Molecular Cloning of a Human Fucosyltransferase Gene That Determines Expression of the Lewis x and VIM-2 Epitopes but Not ELAM-1-dependent Cell Adhesion*

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We have used the human Lewis blood group fucosyltransferase cDNA and cross-hybridization procedures to isolate a human gene that encodes a distinct fucosyltransferase. Its DNA sequence predicts a type II transmembrane protein whose sequence is identical to 193 of 231 amino acids at corresponding positions within the catalytic domain of the Lewis fucosyltransferase. When expressed by transfection in cultured cell lines, this gene determines expression of a fucosyltransferase capable of efficiently utilizing N-acetyllactosamine to form the Lewis x determinant (Galβ1→4[Fucα1→3]GlcNAc). By contrast, biochemical and flow cytometry analyses suggest that the enzyme cannot efficiently utilize the type II acceptor NeuNAcα2→3Galβ1→4GlcNAc, to form the sialyl Lewis x determinant. In Chinese hamster ovary cells, however, the enzyme can determine expression of the α1→3-fucosylated, α1→3-fucosylated structure known as VIM-2, a putative oligosaccharide ligand for ELAM-1. Cell adhesion assays using VIM-2-positive, sialyl Lewis x-negative transfected Chinese hamster ovary cells indicate that surface expression of the VIM-2 determinant is not sufficient to confer ELAM-1-dependent adhesive properties upon the cells. These results demonstrate that substantial structural similarities can exist between mammalian glycosyltransferases with closely related enzymatic properties, thus facilitating isolation of their cognate genes by cross-hybridization methods. The results further suggest that cell surface expression of the VIM-2 determinant is not necessarily sufficient to mediate ELAM-1-dependent cell adhesion.

Oligosaccharides constructed by animal cells are remarkable for their structural diversity. This diversity is not random but rather consists of specific sets of oligosaccharide structures that exhibit precise tissue-specific and developmental expression patterns (1, 2). Molecular mechanisms used by cells to regulate these expression patterns are poorly understood. It can be expected, however, that such patterns are determined largely by the coordinate regulation of expression of the glycosyltransferases that determine these patterns (1, 3). Since many of these enzymes recognize identical nucleotide sugar or oligosaccharide acceptor substrates, it had been anticipated that they would exhibit substantial primary protein and nucleic acid sequence similarities that might facilitate isolation of related glycosyltransferase genes by cross-hybridization strategies. Recent molecular cloning efforts have allowed the isolation of several cloned glycosyltransferase cDNAs (4–8, for example). Comparisons of the primary sequences of these enzymes has revealed that they maintain virtually identical predicted structural topologies (3). With the exception of one pair of distinct glycosyltransferases, however (6, 9, 10), there appear to be no substantial primary sequence similarities between these enzymes, even though many of them exhibit virtually identical requirements for nucleotide sugar or oligosaccharide acceptor substrates. The exceptional pair, an α1,3-galactosyltransferase sequence (6, 9, 10), or its human pseudogene homologue (11), and a human α(1,3)-N-acetylgalactosaminide transferase (6) share substantial primary protein and nucleic acid sequence similarity, even though these enzymes utilize different nucleotide sugar substrates and exhibit distinct oligosaccharide acceptor substrate requirements. Indeed, single amino acid substitutions in this latter enzyme yield striking differences in its ability to utilize different nucleotide sugar substrates (12). Taken together, these observations demonstrate that some glycosyltransferases may be structurally related, but indicate that such relationships cannot necessarily be predicted from knowledge of nucleotide sugar or oligosaccharide acceptor substrate requirements.

We have recently used a mammalian gene transfer procedure to isolate a cloned cDNA that encodes the human Lewis blood group fucosyltransferase (4). Biochemical and genetic data indicate that the human genome contains two or more other structural genes that encode fucosyltransferases competent to construct surface localized Lewis x determinants (Galβ1→4[Fucα1→3]GlcNAc-). (13–17). Specifically, these other enzyme(s) are thought to be polypeptides distinct from the Lewis fucosyltransferase because they exhibit different acceptor substrate specificities and differential sensitivities to divalent cation and N-ethylmaleimide inactivation (13–17). Moreover, their expression is determined by loci distinct from the Lewis blood group fucosyltransferase locus, and they display tissue-specific patterns that are different from expres-
sion patterns determined by the Lewis locus (13-17). Because these enzymes exhibit properties that are very similar to the Lewis blood group antigens, it has been considered implausible that their corresponding genes might be sufficiently related at the primary sequence level to be able to isolate them by cross-hybridization approaches. We report here the isolation and structure of one such cross-hybridizing human gene, and analysis of its enzymatic properties following its expression in transfected mammalian cells. These results indicate that this gene encodes a fucosyltransferase capable of efficiently attaching Fucα1→3 linkage to GlcNAc moieties on type II acceptors with unsubstituted terminal Gal moieties, but that is incapable of acting on type I acceptors. This gene segment can also determine surface expression of the, mammalian-specific sialyloligosaccharide determinant VIM-2 (NeuNAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4[Fucα1→3]GlcNAc- when expressed in CHO cells, as well as surface display of the Lewis x antigen, but not the sialyl Lewis x determinant (NeuNAcα2→3Galβ1→4[Fucα1→3]GlcNAc-). We further demonstrate that these VIM-2-positive, sialyl Lewis x-negative cells were able to bind to vascular endothelium in an ELAM-1-dependent fashion. These results conflict with others' (18) identification of the VIM-2 determinant as a putative ligand for ELAM-1, and suggest that the VIM-2 determinant is not by itself sufficient to mediate ELAM-1-dependent cell adhesion.

EXPERIMENTAL PROCEDURES

Cell Culture—The source and growth conditions of COS-1 cells, CHO cells and transfectants, and A431 cells are as previously described (19-21). The human HL-60 cell line was obtained from Dr. Steve Kunkel (University of Michigan, Ann Arbor). HL-60 cells were grown in 10% fetal calf serum and RPMI-1640 medium. Human umbilical vein endothelial cells (HUVEC) were isolated and propagated exactly as previously described (21).

Antibodies—Anti-Lewis x (anti-SSEA-1, Ref. 22) was provided by Dr. Davor Solter (Wistar Institute, Philadelphia, PA). Anti-H and anti-Lewis a antibodies were purchased from Chemibio, Ltd., Edmonton, Alberta, Canada. Anti-sialyl Lewis x and anti-sialyl Lewis a (23-25) were provided by Dr. Paul Terasaki (UCLA, Los Angeles, CA). The anti-VIM-2 antibody (26) was from Dr. Walter Knapp (Vienna, Austria). A pooled mouse IgG antibody preparation (Mslg) was purchased from Coulter. The monoclonal anti-ELAM-1 antibody (BB-11 (27) was provided by Dr. Roy Lobb (Biogen, Cambridge, MA). An isotype control antibody for BB-11 (IgG2b) was purchased from Coulter. Fluorescein-conjugated goat anti-mouse IgM or IgG antibodies were purchased from Sigma.

Human Genomic Library Construction and Screening—High molecular weight human genomic DNA was prepared from peripheral blood leukocytes as described previously (19, 20). Genomic DNA was subjected to partial digestion with restriction endonucleases, fractionated through a 0.8% agarose gel, and subjected to Southern blot analysis as previously described (19). To aid in the comparison of hybridization patterns obtained with different probes, duplicate blots were prepared from identical sets of restriction digests electrophoresed on a single gel. Southern blots were hybridized exactly as described previously (19). The temperature was maintained at 35 °C. Southern blots were probed with the 1.7-kb XhoI-XbaI fragment isolated from plasmid pCDM7-α(1,3,4)FT (28). Alternatively, Southern blots were probed with a 400-bp AcaII-Po1 fragment isolated from the insert in pFT-3. Following hybridization, blots were rinsed twice in 2 X SSC, 0.5% SDS at room temperature for 10 min, washed according to conditions described in the legend to Fig. 1, and then subjected to autoradiography.

Northern Blot Analysis—Total RNA was prepared from cultured cells using procedures previously described (30). Poly(A)+ RNA was then isolated from total RNA by oligo(dT) cellulose column chromatography using commercially supplied products according to procedures supplied by the manufacturer. RNA samples were electrophoresed through 1.0% agarose gels containing formaldehyde (30) and were then transferred to a nylon membrane (Hybond-N, Amer sham Corp.). Northern blots were prehybridized for 1 h at 61 °C in 1 X SSC, 5 X SSC, 5% sodium dodecyl sulfate, and 5% dry powdered salmon sperm DNA. Blots were then hybridized for at least 16 h at 61 °C in the same hybridization solution. The probe was a radiolabeled (29) 400-bp AcaII-Po1 fragment isolated from the insert in pFT-3. Following hybridization, blots were subjected to three, 10-min room temperature rinses in 2 X SSC, and then washed for 30 min at 62 °C in 2 X SSC, 0.2% SDS.

Transfection and Expression of the Insert in pFT-3—The 3.8-kb PsI insert in plasmid pFT-3 was excised and cloned into the PsI site in the mammalian expression plasmid pCDNA1 (Invitrogen). One plasmid with a single insert in the sense orientation with respect to the plasmid's CMV promoter enhancer sequences was designated pCDNA1-α(1,3,4)FT and was used for subsequent analysis. A second expression construct was prepared (pCDNA1-α(1,3,4)FTs), consisting of a segment of the insert in pCDNA1-α(1,3,4)FT corresponding to sequences between the MluI site at base pair 15 of Fig. 2B, and then extending into the insert's 3' end (Fig. 2A), classifying the sense orientation between the HindIII site and the PsI site in pCDNA1. This plasmid was constructed by digesting the insert in plasmid pCDNA1-α(1,3,4)FT with MluI, and making the ends blunt with the Klenow fragment of E. coli DNA polymerase I. The resulting DNA fragment was then ligated with MluI, and a 691-base pair fragment was isolated, that corresponds to bp 1-38 by bp 654 of the sequence shown in Fig. 2B. The plasmid pCDNA1-α(1,3,4)FT was prepared for receipt of this insert by digestion at the HindIII site proximal to the
The HindIII sites were incubated at 37 °C for periods of time to yield linear rates of reaction.

Concentration of 20 mM (N-acetyllactosamine, lactose, lacto-N-biose I, and lacto-N-tetraose) was assayed with the acceptor N-acetyllactosamine. A control transfected cell line (CHO-V1) was prepared in the same manner using pCDNAI without a CDN insert. CHO-V1 cells do not contain detectable α(1,3)-fucosyltransferase activity. The construction and characterization of stably transfected CHO cell lines containing plasmid pCDM7-(α1,3)FT (CHO-FT) or its control plasmid pCDM7 (CHO-V) have been described previously (21).

FACS Analysis—CHO cells transfected with the various expression plasmids using the DEAE-dextran procedure (30) exactly as previously described (4, 9).

Cell Adhesion Assays—Assays to determine adhesion of transfected CHO cells to HUVECs were performed slightly as described exactly as previously described (21). HUVECs plated in 96-well plates were placed in growth medium without growth factors, with or without 20 ng/ml TNFα (Genentech), for 4–6 h prior to binding assays. In experiments where inhibitory antibodies were used, a saturating concentration of antibody (1 μg/ml) was added at 10 μg/ml. Anti-Lewis x (anti-SSEA-1, mouse monoclonal IgM; ascites) was used at a dilution of 1:1000. Anti-sialyl Lewis x (mouse monoclonal IgM, HPLC purified from ascites) was used at 10 μg/ml. Anti-sialyl Lewis a (mouse monoclonal IgG3, ammonium sulfate precipitate of ascites) was used at a dilution of 1:1000. Anti-VIM-2 antibody (mouse monoclonal IgM, ascites) was used at a dilution of 1:200. Cells were then stained with fluorescein isothiocyanate-conjugated goat anti-mouse IgM or IgG, as appropriate, and were then subjected to analysis on a FACSkan (Becton-Dickinson), as described previously (4).

Fucosyltransferase Assays—Cell extracts containing 1% Triton X-100 were prepared from transfected COS-1 cells or from stably transfected CHO cells, using procedures described previously (4). Fucosyltransferase assays were performed in a total volume of either 20 μl for acceptors N-acetyllactosamine, lactose, lacto-N-biose I, or 2'-fucosyllactose, Sigma), or in 10 μl (NeuNAca2→3Galβ1→4GlcNAc, Oxford Glycosystems) and contained 50 mM sodium cacodylate, pH 6.2, 5 mM ATP, 10 mM fucose, 20 mM MnCl2, 5 mM GDP-[1,4C]Fucose, 0.85). The methyl signals of the GlcNAc N-acetyl group was verified by comparison of their chemical shifts to those of authentic standards (4).

The methyl signals of the GlcNAc N-acetyl group was verified by comparison of their chemical shifts to those of authentic standards (4).
Affinity-purified, protein A-Lewis fucosyltransferase fusion protein (4), or extracts of COS-1 cells transfected with the Lewis fucosyltransferase cDNA expression vector pCDM7-α(1,3,1,4)FT (4), were used to prepare an authentic, radiolabeled NeuNAcα2→3Galβ(1→4)[14C]Fucβ(1→3)GlcNAc standard (42). Enzyme was incubated with 20 mM NeuNAcα2→3Galβ(1→4)[14C]Fucβ(1→3)GlcNAc and 3 μM of GDP-[14C]fucose, under identical conditions used to assay extracts for activity with this sialylated acceptor, as detailed above. The reaction was then diluted with HPLC column buffer (75% acetonitrile, 25% 2 mM sodium acetate, pH 5.5) and fractionated by HPLC on an AX-5 column, as described above. Identities of radiolabeled compounds in the reaction were made by comparison with the elution times of radiolabeled standards (Lt-[14C]fucose, 6 min; GDP-[14C]fucose, 20 min), or according to elution times predicted by the chromatographic method used (the trisaccharide standard Galα(1→4)[14C]Fucα(1→3)GlcNAc elutes at 14 min; the radiolabeled product eluting at 18 min was tentatively identified as the tetrasaccharide chromographic method used (the trisaccharide standard Galα(1→4)[14C]Fucα(1→3)GlcNAc). The compound eluting at 18 min was collected, reduced to dryness with repeated lyophilization from water, and resuspended in 22 μl of water. This was then subjected to digestion with 0.1 units of Streptococcal neuraminidase (EC 3.2.1.18, Genzyme), in a total volume of 40 μl, in 100 mM sodium acetate, pH 6.5, 10 mM CaCl2, and 0.05% bovine serum albumin. The digested product was then fractionated on an AX-5 column as described above and was found to coelute with the trisaccharide standard Galα(1→4)[14C]Fucα(1→3)GlcNAc.

α-1-Fucosidase Digestion—The neutral, chromatographically purified radiolabeled fucosyltransferase product generated with N-acetyllactosamine was subjected to α-L-fucosidase digestion to confirm the α anomeric configuration of the attached fucose. 3-[14C]Fucosyl-N-acetyllactosamine was purified by descending paper chromatography as described above, and an aliquot (7000 cpm) was digested with 40 millimolars of α-L-fucosidase (EC 3.2.1.51, Boehringer Mannheim) in 20 μl of 100 mM sodium citrate, pH 5.5, at 37 °C for 22 h. The reaction was desalted by Dowex column chromatography and subjected to HPLC analysis using conditions described above for preparation of the radiolabeled standard. The product of the digestion was identified by comparison to parallel separations of Lt-[14C]fucose and the 3-[14C]fucosyl-N-acetyllactosamine starting material. Quantitative release of Lt-[14C]fucose was achieved by α-L-fucosidase digestion.

RESULTS

Molecular Cloning of a Human Genomic DNA Segment That Cross-hybridizes with the Lewis Blood Group α(1,3,1,4)-Fucosyltransferase cDNA—Biochemical and genetic studies indicate that the human genome encodes two or more distinct α(1,3)-fucosyltransferases (13-17). We have recently described a cloned cDNA that encodes one of these enzymes and that is thought to represent the product of the human Lewis blood group locus (4). In consideration of the possibility that these α(1,3)-fucosyltransferases might be encoded by a family of structurally related genes, we sought to isolate other such members by cross-hybridization methods, using the cloned Lewis fucosyltransferase cDNA. Low stringency Southern blot hybridization experiments indicate that the coding region of the Lewis α(1,3)-fucosyltransferase cDNA detects strongly hybridizing human DNA restriction fragments, as well as several weakly hybridizing fragments (Fig. 1A). Weakly hybridizing fragments were always detected regardless of the restriction enzyme used, suggesting that these represented one or more DNA sequences distinct from the authentic Lewis gene presumably represented by the strongly hybridizing fragments. To further examine the molecular nature of these sequences, we screened a human λ phage genomic DNA library at low stringency with the Lewis cDNA probe. A total of 18 phages were isolated from phages representing approximately five human genomic equivalents. Southern blot analysis of 16 of these phages allowed them to be placed into three groups, based upon their restriction patterns and hybridization signal intensity strengths (data not shown).2 Six phages representing a class of intermediate hybridization intensity were identified. A 3.6-kb cross-hybridizing PstI restriction fragment was subcloned from a representative phage of this class. To determine the relationship of this fragment to cross-hybridizing fragments detected in human genomic DNA with the Lewis probe, a 400-bp AvaII-PvuII fragment isolated from the insert in plasmid pFT-3 ("Experimental Procedures"), was hybridized with a 1.7-kb Xhol-XbaI fragment of the Lewis fucosyltransferase cDNA ("Experimental Procedures"), washed for 30 min at 65 °C in 1× SSC, 0.1% sodium deoxycholate, and subjected to autoradiography. Panel B, homologous genomic DNA segment probe. The blot was hybridized with a 400 bp AvaII-PvuII fragment isolated from the insert in plasmid pFT-3 ("Experimental Procedures"), washed for 30 min at 65 °C in 2× SSC, 0.5% sodium deoxycholate, and subjected to autoradiography. Relative mobilities of molecular size markers are indicated in kilobases at the left of panel A.

**FIG. 1. Southern blot analysis of human genomic DNA using the Lewis blood group fucosyltransferase probe and a homologous cloned human DNA restriction fragment.** Human genomic DNA was digested with restriction enzymes (Xhol, lane 1; PvuII, lane 2; EcoRI, lane 3; BglIII, lane 4) and subjected to Southern blot analysis using hybridization conditions described under "Experimental Procedures." Blots displayed are duplicates derived from the same agarose gel. Panel A, Lewis fucosyltransferase probe. The blot was hybridized with a 1.7-kb Xhol-XbaI fragment of the Lewis fucosyltransferase cDNA ("Experimental Procedures"), washed for 30 min at 65 °C in 1× SSC, 0.1% sodium deoxycholate, and subjected to autoradiography. Panel B, homologous genomic DNA segment probe. The blot was hybridized with a 400-bp AvaII-PvuII fragment isolated from the insert in plasmid pFT-3 ("Experimental Procedures"), washed for 30 min at 65 °C in 2× SSC, 0.5% sodium deoxycholate, and subjected to autoradiography. Relative mobilities of molecular size markers are indicated in kilobases at the left of panel A.

fragment identified a single long open reading frame within its 3' portion (Fig. 2A), corresponding to sequences that cross-hybridized to the Lewis cDNA probe (Fig. 2B). This reading frame begins with a methionine codon that is found within a 3' portion (Fig. 2A), corresponding to sequences that cross-hybridized to the Lewis cDNA probe (Fig. 2B). This reading frame begins with a methionine codon that is found within a maintain the type hybridized to the Lewis cDNA probe (Fig. 2C). The distal portion of this reading frame shares a substantial amount of

analysis (44) of the protein sequence predicted by this reading frame indicates a single hydrophobic segment at its NH2 terminus, suggesting that the predicted polypeptide would maintain the type II transmembrane orientation typical of mammalian glycosyltransferases (3) (Fig. 2C).

Moreover, hydropathy analysis (44) of the protein sequence predicted by this reading frame shares a substantial amount of

Hydrophobic segments are denoted by

program generates symbols between aligned amino acids, according to the evolutionary distance between them, as measured by Dayhoff (45) and normalized by Gribskov (46). Amino acid sequence identities are assigned a score of 1.5, denoted by

related amino acid residues with scores between 0.1 to

amino acid pairs with scores less than 0.1. The

cross-hybridizing portion of the insert in pFT-3. The DNA sequence of the insert in pFT-3 corresponding to the putative protein coding portion is shown with the derived amino acid sequence

indicated above their locations in the sequence. C, hydropathy analysis of the protein sequence predicted by this reading frame whose sequence is detailed in

endonuclease cleavage sites discussed in the text are indicated above the map (A, AuaII; B, PstII; C, MluI; S, SauI). Distance in base pairs is indicated below the map.

The algorithm of

is aligned with the corresponding amino acid residues of the Lewis

The DNA sequence of the insert in pFT-3 (pFT-3 DNA, corresponding to the putative protein coding portion is shown with the derived amino acid sequence (pFT-3 AA), single-letter code). This is aligned with the corresponding amino acid residues of the Lewis α(1,3/1,4)-fucosyltransferase sequence (Lewis AA). The amino acid sequence alignment was generated using the GAP program of the University of Wisconsin Genetics Computer Group (31), using a gap weight of 0.0, and a length weight of 0.1. This program generates symbols between aligned amino acids, according to the evolutionary distance between them, as measured by Dayhoff (45) and normalized by Gribskov (46). Amino acid sequence identities are assigned a score of 1.5, denoted by “|”; related amino acid residues with scores from 0.5 to 1.4 are denoted by “-”; less strongly related amino acid residues with scores between 0.1 to 0.4 are denoted by “.”; no symbol is placed between dissimilar amino acid pairs with scores less than 0.1. The MluI and SauI sites used to construct cDNA(α,β)FTMI, are indicated above their locations in the sequence. C, hydropathy analysis of the protein sequence predicted by the open reading frame. Hydrophobic segments are denoted by shading above the horizontal axis. The algorithm of Kyte and Doolittle (44) was used, with a window of 11.

FIG. 2. DNA sequence analysis. A, restriction map of the 3.6-kb PstI fragment. The solid area indicates the position of the open reading frame whose sequence is detailed in B, below. The positions of various restriction endonuclease cleavage sites discussed in the text are indicated above the map (A, AuaII; M, MluI; S, SauI; Pu, PstI, PstII). Distance in base pairs is indicated below the map. B, DNA and derived protein sequence of the cross-hybridizing portion of the insert in pFT-3. The DNA sequence of the insert in pFT-3 corresponds to the putative protein coding portion is shown with the derived amino acid sequence (pFT-3 AA, single-letter code). This is aligned with the corresponding amino acid residues of the Lewis α(1,3/1,4)-fucosyltransferase sequence (Lewis AA). The amino acid sequence alignment was generated using the GAP program of the University of Wisconsin Genetics Computer Group (31), using a gap weight of 0.0, and a length weight of 0.1. This program generates symbols between aligned amino acids, according to the evolutionary distance between them, as measured by Dayhoff (45) and normalized by Gribskov (46). Amino acid sequence identities are assigned a score of 1.5, denoted by “|”; related amino acid residues with scores from 0.5 to 1.4 are denoted by “-”; less strongly related amino acid residues with scores between 0.1 to 0.4 are denoted by “.”; no symbol is placed between dissimilar amino acid pairs with scores less than 0.1. The MluI and SauI sites used to construct cDNA(α,β)FTMI, are indicated above their locations in the sequence. C, hydropathy analysis of the protein sequence predicted by the open reading frame. Hydrophobic segments are denoted by shading above the horizontal axis. The algorithm of Kyte and Doolittle (44) was used, with a window of 11.
amino acid sequence identity with the corresponding portion of the Lewis fucosyltransferase (4) (Fig. 2B). This sequence similarity diverges, however, immediately proximal to amino acid 158 within the open reading frame, but begins again at amino acid 127. Inspection of the DNA sequence corresponding to the regions near the discontinuity in amino acid sequence similarity identifies sequences that resemble putative donor and acceptor splice sites (47, 48). Although we cannot yet formally exclude the possibility that these represent functional splice junctions, this seems unlikely since utilization of these sites as a pair would generate an exceptionally short intron (48).

These sequences share the highest degree of similarity between their COOH-terminal portions, within the catalytic domain of the Lewis fucosyltransferase (1). Sequence divergence occurs toward the predicted NH2-end, within the "stem" and transmembrane regions (3) of the latter enzyme. These observations are consistent with interspecies glycosyltransferase sequence comparisons (11) indicating that while glycosyltransferase catalytic domains are highly conserved, stem and transmembrane segments can apparently tolerate substantial differences in primary amino acid sequence. Thus, while this sequence organization might be consistent with the possibility that this segment represents a pseudogene, or that the site of sequence divergence correlates with the acceptor site for a splice site, we instead expected it to be more likely that the segment represents a single exonic sequence that encodes a fucosyltransferase.

The DNA Restriction Fragment Detects mRNA Transcripts in HL-60 Myeloid Cells—To test the possibility that this segment represents a functional α(1,3)-fucosyltransferase gene, a portion of it was used as a probe to identify transcripts in a cell line known to express such enzymes. The HL-60 human cell line was examined since these myeloid lineage cells are known to express one or more α(1,3)-fucosyltransferases that are distinct from the Lewis α(1,3)-fucosyltransferase (13). Northern blot analysis of polyadenylated mRNA isolated from these cells, using the 400-bp AvaII-PvuII segment corresponding to a portion of the open reading frame, identifies four distinct transcripts (Fig. 3). By contrast, no transcripts were detected when the same analysis was performed using the Lewis cDNA (data not shown). These results are consistent with the possibility that the fucosyltransferase(s) expressed by HL-60 cells are encoded by the open reading frame in the cloned PsfI segment.

The Open Reading Frame in the Homologous DNA Restriction Fragment Determines Expression of an α(1,3)-Fucosyltransferase—To determine if this segment encodes an α(1,3)-fucosyltransferase, the 3.6-kb PsfI fragment was cloned into a mammalian expression vector and the resulting plasmid (pCDNA1-α(1,3)FT, "Experimental Procedures") was introduced into two types of mammalian host cells by transfection. Transfected cells were then analyzed for vector-dependent cell surface glycoconjugate expression and for fucosyltransferase activity. COS-1 cells and CHO cells were used as hosts for these experiments since neither cell line normally expresses any detectable α(1,3)- and α(1,4)-fucosyltransferase activities (4, 21). Likewise, COS-1 and CHO cells do not normally express detectable amounts of cell surface Galβ1→4[Fcucα(1→3)]GlcNAc (Lewis x, SSEA-1) moieties, or the α2→3-sialylated derivative (NeuNAcα2→3Galβ1→4[Fcucα(1→3)]GlcNAc-, sialyl Lewis x) (4, 21, 49). These cells do, however, maintain surface display of the non-fucosylated neutral α2→3-sialylated type II oligosaccharides that can function as precursors to such molecules, via the action of the α(1,3)-fucosyltransferase encoded by a transfected Lewis cDNA expression vector (21, 49). COS-1 cells also maintain surface display of the type I precursors to the Lewis α (Galβ1→3[Fcucα(1→3)]GlcNAc-) and sialyl Lewis α (NeuNAcα2→3Galβ1→3[Fcucα(1→4)]GlcNAc-) moieties (22). The vector pCDNA1 was used since this plasmid efficiently transcribes exogenous, subcloned sequences in mammalian hosts by virtue of the cytomegalovirus immediate early promoter sequences in the vector (50).

In initial biochemical analyses, extracts prepared from COS-1 cells transfected with plasmid pCDNA1-α(1,3)FT were tested for the presence of vector-dependent fucosyltransferase activity using several low molecular weight acceptor substrates. In a standard fucosyltransferase assay ("Experimental Procedures"), extracts prepared from pCDNA1-α(1,3)FT-transfected cells, but not from control transfectants, contained a fucosyltransferase activity (296 pmol/mg/h) that utilized the type II disaccharide acceptor N-acetyllactosamine to yield a radiolabeled product with a chromatographic mobility ("Experimental Procedures") characteristic of authentic Galβ1→4[Fcucα(1→3)]GlcNAc (R2 -fucoyl-N-acetyllactosamine = 0.85) However, under these assay conditions, two other neutral type II molecules (2'-fucosyllactose, lactose) did not function as efficiently as N-acetyllactosamine as acceptor substrates for the fucosyltransferase in these extracts (17 and 10 pmol/mg/h, respectively, for 2'-fucosyllactose and lactose). Only a trace amount of transfer could be detected using the type I substrate lacto-N-biose I.

Likewise, we did not detect fucose transfer to the sialylated acceptor NeuNAcα2→3Galβ1→4GlcNAc (less than 1 pmol/ mg/h), even in extracts that exhibited a relatively large amount of activity toward N-acetyllactosamine (474 pmol/mg/h). By contrast, under these same conditions, extracts containing the Lewis blood group fucosyltransferase utilized both the sialylated acceptor (297 pmol/mg/h) and N-acetyllactosamine (526 pmol/mg/h), to form, respectively, the sialyl Lewis x tetrasaccharide and the Lewis x trisaccharide (see "Experimental Procedures"). Thus, the restricted acceptor preference exhibited by this enzyme in vitro contrasts remarkably with that exhibited by the Lewis α(1,3/1,4)-fucosyltransferase, which can efficiently utilize each of the five acceptors tested (4, 42, 51).

COS-1 cells transfected with pCDNA1-α(1,3)FT were also
analyzed by flow cytometry to detect de novo, vector-dependent surface expression of these oligosaccharide products, to allow an assessment of the enzyme’s in vivo acceptor substrate requirements. The transfected COS-1 cells exhibited positive staining with a monoclonal antibody directed against the Lewis x moiety (Gaβ1→4[Fucα(1→3)]GlcNAc-) (Fig. 4), whereas cells transfected with the pCDNA1 vector without insert did not express this determinant. However, COS-1 cells transfected with pCDNA1-α(1,3)FT, or with its control plasmid, did not stain with antibodies specific for the sialyl Lewis x antigen (Fig. 4). Likewise, the transfected cells did not exhibit detectable surface expression of Lewis α or sialyl Lewis α molecules (Fig. 4).

Polylectosaminoglycans with terminal α(2→3)-linked sialic acid also exist that maintain a single internal α(1,3)-linked fucose on the N-acetylgalactosamine residue of the penultimate lactosamine repeat (52). This determinant (NeuNAca2→3Galβ1→4GlcNAcβ1→3Galβ1→4[Fucα(1→3)]GlcNAc-) can be detected on the surfaces of myeloid cells by the monoclonal antibody VIM-2 (26) and may be constructed by the action of α(1,3)-fucosyltransferase(s) on type II polylectosamine acceptors whose terminal galactose residues are substituted with α(2,3)-sialic acid moieties (53). Neither COS-1 cells transfected with the Lewis α(1,3/1,4)-fucosyltransferase nor COS-1 cells transfected with plasmid pCDNA1-α(1,3)FT did not exhibit detectable surface expression of Lewis α or sialyl Lewis α molecules 2'-fucosyllactose and lactose with substantially lower efficiency (5.8 pmol/mg/h and 2.0 pmol/mg/h, respectively). Virtually no transfer could be detected when these extracts were tested with the type I substrate lacto-N-biose I (< 1 pmol/mg/h) or with the sialyl Lewis x precursor NeuNAca2→3Galβ1→4GlcNAc (< 1 pmol/mg/h). These results confirm those obtained with extracts prepared from the transfected COS-1 cells, and indicate that, to a first approximation, the COS-1 and CHO genetic backgrounds do not strongly influence the enzyme’s ability to utilize these low molecular weight acceptor substrates.

With one striking and important exception, flow cytometry analyses with the CHO-FT3 cells were virtually identical to those obtained with the transfected COS-1 cells (Fig. 5). CHO-FT3 cells exhibit uniform, bright staining with anti-Lewis α antibody but not with antibody directed against the sialyl Lewis x molecule (Fig. 5). Control transfected cells do not stain with either antibody (Fig. 5). As expected, neither cell line stained with antibodies against the neutral and α2→3-sialylated Lewis α isoforms (data not shown), since CHO cells do not construct type I precursors (21, 49). However, these cells differed in an important way from the transfected COS-1 cells, in that, like CHO cells transfected with the Lewis α(1,3/1,4)-fucosyltransferase cDNA (pCDM7-α(1,3/1,4)FT), these cells expressed substantial amounts of the VIM-2 determinant (Fig. 5).

Taken together with the results of the biochemical analyses

**Fig. 4. Flow cytometry profiles of transfected COS-1 cells.** COS-1 cells were transfected with the fucosyltransferase expression vectors pCDNA1-α(1,3)FT or pCDM7-α(1,3/1,4)FT, labeled, respectively, α(1,3)FT, and α(1,3/1,4)FT, or with their respective control vectors pCDNA1 and pCDM7. Transfected cells were then subjected to flow cytometry analysis with the monoclonal antibodies detailed in the inset, using methods detailed under “Experimental Procedures.” Between 15 and 28% of the cells transfected with pCDNA1-α(1,3)FT or with pCDM7-α(1,3/1,4)FT expressed, respectively, the Lewis x determinant only, or the Lewis x and sialyl Lewis x determinants, relative to background staining with anti-H antibody (data not shown). The data presented here are the mean (linear) fluorescence intensities of the antigen-positive population of transfected cells.

**Fig. 5. Flow cytometry profiles of stably-transfected Chinese hamster ovary cells.** Stably transfected CHO cell lines were generated (“Experimental Procedures”) with the fucosyltransferase expression vectors pCDNA1-α(1,3)FT (CHO-FT3 cells) and pCDM7-α(1,3/1,4)FT (CHO-FT7 cells), or with their respective control vectors pCDNA1 (CHO-V1 cells) and pCDM7 (CHO-V7 cells). The cell lines were subjected to flow cytometry analysis with the monoclonal antibodies detailed in the figure and as described under “Experimental Procedures.” These data are the mean fluorescence intensities of the entire population of these transfected cells. Virtually 100% of the CHO-FT3 cells stain with anti-Lewis x antibody (anti-Lex) and anti-VIM-2 antibodies (anti-VIM-2) (data not shown), but not with anti-H, anti-sialyl Lewis x (anti-sLex), and anti-Lewis x (anti-Lea) antibodies. Likewise, essentially 100% of the CHO-FT7 cells stain with anti-Lex, anti-sLex, and anti-VIM-2 antibodies. The entire population of CHO-V1 cells and CHO-V7 cells do not stain with any of the six antibodies.
performed with extracts from the transfected cells, the flow cytometry analyses presented in Figs. 4 and 5 and DNA sequence analyses indicate that plasmid pCDNA1-α(1,3)FT encodes an α(1,3)-fucosyltransferase. Transfection results obtained with plasmid pCDNA1-α(1,3)FTm0, demonstrate that this enzyme is encoded by the open reading frame displayed in Fig. 2B. The results further indicate that this enzyme can utilize type II precursors, but not type I precursors, and suggest that the enzyme cannot efficiently utilize α2→3-sialylated type II glycoconjugates to form the sialyl Lewis x determinant. These results also show that the host cell background has an important influence on the ability of fucosyltransferase expression vectors to determine biosynthesis of surface-localized VIM-2 determinants. In particular, whereas CHO cells exhibit VIM-2 expression when transfected with either plasmid pCDM7-α(1,3,1,4)FT or plasmid pCDNA1-α(1,3)FT (Fig. 5), COS-1 cells are apparently not competent to construct the VIM-2 determinant when transfected with either plasmid (Fig. 4). The mechanism(s) responsible for the observed cell-type specific VIM-2 expression patterns observed with these transfected DNA segments remain undefined (see "Discussion").

**Surface Expression of the VIM-2 Determinant, in the Absence of Sialyl Lewis x Expression, Does Not Confer ELAM-1-dependent Cell Adhesion—**Recent evidence from our group (21) and others (18, 55, 56) indicates that one or more members of the family of α2→3-sialylated, α1→3-fucosylated lactosaminoglycans represent oligosaccharide ligands for ELAM-1 (reviewed in Ref. 57). These candidate molecules include the sialyl Lewis x tetrasaccharide (21, 55, 56), its difucosylated analogue (21, 55), and the VIM-2 determinant (18, 21). Identification of the VIM-2 determinant as an ELAM-1 ligand (18) conflicts with data presented by Walz et al. (56), who suggest that VIM-2 does not participate in ELAM-1-dependent cell adhesion. These latter authors demonstrated that pretreatment of HL-60 cells with anti-VIM-2 antibody does not diminish the ability of these cells to adhere to ELAM-1, under conditions whereby pretreatment of the cells with anti-sialyl Lewis x antibody does block such adhesion (56). We expected that cell line CHO-FT3, that exhibits surface-localized expression of the VIM-2 determinant in the absence of sialyl Lewis x expression, would allow us to further address this apparent discrepancy. Using a standard adhesion assay based upon ELAM-1 expression by HUVECs ("Experimental Procedures"), we found that the VIM-2-positive, sialyl Lewis x-negative line CHO-FT3 does not exhibit binding to ELAM-1-expressing HUVEC monolayers (Fig. 6), under conditions whereby ELAM-1-dependent HUVEC adhesion can be demonstrated with the sialyl Lewis x-negative cell line CHO-FT (Fig. 6 and Ref. 21). These results are consistent with the observations of Walz et al. (56) and provide additional evidence contrary to others' proposal (18) that the VIM-2 oligosaccharide determinant represents a ligand for ELAM-1.

**DISCUSSION**

Biochemical and genetic considerations indicate that the human genome maintains three, or more, distinct α(1,3)-fucosyltransferase genes (13–17, and references therein. In an effort to isolate such genes, we identified and isolated human genomic sequences that cross-hybridized with a previously described cloned human α(1,3,1,4)-fucosyltransferase cDNA. Structural and functional analyses reported here using this isolated DNA segment indicate that it encodes an α(1,3)-

![Fig. 6. Adhesion of stably transfected Chinese hamster ovary cell lines to endothelial cell monolayers.](image)

fucosyltransferase, that exhibits enzymatic properties distinct from the Lewis fucosyltransferase encoded by the cDNA used for the cross-hybridization cloning approach. The enzymatic properties displayed by this enzyme, and the presence of its transcript(s) in the HL-60 promyelocytic cell line as well as in normal human cells of the myeloid lineage suggest that it represents the "myeloid type" of α(1,3)-fucosyltransferase (13, 14). Our preliminary results from Southern blot hybridization analyses are also consistent with others' conclusions that this gene is found on human chromosome 11 (58, 59). It remains to be determined if this gene segment corresponds to the one identified by Potvin et al. (14), that determines expression of an α(1,3)-fucosyltransferase in transfected CHO cells.

Cloned cDNAs (termed ELFT, for ELAM-1 ligand fucosyl transferase) corresponding to this gene have been isolated recently by Goelz et al. (60). The coding portions of these cDNAs are colinear with the coding portions of the gene segment reported here, confirming our sequence and expression analyses suggesting that the α(1,3)-fucosyltransferase polypeptide is encoded by a single exon. The conclusions of Goelz et al. (60) regarding the enzyme's neutral acceptor substrate specificities are essentially identical to ours. However, these investigators isolated ELFT cDNAs by virtue of their ability, when transfected into COS-1 and CHO cells, to direct surface-localized expression of molecules recognized by antibodies that inhibit binding of HL-60 cells to ELAM-1 (60). Goelz et al. (60) further demonstrated that CHO cells transfected with these cDNAs can bind to ELAM-1. Those observations are at odds with our own results, and the apparent discrepancy remains unresolved.

While it is possible that the CHO cell lines used by our group and theirs differ in genetic background, it seems unlikely that the sets of glycoconjugate molecules made by each line are sufficiently different to account for differences in fucosyltransferase-dependent synthesis and display of protein expression.

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3 B. W. Weston and J. B. Lowe, manuscript in preparation.

4 J. B. Lowe and C. B. Thompson, unpublished data.

5 R. D. Larsen and J. B. Lowe, unpublished data.
ELAM-1 ligands. Nonetheless, our own observations concerning the discordance between the ability of COS-1 cells and CHO cells to express the VIM-2 determinant provide a precedent for the possibility that distinct cell lines will differ in their capacity to construct such fucosylated molecules. While we do not yet understand the biochemical reasons why COS-1 and CHO cell lines differ in their ability to construct the VIM-2 determinant, it seems probable that this reflects cell-specific differences in the complement of oligosaccharide structures that are presented \textit{in vivo}. For example, the CHO host cells used for these experiments are known to express significant amounts of surface-localized α2-3-sialic acid-substituted polylactosamines on glycoproteins but not on glycolipids (49). COS-1 cells may instead maintain smaller amounts of such VIM-2 precursors, for example, or may not construct them at all. Biochemical characterization of potential VIM-2 precursors in these two cell lines should allow a resolution of this question.

In this context, it would also be useful to know the types and amounts of cell surface oligosaccharide antigens expressed by the ELFT-transfected CHO cells reported by Goelz et al. (60) and compare them to those displayed by the transfected CHO cells we report here. It will also be useful to determine if the apparent difference in specific activities of the α(1,3)-fucosyltransferase in our cells, and in theirs, is sufficient to yield qualitatively or quantitatively distinct arrays of cell surface oligosaccharide antigens. In this regard, it is believed that some mammalian α(1,3)-fucosyltransferases will fucosylate the GlcNAc moiety on the penultimate lactosamine repeat of an α(2,3)-sialylated polylactosamine glycolipid acceptor to form the VIM-2 determinant, in preference to monofucosylation of the GlcNAc moiety on the terminal lactosamine unit to form a sialyl Lewis x molecule (53). Evidence also exists to support the possibility that α(1,3)-fucosyltransferases in the human myeloid lineage exhibit this preference for internal GlcNAc residues and that VIM-2 determinants represent precursors to a difucosylated sialyl Lewis x molecule wherein the terminal GlcNAc residue is fucosylated secondarily (61). If this pathway is operative, it may be speculated that in a cell that constructs appropriate precursors, low level expression of an α(2,3)-sialyltransferase will largely yield VIM-2 determinants, whereas in cells that maintain high fucosyltransferase expression levels, many of these VIM-2 determinants will also be converted to difucosylated moieties with sialyl Lewis x activity. Assuming that the ELFT transfectants bind to ELAM-1 because they express sialyl Lewis x determinants (55, 56), this proposed pathway for an ordered synthetic scheme for (difucosylated) sialyl Lewis x determinants is consistent with the apparently high α(1,3)-fucosyltransferase specific activity (N-acetyllactosamine acceptor) in the ELFT transfectants (1110 pmol/mg/h; Ref. 60), relative to the activity present in the sialyl Lewis x-negative, VIM-2-positive CHO-PT3 cells reported here (69.1 pmol/mg/h). Confirmation of this possibility will require biochemical analyses of the type reported in Howard et al. (53) and in Holmes et al. (54) to determine this enzyme’s relative preference for alternate GlcNAc residues on α(2,3)-sialylated polylactosamine acceptors. Such studies are currently in progress in our laboratory.

Careful biochemical analysis of the cell surface glycoproteins in CHO cells transfected with ELFT or with pCDNA1-α(1,3)FT, or in transfected but otherwise isogenic cell lines with different α(1,3)-fucosyltransferase expression levels, will also be instructive in this regard. Such studies would most probably also contribute to a further understand-
consistent with the notion that the cell surface carbohydrate ligand for ELAM-1 is a mono- (55, 56), or di-fucosylated (55) sialyl Lewis x-reactive molecule.

Barring technical errors, the apparent discrepancy between the conclusions of Tiemeyer et al. (18) and those reported by us here and by Walz et al. (56) might be a consequence of conformational differences between VIM-2 determinants presented by glycolipids (18, 26) versus glycoproteins and/or by cell membranes (21, 52, 56, and this work) versus solid-phase matrices (18), that may be sensed by ELAM-1 but not necessarily by the anti-VIM-2 antibody used in these studies. Alternatively, the VIM-2 determinant may have a low affinity for ELAM-1, relative to the sialyl Lewis x moiety, and the abundance of the VIM-2 epitope displayed by a cell will strongly influence the ability of that cell to be recognized by ELAM-1. Resolution of these issues will await the isolation and testing of homogeneous preparations of molecules that display the sialyl Lewis x and VIM-2-determinants to determine their relative and absolute affinities for ELAM-1, and to determine contributions to binding made by the lipid, protein, and oligosaccharide molecules that display these determinants.

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