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The $\alpha 1 \rightarrow 3$ Fucosylation at the Penultimate GlcNAc Catalyzed by Fucosyltransferase VII Is Blocked by Internally Fucosylated Residue in Sialosyl Long-Chain Poly-LacNAc: Enzymatic Basis for Expression of Physiological E-Selectin Epitope

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Sialosyl-fucosyl poly-LacNAc without sialosyl-Le^x epitope in myeloid cell line HL60 was shown to be the ligand for E-selectin-dependent adhesion, particularly under dynamic flow conditions, in our previous study (Handa K, Stroud MR, Hakomori S, *Biochemistry* 36, 12412–12420, 1997). HL60 cells express only fucosyltransferase (FT) IV and VII. X^{3}NeuAcVII^{3}FucnLc$_{10}$, a representative component showing E-selectin-dependent binding under dynamic flow conditions, is not $\alpha 1 \rightarrow 3$ fucosylated at the penultimate GlcNAc catalyzed by FT-VII, but is $\alpha 1 \rightarrow 3$ fucosylated at the internal GlcNAc catalyzed by FT-IV. VI^{3}NeuAcnLc$_6$ is converted to VI^{3}NeuAcIII^{3}FucnLc$_6$ by FT-IV, but is also converted to VI^{3}NeuAcV^{3}FucnLc$_6$ by FT-VII. Thus, penultimate fucosylation catalyzed by FT-VII is not restricted for nLc$_6$ backbone, but is highly restricted for nLc$_{10}$ backbone. The cooperative effect of FT-IV and FT-VII for synthesis of poly-LacNAc having sialosyl-Le^x with internal fucosylation may be blocked or highly restricted in poly-LacNAc having more than two LacNAc units, because preferential $\alpha 1 \rightarrow 3$ fucosylation by FT-IV takes place at internal GlcNAc, inhibiting penultimate fucosylation by FT-VII. © 1998 Academic Press

Sialosyl and fucosyl residues are essential for selectin ligands (1), and sialosyl-Le^x (SLe^x) binds to E- and P- selectin or inhibits E- and P-selectin-dependent adhesion (2,3). It is therefore generally believed that SLe^x is the binding epitope for these selectins. Since SLe^x was originally discovered as a tumor-associated antigen (4,5), this epitope may be a target of E-selectin-dependent adhesion of tumor cells (6–8). Although human neutrophils and myeloid cells (*e.g.* HL60, U937) are reactive with antibodies originally claimed to be directed to SLe^x epitope (*e.g.* CSLEX, FH6, SNH3, SNH4), these cells contain only trace quantities of SLe^x. The major sialosyl-fucosyl structures that cross-react with these antibodies were identified as having poly-LacNAc backbone, *i.e.* nLc$_8$, nLc$_{10}$ or higher number of monosaccharide units with $\alpha 2 \rightarrow 3$ terminal sialylation and internal (but not penultimate) fucosylation (9,10). Among these structures, sialosyl poly-LacNAc with single internal fucosylation (class I poly-LacNAc, *e.g.* V^{3}FucVIII^{3}NeuAcnLc$_8$; VII^{3}FucX^{3}NeuAcnLc$_{10}$) is the major membrane component of HL60 cells, and showed strong E-selectin-dependent adhesion under dynamic flow conditions but no adhesion under static conditions (11) (see "Discussion" and Table 1A). Interestingly, sialosyl poly-LacNAc with nLc$_8$, nLc$_{10}$, or higher monosaccharide number and having SLe^x epitope without internal fucosylation was not found in HL60 cells. Instead, sialosyl poly-LacNAc with nLc$_8$ or nLc$_{10}$ having internal double fucosylation is the second most common component (class II poly-LacNAc) (Table 1B). There was a trace quantity of poly-LacNAc having internal double fucosylation as well as penultimate fucosylation, *i.e.* SLe^x with internal fucosylation (class III poly-LacNAc) (Table 1C). Class II and class III showed E-selectin-dependent adhesion under both static and dynamic flow conditions (11). Although both FT-IV (12,13) and FT-VII (14,15) are expressed in myeloid cells, the enzymatic basis for expression of these three classes of poly-LacNAc and absence of poly-

Abbreviations: C-M, chloroform-methanol; C-M-W, chloroform-methanol-water; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; FT, fucosyltransferase; GSL, glycosphingolipid; I-H-W, isopropanol-hexane-water; LacNAc, N-acetyllactosamine; poly-LacNAc, tandem repeat of LacNAc having >2 LacNAc units; SLe^x, NeuAc$\alpha 2 \rightarrow$ 3Gal$\beta 1 \rightarrow$ 4(Fuc$\alpha 1 \rightarrow$ 3)GlcNAc$\beta 1 \rightarrow$ R. Glycosphingolipids are abbreviated according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Lipids* **12,** 455–463, 1977). Gangliosides are abbreviated according to Svennerholm (*Eur. J. Biochem.* **79,** 11–21, 1977).

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TABLE 1

Classes of Sialosyl-Fucosyl Poly-LacNAc*

A. Class I. Sialosyl-poly-LacNAc having a single $\alpha 1 \rightarrow 3$ fucosyl residue at internal GlcNAc. Shows E-selectin-dependent adhesion **under dynamic flow, but no adhesion under static conditions. Comprises ~60% of total poly-LacNAc.**

Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4Glc
3 3
↑ ↑
SAα2 Fucα1

B. Class II. Sialosyl-poly-LacNAc having two or more $\alpha 1 \rightarrow 3$ fucosyl residues at internal GlcNAc. Shows E-selectin-dependent adhesion **under both dynamic flow and static conditions. Comprises ~30% of total poly-LacNAc.**

Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4Glc
3 3 3 3
↑ ↑ ↑ ↑
SAα2 Fucα1 Fucα1 ±Fucα1

C. Class III. Sialosyl-poly-LacNAc having penultimate fucosylation and one or more $\alpha 1 \rightarrow 3$ fucosyl residues at internal GlcNAc. Shows E-selectin-dependent adhesion **under both dynamic flow and static conditions. Comprises ~1% of total poly-LacNAc.**

Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4Glc
3 3 3 3 3
↑ ↑ ↑ ↑ ↑
SAα2 Fucα1 Fucα1 Fucα1 ±Fucα1

D. Structures **absent or minimally expressed** in HL60 cells (and presumably in human neutrophils). Short-chain SLe^x or short-chain sialosyl dimeric Le^x.

Gaβ4GNβ3Gaβ4Glc Gaβ4GNβ3Gaβ4GNβ3Gaβ4Glc
3 3 3 3 3
↑ ↑ ↑ ↑ ↑
SAα2 Fucα1 SAα2 Fucα1 Fucα1

E. Structures **completely absent** in HL60 cells and neutrophils. poly-LacNAc having SLe^x without internal fucosylation.

Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4GNβ3Gaβ4Glc
3 3
↑ ↑
SAα2 Fucα1

* The number of LacNAc units per poly-LacNAc structure could be 3 or more (>8 monosaccharides). The structures shown above as examples consist of 4 LacNAc units (10 monosaccharides).

LacNAc having SLe^x epitope without internal fucosylation is unclear, and provides the basis for the present study. Class I poly-LacNAc is not fucosylated at the penultimate GlcNAc to convert it to class III (*i.e.* SLe^x-containing structure) as catalyzed by FT-VII, but it appears to be converted to class II as catalyzed by FT-IV. Penultimate fucosylation of a short-chain sialosyl-LacNAc catalyzed by FT-VII was reported recently (16), although such structures are minimally or not expressed in HL60 cells and neutrophils (Table 1D) (9,10).

MATERIALS AND METHODS

Preparation of sialosyllactonorhexaosylceramide ($VI^3NeuAcnLc_6$) and $X^3NeuAcVII^3FucnLc_{10}$. $VI^3NeuAcnLc_6$ and $X^3NeuAcVII^3FucnLc_{10}$ were prepared from monosialosyl ganglioside fraction of human placenta and HL60 cell extract, respectively. Components of monosialosyl ganglioside fraction were separated further by HPLC using Iatrobeads 6RS-8010 column (Iatron Laboratories Inc., Kanda, Tokyo, Japan) in I-H-W system, as described originally by Watanabe & Arao (17) and used extensively in this laboratory (9,18–20). The column was pre-equilibrated with I-H-W 55:40:5, and ganglioside fraction dissolved in this solvent was coated onto the column by injection and eluted by linear gradient from I-H-W 55:40:5 to 55:25:20 with a flow rate of 1 mL/ min. The fraction containing $VI^3NeuAcnLc_6$ (or $X^3NeuAcVII^3FucnLc_{10}$) was separated and purified further by HPTLC in C-M- 0.5% CaCl2 50:40:10 or C-M- 0.5% CaCl2 50:55:19. Ganglioside bands were visualized by spraying with 0.03% Primuline (Aldrich Chemical Co., St. Louis, MO) in 80% acetone. Bands revealed under UV light were scraped, and gangliosides were extracted from TLC silica gel by repeated sonication in I-H-W 55:25:20 for 20 min, followed by purification on C18 Sep-Pak cartridge (Waters Corp., Milford, MA) as described previously (9). Structures of these compounds were identified by ^{1}H-NMR and electrospray mass spectrometry with

collision-induced dissociation of permethylated compounds as described previously (10).

Preparation of FT-IV and FT-VII, and their fusion protein with Fc domain of human Ig. A FT-IV encoding clone was isolated from a human placental genomic DNA library (Stratagene) by cross hybridization to a FT-III probe (Larson G, Masamune A, Hakomori S, unpubl. data). FT-VII sequences were recovered by reverse transcriptase polymerase chain reaction (RT-PCR). Briefly, RNA from HL60 cell line was reverse transcribed using MoMuLV reverse transcriptase (Gibco/Life technologies) and the FT-VII 3′ primer (CTCAGGCCTGAAACCAACCC) (14). FT-VII 5′ primer (GCACCCCAGCCCACGATCACC) (14) was added and the reaction adjusted according to the Gene Amp RT-PCR kit (Perkin-Elmer Corp.) instructions. Amplification, using Taq polymerase (Promega Corp.), occurred over forty cycles of 1 minute at 95°C, two minutes at 58°C and three minutes at 72°C. The resulting fragment, encoding residues 42 through 342, was cloned into Bluescript SK- (Stratagene) SmaI site (T/A modified) and sequenced. DNA fragments encoding FT-IV and FT-VII catalytic domains were fused in frame with S. Aureus protein A encoded sequence contained in the vector pPROTA (21,22) (a kind gift of J. Lowe, HHMI, Ann Arbor, MI) by the following steps. For FT-IV: a 1235 bp NruI/NheI (Klenow filled) DNA fragment coding for residues 62 through 405 was isolated and ligated to EcoRI linkers and cloned into the EcoRI site of pPROTA. For FT-VII: a Bluescript clone containing FT-VII insert of the desired orientation was excised using BamHI (blunted and ligated to EcoRI linkers) and EcoRI, then cloned into the EcoRI site of pPROTA. FT-IV and FT-VII clones of the desired orientation were recovered and sequenced to confirm proper reading frame.

Enzyme preparation. Enzyme-protein A fusion proteins were prepared as described previously (21) with some modification. Briefly, 15 μg of plasmid DNA encoding the fusion protein was transfected separately into COS-1 cells (~3,000,000 per 150 mm dish) by DEAE-dextran method (23). After 72 h incubation, the medium was harvested from each plate and centrifuged at 1000 ×g for 10 min and then 100,000 ×g for 60 min. Tween 20 (0.05%) was added to the supernatant. IgG-Sepharose 6FF (Pharmacia) was pre-equibrated as per manufacturer's instruction and then pre-equibrated with 10% FCS-DMEM. 5 mL of supernatant containing the fusion protein was mixed with pre-equibrated IgG-Sepharose (~50 μL of bed volume) at 4°C overnight. The IgG-Sepharose was washed 5× with 50 mM Tris-HCl, pH 7.5, and 1× with 10% FCS-DMEM-0.05% Tween 20, then resuspended in 100 μL 10% FCS-DMEM. Aliquots of enzyme-IgG-Sepharose suspension, thus prepared, were used for enzymatic reaction.

To prepare Colo205 crude cell lysate for FT-III source, 2 mL of Colo205 cell pellet was homogenized with 2 vol of 50mM Hepes- 0.5 M sucrose- 1 mM EDTA, pH 7.4, and centrifuged at 1000 ×g for 15 min. The resulting pellet was homogenized in 2 vol of the above buffer containing 1% Triton CF-54, and the homogenate was centrifuged at 100,000 ×g for 60 min. The supernatant were frozen at −70°C until use. 50 μg of protein per enzyme reaction was used.

Conditions for enzymatic reaction. Preliminary studies revealed the following optimal conditions for enzymatic fucosylation of sialosyl type 2 chain GSL ($VI^3NeuAcnLc_6$ as a model) catalyzed by FT-III, FT-IV, and FT-VII.

(i) For FT-III and FT-IV: Mix 0.1 - 1.0 nmole GSL substrate dissolved in 20-50 μL C-M with 6 μL sodium deoxytaurocholate solution (5 mg/ mL C-M). Evaporate C-M to dryness. Mix residue with 5 μL 250 mM HEPES buffer, pH 7.5, 5 μL 0.15 M $MnCl_2$, and 5 μL H_2O, and sonicate for 10 sec in a Branson waterbath sonicator. Add 5 μL water solution containing 7.5 nmole cold GDP-fucose plus small amount of GDP-^{14}C-fucose (~50,000 cpm) and 10 μL enzyme-IgG-Sepharose suspension for FT-IV, or Colo205 cell lysate for FT-III, as described under "Enzyme preparation".

(ii) For FT-VII: Mix 0.1 - 1.0 nmole GSL substrate dissolved in 20-50 μL C-M with 15 μL ethanol solution of 1% Triton X-100. Dry under nitrogen stream. Combine residue with 3 μL 500 mM cacodylate buffer, pH 6.5, 5 μL 0.15 M $MnCl_2$, and 7 μL H_2O, and sonicate for 10 sec. To mixture, add 5 μL GDP-fucose solution (with small amount of ^{14}C label as above) and 10 μL enzyme-IgG-Sepharose suspension.

The reaction mixtures were incubated at 37°C for 20 h, then added with C-M-W 0.2: 5: 10, sonicated, passed through a C18 silica gel column, and washed extensively with C-M-W 0.2: 5: 10. The pass-through and washing eluate were discarded, and GSLs were eluted with C-M 2:1. C-M was evaporated to dryness, and the residue was subjected to HPTLC and autoradiography if necessary. Radioactive bands were scraped from TLC plates and counted by scintillation counter.

For enzymatic preparation of $VI^3NeuAcIII^3FucnLc_6$, 20 nmole $VI^3NeuAcnLc_6$ was dissolved in solutions containing the same buffer and detergent as above, and added with 200 nmole cold GDP-fucose, 30 μL FT-IV (see "Enzyme preparation"), and incubated 20 h at 37°C. The reaction mixture was treated as above and purified by C18 silica gel column. Purified GSL fraction was subjected to preparative HPTLC and bands were stained with Primuline and the product was extracted from lower band (see first paragraph of this section). The enzymatic product was structurally characterized as $VI^3NeuAcIII^3FucnLc_6$, as will be described in detail elsewhere.

RESULTS

Absence of α1 → 3 fucosylation at penultimate GlcNAc or any other position catalyzed by FT-VII in Fraction 12-2 ($X^3NeuAcVII^3FucnLc_{10}$). Incubation of Fr. 12-2 (having structure $X^3NeuAcVII^3FucnLc_{10}$) with FT-VII and ^{14}C-labeled GDP-fucose under the conditions described in Materials and Methods did not give any radioactive band (Fig. 1B, lane 2). In contrast, incubation of $VI^3NeuAcnLc_6$ with the same enzyme under the same conditions gave a strong band corresponding to $VI^3NeuAcV^3FucnLc_6$ (Fig. 1B, lane 4).

Occurrence of multiple α1 → 3 fucosylation catalyzed by FT-IV at internal GlcNAc in Fraction 12-2 ($X^3NeuAcVII^3FucnLc_{10}$). Incubation of Fr. 12-2 with FT-IV plus ^{14}C-labeled GDP-fucose under the conditions described in Materials and Methods gave two major bands with slower TLC mobility (Fig. 1B, lane 1) than the original $X^3NeuAcVII^3FucnLc_{10}$ (Fig. 1A, lane 1). The TLC mobility of these components corresponds to that of $X^3NeuAcVII^3FucV^3FucnLc_{10}$ or $X^3NeuAcVII^3FucV^3FucIII^3FucnLc_{10}$; however, details of characterization of the FT-IV-dependent product of $X^3NeuAcVII^3FucnLc_{10}$ will be described elsewhere. Incubation of $VI^3NeuAcnLc_6$ (Fig. 1A, lane 2) with FT-IV resulted in a strong radioactive band with slightly slower motility (Fig. 1B, lane 3), which was identified as $VI^3NeuAcIII^3FucnLc_6$.

Penultimate α1 → 3 fucosylation catalyzed by FT-VII and internal α1 → 3 fucosylation by FT-IV of sialosyl-nLc_6 ($VI^3NeuAcnLc_6$). Cold $VI^3NeuAcIII^3FucnLc_6$ was prepared in sufficient quantity by incubation of VI^3-$NeuAcnLc_6$ with FT-IV as described in Materials and Methods. Incubation of this cold compound with FT-VII plus ^{14}C-labeled GDP-fucose gave a slow-migrating radioactive band (Fig. 2, lane 4). *I.e.* α1 → 3

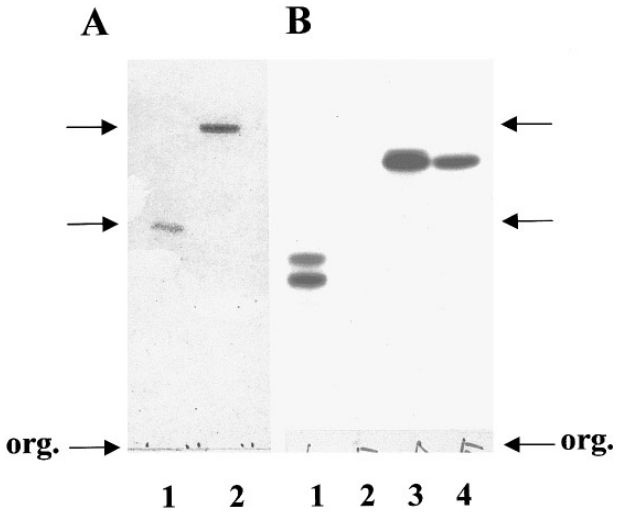

FIG. 1. HPTLC pattern of $X^3NeuAcVII^3FucnLc_{10}$, $VI^3NeuAcnLc_6$, and their enzymatic reaction products with FT-VII and FT-IV. HPTLC was developed in C-M- 0.5% $CaCl_2$ in H_2O 50:55:19. Panel A: bands revealed by orcinol-sulfuric acid staining. Lane 1, 0.1 nmole $X^3NeuAcVII^3FucnLc_{10}$. Lane 2, 0.5 nmole $VI^3NeuAcnLc_6$. Panel B: autoradiogram of products after enzymatic reaction with defined substrate and enzyme, plus ^{14}C-labeled GDP-fucose. Lane 1, $X^3NeuAcVII^3FucnLc_{10}$ with FT-IV. Lane 2, $X^3NeuAcVII^3FucnLc_{10}$ with FT-VII. Lane 3, $VI^3NeuAcnLc_6$ with FT-IV. Lane 4, $VI^3NeuAcnLc_6$ with FT-VII. Arrows indicate position of $VI^3NeuAcnLc_6$ (top), $X^3NeuAcVII^3FucnLc_{10}$ (middle), and origin of chromatography (bottom). Approximately 0.1 nmole $X^3NeuAcVII^3FucnLc_{10}$, incubated with FT-IV, and half of the reaction product were placed in lane 1. 0.1 nmole $X^3NeuAcVII^3FucnLc_{10}$, incubated with FT-VII, and all the reaction product were placed in lane 2. Note that two clear radioactive bands, having 120 and 100 cpm respectively, are evident in lane 1, whereas no radioactive bands are detectable in lane 2; all areas of lane 2 showed only background count ($\leq$10 cpm). Approximately 0.5 nmole $VI^3NeuAcnLc_6$, incubated with FT-IV, and half of the reaction product were placed in lane 3. Approximately 0.5 nmole $VI^3NeuAcnLc_6$, incubated with FT-VII, and all the reaction product were placed in lane 4. Both lanes 3 and 4 gave a strong radioactive band with mobility similar to each other but slower than original $VI^3NeuAcnLc_6$. Radioactivity counts of the bands from lanes 3 and 4 were 300 and 170 cpm respectively.

fucosylation catalyzed by FT-VII at penultimate GlcNAc is not blocked by internal $\alpha 1 \rightarrow 3$ fucosylation at III GlcNAc in short-chain sialosyl-LacNAc ($VI^3NeuAcnLc_6$). Incubation of $VI^3NeuAcnLc_6$ with FT-VII gave a band with the same motility as $VI^3NeuAcIII^3FucnLc_6$ (Fig. 2, lane 3). However, the compound has $\alpha 1 \rightarrow 3$ fucosylation at penultimate GlcNAc ($VI^3NeuAcV^3FucnLc_6$), as will be described elsewhere. Incubation of cold $VI^3NeuAcIII^3FucnLc_6$ with FT-III gave a slow-migrating band which has the structure $VI^3NeuAcV^3FucIII^3FucnLc_6$ (Fig. 2, lane 2).

DISCUSSION

Our systematic studies revealed the presence in HL60 cells and human neutrophils of sialosyl-fucosyl unbranched poly-LacNAc gangliosides as E-selectin ligands (10), although the same structure present in O-linked glycoprotein as physiological ligand remains to be clarified. The term "poly-LacNAc" as used here refers to an unbranched carbohydrate chain having 3 or more LacNAc units. Three classes of such structures, capable of causing E-selectin-dependent cell adhesion under dynamic flow or static conditions (11), are shown in Table 1.

Classes I and II are the major components (>90%) of total poly-LacNAc gangliosides in HL60 cells and neutrophils. Class III comprises ~1% of the total. Classes I and II must therefore be the major physiological ligands for E-selectin-dependent adhesion. This conclusion conflicts with the general belief that SLe^x-

FIG. 2. HPTLC pattern of enzymatic reaction products from $VI^3NeuAcnLc_6$ and $VI^3NeuAcIII^3FucnLc_6$ catalyzed by FT-III, FT-IV, and FT-VII. Enzymatic reaction products as below after incubation of substrate with enzyme and ^{14}C-labeled GDP-fucose and purification with C18 silica gel column (see Materials and Methods) were placed in HPTLC plates, developed in C-M- 0.2% $CaCl_2$ in H_2O (50:40:10), and bands were revealed by autoradiography. Lane 1: 0.5 nmole $VI^3NeuAcnLc_6$ incubated with Colo205 FT (mainly FT-III and FT-IV). Lane 2: 0.5 nmole $VI^3NeuAcIII^3FucnLc_6$ incubated with Colo205 FT. Lane 3: 0.5 nmole $VI^3NeuAcnLc_6$ incubated with FT-VII. Lane 4: 0.5 nmole $VI^3NeuAcIII^3FucnLc_6$ incubated with FT-VII. Two bands (upper and lower) in lane 1 were 1660 and 2090 cpm. The bands in lanes 2, 3, and 4 were 1170, 400, and 290 cpm respectively.

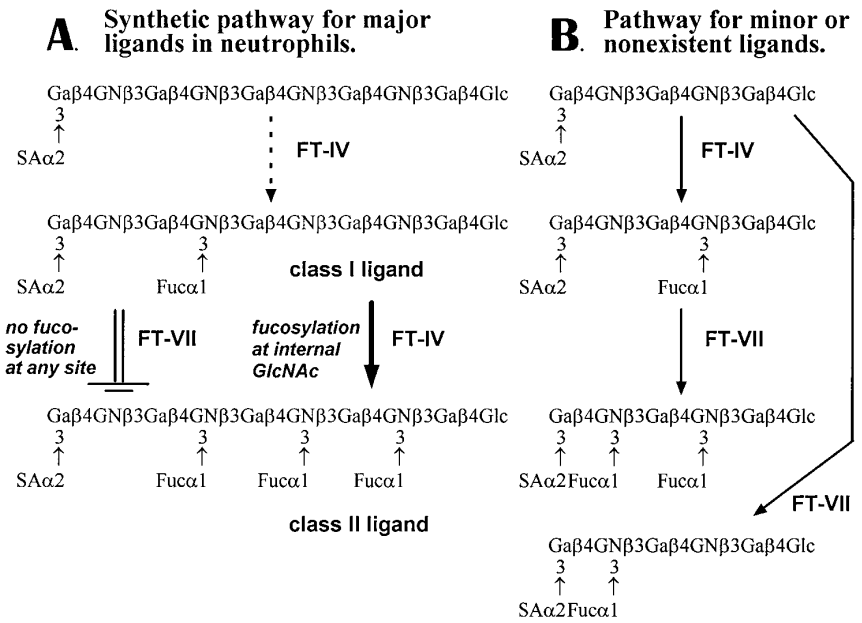

FIG. 3. Schematic illustration of differential enzymatic effect of FT-IV vs. FT-VII on class I sialosyl-fucosyl poly-LacNAc (panel A) and on sialosyl short-chain LacNAc (panel B). Ga, galactose. GN, N-acetyl-glucosamine. SA, sialic acid. Panel A: Synthetic pathway for major ligands in neutrophils. Panel B: Pathway for ligands which are minor or nonexistent in neutrophils, but are the major component in gastrointestinal tumors. In these tumors, however, synthesis is catalyzed by cooperative effect of FT-III and FT-IV.

containing structures are the major E-selectin ligands (2) and many reviews as cited in (10). Importantly, HL60 cells (and presumably human neutrophils) do not contain short-chain SLe^x or sialosyl dimeric Le^x (Table 1D), which are expressed abundantly in human tumors (4,5). Poly-LacNAc containing SLe^x without internal fucosylation (Table 1E) is completely absent in HL60 cells.

Since HL60 cells and neutrophils express only FT-IV and FT-VII (12–15), enzymatic synthesis of class I and II structures must result from cooperative catalytic function of these enzymes. However, exact substrate specificity of FT-IV and FT-VII has not been studied. We therefore undertook the initial step of analysis along this line, using chemically well-defined VI^3-$NeuAcnLc_6$, $VI^3NeuAcIII^3FucnLc_6$, and $X^3NeuAcVII^3$-$FucnLc_{10}$. The following findings are of interest.

i. $\alpha 1 \rightarrow 3$ fucosylation catalyzed by FT-IV always occurs at internal GlcNAc, not at penultimate GlcNAc, regardless of carbohydrate chain length.

ii. $\alpha 1 \rightarrow 3$ fucosylation catalyzed by FT-VII occurs at penultimate GlcNAc, *i.e.* adjacent to $2 \rightarrow 3$ sialosyl residue, only when the substrate has one or two LacNAc units, *i.e.* $IV^3NeuAcnLc_4$ or $VI^3NeuAcnLc_6$.

iii. FT-VII does not catalyze $\alpha 1 \rightarrow 3$ fucosylation at any GlcNAc position when internal $\alpha 1 \rightarrow 3$ fucosylation is present in poly-LacNAc having three or more LacNAc units. FT-IV may fucosylate preferentially at internal GlcNAc in sialosyl poly-LacNAc. The internal $\alpha 1 \rightarrow 3$ Fuc residue may block penultimate fucosylation catalyzed by FT-VII.

Poly-LacNAc, or $\beta 1 \rightarrow 3$, $\beta 1 \rightarrow 4$ linear chain, forms a helical spatial structure (24, 25). Therefore, the spatial relationship between the terminal sialosyl and the internal Fuc residue present along the helical poly-LacNAc is important for defining E-selectin-dependent cell tethering and rolling under dynamic flow conditions. SLe^x does not cause rolling under physiological shear stress conditions (5-10 dynes/ cm^2) (11). Fucosylation at penultimate GlcNAc as catalyzed by FT-VII is restricted to long-chain poly-LacNAc. This may be ascribable to internal $\alpha 1 \rightarrow 3$ fucosylation along the helical structure. In short-chain LacNAc, internal Fuc residue does not block penultimate fucosylation, presumably because of the absence of helical structure. Contrasting fucosylation catalyzed by FT-IV and FT-VII in sialosyl poly-LacNAc vs. short-chain LacNAc is illustrated in Fig. 3.

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