Carbohydrate Research, 228 (1992) 265–276
Elsevier Science Publishers B.V., Amsterdam

Five specificity patterns of (1→3)-α-L-fucosyltransferase activity defined by use of synthetic oligosaccharide acceptors. Differential expression of the enzymes during human embryonic development and in adult tissues*

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(Received February 21st, 1991; accepted June 17th, 1991)

ABSTRACT

The use of synthetic trisaccharides as acceptors led to the definition of five main (1→3)-α-L-fucosyltransferase activity patterns in human adult tissues: (*I*). *Myeliod cells, granulocytes, monocytes,* and *lymphoblasts,* transfer an α-L-fucopyranosyl group to O-3 of a 2-acetamido-2-deoxy-D-glucosyl residue of H blood-group Type 2 oligosaccharide [α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc→R] with Mn^{2+} as activator. (*II*) *Brain* has the same acceptor specificity pattern as myeloid cells, but can also use Co^{2+} as activator. (*III*) *Plasma* and *liver* transfer an α-L-furopyranosyl group to H blood-group Type 2 and to sialyl-*N*-acetyllactosamine [α-NeuAc-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc→R]. (*IV*) *Intestine, gall bladder, kidney,* and *milk* have the same activity as (*III*), but also transfer an α-L-fucopyranosyl group to O-4 of a 2-acetamido-2-deoxy-D-glucose residue of H blood-group Type 1 [α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc→R] and sialyl Type 1 [α-NeuAc-(1→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc→R]. (*V*) *Stomach* mucosa is not able to use sialyl-*N*-acetyllactosamine, but can transfer an α-L-fucopyranosyl group to the other Type 1 and Type 2 acceptors. Unlike in adult tissue, a single *myeloid*-like pattern of (1→3)-α-L-fucosyltransferase activity was found at early stages of development in all tissues tested. This embryonic enzyme is later progressively replaced by enzymes or mixtures of enzymes having the corresponding adult patterns of enzyme expression. All lymphoblastoid cell lines and half of the tumor epithelial cell lines tested expressed the *myeloid*-like pattern of enzyme found in normal embryonic tissues. The remaining tumor epithelial cell lines expressed different forms of (1→3/4)-α-L-fucosyltransferase acceptor specificity patterns.

INTRODUCTION

With the use of synthetic trisaccharides linked to an hydrophobic arm as acceptors, three main patterns of (1→3)-α-L-fucosyltransferase activity in human normal adult tissues were previously brought to light[1]: (*I*) *Myeloid*, found in brain and leukocytes, which is characterized by a strong transfer of an α-L-fucopyranosyl group to O-3 of a 2-acetamido-2-deoxy-D-glucose residue of H blood-group Type 2 oligosaccharide

* Dedicated to Professor Serge David on the occasion of his 70th birthday.

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0008-6215/92/$ 05.00

(**1**). (*II*) *Plasma,* found in plasma or serum and liver, which is characterized by a similar transfer of an α-L-fucopyranosyl group to both H blood-group Type 2 (**1**) and sialyl-*N*-acetyllactosamine (**2**) compounds. (*III*) *Lewis,* or (1→3/4)-α-L-fucosyltransferase, found in kidney, gall bladder, and milk, which is characterized by an efficient transfer of an α-L-fucopyranosyl group to O-3 of a 2-acetamido-2-deoxy-D-glucose residue of H Type 2 (**1**) and sialyl-*N*-acetyllactosamine (**2**), and to O-4 of a 2-acetamido-2-deoxy-D-glucose residue of H blood-group Type 1 (**3**) and sialyl Type 1 (**4**) acceptors. Kinetic analysis showed additional differences in K_m, optimum pH, heat resistance, and sensibility to *N*-ethylmaleimide for each of these three patterns of enzyme activity, expressed in different tissues[1]. Substrate competition experiments with different synthetic trisaccharide acceptors suggested that tissues expressing *plasma* or *Lewis* acceptor specificity patterns contained mixtures of more that one enzyme[1].

On the other hand, it was also known that a single, highly purified (1→3/4)-α-L-fucosyltransferase or Lewis enzyme from milk was able to use both Type 1 and Type 2 acceptors[2,3]. In good agreement with our results[1], addition of an α-L-fucopyranosyl group linked to O-2 of the nonreducing terminal D-galactosyl group, to form the H acceptor structure, markedly enhanced the capacity of the acceptors to receive a second α-L-fucopyranosyl group linked to O-3 of the 2-acetamido-2-deoxy-D-glucose residue[3]. On the contrary, the efficiency of acceptor compounds containing a terminal, (2→3)-linked α-sialyl group was controversial since both negative[3] and positive[4,5] results were reported.

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc→R

1 (Type 2)

α-NeuAc-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc→R

2 (Type 2)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc→R

3 (Type 1)

α-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc→R

4 (Type 1)

R = $O(CH_2)_8CO_2Me$

EXPERIMENTAL

Synthetic acceptors. — α-L-Fucosyl- and sialyl-oligosaccharide acceptors that cannot be further fucosylated by any of the (1→2)-α-L-fucosyltransferases[6] found in human tissues were selected for this study. Neutral H blood-group Type 1 and H blood-group Type 2 oligosaccharides were made according to Lemieux[7] and Hindsgaul *et al.*[8] Sialic acid derivatives of Type 1 and Type 2 were prepared by Venot and Ratcliffe[9].

All oligosaccharide acceptors synthesized as 8-methoxycarbonyloctyl glycosides were obtained from Chembiomed Ltd. (Edmonton, Canada).

Enzyme activity.— (1→3)- And (1→3/4)-α-L-fucosyltransferase activities were measured with the aforementioned hydrophobic hapten acceptors by the general method using glycoside acceptors attached to an hydrophobic aglycon[10]. The reaction mixture was prepared at 4° and contained, in a total volume of 60 μL, 30 μg of protein of tissue extract or 300 μg of cell line extract in 35mM Tris · HCl (pH 7.5) buffer, 4mM ATP, 20mM $MnCl_2$, 5 μL of a 1 mg/mL solution of the oligosaccharide-$O(CH_2)_8CO_2Me$ acceptor, and GDP-L-[^{14}C]fucose (100 000 d.p.m., 10 GBq/mmol, Amersham, U.K.). The mixtures containing tissue extracts were incubated for 16 h at 37° and those containing the cell line extracts for 4 h at the same temperature. The reaction was stopped by addition of cold water (3 mL). After centrifugation (5 min at 2000 *g*), the supernatant containing the final ^{14}C-labeled product was adsorbed on a preconditioned reverse-phase Sep-Pak C_{18} cartridge (Waters, Milford, U.S.A.), washed with water (2 × 10 mL), and eluted with methanol (2 × 5 mL) directly into scintillation vials. The radioactivity in the vials was counted, after addition of Instagel (1 vol.; Packard, Illinois, U.S.A.) in a liquid-scintillation counter (counting efficiency 86%)[1].

Organs and tissues. — Human embryos of five- to ten-week gestational age were obtained from legal abortions performed at the Maternity Centre of the Hôpital de Pontoise. Organs and tissues were dissected under stereomicroscope (magnification × 10 and × 20). Fetuses of 20- to 42-week gestational age were obtained from spontaneous or therapeutic abortions. Adult tissues were obtained from postmortem examinations. All tissues were dissected, rinsed in phosphate buffered saline (PBS), and kept at −80° until used for enzyme tests. The tissue sample (1 g) was extracted in a Potter homogenizer with the extraction buffer (3 mL; 2% Triton X-100, 50 mM β-mercaptoethanol, and 50 mM Tris · HCl, pH 7.4). Reduced-scale microextractions with only 30–300 μL of the same buffer were used for embryonic tissues. Homogenates were centrifuged for 30 min at 2000 *g*, and the protein concentration of supernatants was measured by the method of Bradford[11]. ABO and Lewis phenotypes of tissue donors were determined by immunofluorescence on fixed and paraffin-embedded tissues with specific antibodies (anti-A, anti-B, anti-Le^a, and anti-Le^b)[12,13].

Cell lines. — Adherent cell lines from kidney (Caki I and Caki II); colon (Caco 2), differentiated HT29 cells cultured in D-glucose-free medium supplemented with inosine (HT29Ino), and the same cells, but undifferentiated, cultured in the same medium but with glucose (HT29IG)[14]; liver (HepG2); ovary (OV1P)[15]; and mammary gland (MCF7, MDA, T47D, and ZR-751)[16] were cultured in 75-cm^2 flasks until confluence, washed with PBS, and kept frozen at −80° until used. They were scraped off with a disposable cell scraper (Costar, Cambridge, U.S.A.) in 3 mL of tissue extraction buffer and centrifuged for 5 min at 2000 *g*.

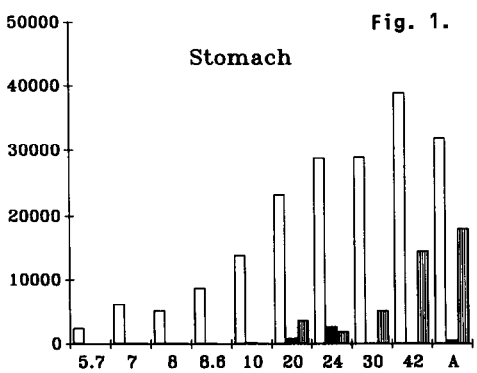
Suspension-growing cells (1–10 × 10^6; T line CEMT4, pre-B lines Reh and KM3, and EBV-lymphoblastoid cell lines[17]) were washed in PBS and stored frozen at −80°. Frozen pellets of both adherent and nonadherent cells were suspended in tissue extrac-

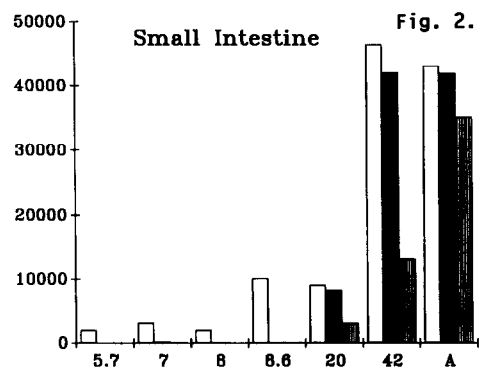
tion buffer (50 μL), and the protein concentration was adjusted to 30 mg/mL; 10 μL of this suspension were used for determination of (1→3)-α-L-fucosyltransferases.

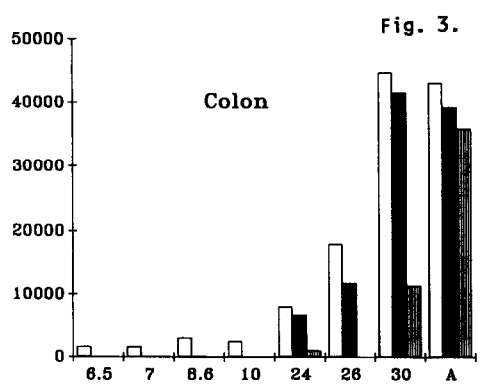
RESULTS AND DISCUSSION

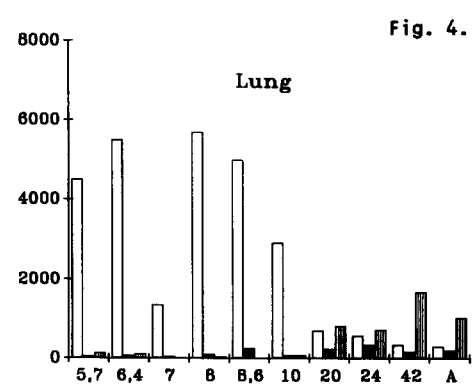
New acceptor specificity pattern of (1→3/4)-α-L-fucosyltransferase activity in the stomach. — Originally, all the normal human tissues tested seemed to fit into one of the three acceptor-specificity patterns summarized in the Introduction[1]. However, tests of normal digestive mucosa with the same synthetic acceptors brought to light the existence of a new pattern for stomach mucosa. This tissue failed to transfer significant amounts of fucose to sialyl-*N*-acetyllactosamine (**2**), but was able to use H-Type 2 compound (**1**). The two Type 1 acceptors, H-Type 1 (**3**) and sialyl Type 1 (**4**), incorporated some fucose, but were not as good acceptors as H-Type 2 compound (**1**) (Table I). These results suggested that more than one (1→3)-α-L-fucosyltransferase can be expressed in normal exocrine secretions and may help to interpret some of the discordant results reported by investigators working with human-milk Lewis enzyme[3-5].

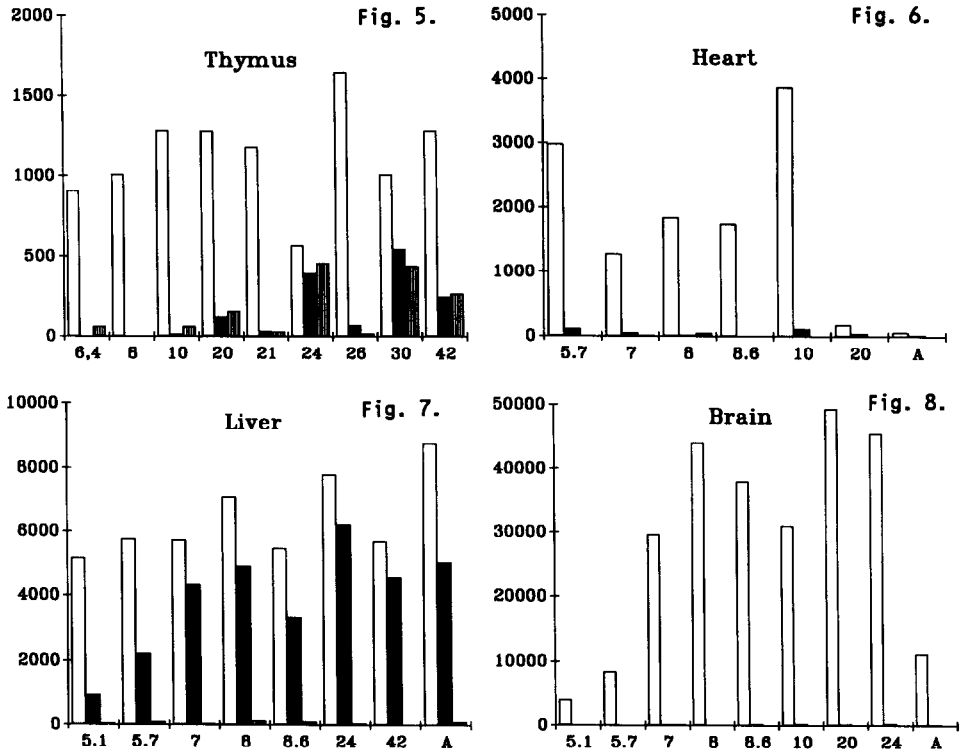
Normal human small intestine and colon mucosae transferred fucose to H-Type 1 (**3**) and H-Type 2 (**1**) compounds, as well as to sialyl-Type 1 (**4**) and Type 2 (**2**) acceptors,











Figs. 1–8. Human embryofetal and adult expression of (1→3)- and (1→3/4)-α-L-fucosyltransferase activities in different organs. Abscissa: Gestation ages in weeks. Ordinate: Incorporation of L-[^{14}C]fucose (d.p.m.) into: H-Type 2 compound (**1**) (empty white columns), sialyl-*N*-acetyllactosamine (**2**) (solid black columns), and H-Type 1 compound (**3**) (striped columns). Five main (1→3)-α-L-fucosyltransferase acceptor specificity patterns can be distinguished in adult tissue (A): (*I*) *Myeloid*, specific transfer of fucose into H-Type 2 compound (**1**) only (leukocytes, not shown). (*II*) *Brain*, with the same myeloid acceptor specificity pattern as leukocytes, but can use Co^{2+} as activator. (*III*) *Plasma*, transfer of fucose into H-Type 2 compound (**1**) and sialyl-*N*-acetyllactosamine (**2**) (liver). (*IV*) *Intestinal* variant of *Lewis* or (1→3/4)-α-L-fucosyltransferase pattern, which can transfer fucose into H-Type 2 (**1**) and H-Type 1 (**3**) compounds, sialyl-*N*-acetyllactosamine (**2**), and sialyl Type 1 compound (**4**) (not shown). (*V*) *Stomach* variant of *Lewis* or (1→3/4)-α-L-fucosyltransferase pattern, which can transfer fucose into H-Type 2 (**1**), H-Type 1 (**3**), and sialyl Type 1 (**4**) acceptors. The early developmental stages of all tissues express only a *myeloid*-like pattern of enzyme activity. Later on, each tissue modifies progressively the expression of (1→3)- and (1→3/4)-α-L-fucosyltransferase activities according to its own particular maturation process.

corresponding to the *Lewis* pattern of (1→3/4)-α-L-fucosyltransferase previously described[1]. The existence of a new and different pattern of acceptor specificity for the (1→3/4)-α-L-fucosyltransferase activity of the stomach, which is unable to use the sialyl-Type 2 acceptor (**2**), suggested that other tissues showing a (1→3/4)-α-L-fucosyltransferase pattern of enzyme activity (small intestine, colon, kidney, and gall bladder) might contain mixtures of different (1→3)-α-L-fucosyltransferases.

Acceptor specificity patterns of the (1→3)-α-L-*fucosyltransferase activities found during human development.* — Various tissues were obtained from abortions covering

TABLE I

Transfer of L-[^{14}C]fucose (d.p.m.) into synthetic trisaccharide acceptors by the (1→3)- and (1→3/4)-α-L-fucosyltransferases present in extracts of human normal adult stomach mucosa.

Oligosaccharide acceptors	*Adult stomach mucosae*		
	ALe[b]	*OLe*[a]	*BLe*[a]
Type 2			
1	65 000	53 000	49 000
2	840	960	870
Type 1			
3	28 000	9 000	20 400
4	5 850	12 000	9 500

the period from 5 to 42 weeks of gestation. The values obtained on the corresponding adult tissue (A) were added to the right of each figure for comparison (Figs. 1–8).

(a) Stomach. A progressive increase of enzyme activity for the H-Type 2 acceptor (**1**) occurred during embryo-fetal development, and adult values were reached at the end of gestation. Very small amounts of enzyme activity were detected with sialyl-*N*-acetyllactosamine (**2**) at 20 and 24 weeks. Enzyme activity for the H-Type 1 compound (**3**) began at week 20, increased progressively, and reached a value close to adult levels at 42 weeks (Fig. 1). Only H-Type 2 compound (**1**) was an acceptor for the enzyme detected between 5 and 10 weeks. This early embryonic pattern corresponds to the *myeloid* pattern of (1→3)-α-L-fucosyltransferase activity described for leukocytes and brain in the adult[1]. In contrast, the acceptor specificity pattern at late fetal stages (30 and 42 weeks) was similar to the pattern of (1→3/4)-α-L-fucosyltransferase activity described for adult stomach.

(b) Intestine. Enzyme activity in small intestine (Fig. 2) and colon (Fig. 3) mucosae had a trait in common with the enzyme activity found in stomach mucosa during the first 5–10 weeks of gestation, only H-Type 2 compound (**1**) being an acceptor, as described for the adult *myeloid* pattern of (1→3)-α-L-fucosyltransferase. After this early embryonic stage, the two intestinal mucosae activities had similar development patterns, but both were different from that of the stomach.

Starting at week 20, sialyl-*N*-acetyllactosamine (**2**) and H-Type 1 compound (**3**), also incorporated some fucose, but for each age the incorporation into sialyl-*N*-acetyllactosamine (**2**) was greater as compared to that into H-Type 1 compound (**3**), although the difference between sialyl-*N*-acetyllactosamine (**2**) and H-Type 1 compound (**3**) tended to decrease and was minimal in the adult. This evolution of acceptor-specificity pattern confirmed that the intestine has a *plasma*-like (1→3)-α-L-fucosyltransferase which can use sialyl-*N*-acetyllactosamine (**2**) as acceptor, and suggested that the appearance of this enzyme precedes the appearance of *Lewis* (1→3/4)-α-L-fucosyltransferase. Later on, these two types of enzymes may coexist in adult tissue, giving rise to what was previously called the *Lewis* or (1→3/4)-α-L-fucosyltransferase pattern[1],

which is different from the stomach pattern of (1→3/4)-α-L-fucosyltransferase activity described above.

The 26-week colon sample was from a fetus devoid of Le^a and Le^b antigens in all tissues and, therefore, was expected to be deficient in Lewis enzyme and to have the homozygous recessive genotype *le/le*. The incorporation of L-fucose into sialyl-*N*-acetyllactosamine (**2**) by this sample confirmed that this activity can be expressed independently of the Lewis phenotype of the individual, as previously shown for milk (1→3)-α-L-fucosyltransferase activity[1].

(c) Lung. Strong (1→3)-α-L-fucosyltransferase activity with a *myeloid*-like acceptor specificity pattern was found in the 5–10 week embryonic period. This transfer into H-Type 2 compound (**1**) decreased dramatically, at the 20–42-week period, to the low levels found in adult lung. Very low levels of enzyme activity were found with sialyl-*N*-acetyllactosamine (**2**) at 20, 24, and 42 weeks. Enzyme activity with the H-Type 1 compound (**3**) also appeared at week 20 but, although higher than that for sialyl-*N*-acetyllactosamine (**2**), it remained low and close to adult levels at 24 and 42 weeks (Fig. 4).

(d) Thymus. Low overall enzyme activity was found in this organ, but again a mainly *myeloid* pattern of (1→3)-α-L-fucosyltransferase activity was present during the early embryonic 5–10-week period. From then onwards, small amounts of fucose were also transferred into sialyl-*N*-acetyllactosamine (**2**) and H-Type 1 compound (**3**) (Fig. 5). The two samples corresponding to 21 and 26 weeks were from homozygous *le/le* fetuses.

The *Lewis*-like enzyme activity of fetal stages of this organ fits well with the presence of ABH and Lewis antigens in the epithelial cells and Hassals' bodies of the thymus. The presence of (1→3)-α-L-fucosyltransferase activity for the H-Type 2 acceptor (**1**), in both Lewis-positive and -negative individuals, correlated well with the presence of Le^x antigen in Hassals' bodies of Lewis-positive and -negative individuals[18]. The persistence of *myeloid*-like enzyme activity in the thymus might be related to the different waves of hematopoietic cell migration, received by this organ during development.

(e) Heart. A similar early embryonic period with *myeloid*-like activity at 5–10 weeks was followed by a dramatic decrease of (1→3)-α-L-fucosyltransferase activity at 20 weeks, and its almost complete disappearance in adult hearts (Fig. 6). Similar results were obtained with striated muscle (not shown).

(f) Liver. This organ presented a pattern different from all the other organs studied. Two compounds, H-Type 2 compound (**1**) and sialyl-*N*-acetyllactosamine (**2**), were active acceptors at all developmental stages, including adult livers. The only changes during development were a relative lower activity for sialyl-*N*-acetyllactosamine (**2**), as compared to H-Type 2 compound (**1**), in the earliest stages of development, suggesting that a *myeloid* pattern of enzyme activity may exist before the fifth week (Fig. 7). It is well known that liver has hematopoietic functions in the early stages of development. However, the *myeloid*-like pattern of (1→3)-α-L-fucosyltransferase activity of the fifth week cannot be only a consequence of these functions, since liver

hematopoiesis starts at seven weeks, reaches its maximum activity at 10–14 weeks, and continues during later stages of development, until it is progressively replaced by bone marrow hematopoiesis.

(g) Brain. A single acceptor, H-Type 2 compound (**1**), was active at all stages including the adult. Quantitatively, this *myeloid*-like activity was stronger between seven and 24 weeks as compared to the two extreme, embryonic five weeks and adult stages (Fig. 8). The embryofetal evolution of brain enzyme acceptor activity was originally taken as an example of maturation of the *myeloid* pattern, because brain samples were easier to obtain than leukocyte samples, and the same *myeloid* pattern of (1→3)-α-L-fucosyltransferase activity, restricted to H-Type 2 structure, was found in adult leukocytes and brain. The enzymes expressed in both tissues had similar K_m values for GDP-L-fucose and H-Type 2 compound (**1**), the same optimum pH, and both were resistant to *N*-ethylmaleimide. However, enzymes from these two sources behaved differently when divalent cations were tested as enzyme activators. Adult brain had the same activity in the presence of 20 mM Mn^{2+} or Co^{2+}, whereas leukocytes had only 15% of the activity in the presence of Co^{2+} as compared to Mn^{2+}. Therefore, Co^{2+} was confirmed as a poor activator for leukocyte enzyme[1].

Since brain at all developmental stages had the same *myeloid*-like acceptor specificity pattern with Mn^{2+} (Fig. 8), it was interesting to determine its behavior with Co^{2+} as activator at different ages. Brains of 5.1-, 5.7-, and 7-week gestation age were tested for their relative enzyme activities in the presence of Co^{2+} and Mn^{2+}. The activity of the 5.1- and 5.7-week samples was strongly inhibited by the presence of Co^{2+}, like the (1→3)-α-L-fucosyltransferase activity found in other embryonic organs and adult leukocytes, whereas the same activity was obtained with both cations in the seven-week sample, as in adult brain. This experiment suggested that, at the five-week stage, brain expresses the *myeloid* form of enzyme also found in other embryonic organs, whereas later on it is replaced by another enzyme, the adult form of brain (1→3)-α-L-fucosyltransferase; this enzyme also has a *myeloid*-like acceptor specificity pattern, but can be distinguished from the embryonic enzyme because it can use Co^{2+} as activator. The only adult cells that seem to conserve a form of (1→3)-α-L-fucosyltransferase indistinguishable from the embryonic enzyme are leukocytes. This might be related to the short life of leukocytes and their rapid turnover during their entire life.

The striking and unexpected new observation of this study is that, during early embryonic stages, all organs seem to express a similar *myeloid* pattern of (1→3)-α-L-fucosyltransferase activity, strictly restricted to the H-Type 2 acceptor and not activated by Co^{2+}, which is later replaced by other *myeloid, plasma*, or *Lewis*-like enzymes, according to different maturation processes, specific for each organ or tissue. This general process seems to occur earlier in brain and liver, as compared to other organs. Both the neural tube and liver are starting points of important cell migrations which colonize distant areas, in early phases of development. In this respect, it is interesting to note that the ligands of some adhesion molecules found in inflammatory processes (PADGEM[19] and ELAM-1[20,21]) have been recently identified as oligosaccharides, products of (1→3)-α-L-fucosyltransferases. Therefore, it is also possible to imagine that some

forms of (1→3)-α-L-fucosyltransferase, specially the embryonic *myeloid*-like form which seems to be present in all subjects, might play a role in cell–cell recognition phenomena during cell migrations occurring in normal development. Other, *plasma* or *Lewis*-like enzymes are less likely candidates for this kind of function, because they are genetically polymorphic and, therefore, lacking in certain otherwise apparently normal people.

In working with whole tissue extracts, the possibility cannot be ruled out that L-fucosidases or neuraminidases remove fucosyl or sialyl groups from the acceptors and expose terminal β-D-galactosyl groups, which might function as acceptors for (1→2)-α-L-fucosyltransferases. Thus, (1→2)-α-L-fucosyltransferase activity was investigated with acceptors specific for these enzymes (phenyl β-D-galactopyranoside, *O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2,4-dideoxy-D-*xylo*-pentose, and *O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2,3-dideoxy-D-*ribo*-pentose)[6], but none was detected in any tissue during the embryonic 5–10-week period. Therefore, incorporation of radioactive fucose by tissues at this early period cannot be due to (1→2)-α-L-fucosyltransferases and is the result of a (1→3)-α-L-fucosyltransferase activity. Later

TABLE II

Transfer of L-[^{14}C]fucose (d.p.m.) onto synthetic acceptors by (1→3)- and (1→3/4)-α-L-fucosyltransferases present in extracts of immortalized human cell lines

Cell lines	*Oligosaccharide acceptors*			
	Type 2		*Type 1*	
	1	**2**	**3**	**4**
(1→3)-α-L-Fucosyltransferases				
Caki I	4900	130	150	40
MDA	6200	35	20	0
T47D	11200	20	0	0
OV1P	11400	320	160	20
EBVF5	13000	400	0	0
Caki II	13400	680	0	0
EBV29930	15800	270	0	0
EBV29874	19000	1900	0	0
Reh	35000	390	90	0
CMT4	39700	170	0	0
KM3	40900	380	110	100
(1→3/4)-α-L-Fucosyltransferases				
MCF7	6100	30	1200	330
ZR-751	9500	420	18300	5400
Caco2	20300	630	950	290
HT29IG	37000	500	15200	6900
HT29Ino	52800	4300	52000	25000
HepG2	30600	23400	4200	2000

on, (1→2)-α-L-fucosyltransferase activities appeared in some tissues, but they were always lower than the (1→3)-α-L-fucosyltransferase activities for H-Type 2 compound (**1**). The final answer as to the number and characteristics of the fucosyltransferases present in various tissues will be obtained by chemical characterization of the end products, purification of enzymes, and study of the corresponding gene probes. This last approach seems more promising, as the small amounts of tissue available from embryos preclude most attempts at enzyme purification.

Acceptor specificity patterns of (1→3)-α-L-fucosyltransferase activity in immortalized human cell lines. — All lymphoblastoid cell lines had the same *myeloid* acceptor specificity pattern, previously described for normal leukocytes[1], irrespective of their origin [T cell leukemia (CMT4), B cell leukemia (Reh and KM3), or Epstein–Barr virus-transformed cell lines (F5, 29930, and 29874)] (Table II). Like the lymphoblastoid cell lines, half of the tumor epithelial cell lines had the same *myeloid*-like acceptor specificity pattern and were also not related to a specific organ origin, since there were two kidney lines (Caki I and Caki II), two mammary gland lines (MDA and T47D), and one ovary cell line (OV1P).

The other half, two mammary gland cell lines (MCF7 and ZR-751) and three tumor colon lines (Caco2, HT29IG, and HT29Ino), expressed (1→3/4)-α-L-fucosyltransferase activity (Table II). The acceptor specificity pattern of these lines was more like the *stomach* than the *intestinal* variant of (1→3/4)-α-L-fucosyltransferase, since they incorporated fucose efficiently into H-Type 2 (**1**) and H-Type 1 (**3**) compounds, and poorly into sialyl-*N*-acetyllactosamine (**2**). One of the mammary gland cell lines (ZR-751) incorporated considerably more fucose into H-Type 1 (**3**) than into H-Type 2 compound (**1**). This is the only case found so far showing this pattern.

Lastly, HepG2, an hepatocarcinoma cell line, was able to incorporate fucose almost as well into H-Type 2 compound (**1**) as into sialyl-*N*-acetyllactosamine (**2**), and ten times less efficiently into the Type 1 acceptors (**3** and **4**) (Table II). This pattern of acceptor specificity is close to the *plasma* pattern, displayed by normal hepatocytes, although it might also contain a small component of *Lewis* activity, possibly of biliary origin[1]. A similar *plasma*-like specificity pattern has been recently described for a tumor colon cell line, Colo205, and was shown to be different from the pattern of activity of the (1→3/4)-α-L-fucosyltransferase expressed[22] by HT29.

Most established cell lines have undergone chromosomal rearrangements and tend to favor expression of certain genes. Therefore, they cannot be expected to express the whole range of molecules present in the original normal tissue, but only a selected subpopulation. In this sense, cell lines may be very useful tools to dissect the complex mixtures of molecules present in normal tissue. However, for each specific system, no cell line was found to predominantly express a single enzyme. Rather complex mixtures, in different proportions, were found, as in whole tissue extracts[1].

Our overall conclusion is that there must be a family of (1→3)- and (1→3/4)-α-L-fucosyltransferase genes encoding for different categories of enzymes, *i.e.*, *myeloid, plasma,* and *Lewis*-like. Some of them have already been cloned, such as the Lewis (1→3/4)-α-L-fucosyltransferase gene[23], located on the short arm of chromosome 19, or

the ELFT gene[21] encoding for a (1→3)-α-L-fucosyltransferase. A gene, probably encoding for another (1→3)-α-L-fucosyltransferase, expressed in granulocytes, monocytes, and lymphoblasts, has been located[24,25] by somatic hybridization on the long arm of chromosome 11. On the basis of family and population data of recent studies in Indonesia, we have proposed[26,27] the existence of a gene encoding for the *plasma* (1→3)-α-L-fucosyltransferase, which might be closely linked to the Lewis gene on chromosome 19.

ACKNOWLEDGMENTS

The authors are greatly indebted to F. Narcy (Service of Pr. M. Forest, Hôpital Cochin, Paris, France) for her assistance in the obtention of fetal tissues. Cell lines were kindly provided by F. Calvo, INSERM U.204, Hôpital St. Louis, Paris (MDA, T47D, MCF7, and ZR-751); A. Zweibaum, INSERM U.178, Villejuif (Caco2, HT291G, and HT29Ino); C. Boucheix, INSERM U.268, Villejuif (Reh. and KM3); L. Edelman, Institut Pasteur, Paris (CEMT4); M.L. Pourci, Biochimie, Châtenay-Malabry (HepG2); and J. Benard, Institut Gustave Roussy, Villejuif (OV1P).

This work was supported, in part, by a grant to R.O. from the Ministère de la Recherche et la Technologie (88.C.0782), and by INSERM and CNRS.

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