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THE CLONING AND EXPRESSION OF A HUMAN α-1,3 FUCOSYLTRANSFERASE CAPABLE OF FORMING THE E-SELECTIN LIGAND

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The polymerase chain reaction was used to amplify a novel fucosyltransferase cDNA (FucT-VI) from A431 and from HL60 cells . The amplified cDNA has a high degree of sequence identity to FucT-V and to FucT-III, and a much lower level of similiarity to FucT-IV. Transfection of the FucT-VI gene into mammalian cells confers α-1,3 fucosyltransferase activity to the cells, resulting in cell surface expression of Lewis x and sialyl-Lewis x carbohydrates. In contrast to FucT-IV activity, FucT-VI catalyzes the transfer of fucose from GDP-β-fucose to α-2,3 sialylated substrates. The substrate specificity of the FucT-VI gene product suggests that FucT-VI may be an enzyme involved in the biosynthesis of the E-Selectin ligand, sialyl-Lewis x, in myeloid cells.

The Selectin family of cell surface adhesion molecules includes receptors which bind ligands incorporating sialylated, fucosylated lactosamines (1). In particular, the ligand for E-selectin on human neutrophils is sialyl-Lewis x (2,3). The adhesive interaction between the E-selectin protein on inflamed endothelial cells and the sialyl-Lex carbohydrate on human neutrophils plays a key role in the recruitment of neutrophils to sites of inflammation. While the regulation of E-selectin expression has been examined (4), the enzymes responsible for the biosynthesis of the E-selectin ligand, sialyl-Lewis x, remain unidentified.

The sialyl-Lewis x carbohydrate (NeuNAc-α2,3-Gal-β1,4-(Fuc-α1,3)-GlcNAc-β1,-) is a tetrasaccharide consisting of N-Ac-lactosamine core which has an N-acetyl neuraminic acid moeity linked to the galactose and a fucose pyranoside linked α1,3 to the N-Ac-glucosamine. Because most mammalian

Abbreviations and trivial names: pcr, polymerase chain reaction; Lex, Lewis x; sLex, sialyl-Lewis x; MMLV RT, Moloney murine leukemia virus reverse transcriptase; FucT, fucosyltransferase; PAGE, polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate; FACS, fluorescence activated cell sorting; N-Ac-lactosamine, Gal-β1,4-GlcNAc; Lactose, Gal-β-1,4-Glc; Fucosyllactose, Fuc-α1,2-Gal-β1,4-Glc; Sialyllactosamine, NeuNAc-α2,3-Gal-β1,4-GlcNAc; Lacto-N-biose, Gal-β1,3-GlcNAc.

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cells are capable of synthesizing carbohydrates terminating in sialyl-α2,3-N-Ac-lactosamine, cell-specific expression of an α1,3-fucosyltransferase is thought to determine sLex biosynthesis and, therefore, the capacity to bind E-Selectin (5). Consistent with this hypothesis, Goelz, et al. (6), describe a human α1,3-fucosyltransferase (ELFT or FucT-IV) cDNA which imparts E-Selectin binding to transfected COS cells. Other workers, however, have shown that although FucT-IV efficiently catalyzes the formation of low affinity E-Selectin ligands such as Lewis x and VIM-2, FucT-IV appears incapable of catalyzing the formation of sialyl-Lewis x in vitro (7,8). Similarly, transfection of FucT-IV expression constructs into COS cells does not result in cell-surface expression of sLex. Thus, in contrast to FucT-III (9) and FucT-V (10), FucT-IV does not fucosylate sialyl-N-Ac-lactosamine.

The question remains open as to which fucosyltransferase isoenzyme is responsible for the biosynthesis of sLex in leukocytes. Of the three cloned human α1,3-fucosyltransferases described, only FucT-V possesses the appropriate enzymatic properties and tissue localization to give rise to the biologically relevant E-selectin ligand. This communication describes the serendipitous discovery of a fourth human α1,3-fucosyltransferase, FucT-VI, which appears to be a suitable candidate for the fucosyltransferase giving rise to sLex in myeloid cells.

Methods and Materials

PCR--Total RNA was isolated from A431 cells and from HL60 cells by guanidinium isothiocyante disolution of cells and ethanol precipitation. cDNA was prepared using MMLV reverse transcriptase and oligo-dT priming. The following pcr primers were synthesized by the phosphoramidite method and were purified by denaturing PAGE:

A 5'-GAGATACTCTGACCCATGG-3'
B 5'-AGCCTCTCAGGTGAACCAAGC-3'
C 5'-GACCCTGGCAGCCCAGGCCCCATGCCGGCC-3'

For pcr amplification, 1 μg cDNA was combined with the appropriate primers in a Gene-Amp (Perkin Elmer-Cetus) buffer containing 1.5 mM $MgCl_2$ and 5% DMSO. Thirty cycles (1 min at 94°, 1 m at 42°, 1.5 min 72°) were run and the products were isolated from agarose gels for subcloning into pCR1000 (Invitrogen). The clones were sequenced using plasmid dna and Sequenase (United State Biochemical).

FACS assay--cDNAs of FucT-IV, FucT-V, and FucT-VI were subcloned into CDM8 (11) and were transfected into COS-7 cells by the DEAE-Dextran method. The cells were stained with 3 μg/mL anti-CD15 IgM mAb (anti-Leu-M1, Becton-Dickinson) or with neat supernatant (approximately 10 μg/mL IgM by ELISA) of CSLEX1 hybridoma (American Type Culture Collection HB8580); these antibodies recognize Lex and sLex determinants, respectively. The cells were washed and then mixed with FITC-goat anti-mouse Ig for analysis by

FACS. Nearly 40% of the transfected cells displayed fucosylated product on their surface.

Fucosyltransferase assay--COS-7 cells which had been transfected with FucT genes in CDM8 were lysed in 0.2 mL 1% Triton X-100 72 hours after transfection. The lysates were analyzed for fucosyltransferase activity essentially as described (6). Typically, transfectant cells from a single 150 cm^2 tissue culture dish gave sufficient fucosyltransferase activity to transfer 10 picomoles per minute of fucose from GDP-β-fucose to N-Ac-lactosamine. Acceptor carbohydrates were purchased from Pfanstiehl Laboratories, Sigma Chemicals, or Oxford GlycoSystems and were used at a 10 mM concentration; GDP-β-[^{14}C]fucose was acquired from New England Nuclear. When charged acceptor oligosaccharides were assayed, we used 2 mL 0.5 M acetic acid instead of water to elute the fucosylated product from the Dowex A resin. We found that this modification served to elute weak acids, i.e., the sLex tetrasaccharide, from Dowex A, but did not elute strong acids (GDP-β-fucose) from the resin.

Results and Discussion

We initially sought to clone FucT-III by pcr amplification of A431 cell cDNA using oligonucleotides A and B. During agarose gel purification of the expected 1.2 kb pcr product, we noted a size heterogeneity in this band. After cloning the pcr products, we found two coding regions distinct from FucT-III. Each of these cloned pcr products was sequenced, and one proved to be identical to FucT-V (10). Sequence analysis revealed the other coding region to be similar but not identical to FucT-III or FucT-V; following the suggested nomenclature of Lowe, we named this clone FucT-VI. Because the FucT-VI clone was isolated by pcr, we were concerned that the coding region represents a mutated version of FucT-V. We therefore isolated three additional, independent FucT-VI clones by pcr amplification of separate HL60 cDNA preparations. Moreover, to ensure that the stop codon for the open reading frame is authentic and was not introduced by pcr primer B, we used oligonucleotide primer C, which primes outside of the putative FucT-VI open reading frame, for the confirming amplifications.

Consistent with the documented infidelity of Taq polymerase (12), every clone had one or two sequence non-identities when compared to the other clones. However, the near identity of the additional clones supports our claim that FucT-VI is a gene distinct from other described fucosyltransferases. The sequence shown in figure 1 represents a consensus sequence of the cloned FucT-VI amplification products.

The FucT-VI nucleotide sequence and the deduced amino acid sequence is typical of glycosyltransferases and other type II membrane proteins. The predicted 359 amino acid protein appears to have an amino-terminal signal anchor sequence as well as a luminal catalytic domain with high sequence

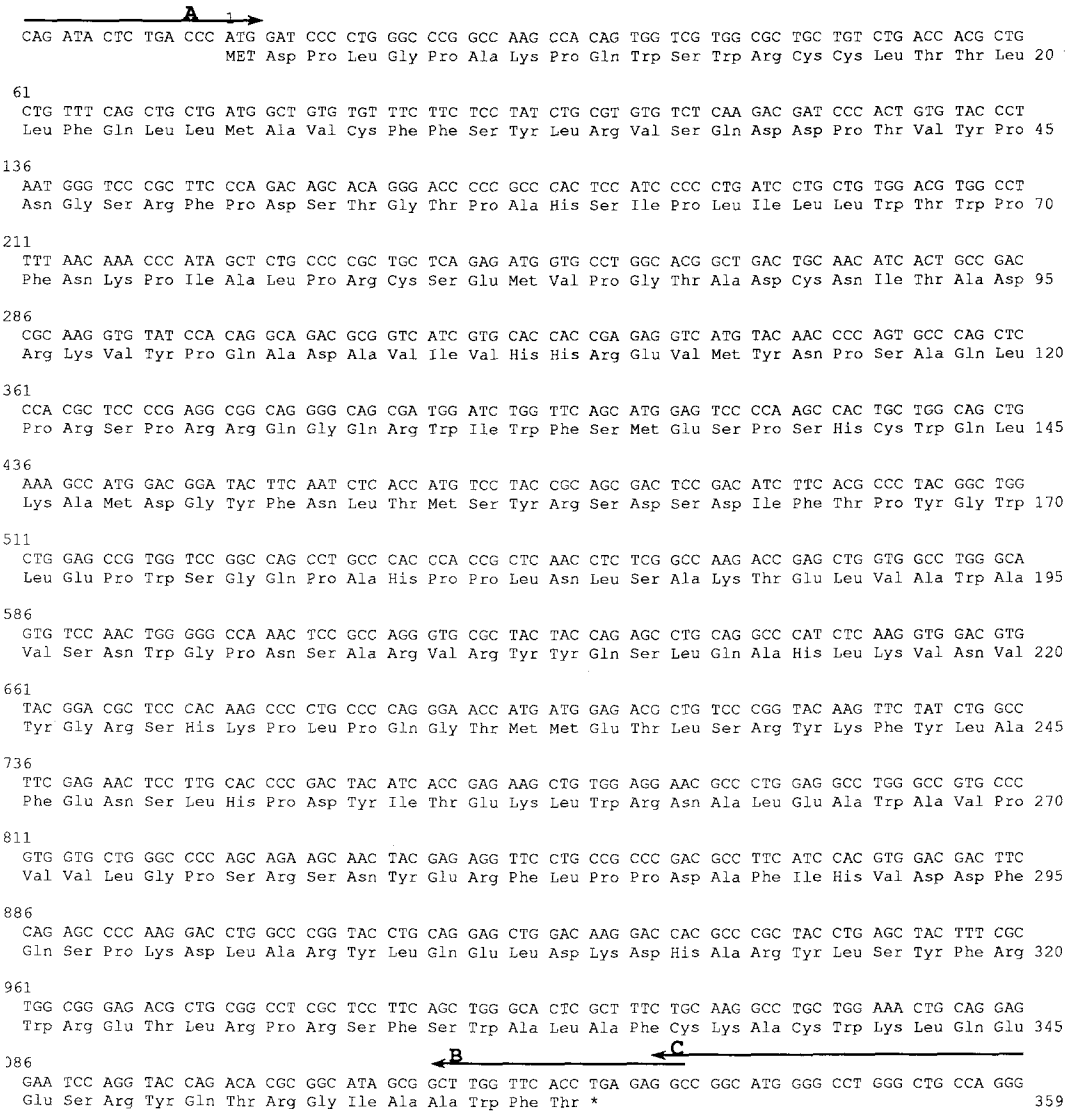

Figure 1. cDNA sequence and deduced amino acid sequence of FucT-VI. The arrows indicate pcr primers described in Methods and Materials.

similarity to other cloned human α1,3-fucosyltransferases. FucT-VI cDNA is 90% identical to the nucleic acid sequence of FucT-III. At the amino acid level, FucT-III and FucT-VI are 84% identical. The similiarity is much higher in the C-terminal, catalytic region (95%) than in the N-terminal half of the proteins (71%).

To establish that FucT-VI is indeed a fucosyltransferase, we expressed the gene in COS-7 cells using the transient expression vector CDM8. We stained the transfectants with antibodies against Lex and against sLex, analyzed the cells by FACS, and observed that FucT-VI transfection imparts cell surface expression of both Lex and sLex to the COS cells (data not shown). In parallel

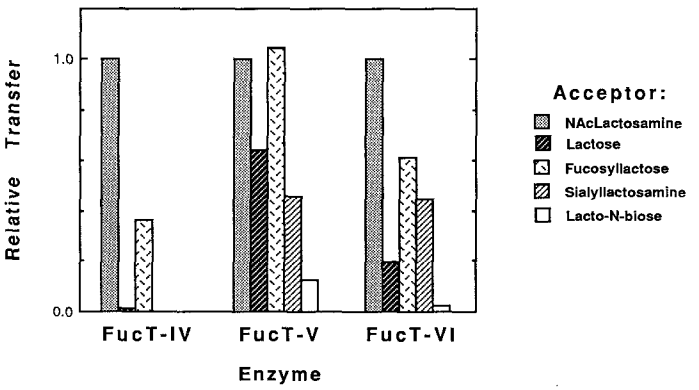

Figure 2. Fucosyltransferase activity of COS-7 cells transfected with the indicated gene against various acceptor molecules (10 mM). The results are normalized to the measured value for N-Ac-lactosamine.

experiments, we detected surface expression of Lex but not sLex in FucT-IV transfectants. Next, to characterize the substrate specificity of the FucT-VI gene product, we lysed transfected cells in detergent and assayed the lysates for fucosyltransferase activity against a panel of oligosaccharide acceptor molecules. As shown in figure 2, we found that FucT-VI, like FucT-V, catalyzes the transfer to both sialylated and non-sialylated N-Ac-lactosamine. Furthermore, the finding that FucT-VI does not transfer fucose efficiently to lacto-N-biose suggests that the enzyme has minimal α1,4-fucosyltransferase activity.

These results indicate that FucT-VI represents a human gene encoding a fucosyltransferase isoenzyme capable of catalyzing the transfer of fucose from GDP-β-fucose to various acceptors substrates. By FACS analysis of FucT-VI transfectants, we established that the enzyme produces Lex and sLex determinants, indicating that FucT-VI forms α1,3 anomeric linkages to the GlcNAc moeity of N-Ac-lactosamine. Additionally, both FACS analysis and enzymatic assays show that FucT-VI effects this transfer to sialylated precursors, as is required in the biosynthesis of the E-Selectin ligand, sLex. The present data suggest that FucT-VI represents a candidate for the fucosyltransferase isoenzyme responsible for biosynthesis of sLex carbohydrate structures on human leukocytes.

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