiple protein bands of relatively high molecular mass (see Fig. 1 and Table 1). Most of the radiolabeled FBB 2/29 and FBB 4/2 antigens barely entered the separation gel (Fig. 1, lanes 5–7 and 11–13, respectively), suggesting that these antigens either have a high molecular mass (in excess of 400 kDa) or are composed of subunits that remain aggregated in the presence of $NaDodSO_4$ and dithiothreitol. The FBB 3/4 and FBB 3/24 antigens appeared in all cases as a major protein band of 150 kDa and a minor band of >200 kDa detectable in some samples (data not shown). Sample-to-sample variability in the relative intensity of multiple antigen bands was observed, but no reproducible differences were noted among antigens purified from fetal, suckling, or adult rat small intestines. To determine the ability of the nine monoclonal antibodies listed in Table 1 to bind to known brush border enzymes, Triton X-100-solubilized luminal membrane proteins were incubated with antibodies bound to Sepharose 4B. After extensive washing, the beads were tested for the presence of various enzyme activities (maltase, glucoamylase, lactase, sucrase, alkaline phosphatase, trehalase, aminopeptidase, γ-glutamyltransferase). By using this assay, two antibodies specific for lactase (FBB 3/4 and FBB 3/24; see Table 1) were identified. None of the enzyme activities tested was found to be associated with any of the other antigens.

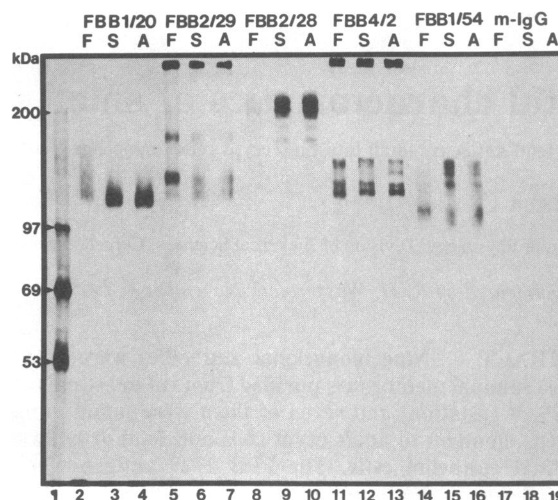

FIG. 1. Identification of antigens recognized by monoclonal antibodies in luminal membranes from fetal (day 20 of gestation) (lanes F), suckling (age 9 days) (lanes S), and adult (age 45 days) (lanes A) enterocytes. After labeling by reductive alkylation with [^{14}C]formaldehyde (10, 15), Triton X-100-solubilized membrane proteins were immunopurified with monoclonal antibodies bound to Sepharose 4B (10) and analyzed by $NaDodSO_4$ slab gel electrophoresis on 7.5% acrylamide gels under reducing conditions. Labeled antigens were visualized by fluorography. m-IgG, control (nonimmune mouse IgG bound to Sepharose 4B). ^{14}C-labeled molecular mass markers (arrowheads in lane 1) are myosin (200 kDa), phosphorylase B (97 kDa), bovine serum albumin (69 kDa), immunoglobulin heavy chain (53 kDa).

Antigen Expression During Development of Fetal Rat Small Intestine. At day 13 of gestation, the intestinal epithelium consists of a single layer of tall, columnar cells, surrounded by an undifferentiated mesenchyme (8). At this time, no

Table 1. Summary: Characterization of monoclonal antibodies to fetal intestinal epithelial cells

		Antigen		Immunofluorescence staining of small intestine§	
Antibody	Ig subtype*	Bands on gel,† $M_r \times 10^{-3}$	Enzyme activity‡	Fetal	Adult
FBB 1/20	IgM	112	ND	LM, entire epithelium	LM, crypts
FBB 2/29	IgM	>400, 160, 130	ND	LM(+BLM), entire epithelium	LM(+BLM), crypts
FBB 4/2	IgG1	>400, 137, 128, 122, 144	ND	LM, entire epithelium	LM, crypts
FBB 2/28	IgG1	195, 185	ND	LM, entire epithelium	LM, entire epithelium
FBB 1/54/1	IgG1	135, 128, 120, 100	ND	Base of villi	LM, crypts, lower villus
FBB 3/46	IgG1	ND	ND	Base of villi	LM, crypts, lower villus
FBB 3/78/9	IgG1	ND	ND	Base of villi	LM, crypts, lower villus
FBB 3/4	IgG1	150	Lactase	LM, entire epithelium	LM, villus cells
FBB 3/24	IgG1	150	Lactase	LM, entire epithelium	LM, villus cells

ND, none detected; LM, luminal (brush border) membrane; BLM, basolateral membrane.

*Typing of monoclonal antibodies in serum-free hybridoma-conditioned medium was done by using a "Mouse Immunoglobulin Subtype Identification Kit" from Boehringer Mannheim following the protocol suggested by the manufacturer.

†Molecular weights of protein (subunits) on $NaDodSO_4$/polyacrylamide gels under reducing conditions (see Fig. 1 and the text for details of the methods used).

‡Triton X-100-solubilized membrane proteins from fetal, newborn, or adult intestinal cells were incubated with monoclonal antibodies covalently bound to Sepharose 4B. Specifically bound antigens were tested for maltase, sucrase, lactase, trehalase, aminopeptidase, alkaline phosphatase, glucoamylase, γ-glutamyltransferase activities as described (10, 15).

§Immunofluorescence staining of proximal small intestine from fetal (day 19 of gestation) or adult 60-day-old rats fixed with 1% formaldehyde and stained by the double-antibody fluorescence technique as described in the legend to Fig. 2 and text.

staining of the intestinal mucosa was observed with any of the antibodies listed in Table 1 or with others previously produced and characterized (10, 15). During days 14 and 15 of pregnancy, a stratified epithelium several cells thick is formed; at the same time, adjacent mesenchymal cells become oriented parallel to it, and maturation of the muscular layers occurs. This stage of intestinal development was characterized by the appearance of the FBB 2/29 antigen; intense staining of the epithelial–mesenchymal junction and weaker staining of the entire epithelium were observed (Fig. 2b). Between days 15 and 17 of gestation, this antigen was also present around the first and second layers of mesenchymal cells surrounding the epithelium (Fig. 2 *b* and *c*), suggesting the possibility that it is at least in part secreted and capable of diffusing into the mesenchyme. The FBB 2/29 antigen represents the earliest differentiated cell product so far identified during intestinal development. It should be noted that, at days 14–15 of gestation, analysis of serial sections obtained from entire rat fetuses localized this antigen exclusively to the intestinal tract. At later stages of fetal intestinal development, the FBB 2/29 antibody stained mostly the luminal membrane of the intestinal epithelial cells (Fig. 2 *c* and *d*), although a weaker fluorescence was observed at the lateral and basal sides of the cells in most cases and sometimes also apparently in the cytoplasm (Fig. 2*e*).

The FBB 1/20, FBB 2/28, and FBB 4/2 antigens and lactase were first detectable by immunofluorescence staining at the surface membrane of the entire epithelial population in fetal intestine at days 18–19 of gestation (see data summarized in Fig. 4). Their appearance coincided with a period of rapid maturation and differentiation of the intestinal mucosa, which is associated with a transition from stratified to simple epithelium (8), formation of the villi, and appearance of

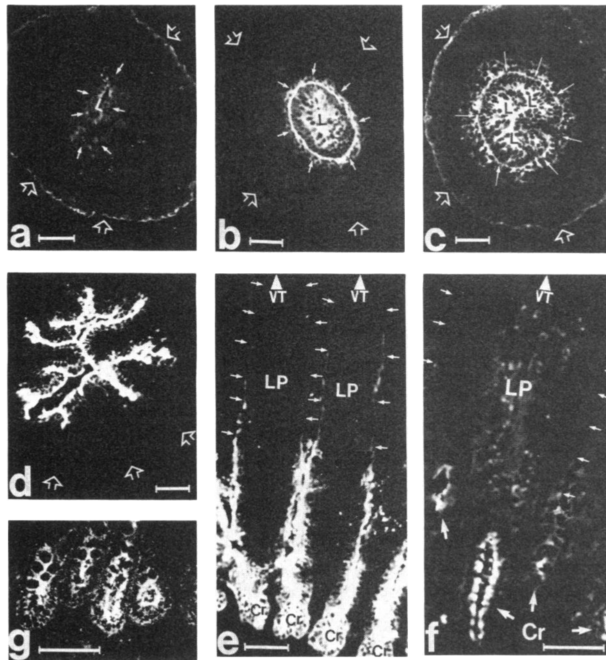

FIG. 2. FBB 2/29 antigen expression in fetal (*a–d*) suckling (*e*), and adult (*f* and *g*) rat small intestine. In all cases, frozen sections of proximal jejunum were fixed with 1% formaldehyde and stained with monoclonal antibodies by the double-antibody fluorescence technique (10). (*a*) Fetal small intestine, day 13 of gestation: the epithelium (small arrows) consists of a single layer of columnar cells arranged radially around the lumen (L); only a faint, nonspecific fluorescence (also observed in control sections stained with nonimmune mouse serum) is present on the epithelial cells and at the outer edge of the tissue sections (large arrows). (*b*) Fetal small intestine, day 15 of gestation: the epithelium is stratified, two to three cells thick, and surrounds a tiny lumen (L); strong, specific fluorescence is present over the entire epithelium and is most intense at the epithelial–mesenchymal junction (small arrows), extending to the first and second mesenchymal cell layers; the surrounding mesenchyme (delimited by large arrows) is unstained. (*c*) Fetal small intestine, day 17 of gestation: the epithelium is now up to 6–8 cells thick, but the lumen (L) is still very narrow; specific fluorescence is present over the entire epithelium and is most intense in correspondence with the epithelial cells facing the lumen; staining of the epithelial–mesenchymal junction and adjacent mesenchymal cells (small arrows) is less intense and sharply defined than at day 15 of gestation. (*d*) Fetal small intestine, day 19 of gestation: well-formed villi lined by a single layer of epithelium are present; the luminal aspect of the epithelial cells is intensely stained, but weak fluorescence is also present at the lateral and basal sides of the epithelial cells. (*e*) Suckling rat intestine 8 days after birth: the region of the crypts (Cr) is characterized by intense fluorescence, which extends to the lower half of the villi; no staining of the upper villus cells (small arrows) or of the lamina propria (LP) is apparent; arrowheads point to the position of the villus tips (VT). (*f*) Adult small intestine: strong, specific fluorescence is only present in correspondence with the crypt cells (Cr); the villus cells (small arrows) and the lamina propria (LP) are negative. (*g*) Adult crypts: staining is most intense at the luminal aspect of the epithelial cells, but specific, somewhat irregular staining of the lateral and basal sides of the cells is also observed. (Bars in *a–d* = 100 μm and in *e–g* = 50 μm.)

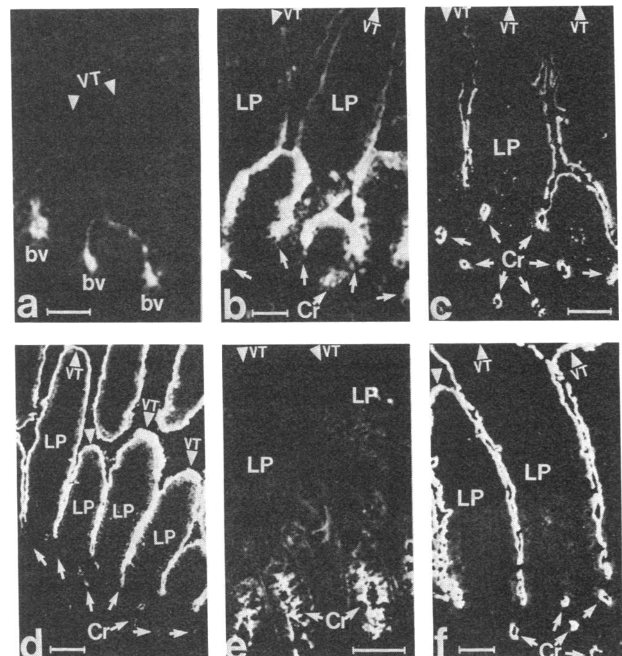

FIG. 3. Indirect immunofluorescence staining of fetal (*a*), suckling (*b* and *d*), and adult (*c*, *e*, and *f*) small intestines with monoclonal antibodies to fetal intestinal antigens. (*a*) Fetal intestine, day 18 of gestation, stained with the FBB 1/54/1 antibody: staining is present only at the base of the villi (bv). (*b*) Suckling rat jejunum, 8 days after birth, stained with the FBB 1/54/1 antibody: strong fluorescence is present in the region of the crypts (Cr, see arrows) and lower portions of the villi; the upper portions of the villi and the lamina propria (LP) are unstained. (*c*) Adult small intestine, stained with the FBB 1/54/1 antibody: the luminal membrane of the crypt (Cr, see arrows) and lower villus epithelial cells is specifically stained; the intensity of the fluorescence decreases towards the upper portions and tips (arrowheads, VT) of the villi. (*d*) Suckling rat jejunum, 6 days after birth, stained with the FBB 1/20 antibody: the luminal aspect of the epithelial cells covering the villi is intensely stained; staining of the crypt cells (Cr, see arrows) is faint and irregular. (*e*) Adult small intestine, stained with the FBB 1/20 antibody: specific fluorescence is confined to the luminal membrane of the crypt cells (Cr, see arrows). (*f*) Adult intestine, stained with the FBB 2/28 antibody: the luminal aspect of both crypt (Cr, see arrows) and villus epithelial cells is about equally intensely stained. (Bars in *a*, *b*, *e*, and *f* = 50 μm and in *c* and *d* = 100 μm.)

goblet and endocrine cells and of most typical brush border enzymes (12).

The FBB 1/46, FBB 1/54/1, and FBB 3/78/9 antigens were not detected until shortly before birth (days 20–21 of gestation), when staining of the epithelial cells present at the base of the villi was observed (Fig. 3*a*) (both surface membrane and cytoplasm appeared to be stained). Expression of the above antigens coincided with the restriction of the proliferative cell population to the lower region of the villi (7).

Antigen Distribution in Suckling and Adult Intestine. Previous studies (10, 12) have shown complex patterns of changes in the cellular distribution of many antigens during development of the small intestinal mucosa in suckling rats. Similar observations were made in the present study with most of the monoclonal antibodies listed in Table 1. By the end of week 1 after birth, the FBB 2/29 (Fig. 2*e*), FBB 1/46, FBB 1/54/1 (Fig. 3*b*), FBB 3/78/9, and FBB 4/2 antigens were found to be localized in the crypt and lower villus cells. The FBB 1/20 (Fig. 3*d*) and FBB 2/28 (data not shown) antibodies stained the luminal membrane of both crypt and villus cells until weaning (20–22 days after birth). As expected, antibodies to lactase stained exclusively the brush border membrane of the villus cells at all times (data not shown).

With the exception of the two lactase antibodies, all other antibodies prepared to fetal intestinal membranes were found to stain the crypts in adult intestine. The FBB 2/29 (Fig. 2*f*), FBB 1/20 (Fig. 3*e*), and FBB 4/2 (data not shown) antibodies were specific for the crypt cells and stained their luminal membrane. Weaker and irregular staining of the lateral–basal sides of the cells was also observed with the FBB 2/29 antibody (Fig. 2*g*). The FBB 1/54/1 (Fig. 3*c*), FBB 3/46, and FBB 3/78/9 antibodies stained the luminal membrane of the crypt and lower villus cells, and the FBB 2/28 antibody stained the entire epithelial population with about equal intensity (Fig. 3*f*).

The results of these studies are summarized in Fig. 4.

FBB 2/29 Antigen Expression in Nonintestinal Tissues and in Tumor Colonic Cells. This antigen was found to be absent in most adult nonintestinal tissues examined by immunofluorescence staining, which included skin, lung, bladder, pancreas, spleen, liver, heart, and skeletal muscle. All regions of adult large intestine were also negative. Staining of specific portions of the fetal kidneys was observed starting from days 16–17 of gestation, and in adult rats this antigen was found to be expressed by the kidney epithelial cells lining the thick ascending limbs and the distal convoluted tubuli. Staining appeared to be restricted to the apical cell surface. The only other adult rat tissue in which the FBB 2/29 antigen could be detected was the stomach, where ≈50–70% of the parietal cells localized in the upper (less mature) region of the gastric glands were stained.

Like the previously described YBB 3/10 antigen (9, 17), the FBB 2/29 antigen was found to be expressed by most human tumor colonic cell lines examined and by subpopulations of tumor intestinal cells in both human colonic adenocarcinomas and 1,2-dimethylhydrazine-induced rat intestinal tumors (unpublished data).

DISCUSSION

The results obtained in this study provided evidence for a close similarity in surface membrane components between adult crypt cells and fetal intestinal epithelial cells. By immunizing mice with luminal membrane fractions purified from fetal rat intestine, seven monoclonal antibodies were produced that define five surface membrane antigens that are expressed in adult crypt cells. In contrast, when luminal membranes obtained from adult intestinal epithelial cells were used previously to produce monoclonal antibodies to brush border membrane antigens, only 3 of 25 antibodies produced stained the crypt cells (15).

In adult animals, the intestinal crypts are populated by a heterogeneous cell population, including stem cells, committed but not yet differentiated cells, differentiated proliferative cells, and differentiated nonproliferative cells that are functionally different from villus cells (2–4). None of the monoclonal antibodies prepared and characterized as described in this paper appeared to be able to distinguish different crypt cell populations in rat small intestine at all stages of development.

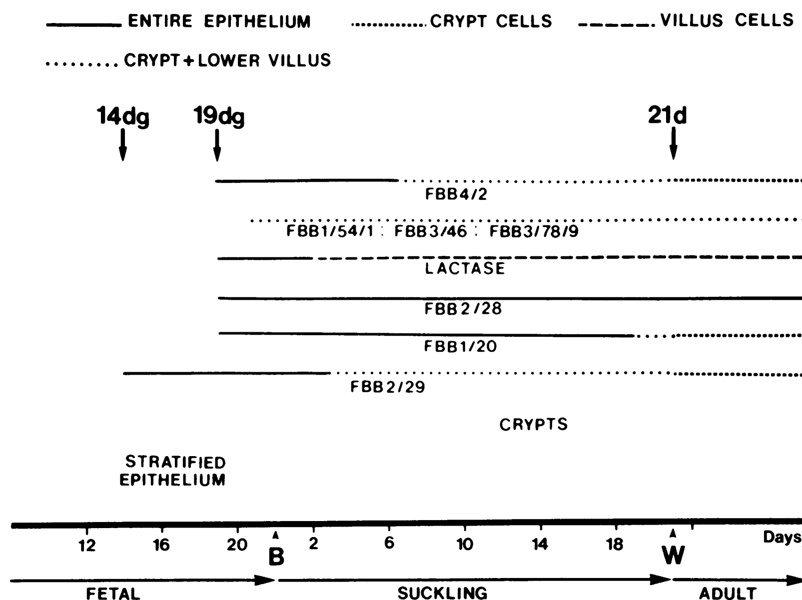

FIG. 4. Developmental changes in the distribution in rat small intestine of the antigens recognized by the monoclonal antibodies to fetal intestinal membrane components described in the text. This figure summarizes the results obtained by immunofluorescence staining of intestinal frozen sections (proximal jejunum). In all cases, staining was limited to the epithelial cells. Presence of the antigens over the entire epithelium, the crypt or villus cells exclusively, or the crypt and lower villus cells (see Figs. 2 and 3 for examples) is indicated. Also noted is the presence of a stratified epithelium (days 15–18 of gestation in the fetus) and of morphologically distinguishable crypt cells (starting from the first few days after birth). B, birth (at days 21 or 22 of gestation); W, weaning (around day 21 after birth).

The newly prepared and characterized monoclonal antibodies listed in Table 1 have provided important information regarding the expression of differentiated characteristics in the small intestinal epithelium during embryonic and fetal life. Previous studies (8) have identified a sequence of morphological events involved in the maturation of the fetal rat intestinal mucosa from a simple epithelium (present at day 13 of gestation) to a primitive stratified epithelium (between days 15 and 18 of gestation) to a fairly well-differentiated mucosa with villi lined by a single layer of columnar cells. The transition from stratified to simple epithelium, occurring at days 18–19 of gestation, has been identified as a major step in the maturation of the intestinal mucosa (8, 11). This morphological change is accompanied by formation of the villi and appearance of goblet and endocrine cells and of typical brush border enzymes (alkaline phosphatase, lactase, maltase), which are markers for absorptive villus cells in newborn and adult intestine. Note that this period of intestinal development was also found to correspond to the appearance of the FBB 1/20 and FBB 4/2 antigens, which are specifically expressed by the crypt cells in adult intestine (Fig. 4).

Earlier stages of fetal and embryonic development of the intestinal mucosa have been previously defined exclusively in morphological terms: the FBB 2/29 and YBB 3/10 (10) antigens are the only cell- and tissue-specific components that have been found associated with the intestinal epithelial cells before day 18 of gestation. In this study, the FBB 2/29 antigen was first found to be expressed at day 15 of gestation, when intense staining of the epithelial–mesenchymal junction and a weaker staining of the entire epithelium were observed (Fig. 2*b*). At this time, the FBB 2/29 antigen was exclusively localized in the intestinal tract. It has been previously shown that, at this stage of intestinal development, the epithelial cells undergo apparently rapid proliferation and form a stratified epithelium several cells thick (8). At the same time, maturation of the surrounding mesenchyme has been noted, supporting the conclusion from *in vivo* and *in vitro* studies (18, 19) that differentiation of the gut is, at least in part, controlled by the inductive action of the mesenchyme. Thus, the appearance of the FBB 2/29 antigen at day 15 of gestation in rat intestine, when the epithelial cells are still relatively undifferentiated morphologically and functionally, is suggestive of a role in the intense morphogenetic events occurring at that stage of intestinal development. The presence of this antigen at the basal aspects of the crypt cells in newborn (Fig. 2*e*) and adult (Fig. 2*g*) intestine would be consistent with its involvement in epithelial–mesenchymal interactions in mature intestine as well.

The FBB 2/29 antibody has many properties in common with the previously described YBB 3/10 antibody (10) and with a set of five monoclonal antibodies (CaCo 3/53, CaCo 3/61, CaCo 4/8, CaCo 4/23, and CaCo 4/94/2) prepared against brush border membranes purified from the human tumor colonic cell line CaCo-2 (17). Thus, all seven antibodies are (*i*) of IgM class, (*ii*) crossreact with human and rat antigens, (*iii*) recognize antigens of similar molecular mass in the human tumor colonic cell line CaCo-2 (17), (*iv*) stain the crypt cells in both rat and human (17) adult small intestine, (*v*) stain the rat large intestine exclusively during a limited period of development comprising the last few days of fetal life and the first 7–10 days after birth (9), (*vi*) bind specifically to most human tumor colonic cell lines examined, and (*vii*) stain rat and human tumor intestinal cells *in situ* (unpublished data). However, these seven antibodies differed markedly in their ability to bind to different human tumor intestinal cells in culture, suggesting that they have similar but not identical antigen specificities. The antigen(s) these antibodies define are not expressed in normal adult large intestine, and may represent oncofetal markers for rat and human tumor colonic cells.

Three of the monoclonal antibodies prepared and characterized in the course of this study (FBB 1/54/1, FBB 3/46, and FBB 3/78/9) appear to represent useful markers to study the early development of crypt cells. Discrete, well-defined crypts are shown to develop only shortly after birth in the rat (5), but in the duodenum of fetal rats, DNA synthesis and cell proliferation become confined to the epithelial cells present at the extreme base of the villi at days 21–22 of gestation (7). This time period coincided with the appearance of the FBB 1/54/1, FBB 3/46, and FBB 3/78/9 antigens (Fig. 4), which were also confined to the base of the villi (Fig. 3*a*) and then were expressed by the crypt cells at all stages of intestinal development (Fig. 3 *b* and *c*). These results suggest that compartmentalization of the proliferative cells at the base of the fetal villi immediately before birth is accompanied by the expression of new cell-surface antigens specific for these cells.

Taken together, the results obtained in this study suggest that formation of the crypts and development of the crypt cells are gradual processes during which embryonic-like intestinal cells are segregated in mucosal indentations and retain the ability to express fetal antigens and a rapid rate of proliferation.

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