LOCALIZATION AND ALTERATION OF MONO-, DI-, AND TRIFUCOSYL $\alpha 1 \rightarrow 3$ TYPE 2 CHAIN STRUCTURES DURING HUMAN EMBRYOGENESIS AND IN HUMAN CANCER

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Development and differentiation could be mediated by continuous changes of cell surface glycoconjugates through which cell-cell interaction may take place (1). Rapid, dramatic changes of specific carbohydrate structures defined by various antibodies during the development of the preimplantation (2–11) as well as the postimplantation (12) mouse embryo have suggested that these structures are signals for cell recognition. Despite the fact that a great deal of information has accumulated on cell surface carbohydrate changes during mouse embryogenesis, our knowledge of the cell surface glycoconjugates in human embryos is extremely meager. Only one series of studies by Szulman (13) indicates the occurrence of an orderly appearance and disappearance of blood group A, H, and Lewis antigens during human embryogenesis. A systematic knowledge of the changes of cell surface carbohydrates during organogenesis is important for understanding the oncofetal expression of tumor-associated antigens expressed in many human cancers. A series of glycolipids bearing the X determinant have been found to be accumulated in various types of human adenocarcinoma, and their structures have been identified as shown in Table I. A number of monoclonal antibodies produced by various investigators, originally assigned as being directed to "tumor-specific antigens", have been identified as being directed to the same structure as an embryonic antigen, SSEA-1 (14–16). The glycolipid lactofucopentaosyl(III)ceramide, designated as X hapten (17), was found to be greatly accumulated in various types of human cancer (18) and has the same terminal structure as SSEA-1. However, the antigen defined by the SSEA-1 antibody is widely distributed in normal human tissues, such as gastric epithelia and kidney tubules, as well as in a large variety of tumors (19). More recently, glycolipids with di- or trimeric X determinant, difucosyllacto*nor*hexaosylceramide ($III^3V^3Fuc_2nLc_6$) and trifucosyllacto*nor*octaosylceramide (III^3V^3-$VII^3Fuc_3nLc_8$), have been found to accumulate in colonic and liver cancers (20). Two monoclonal antibodies have been established; one antibody, FH4, is directed

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TABLE I

Reactivity of FH3 and FH4 to Fucosylated Type 2 Chain Structures

Antibodies	Ig class	Reactivities with:			
		$III^3FucnLc_4$*	$V^3FucnLc_6$‡	$III^3V^3Fuc_2nLc_6$§	$III^3V^3VII^3Fuc_3nLc_8$‖
FH3	IgG3	+	+	+	+
FH4	IgG3	−	−	+	+

* Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
3
↑
Fucα1

‡ Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
3
↑
Fucα1

§ Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
3 3
↑ ↑
Fucα1 Fucα1

‖ Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer
3 3 3
↑ ↑ ↑
Fucα1 Fucα1 Fucα1

towards di- or trifucosylated type 2 chains, and the other, FH3, is directed toward the X determinant as the common terminus of various fucosylated type 2 chains (21). Because it was noticed (21) that di- or trifucosylated type 2 chains defined by antibody FH4 were much more restricted than the X hapten structure defined by FH3 and because both FH3 and FH4 antibodies are IgG3 with comparable reactivities, a systematic study of the distribution of the antigens defined by these two antibodies in various stages of human development was undertaken using immunohistological techniques.

Materials and Methods

Tissues. 27 human embryos and fetuses[1] were collected at the Division of Human Embryology and Teratology, Department of Pediatrics, University of Washington. The ages of the embryos and fetuses were between 38 and 127 d, and were organized as described by Shepard et al. (22). Two newborn tissue samples were donated from the Department of Pathology, Children's Orthopedic Hospital, Seattle. Various normal adult tissues, including colon, stomach, ovary, lung, skin, breast, liver, spleen, and skeletal muscle, were obtained from specimens associated with the surgical removal of tumors. In total, 65 cases of various cancer tissues were obtained from the Department of Surgery, Swedish Hospital, Seattle, and from Japan Immunoresearch Laboratories, Takasaki, Japan. Formalin-fixed, paraffin-embedded sections of normal bladder, urethra, and testis were obtained from the Department of Urology, Tohoku University School of Medicine, Sendai, Japan.

Antibodies. IgG3 monoclonal antibodies FH3 and FH4 (21) were purified from culture supernatant or from ascites fluid by affinity chromatography on a protein A-Sepharose

[1] According to the definition of Moore (26), the time from 1 to 8 wk after fertilization is called the embryonic period, and 9–38 wk is called the fetal period. The terms embryo and fetus are approximately distinguished according to this definition.

column. Antibody adsorbed on protein A-Sepharose was eluted with 0.1 M citrate buffer, pH 4.2, dialyzed against phosphate-buffered saline (PBS),[2] and stabilized by addition of 0.1% bovine serum albumin. The final concentration of antibody was adjusted to 100 μg/ml, which was approximately equivalent to a sixfold dilution of ascites. The secondary antibody (rabbit Ig directed to mouse Ig) conjugated with horseradish peroxidase was purchased from Accurate Chemicals Co., Westbury, NY.

Preparation of Tissue Sections. Tissues were embedded in OCT compound (Tissue-Tek II Division, Miles Laboratories, Inc., Naperville, IL), frozen in dry ice–acetone, and stored in a Revco freezer at −80°C until use. Frozen sections (4–6 μm thick) were prepared on a cryostat. Each section was dried on objective glass for 30 min at room temperature, fixed in acetone at 4°C for 10 min, and washed with PBS at 4°C. Tissue sections (6–8 μm thick) were also prepared from formalin-fixed, paraffin-embedded specimens according to established procedure. Sections were deparaffinized in xylene for 5 min at 4°C, dehydrated in ethanol, and washed with PBS. Before antibody labeling, frozen sections and paraffin-embedded sections were blocked by incubation with 15% normal rabbit serum in PBS for 1 h at room temperature.

Immunostaining Procedure. After blocking with normal rabbit serum, sections were incubated at room temperature with the primary antibody solution (FH3 and FH4 as prepared above) for 18 h in a moist chamber. Sections were washed three times with PBS at 4°C (5 min per washing). Sections were then incubated with the peroxidase-conjugated second antibody (diluted 1:30) for 1 h at room temperature in a moist chamber and washed three times in PBS at 4°C as above. Bound antibodies were detected by incubating tissue sections in 0.05 M Tris/HCl buffer, pH 7.6, containing 0.03% 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) and 0.008% hydrogen peroxide. After 10 min, the sections were washed with distilled water, counter-stained with hematoxylin, dehydrated in ethanol, washed with xylene, and mounted. Two controls were performed for each staining experiment: sections treated without the primary antibody and sections treated with normal mouse serum. Specific tissue labeling was not observed after either of the above treatments.

Comparison of Immunostaining of Frozen Sections and Paraffin-embedded Sections. In view of a possible deletion of glycolipid antigens during preparation of sections from paraffin-embedded specimens, staining of the antigens in these sections was carefully compared with cryostat sections from frozen samples. There were no significant differences in immunoreactivity between frozen sections and paraffin sections for both fetal and newborn specimens.

Results

General Developmental Changes of Antigens Defined by FH3 and FH4 Antibodies

Strong staining of embryonic and fetal tissue by both FH3 and FH4 antibodies was limited to gastrointestinal and urogenital epithelia,[3] and the patterns of antibody reactivity showed dramatic changes depending on the stage of the embryo. The X determinant defined by the FH3 antibody showed a wider distribution than the multimeric X antigen defined by the FH4 antibody. Both antigens were absent or present in relatively low concentration during early embryonic development (up to 40–50 d), showed maximum expression at a specific stage of development (mostly 50–70 d), and regressed upon further differentiation and development. The antigen defined by FH3 appeared about

[2] *Abbreviations used in this paper:* PBS, phosphate-buffered saline (10 mM sodium phosphate buffer, pH 7.2, containing 0.9% NaCl).

[3] Since the staining behavior and its change in urogenital epithelia associated with development from pronephros through metanephros are highly complex, and since there was a great deal of variation in the staining of a variety of kidney tumors, this subject will be described elsewhere.

TABLE II

Reactivities of Digestive Organs of Human Embryo with FH3 and FH4

Antibody	Organ	Stages of embryo (in days of gestation)																						
		38	40		42	52	53	53	54			57	58	59	67		69	72	84		110	127	Newborn	
		P*	F‡	P	P	F	P	P	F	P	P	P	P	P	F	P	F	F	F	P	P	P	P	P
	Esophagus	+	−							+				+									+	+
	Stomach	++	++	++	++				++	++				++			++		++	++	+	−,+§	−,+,++‖	−,+,++‖
	Small intestine	+	−	−					++	++	−	+	+	+	−,++¶	+	−,+¶	−,+	−,+¶	−,+	−,±	−,+	−,++**	−,++**
FH3	Colon	±	−			±		+	±	±				−	−		±					−	−	−
	Liver											−						−					−	
	Gall bladder											±												
	Pancreas	±	−		+		±			±				−								±		−,±##
	Esophagus	−	−							±				−									±	−
	Stomach	++	++	++	++				++	++				++			++		++	++	+	−,+§	−,+,++‖	−,+,++‖
	Small intestine	−	−	−					−	±	−	±	++	+	−,++¶	+	−,+	−,++	−,++	−,+	−,±	−,+	−,++**	−,++**
FH4	Colon	−	−			−		+	−	+				−	±		−					−	−	−
	Liver											−						−					−	
	Gall bladder											±												
	Pancreas	−	−		−		±			±				−								−		−

* Paraffin section.

‡ Frozen section.

§ +, Deeply folded portion; −, superficial portion.

‖ −, chief cells; +, surface mucous cells; ++, parietal cells and other epithelial cells.

¶ Some unidentified cells are strongly positive.

** ++, Paneth's cell, basal granular cells; −, other epithelial cells.

±, Cells of Islets of Langerhans; −, other cells.

TABLE III

Reactivities of Circulatory, Respiratory Organs, Adrenal, Skin, and Nervous System of Human Embryo with FH3 and FH4 Antibodies

Antibody	Organ	Stage of embryo (in gestation days)																Newborn	
		38	40	52	53	54		58	59	64	67	69	72		84	110	127		
		P	F	F	P	F	P	P	P	P	F	F	P	F	P	P	P	P	F
FH3	Heart, artery and vein	−	−	−	−	−	−	−	−								−	−	
	Lung airway					+	+		−		−	−	±	±			−	−	
	Trachea													+		+		+	
	Cerebrum, cerebellum and pons			−	−							−	−			−	−		−
	Spinal cord	−	−	−	−							−	−				−		−
	Adrenal	−	−	−		−	−,±*	−,±*	−,±*	−,+*	−,±*			−,±*	−,±*			−,+‡	
	Spleen														−			−	
	Skin	+§			+§		+§		+§									+¶	
	Bone and Muscle	−	−	−	−			−	−				−					−	
FH4	Heart, artery and vein	−	−	−	−	−	−	−	−								−	−	
	Lung airway					−	±		−		−	−	−				−	−	
	Trachea												+			±	−	−	
	Cerebrum, cerebellum and pons			−	−							−	−			−	−		−
	Spinal cord	−	−						−			−	−				−		−
	Adrenal	−	−	−		−	−,±*	−,±*	−,±*	−,+‡	−,±*			−,±*	−,±*			−,±*	
	Spleen														−			−	
	Skin	+§			+§		+§		+§									−¶	−
	Bone and muscle	−	−	−	−			−	−				−					−	

* Adrenal cortex, −; medulla, ±.
‡ Adrenal cortex, −; medulla, +.
§ Entire layers of skin, +.
‖ Duct of ecrine sweat gland, +; epidermis, +; corium, −.
¶ Entire layer, −.

2 wk earlier than the antigen defined by FH4 and regressed later than that defined by FH4 or was continuously expressed after birth. Thus, the FH4 antigen was often limited to a specific type of cell in certain epithelial tissues (see below). The reactivities of the FH3 and FH4 antibodies with fetal, newborn, and adult tissues are summarized in Tables II, III, and IV.

Weak staining was also observed in the pulmobronchial epithelia, adrenal medulla, and entire layers of the epidermis by both FH3 and FH4 in embryonic stages. The reactivities of FH4 regressed completely in newborn tissues. A weak staining with FH3 remained, however, in the adrenal medulla as well as in sebaceous and sweat glands of the epidermis (Table III).

The antigens recognized by FH3 and FH4 were not found in connective tissues, nervous tissues (brain and spinal cord), tissues of the circulatory system (heart, arteries, and veins), skeletal tissue (bones and muscles), or other parenchymatous organs, such as liver and spleen, at any stage of development. Distribution of the antigen defined by FH4 in tissues of newborns and adults was limited to specific types of cells in gastric and intestinal epithelia and the antigen was completely absent in colonic epithelia.

TABLE IV
Reactivities of Human Adult Tissues with FH4 Antibody

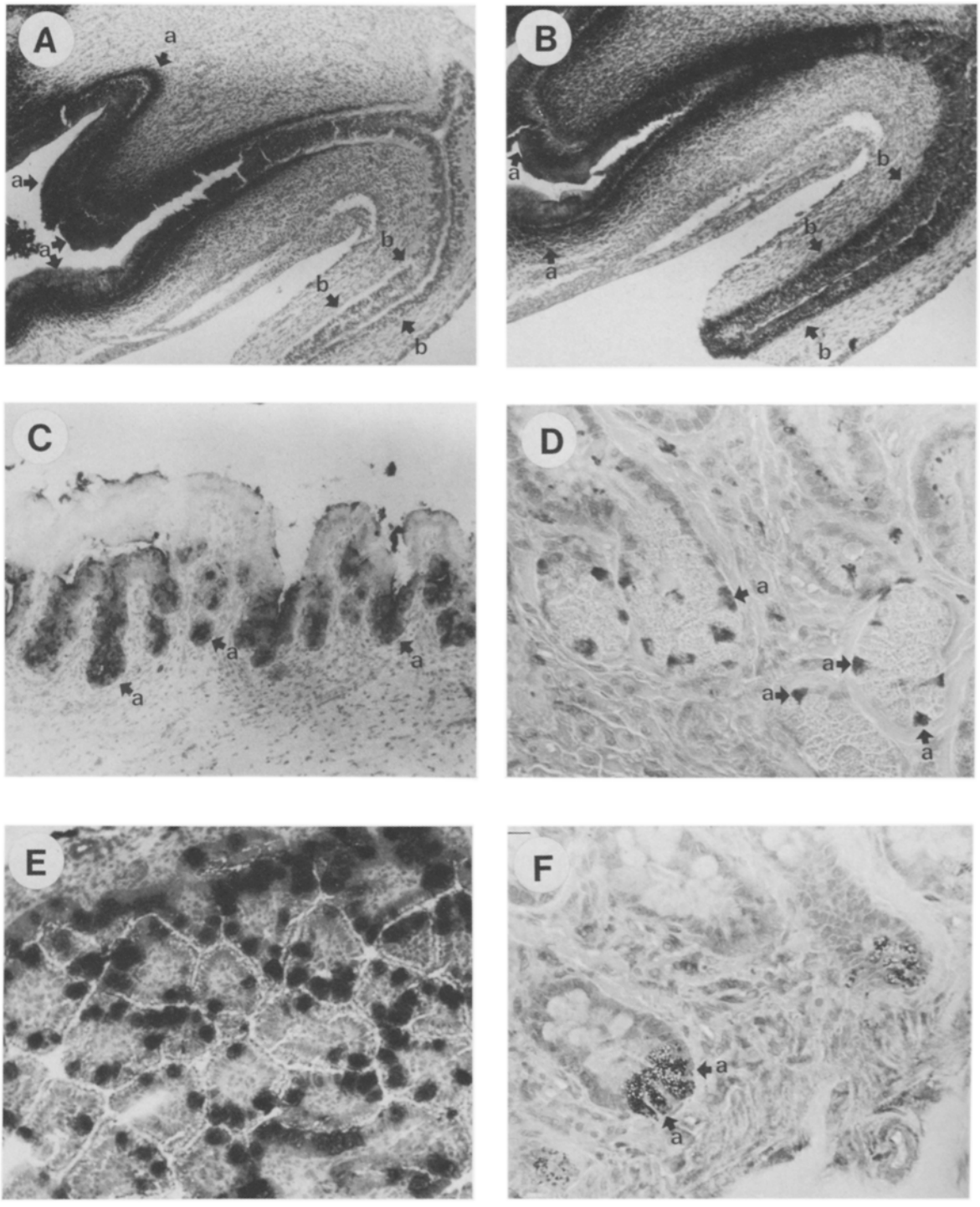
Tissue	Reactivity (number of cases)	
	Paraffin section	Frozen section
Stomach		
Surface mucous cells	−,±*	±
Chief cells	−	−
Parietal cells	++ (4)	+ (3)
Pyrolic gland	++	+
Other cells	−,+‡	±
Small intestine		
Paneth's cells	++	++
Basal granular cell	++	++
Cuticular or brush border cells	− (2)	− (1)
Globlet cell	−	−
Colon		
Crypt cells	−	−,+§
Cuticular cell	− (4)	− (7)
Globlet cells	−	−
Mammary gland		
Parenchyma and duct cells	− (3)	− (4)
Lung	− (3)	− (4)
Skin	− (2)	− (1)
Testis	− (3)	
Prostate	− (4)	
Muscle		− (2)

A single grading indicates that all cases examined showed the same degree of reactivity.
* −, Three of four cases; ±, one of four cases.
‡ −, Three of four cases; +, one of four cases.
§ −, Six of seven cases; +, one of seven cases.

Distribution of Antigens Defined by FH3 and FH4 Antibodies in Gastrointestinal Tissues of the Human Embryo

The strongest expression of the antigens defined by FH3 and FH4 antibodies was found in stomach epithelia. The reactivity was intense in all cell populations of stomach epithelia at 35 d of development (Fig. 1 *A* and *B*, arrow *a*). This intense reactivity continued up to 90 d. After 100 d, the reactivity of many stomach cell populations regressed, became limited to a deep layer at later stages (Fig. 1 *C*, arrow *a*), and was finally limited to the parietal cells (Fig. 1 *D*) and pyloric glands (data not shown) of newborn and adult gastric epithelia. The chief cells and other epithelial cells in stomach epithelia became completely negative in newborn and adult epithelia (Table IV, Fig. 1 *D*).

Small intestinal epithelial cells were not stained by FH4 at 40 d of development (Fig. 1 *A*, arrow *b*), but were strongly stained by FH3 (Fig. 1 *B*, arrow *b*). At later stages, the majority of small intestine epithelial cells became negative; however, a strong reactivity with both FH3 and FH4 was observed in some unidentified cells of fetal tissue (Fig. 1 *E*). The antigens defined by both FH3 and FH4 in the majority of cell populations regressed subsequently, becoming restricted only to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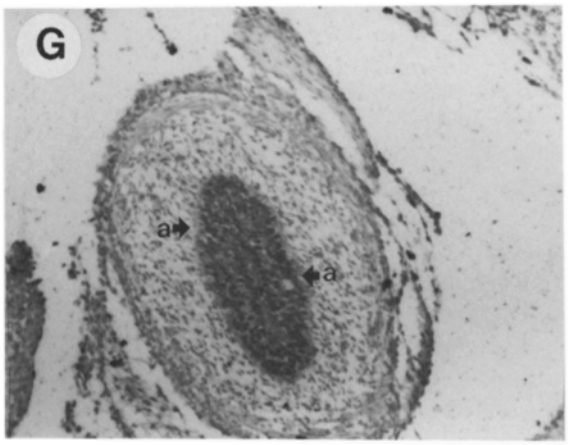
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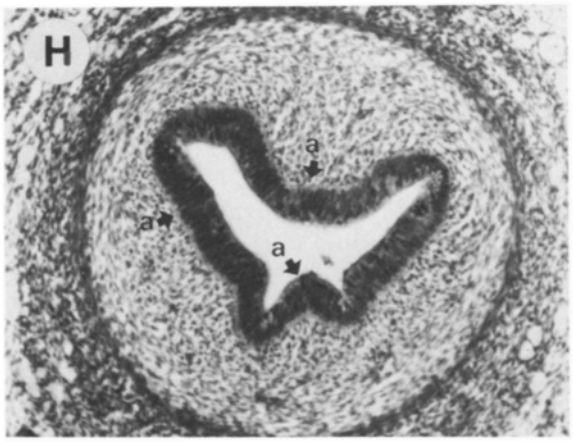
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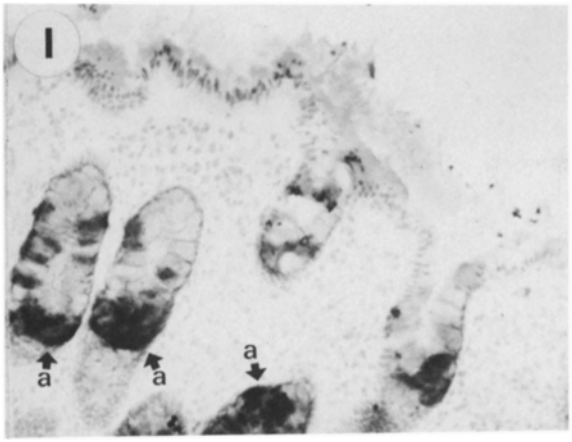
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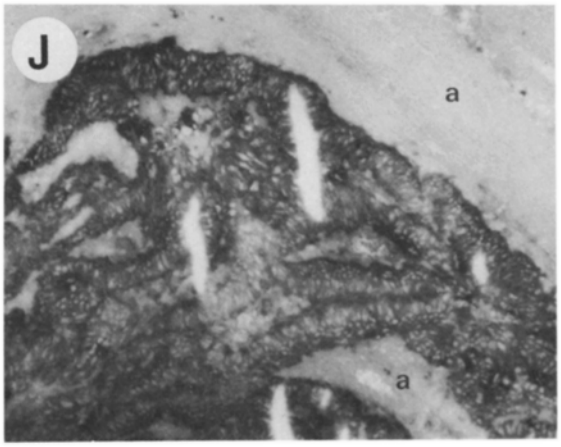
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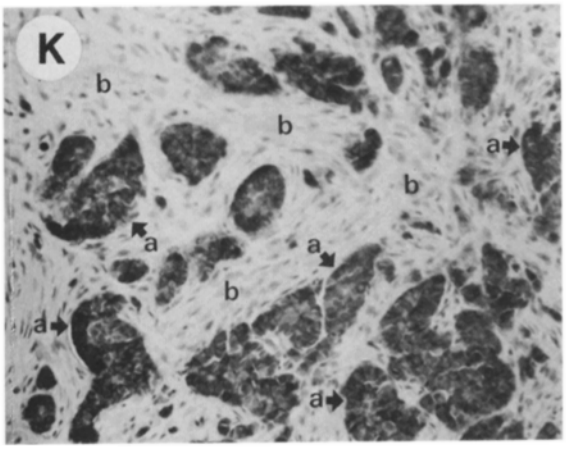
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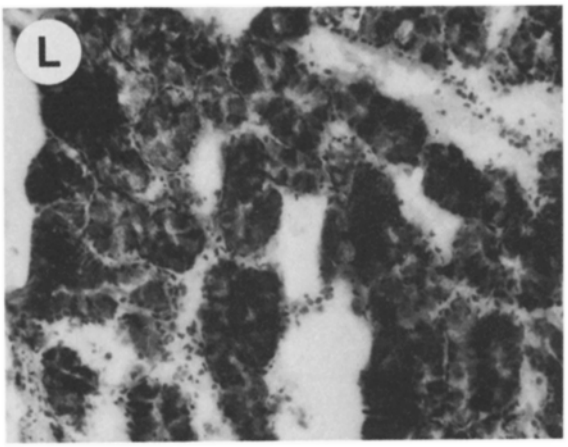
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Paneth's cells and basal granular cells in the cryptic region of the newborn and adult small intestine (Fig. 1 *F*, arrow *a*; Table IV).

The reactivity of FH3 and FH4 with colonic epithelial cells showed a similar pattern. These cells were weakly positive in the early embryo, became positive and maximally expressed between 50 and 60 d (Fig. 1 *H*, arrow *a*), and subsequently regressed. At later stages, FH3 antigen expression was limited to crypt cells in the newborn and adult (Fig. 1*I*, arrow *a*). Crypt cells in newborn and adult tissues were not stained by FH4 (data not shown). The antigen defined by FH3 was present in esophageal epithelia in the early fetus (Fig. 1 *G*) as well as in the newborn and adult (data not shown), but the antigen defined by FH4 was absent in esophageal epithelia throughout all stages of embryonic development examined. A similar distribution was found in the fetal pancreas; the ductal epithelium was weakly stained by FH3, but not by FH4, and became completely negative in the newborn. It is interesting to note that the antigen defined by FH3 was present in cells of Langerhans' islets, but that defined by FH4 was absent (data not shown). Neither antigen was detected in the various stages of liver development (data not shown).

Respiratory Organs and Other Tissues

The epithelial cells of trachea and secretory glands of bronchus were stained by FH3, but not by FH4 in newborns. No fetal tissue of bronchus was available. The epithelial cells of the lung airway found in fetal lung bud were positive with FH3, but were negative with FH4 (data not shown). The entire layer of epidermal tissue of fetal age was positive with both FH3 and FH4, but the reactivity with FH4 became negative in adult tissue. A weakly positive reaction was observed with FH3 in sebaceous glands and in ecrine sweat glands; no reactivity was observed with FH4 (data not shown). Heart, aorta, arteries, veins, cerebrum, cerebellum, pons, spinal cord, muscles, and bones were all negative with both FH3 and FH4 throughout all developmental stages (data not shown).

FIGURE 1. Staining of human gastrointestinal tissue at different stages of development and human cancers by FH3 and FH4 antibodies. (*A*) Paraffin-embedded section of gastric and intestinal tissue of the foregut of a 38-d-old embryo stained with FH4. Arrow *a* indicates gastric epithelia. Arrow *b* indicates intestinal epithelia. Note that only gastric epithelia are strongly stained. (*B*) The same section as in *A* stained by FH3. Note that both gastric and intestinal epithelia are strongly stained. (*C*) Paraffin-embedded section of gastric epithelia of 120-d-old fetus stained by FH4. Only deep foveola are strongly stained (arrow *a*). (*D*) Frozen section of adult gastric epithelia stained by FH4. None of the tissue is stained, except for the parietal cells (arrow *a*). (*E*) Paraffin-embedded section of intestinal epithelia of 70-d-old fetus stained by FH4. Many groups of cells, not identified exactly, are strongly stained, but unstained cells can also be seen.(*F*) Paraffin-embedded section of newborn intestinal epithelia stained by FH4. The majority of cells are negative and only Paneth's cells and basal granular cells (arrow *a*) are stained. (*G*) Paraffin-embedded section of esophagus tissue of 60-d-old embryo stained by FH3. Positive staining is localized in the solid epithelial area as indicated by arrow *a* (internal lumen of esophagus is not yet opened at this stage). The same section was not stained by FH4 (not shown). (*H*) Frozen section of colonic tissue of 50-d-old embryo stained by FH3. Only epithelial tissue (arrow *a*) is stained. The same section was not stained by FH4 (not shown). (*I*) Frozen section of adult colonic epithelia stained by FH3. Crypt cells (arrow *a*) are clearly stained. The same section was not stained by FH4 (not shown). (*J*) Frozen section from adenocarcinoma of colon stained by FH4. Stroma (*a*) is not stained. (*K*) Frozen section from infiltrative ductal carcinoma of breast stained by FH4. Only tumor cells (arrow *a*), but not stroma (*b*) are stained. (*L*) Frozen section of tubular adenocarcinoma of stomach stained by FH4.

Distribution of the Antigen Defined by FH4 in Human Cancer in Comparison with Adult Normal Tissues

Gastric cancer. 8 out of 11 cases of paraffin-embedded sections and four out of five frozen sections of gastric cancers were strongly or clearly positive with FH4 (Table V). All the positive cases were tubular or papillary adenocarcinoma, while all the negative cases were undifferentiated adenocarcinoma (Table V). A typical positive example is shown in Fig. 1*L*. In normal adult gastric epithelia, only parietal cells and pyloric gland cells were consistently positive in both paraffin and frozen sections (Table IV).

Colonic cancer. Three out of three cases of paraffin-embedded sections and seven out of eight cases of frozen sections were strongly or clearly positive with FH4 (Table V). A typical section from colonic cancer is shown in Fig. 1*J*. Only one case was negative, which was not correlated with the histological characteristics of the case. Normal parts of colonic epithelia were all negative, including crypt cells in both paraffin and frozen sections, except for one case that showed a positive reaction in the crypt cells.

Breast cancer. Three out of four cases of both paraffin-embedded and frozen

TABLE V
Reactivities of Human Cancers with FH4 Antibody

	Tumor tissue	Reactivity (number of cases)	
		Paraffin section	Frozen section
Stomach cancer	Adenocarcinoma	++ (5/11)	++ (2/5)
		+ (3/11)	+ (2/5)
		− (4/11)	− (1/5)
Colon cancer	Adenocarcinoma	++ (1/3)	++ (5/8)
		+ (2/3)	+ (2/8)
		− (0/3)	−
Ovary	Clear cell carcinoma		− (1/1)
	Serous cystoadenocarcinoma		− (1/1)
	Mucinary cystoadenocarcinoma		− (1/1)
Testis	Seminoma	− (3/3)	
Breast	Infiltrating ductal carcinoma	++ (3/4)	++ (3/4)
		− (1/4)	− (1/4)
	Lymph node metastasis	++ (2/2)	++ (2/2)
Lung	Squamous cell carcinoma	− (2/2)	− (4/4)
Gall bladder	Adenocarcinoma		+ (1/1)
Prostate	Adenocarcinoma	− (3/3)	
	Benign adenoma	− (3/3)	− (1/1)
Skin	Malignant melanoma	− (1/1)	− (1/1)
Muscle	Leronmyosarcoma		− (1/1)

sections of infiltrating ductal carcinoma were positive. A typical case is shown in Fig. 1 *K*. Metastatic lesions in lymph nodes were also strongly positive (Table V).

Kidney cancer. A great deal of variation in the staining of kidney tumors was observed, which will be described elsewhere.[3] These results may appropriately reflect the normal variation in the reactivity of FH3 and FH4 with urogenital epithelia during their development from pronephros to mesonephros to metanephros (manuscript in preparation).

Other cancers. Various types of ovarial carcinoma, seminoma, lung squamous cell carcinoma, prostate adenocarcinoma, malignant malanoma, and leiomyosarcoma were all negative.

Discussion

The reactivity of FH3 and FH4 with the developing human embryo and fetus can be summarized as follows: (*a*) The antigens detected by both FH3 and FH4 are most strongly expressed in the epithelial cells of the gastrointestinal and urogenital[3] organs at specific stages of development. Expression of these antigens, particularly FH4, regressed upon further development with functional differentiation, and disappeared from most of the epithelial cell populations of those tissues with the exception of a few specific types of cells in normal adult tissue. (*b*) The antigen defined by FH3 appeared at an earlier stage of fetal development than the antigen defined by FH4; however, the antigen defined by FH4 regressed rapidly and completely at later stages of development and its expression became highly limited in adult epithelial tissues. The antigen defined by FH3 remained in a wider variety of cells than the antigen defined by FH4 in developed tissues. (*c*) In adult epithelial tissue, the antigen defined by FH4 was found to be limited to parietal cells and pyloric glands of stomach epithelia, Paneth's cells and basal granular cells of the intestine, and proximal convoluted tubules of the kidney.[3] The antigen was not detected by FH4 in the entire colonic epithelia, including crypt cells. The crypt cells in colonic epithelia were positive with FH3.(*d*) A clear differential reactivity was found between the FH3 and FH4 antibodies in sebaceous and sweat glands of the epidermis, Langerhans' islet of the pancreas, adrenal medulla, esophageal epithelia, bronchial epithelia, airway of lung buds, and vaginal epithelia. Cells in these tissues were clearly or strongly stained by FH3, but were not reactive with FH4 throughout fetal development as well as in newborn and adult tissues.

The antigens defined by FH3 and FH4 may not be expressed or may be expressed weakly in preimplantation human embryos, in contrast to mouse embryos. This possibility is suggested by the absence of SSEA-1 in undifferentiated human teratocarcinoma and its appearance on differentiation (7), in contrast to a strong expression of SSEA-1 in undifferentiated mouse teratocarcinoma and its decline on differentiation (8, 12). There were no cases in which tissues were negative at fetal stages, folllowed by increasing expression in newborn or adult tissues.

Maximum expression of the antigens defined by FH3 and FH4 was found in the epithelia of tissues at a specific developmental stage, mostly 40–80 d. This may indicate that these structures are essential signals for cell adhesion and recognition, which could be an essential step for further differentiation of fetal

epithelial cells into a variety of functionally differentiated adult epithelial cells. Despite our lack of knowledge of a functional role of these structures, such a dramatic change, with maximum expression at a defined stage followed by orderly disappearance, suggests a vital function for these structures in "chemical conversation" (23) between embryonic cells during epitheliogenesis.

Since a majority of human cancers are derived from gastrointestinal, urogenital, and pulmobronchial epithelia, in which X or oligomeric X antigens are strongly expressed at embryonic to fetal age, an intense reexpression of these antigens in a large variety of human cancers strongly suggests that these structures are essentially oncofetal antigens. Only those cancer cells derived from epithelial tissues that express a high level of these antigens during a certain stage of development showed a strong reactivity with FH3 and FH4 antibodies. Interestingly, differentiated papillary adenocarcinoma of stomach expresses the antigen defined by FH4, whereas undifferentiated adenocarcinoma does not express this antigen. Thus, reexpression of the structure defined by FH4 in tumors could be associated with retrogenesis of tumor cells to a certain stage of organogenesis rather than to a stage of the very early embryo. If retrogenesis of cells occurs to a very early, undifferentiated stage of embryonic tissue, tumor cells may not express the FH4 antigen (Fig. 2).

Immunostaining of both X antigen and multimeric X antigen in paraffin-embedded sections gave similar or identical results to those from immunostaining of frozen cryostat sections. This may indicate that (*a*) glycolipid antigens are not diminished during preparation of paraffin sections, or (*b*) the antigens may be carried by glycoproteins that are not diminished by preparation of paraffin sections. Since many lacto-series carbohydrates are carried by both glycolipids and glycoproteins (24), it is reasonable to assume that the antigens detected by immunostaining of fetal tissue sections are, in fact, glycoproteins with properties similar to "embryoglycan" or lactosaminoglycans (25).

Oncofetal expression of both FH3 and FH4 should be based on a common mechanism for activation of $\alpha 1 \rightarrow 3$ fucosyltransferase in fetal epithelial tissue and in certain types of human cancer. The fucosyltransferase that makes FH4 antigen can be distinguished from that for synthesis of FH3 antigen, and the genetic regulation of these enzymes is a crucial mechanism controlling embryogenesis as well as oncogenesis.

Summary

Distribution patterns of specific fucose-containing antigens having X determinant (Galβ1 $\rightarrow$ 4[Fucα1 $\rightarrow$ 3]GlcNAc) as well as the di- or trimeric X determinants (Galβ1 $\rightarrow$ 4[Fucα1 $\rightarrow$ 3]GlcNAcβ1 $\rightarrow$ 3Galβ1 $\rightarrow$ 4[Fucα1 $\rightarrow$ 3]-GlcNAc) in the developing human embryo and fetus and in human cancer have been examined using immunohistological techniques. Tissue sections were stained with monoclonal antibody FH3, which defines X determinant, and with monoclonal antibody FH4, which defines di- or trimeric X determinant. The following general trends in the expression of the antigens defined by FH3 and FH4 have been observed: (*a*) A well-organized, orderly appearance and disappearance of the antigens was observed during the histogenesis of various epithelia of gastrointestinal and other organs. The developmental stage exhibiting the

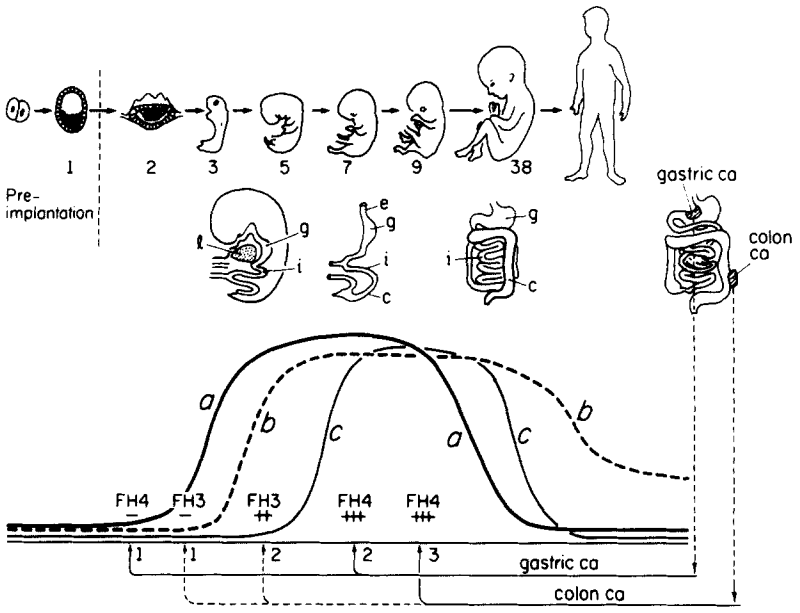

FIGURE 2. Stage-dependent expression of X antigen (defined by FH3) and di- or timeric X antigen (defined by FH4) in gastrointestinal epithelia during human development, and retrogenetic expression of the antigens in gastrointestinal tumors. X and di- or trimeric X antigens may not be expressed in preimplantation embryo, although the X antigen is highly expressed in mouse preimplantation embryo (8–10). This possibility is suggested by the absence of the X antigen in undifferentiated human teratocarcinoma and the induction of antigen synthesis on differentiation, which is the opposite of mouse teratocarcinoma (7). The antigens, however, are expressed in various tissues of human postimplantation embryos and fetuses. Curves *a* and *c* illustrate the change in FH4 antigen expression in gastric and colonic epithelia, respectively. Curve *b* represents the change in FH3 antigen expression in colonic epithelia. FH4 expression in colonic epithelia reaches its maximum between 7 and 9 wk, then regresses almost completely, while the FH3 antigen does not regress and remains in the crypt cells. The FH4 antigen is strongly expressed in differentiated gastric cancer, suggesting that antigen retrogenesis occurs to the point at which FH4 expression is at its maximum (arrow *2*). However, FH4 antigen expression is negative in undifferentiated gastric cancer because antigen retrogenesis occurs to a point at which FH4 is not yet expressed at the very early stages of embryogenesis (arrow *1*). Both FH3 and FH4 antigen expression in colonic cancer could be strong if retrogenesis of the antigen expression occurs to the point at which the FH3 antigen is active. The numbers at each stage of development represent the number of weeks from fertilization. *g*, gastric epithelia; *l*, liver; *i*, intestine; *c*, colon. The ordinate indicates an arbitrary unit of antibody reactivity.

maximum antigen expression is different for each organ. (*b*) The X determinant defined by FH3 was expressed ~2 wk earlier than the di- or trimeric X determinant defined by FH4, and the antigen defined by FH4 regressed more rapidly and more completely than the X determinant defined by FH3 on further development of epithelial tissue. Thus, expression of the FH4 antigen is highly limited to specific types of cells in newborn and adult epithelial tissues. (*c*) The antigen defined by FH4 was strongly expressed in the majority of tubular and papillary adenocarcinoma of stomach, adenocarcinoma of colon, and infiltrating ductal carcinoma of breast and its metastatic lesions. No antigen was found in poorly differentiated stomach adenocarcinoma, squamous lung carcinoma, and many other types of tumors from ovary, testis, prostate, skin, and muscle. The

presence of the antigen defined by FH4 is therefore limited to carcinoma of the stomach, colon, and breast and can be regarded as a retrograde expression of the antigen to a certain stage of fetal development in which expression of this antigen was maximal.

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