The Fucosyltransferase FucT-VII Regulates E-Selectin Ligand Synthesis in Human T Cells

Randall N. Knibbs,* Ronald A. Craig,* Shunji Natsuka,§ Alfred Chang,‡ Mark Cameron,‡ John B. Lowe,* and Lloyd M. Stoolman*

*Departments of Pathology, ‡Surgery, and the §Howard Hughes Medical Research Institute, University of Michigan, Ann Arbor, Michigan 48109-0602

Abstract. Selectin-ligands on T cells contribute to the recruitment of circulating cells into chronic inflammatory lesions in the skin and elsewhere. This report provides the first evidence that a single fucosyltransferase, termed FucT-VII, controls the synthesis of E-selectin ligands in human T-lymphoblasts. The FucT-IV transferase (the ELFT enzyme), in contrast, constructs lower avidity E-selectin ligands and requires enzyme levels found only in myeloid cells. Treatment of Jurkat cells with phorbol myristate acetate increased the expression of sialylated LewisX-related (sLeX-related) epitopes and induced the synthesis of E-selectin ligands functional at physiologic levels of linear shear-stress. Northern analysis revealed a parallel increase in the steady-state level of FucT-VII mRNA, but there were no increases in the two other leukocyte-associated fucosyltransferases (FucT-IV and VI). The stable transfection of the FucT-VII gene into Jurkat cells induced high levels of the sLeX-related epitopes and the synthesis of E-selectin ligands which equaled or exceeded the avidity of those on circulating lymphocytes. The growth of T-lymphoblasts under conditions which induced expression of the sLeX,a epitopes increased the level of FucT-VII mRNA, the synthesis of sialylated-LewisX structures by cell-free extracts and the synthesis of E-selectin ligands equal in avidity to those on FucT-VII transfectants. In contrast, neither the mRNA levels nor activities of the FucT-IV and VI enzymes increased in association with E-selectin ligand synthesis in T-lymphoblasts. Myeloid cell lines, unlike lymphoblasts, expressed high levels of both the FucT-VII and IV enzymes in conjunction with E-selectin ligands raising the possibility that both enzymes contributed to ligand synthesis. FucT-IV transfected Jurkat cells synthesized low avidity ligands for E-selectin but only in association with the CDw65 (VIM-2) carbohydrate epitope. Only blood neutrophils and myeloid cell lines expressed this epitope at the levels associated with E-ligand synthesis in the transfectants. In contrast, native Jurkat cells, blood monocytes, blood lymphocytes, and cultured T-lymphoblasts expressed low levels or none. We conclude that FucT-VII is a principal regulator of E-selectin ligand synthesis in human T-lymphoblasts while both FucT-VII and FucT-IV may direct ligand synthesis in some myeloid cells.

Functional ligands for the endothelial selectins are expressed on 5–20% of circulating αβ memory T cells (20, 33), a large percentage of γδ T cells (13, 32), natural killer cells (25), and activated B cells (36). Correlative studies in several species suggest that these molecules participate in recruitment. T-lymphocytes expressing carbohydrate epitopes associated with ligands for E-selectin are preferentially recruited into inflammatory and malignant diseases involving human skin (7, 39). In addition, E-selectin is expressed on the microvasculature in chronic rheumatoid synovitis (10) and lymphocytes isolated from the synovial pannus bind to E-selectin more efficiently than circulating lymphocytes from the same patient (35). Temporal associations between E-selectin expression and lymphocyte recruitment have been documented in human skin (11, 16), ruminant skin (44), and in the peribronchial tissue of human xenografts in SCID mice (34). Finally, carbohydrate-ligands for E-selectin have also been identified on the activated B-lymphocytes infiltrating the thyroid in Hashimoto's thyroiditis (36). Thus, lymphocytes synthesize glycoconjugate ligands for E-selectin and may use them during recruitment into inflammatory lesion.

Please address all correspondence to L.M. Stoolman, Department of Pathology, University of Michigan, Medical Sciences Bldg. 1, Rm. 4224, 1301 Catherine Road, Ann Arbor, MI 48109-0602. Tel.: (313) 936-2459. Fax: (313) 763-6476. E-mail: STOOLMAN@path.umich.edu

Published in abstract form: Knibbs, R.N., R.A. Craig, S. Natsuka, A. Thall, M. Cameron, J.B. Lowe, and L.M. Stoolman. 1995. FASEB (Fed. Am. Soc. Exp. Biol.) J. 9(3):A34.
Studies by Picker and colleagues show that the synthesis of E-selectin ligands on αβ T cells occurs during the naive to memory transition after antigen-induced proliferation (33). The prevalence of carbohydrate epitopes associated with selectin ligands (e.g., CSLEX1 and HECA452, also known as the cutaneous lymphocyte antigen or CLA) depend on the lymphoid organ in which proliferation occurs implying that the microenvironment of the tissue regulates ligand synthesis. In vitro experiments support this hypothesis by showing that the mix of growth factors in the medium influences the expression of the HECA452 epitope (33). Thus, αβ T cells regulate selectin-mediated recruitment, in part, through control of ligand synthesis at the cell surface.

The synthetic pathway and control points for construction of selectin ligands on T cells are completely unknown. Structure/function studies of endogenous selectin ligands imply that multiple control points may exist. All selectin ligands consist of a protein or lipid “core” coupled to multiple O- or N-linked oligosaccharide chains (23, 37, 42). The oligosaccharide portion of the molecules is essential for function. For example, all ligands contain terminal sialic acid and the removal of this sugar alone will inactivate the molecule (15, 26, 29, 38). Similarly, all ligands contain fucose, and addition of this sugar alone to the appropriate sialylated precursor will generate a functional molecule (19). However, recent studies with the ligand for P-selectin known as PSGL-1 show that expression of the appropriate glycosyltransferases is not sufficient for synthesis of high affinity molecules (40). As a consequence, the expression of functional E-selectin ligands on T cells could be regulated through synthesis of the core structures, the oligosaccharides, or both.

Finally, the glycosyltransferases which synthesize the oligosaccharide portion of the selectin ligands on leukocytes have not been identified. The finding that glycosyltransferases capable of synthesizing sialylated Lewisα (sLeα) result in expression of functional ligands for E-selectin in COS and CHO cells suggests that one or more of these enzymes participates in the synthesis of ligands on leukocytes (18, 19). Cloning of the ELFT fucosyltransferase (now designated fucosyltransferase-IV or FucT-IV) from the HL-60 cell line led to the hypothesis that this enzyme regulates the synthesis of ligands in leukocytes (9, 24). However, the low efficiency of sLeα synthesis and E-selectin binding activity in some FucT-IV transfected CHO strains (17), and the subsequent identification of two additional fucosyltransferases in leukocytes (41) raises the possibility that other enzymes are involved.

This study examines the role of fucosyltransferases in the synthesis of E-selectin ligands on normal and malignant T-lymphoblasts. The investigation reveals that control of terminal glycosylation regulates adhesive activity and that the fucosyltransferase FucT-VII, rather than FucT-IV, is the key enzyme.

Materials and Methods

Cell Culture

Jurkat cells were grown in RPMI-1640 (GIBCO-BRL, Grand Island, NY) supplemented with 10% FCS. Cells were stimulated with 10 nM PMA (Sigma Chem. Co., St. Louis, MO) for 6-8 d before analysis of fucosyltransferase activity and selectin-ligand expression. The fucosyltransferase transfectants of a T-lymphoblastic cell lines were grown in RPMI-1640 with 10% FCS and G418 (GIBCO-BRL) to maintain the transfected phenotype. Human lymphocytes isolated from vaccine-primed lymph node cells (5) were activated by cross-linking of CD3 and expanded in interleukin-2 (10 U/ml, recombinant-human, a gift from Genentech, South San Francisco, CA) supplemented media as described in the Results section.

Flow Cytometry

Indirect immunofluorescence staining and flow cytometry were conducted as previously described using light-scatter gating to identify the populations of interest (14). The anti-VIM-2 (Immunotech, Westbrook, ME), CSLEX1 (Amer. Type Culture Collection, Rockville, MD), HECA-452 (Dr. Louis Picker, University of Texas Southwestern School of Medicine, Dallas, TX), anti-Lewisα (MMA, Becton Dickinson, San Jose, CA), and isotype-matched control antibodies were used at concentrations ranging from 10-20 µg/ml. The FITC- and phycocerythrin (PE)-conjugated secondary reagents (Immunotech) were used at saturating concentrations, generally at a 1:50-1:100 dilution of the commercial stock solution.

The FACScan photomultiplier was set to a mean fluorescence intensity (MFI) of 6-10 for the isotype-matched control population (on a four-decade log scale) before reading the test cells. The fluorescence intensity measurements and the histograms were made with the Winlist Listmode analysis program (Verity Software, Topsham, MA). The histograms in the figures are plotted on four-decade logarithmic scales with maximal peak heights normalized to the negative control population so that differences in the prevalence of positive cells can be compared. In Table I, the “positive cells” were defined as the fraction which exceeded the fluorescence intensity of 98% of the cells in the isotype-matched control. The MFI was the numerical mean of the fluorescence histograms for the positive population.

RNA Preparation and Analysis

Total RNA preparation, Northern blotting, and analysis were performed as described (2). Briefly, cells grown in suspension culture were harvested by centrifugation. The cells were triturated in the Trizol reagent (GIBCO-BRL) which both lysed cells and extracted the RNA. Total RNA was solubilized in chloroform and precipitated with isopropanol. The RNA pellet was washed with 75% ethanol, dried under a vacuum, dissolved in DEPC-treated water, and stored at −70°C until used.

 Twice-purified mRNA was used in the Northern analyses due to the low abundance of transcripts for the glycosyltransferases. Poly (A) mRNA was separated from total RNA over an oligo-(dT)-cellulose column (Boehringer Mannheim Biochemicals, Indianapolis, IN). The bound mRNA was eluted with 0.1 M KOH, reapplied to the oligo-(dT)-cellulose column, eluted, and precipitated in ethanol/potassium containing 20 µg of glycerogen (Boehringer Mannheim Biochemicals). The RNA precipitates were dried on a rotary evaporator, resuspended in DEPC-treated water, electrophoresed (7-10 µg/ lane) on a denaturing 1% agarose-formaldehyde gel and analyzed by Northern blotting.

Northern Blot Analysis

Northern blotting was performed following capillary transfer of mRNA from the agarose-formaldehyde gels to nylon membranes (Hybond-N, Amersham Corp., Arlington Heights, IL). The mRNA was cross-linked to the membrane (UV light, 5 min), rinsed in 6X SSC buffer, and baked for 2 h at 80°C. The blot was prehybridized for 6-20 h at 42°C in a buffer consisting of 5X SSC, 5X Denhardt’s solution, salmon sperm DNA, 50% formamide and 50 mM sodium phosphate, pH 6.5. The fucosyltransferase and β-actin probes were labeled with [32P] using a Random Primed labeling kit (Boehringer Mannheim Biochemicals) and α-[32P]dCTP (Amersham Corp.) following the manufacturer’s protocol. The blot was hybridized with the 32P-labeled probe at a concentration of at least 10 x 106 cpm/ml in the prehybridization buffer for 24 h at 42°C, followed by four washes for 5 min each in 2X SSC containing 0.2% SDS. The blot was then washed.

The Journal of Cell Biology, Volume 133, 1996 912
Enzyme Assay for Fucosyltransferase-VII and Fucosyltransferase-IV

Fucosyltransferase assays were carried out as described (27). Briefly, cells grown in suspension culture were collected by centrifugation, washed twice in Dulbecco's PBS, and resuspended in 1% Triton X-100 at 50-100 \times 10^6 cells/mL. The reaction cocktails contained the following constituents in a total volume of 20 μL: extract from 1-5 \times 10^6 cells, 3 μM GDP-[3H]Fucose or [14C]Fucose (American Radiolabeled Chemicals, Inc., St. Louis, MO); 5 mM of the carbohydrate acceptor, 5 mM ATP, 10 mM L-fucose; 10 mM MnCl_2; 0.5% Triton X-100 and 25 mM cacodylate buffer, pH 6.2. The carbohydrate acceptor for the FucT-IV assay was N-acetyllactosamine (LacNAc, provided by I.J. Goldstein, University of Michigan, Ann Arbor, MI) and the acceptor for the FucT-VII assay was sialyl α2,3-LacNAc (OxFord Glycosystems, Rosedale, NY). Incubations were carried out at 37°C for 2 h (FucT-IV) or 3 h (FucT-VII). The reactions were stopped by the addition of 20 μL of ethanol and 560 μL of water (FucT-IV) or 580 μL of 5 mM PO_4, pH 6.8 (FucT-VII). The reaction products were isolated over Dowex 50W-X8 form (FucT-IV) and Dowex 1X-8-400, phosphoric form (FucT-VII), and analyzed by scintillation counting. Results are reported as picomoles product formed per hour per 10^6 cells.

Parallel-Plate Flow Assays

The parallel-plate flow chamber used in these studies and the general technique has been described (1). Monolayers of either human umbilical vein endothelium (HUVE) passage 3-5 or transfected CHO cells expressing E-selectin (CHO-E) were grown on 35-mm Petri-plates. The HUVE cells were treated with recombinant human tumor necrosis factor (TNF-α) nanograms/ml, PeproTech, Inc., Rocky Hill, NJ) for 4-6 h before conducting experiments. Experiments on TNF-treated HUVE were conducted with the α4- and β2-integrins blocked by the monoclonal antibodies HP2/1 (IgG2a, provided by JI Goldstein, University of Michigan, Ann Arbor, MI) and the acceptor for the FucT-VII assay was sialyl α2,3-LacNAc (Oxford Glycosystems, Rosedale, NY). Incubations were carried out at 37°C for 2 h (FucT-IV) or 3 h (FucT-VII). The reactions were stopped by the addition of 20 μL of ethanol and 560 μL of water (FucT-IV) or 580 μL of 5 mM PO_4, pH 6.8 (FucT-VII). The reaction products were isolated over Dowex 50W-X8 form (FucT-IV) and Dowex 1X-8-400, phosphoric form (FucT-VII), and analyzed by scintillation counting. Results are reported as picomoles product formed per hour per 10^6 cells.

Transfection of T-Lymphoblastic Cell Lines

The construction of the FucT-IV or FucT-VII expression vectors has been described (27, 45). Transfection of Jurkat cells with linearized vectors containing FucT-IV or FucT-VII was carried out using a Cell-Porator® Electroporation system (GIBCO-BRL) as follows: 800 μL of 10^7 cells/mL in HEBS buffer (20 mM Hepes, pH 7.05, 137 mM NaCl, 5 mM KCl, 0.7 mM PO_4, 6 mM dextrorose) were pulsed at 280 V, 880 μF at a sterile electroporation cell. Aliquots of the electroporated cells were added to normal medium in T25 flasks and grown for 3 d. After 3 d, 2 mg/mL of G418 was added to select for stable transfectants. Resistant cells appeared within 2-4 wk at which time the bulk cultures were examined for expression of carbohydrate epitopes and functional selectin ligands. Limited-dilution cloning was conducted to stabilize and enhance the prevalence of transfectants with the desired phenotype. The selected bulk cultures and clones were maintained in media with 0.4 mg/mL of G418.

Results

Previous studies indicated that selectin ligands on T cells are synthesized in response to mitogenic stimuli. Since protein kinase C regulates many transcriptional responses in T cells during mitogenesis (22), we examined the effects of the agonist phorbol myristate acetate (PMA) on the Jurkat cell line. This line retains many of the T cell responses to mitogens including transcriptional induction of IL-2, IL-2 receptors, and L-selectin (14). Addition of PMA to the growth medium increased the prevalence and density of two epitopes defined by terminal sialylated, fucosylated N-acetyllactosamine structures, HECA452 and CSLEX1. The HECA452 antibody reacts with glycoconjugates carrying sialylated Lewis^a (sLe^a), sialylated Lewis^b and other epitopes to be defined capping structures (3). The CSLEX1 antibody reacts with glycoconjugates carrying the sLe^a tetrasaccharide primarily (6). These two epitopes will subsequently be referred to as sLe^a-related. The response varies markedly in different clonal isolates of Jurkat. Fig. 1 shows the time course for upregulation in the JS9-78 clone used for the experiments depicted in Figs. 2 and 3. Expression of the HECA452 epitope increased within 24-48 h and then
rose steadily over the next 3–8 d. Expression of the CSLEX1 epitope lagged behind HECA452; however, the prevalence and densities of both were significantly elevated after 6–8 d of culture in 5–10 nM PMA. Jurkat cells which survived and proliferated in the presence of 5 nM PMA for 30–60 d showed the highest densities of both carbohydrate epitopes.

Fig. 2 shows that 6–8 d of treatment with PMA upregulated expression of the sLe^α-related epitopes (A) and E-selectin mediated rolling activity in Jurkat cells (B). These experiments used human umbilical vein endothelium treated with tumor-necrosis factor for 4–6 h (TNF-HUVE). The velocity measurements were made with the α4- and β2-integrins on the Jurkat cell blocked. The adhesive interactions observed under these conditions are completely inhibited by the E-selectin specific monoclonal antibody 7A9. The untreated Jurkat cells showed virtually no interactions with TNF-HUVE. PMA treatment increased the prevalence of E-selectin–dependent adhesion. The low number of adhesion events indicated that only a subset of the PMA-treated cells expressed high avidity ligands. This behavior is consistent with the observation that high level expression of sLe^α-related epitopes was confined to a subset of the treated cells (A).

PMA treatment selectively increased the steady-state level of the FucT-VII mRNA in Jurkat cells. The increase was readily detected by Northern analysis at the 6–8 d time point (Fig. 3) and in Jurkat cells treated for >60 d with PMA (not shown). In contrast, FucT-IV (ELFT) transcripts were barely detectable by Northern blot analysis despite using relatively large amounts of purified mRNA (7 μg poly-A purified mRNA per lane) and prolonged expositions of the 32P-labeled blot in the PhosphorImager (14 d).

The capacity of the FucT-VII gene product alone to regulate E-selectin ligand synthesis in T cells was explored by constructing stable FucT-VII transfectants in the Jurkat cell line (FucT-VII-Jurkat). The subcloned transfectants expressed the sLe^α-related epitopes at the highest levels observed on peripheral blood lymphocytes (Fig. 4 A).
FucT-VII-Jurkat cell line also expressed high avidity E-selectin ligands capable of mediating slow rolling on TNF-HUVE at physiologic levels of linear shear-stress (Fig. 4B). In addition, the FucT-VII-Jurkat showed a higher prevalence of adhesive interactions (i.e., eightfold more events) and a slower mean rolling velocity than monocyte-depleted peripheral blood lymphocytes (MDPBL). This behavior mirrored the overall prevalence, and, to some extent, the level of sLeα-related epitopes in the two populations. Transfection of the vector alone did not result in the expression of either the sLeα-related epitopes or functional E-selectin ligands (not shown).

The next series of experiments capitalized on the discovery that minor changes in the growth media dramatically altered the expression of selectin ligands on cultured normal T-lymphoblasts. The growth protocols consisted of two activation/expansion cycles as described for production of vaccine-primed lymph node cells before infusion for the adoptive immunotherapy of renal cell carcinoma (5, 43). Each cycle began with cross-linking of the T cell receptor on plate-immobilized CD3 (48 h) followed by expansion in IL-2 supplemented media for 6 d in gas-permeable bags. This protocol resulted in a 50-100-fold increase in the absolute number of CD8 cells with >90% viability after 15 d. At 15 d, the populations consisted of 80-90% CD8 cells and 10-20% CD4 cells.

Fig. 5 shows the carbohydrate epitopes and E-selectin binding activity for lymph node lymphocytes expanded in the serum-free medium XVIVO15 (Bio-Whittaker, Walkersville, MD), XVIVO15 supplemented with 10% human AB serum (HAB-Sigma Chemical Co.) or RPMI with 10% HAB. Growth in XVIVO15 produced CD8 blasts uniformly positive for CSLEX1 and HECA452 epitopes (Fig. 5A). These cells rolled and arrested on E-selectin transfected CHO cells at 1.9 dynes/cm² of shear-stress confirming the presence of functional ligands (Fig. 5B). Northern analysis of twice purified poly-A messenger RNA showed massive induction of mRNA specific for the fucosyltransferase FucT-VII but no evidence for induction of mRNAs for FucT-III-VI (Fig. 6A). In contrast, growth in HAB-supplemented mediums resulted in cell populations with a low prevalence of CSLEX1 and HECA452 epitopes, low E-selectin binding activity and much lower levels of FucT-VII mRNA by Northern analysis (Figs. 5 and 6). Finally, the level of sLeα-related epitopes and functional E-selectin ligands correlated with the level of sLeα synthesis by cell-free extracts (Fig. 6B). The Leα synthetic activity, in contrast, was the same regardless of the prevalence of sLeα-related epitopes and E-selectin ligands in the blast populations.

As noted above, FucT-IV activity was not significantly different in lymphoblasts with and without E-selectin ligands. However, recent intravital studies in FucT-IV knockout animals showed an increase in selectin-dependent neutrophil rolling velocities in cremasteric venules implying that the selectin-ligand density/affinity was reduced in enzyme deficient cells (Thall, A., U. von Andrian, and J.B. Lowe, manuscript in preparation). We, therefore, conducted several experiments to determine whether this enzyme directed the synthesis of selectin ligands in human leukocytes.

Stable FucT-IV transfectants constructed in Jurkat cells (FucT-IV-Jurkat) rolled on E-selectin under shear; however, the mean velocity was higher than either the FucT-VII-Jurkat cells or the myeloid cell lines (Fig. 7). The
figure 5. coregulation of carbohydrate epitopes and e-selectin ligands during t cell proliferation in vitro. vaccine-primed lymph node cells (vplns) were expanded in serum-free or serum-supplemented media. the figure shows the results of flow cytometry (a) and parallel-plate adhesion assays (b) on vplns grown in the serum-free medium xvivo15 (xvivo), xvivo15 with 10% human ab serum (xvivo+hab) or rpmi with 10% human ab serum (rpmi+hab). the assays were conducted as described in fig. 1 except that chinese hamster ovary cells transfected with the full-length e-selectin gene (cho-e) were substituted for tnf-huve.

fucT-IV-Jurkat cells showed a 10–15-fold increase in expression of the CDw65 epitope (originally known as VIM-2: [21]) but not the structurally distinct CSLEX1 or HECA452 epitopes (table 1). the level of CD15, the Le6 structure, increased as well. the myeloid cells lines (HL60 and U937) and blood neutrophils were the only cells constitutively expressing the CDw65 epitope at or above the levels on the FucT-IV-Jurkats. monocytes and nontransfected Jurkat cells expressed the epitope to a lesser degree while blood lymphocytes and T-lymphoblasts cultured in XVIVO15 were essentially devoid of the structure. the constitutive expression of CD15 in cell lines and blood leukocytes varied in concert with CDw65; however, its level was consistently higher. in contrast, the CSLEX1 and HECA452 epitopes were expressed on all leukocytes with functional ligands for E-selectin and did not vary in concert with either CDw65 or CD15. in fact, overexpression of FucT-VII in Jurkat cells eliminated the CDw65 epitope and markedly reduced CD15 expression.

in the final experiment, the levels of fucosyltransferase mRNA and enzyme activity in the two CSLEX1/CDw65 positive myeloid cell lines were compared to the CSLEX1 positive, CDw65 negative lymphoblasts grown in XVIVO15 (fig. 8). the Northern blots showed the relative levels of

fucT-VII
fucT IV
fucT III/VII
β actin

sLeaX
LeaX

xvivo xvivo+hab rpmi+hab

0.55(0.07) 0.06(0.015) 0.10(0.05)
1.05(0.08) 0.73(0.05) 1.02(0.04)

figure 6. induction of FucT-VII mRNA and sialyl-Lewisx ligands during t cell proliferation in vitro. vaccine-primed lymph node cells (vplns) were expanded in serum-free or serum-supplemented media. the figure shows the steady-state prevalence of mRNA species (a) and the fucosyltransferase activities in cell-free extracts (b) of vplns grown in the serum-free medium xvivo15 (xvivo), xvivo15 with 10% human ab serum (xvivo+hab) or rpmi with 10% human ab serum (rpmi+hab). the rate of sialylated Lewisx and Lewisx synthesis on exogenous sialyl-LacNAc and LacNAc acceptors (in pmol/h/10^6 cells, standard deviation in parentheses) provided measures of fucosyltransferase activities.
Figure 7. Velocity histograms of Jurkat transfectants and myeloid cell lines on CHO-E. Jurkat cells were transfected with expression vectors containing either the FucT-VII or FucT-IV genes as described in Fig. 2. The bulk FucT-VII transfectants expressed the CSLEX1 epitope while the FucT-IV transfectants expressed the VIM2 epitope exclusively (see text). Therefore, the lines were subcloned for optimal expression of the CSLEX1 and VIM2 epitopes, respectively, before functional analysis. The figure compares the velocity distributions of the FucT-IV transfectants (FucT-IV-Jurkat), the FucT-VII transfectants (FucT-VII-Jurkat), and the myeloid cell lines U937 (U937) and HL-60 (HL-60). See Fig. 1 for additional information on the velocity measurements.

Figure 8. Comparison of fucosyltransferases in myeloid cell lines and T-lymphoblasts expressing functional ligands for E-selectin. The figure shows the steady-state prevalence of mRNA species (A) and the fucosyltransferase activities in cell-free extracts (B) of two myeloid cell lines (U937, HL-60) and VPLNs grown in the serum-free medium XVIVO15 (XVIVO-blasts). See Fig. 2 for additional information on the Northern analyses and enzyme assays.

Discussion

This report establishes a role for the fucosyltransferase FucT-VII in the synthesis and regulation of E-selectin ligands on human T-lymphoblasts. First, in PMA-treated Jurkat cells and cultured T-lymphoblasts, the steady-state level of FucT-VII mRNA correlates with the prevalence of sLeα-related epitopes and the construction of physiologically relevant E-selectin ligands. In contrast, transcripts for the leukocyte-associated fucosyltransferases FucT-IV and FucT-VI are essentially undetectable by Northern analysis in up to 7 μg of purified messenger RNA regardless of the level of E-selectin ligands on T-lymphoblasts. Second, the pattern of sialylated N-acetyllactosamine epitopes on the surface of FucT-VII transfected Jurkat cells (CSLEX1 and HECA452 positive, CD15 low and CD665 negative) recapitulates the phenotype observed on cultured T-lymphoblasts (Table I) and reported for T-lymphoblasts synthesizing E-selectin ligands in vivo (33). Fuc-T-IV transfectants, in contrast, express high levels of CDw65 and CD15 and no CSLEX1 or HECA452. Third, the avidity of the ligands synthesized by the FucT-VII transfectants and the cultured T-lymphoblasts are similar. Fuc-T-IV transfectants, on the other hand, produce only low avidity ligands. Finally, the fucosyltransferase activity associated with the synthesis of E-selectin ligands in T-lymphoblasts best fits the substrate specificity of the FucT-VII enzyme. The cell-free assays found that synthesis of sLeα on the 2,3 sialyl-LacNAc substrate increases in conjunction with E-selectin binding activity. The synthesis of Leα on the LacNAc sub-
The current study shows that in human leukocytes, FucT-IV constructs E-selectin ligands in association with high levels of the CDw65 epitope exclusively. The sLe^a-related epitopes CSLEX1 and HECA452 are not expressed by FucT-IV transfected Jurkat cells; thus the E-selectin "ligands" synthesized by this enzyme may differ structurally from those specified by the FucT-VII enzyme. Furthermore, the ligands appear to be low in density and/or avidity since the FucT-IV transfectants show fewer interactions and roll faster than the FucT-VII transfectants or myeloid cells (Fig. 7). Whether the CDw65 epitope forms part of an E-selectin ligand on leukocytes cannot be determined from these transfection studies. However, the findings indicate that the FucT-IV enzyme synthesizes low avidity E-selectin ligands when expressed at high levels and that the sLe^a-moicity is probably not the active structure.

The contributions of the two fucosyltransferases to E-selectin ligand synthesis appears to be lineage dependent. Granulocytes, monocytes, myeloid cells lines, and E-selectin ligand-positive lymphoid cells all express the sLe^a-related epitopes at levels found on the FucT-VII transfectants. In contrast, only neutrophils and myeloid cell lines express the CDw65 epitope at the level found on the FucT-IV transfectants (Table I). In keeping with their phenotype, the myeloid cell lines contain very high levels of mRNA and enzymatic activity for both fucosyltransferases. The E-ligand positive T-lymphoblasts, on the other hand, express high levels of FucT-VII activity but 10-fold lower levels of FucT-IV activity than the myeloid cells (Fig. 8). Thus, the level of FucT-IV activity in T-lymphoblasts is sufficient to synthesize the Le^a epitope but not high enough to synthesize the CDw65 epitope or E-selectin ligands in association with high levels of the CDw65 epitope exclusively. The sLe^a-related epitopes CSLEX1 and HECA452 are not expressed by FucT-IV transfected Jurkat cells; thus the E-selectin "ligands" synthesized by this enzyme may differ structurally from those specified by the FucT-VII enzyme. Furthermore, the ligands appear to be low in density and/or avidity since the FucT-IV transfectants show fewer interactions and roll faster than the FucT-VII transfectants or myeloid cells (Fig. 7). Whether the CDw65 epitope forms part of an E-selectin ligand on leukocytes cannot be determined from these transfection studies. However, the findings indicate that the FucT-IV enzyme synthesizes low avidity E-selectin ligands when expressed at very high levels and that the sLe^a-moicity is probably not the active structure.

The contributions of the two fucosyltransferases to E-selectin ligand synthesis appears to be lineage dependent. Granulocytes, monocytes, myeloid cells lines, and E-selectin ligand-positive lymphoid cells all express the sLe^a-related epitopes at levels found on the FucT-VII transfectants. In contrast, only neutrophils and myeloid cell lines express the CDw65 epitope at the level found on the FucT-IV transfectants (Table I). In keeping with their phenotype, the myeloid cell lines contain very high levels of mRNA and enzymatic activity for both fucosyltransferases. The E-ligand positive T-lymphoblasts, on the other hand, express high levels of FucT-VII activity but 10-fold lower levels of FucT-IV activity than the myeloid cells (Fig. 8). Thus, the level of FucT-IV activity in T-lymphoblasts is sufficient to synthesize the Le^a epitope but not high enough to synthesize the CDw65 epitope or E-selectin ligands in association with high levels of the CDw65 epitope exclusively. The sLe^a-related epitopes CSLEX1 and HECA452 are not expressed by FucT-IV transfected Jurkat cells; thus the E-selectin "ligands" synthesized by this enzyme may differ structurally from those specified by the FucT-VII enzyme. Furthermore, the ligands appear to be low in density and/or avidity since the FucT-IV transfectants show fewer interactions and roll faster than the FucT-VII transfectants or myeloid cells (Fig. 7). Whether the CDw65 epitope forms part of an E-selectin ligand on leukocytes cannot be determined from these transfection studies. However, the findings indicate that the FucT-IV enzyme synthesizes low avidity E-selectin ligands when expressed at very high levels and that the sLe^a-moicity is probably not the active structure.

The contributions of the two fucosyltransferases to E-selectin ligand synthesis appears to be lineage dependent. Granulocytes, monocytes, myeloid cells lines, and E-selectin ligand-positive lymphoid cells all express the sLe^a-related epitopes at levels found on the FucT-VII transfectants. In contrast, only neutrophils and myeloid cell lines express the CDw65 epitope at the level found on the FucT-IV transfectants (Table I). In keeping with their phenotype, the myeloid cell lines contain very high levels of mRNA and enzymatic activity for both fucosyltransferases. The E-ligand positive T-lymphoblasts, on the other hand, express high levels of FucT-VII activity but 10-fold lower levels of FucT-IV activity than the myeloid cells (Fig. 8). Thus, the level of FucT-IV activity in T-lymphoblasts is sufficient to synthesize the Le^a epitope but not high enough to synthesize the CDw65 epitope or E-selectin ligands in association with high levels of the CDw65 epitope exclusively. The sLe^a-related epitopes CSLEX1 and HECA452 are not expressed by FucT-IV transfected Jurkat cells; thus the E-selectin "ligands" synthesized by this enzyme may differ structurally from those specified by the FucT-VII enzyme. Furthermore, the ligands appear to be low in density and/or avidity since the FucT-IV transfectants show fewer interactions and roll faster than the FucT-VII transfectants or myeloid cells (Fig. 7). Whether the CDw65 epitope forms part of an E-selectin ligand on leukocytes cannot be determined from these transfection studies. However, the findings indicate that the FucT-IV enzyme synthesizes low avidity E-selectin ligands when expressed at very high levels and that the sLe^a-moicity is probably not the active structure.
tin ligands. Only FucT-IV transfectants, myeloid cell lines, and neutrophils (or their precursors) contain enough enzyme to synthesize E-selectin ligands functional at physiologic levels of linear-shear stress. Consequently, we conclude that both fucosyltransferases may regulate E-selectin binding activity in myeloid cells but that FucT-VII is the predominant enzyme in T-lymphoblasts.

The synthetic pathway identified in this study may regulate the trafficking of both normal and malignant T cells in vivo. Picker and colleagues report that the prevalence of the HECA452 epitope (also known as CLA) is highest on T-lymphoblasts generated in lymphoid organs draining the skin (33). Both the HECA452 and CSLEX1 epitopes are strongly expressed on a subset of T-lymphoblasts undergoing the naïve to memory transition in these organs. In contrast, memory T-lymphocytes with E-selectin ligands in the circulation frequently express the HECA452 epitope at higher levels than CSLEX1. HECA452-positive memory T cells also accumulate in cutaneous inflammatory/immunologic lesions in association with E-selectin positive venules (28, 30, 31). In addition, the HECA452 epitope is strongly expressed on T cell lymphomas infiltrating skin (4) and the prevalence of the epitope on the circulating malignant T cells correlates with the extent of skin involvement (12). Thus, E-selectin may promote the recruitment of HECA452 positive normal and malignant cells from the bloodstream into the skin.

The current study shows that the FucT-VII enzyme controls expression of both the HECA452 epitope and E-selectin ligands on T-lymphoblasts. Furthermore, it demonstrates that the medium in which T cell activation and proliferation occurs influences HECA452 and E-ligand synthesis through pretranscriptional regulation of the FucT-VII gene product. Consequently, we propose that modulation of the FucT-VII gene product during the antigen-driven proliferation of T-lymphoblasts in vivo determines the level of E-selectin ligands on the memory cells reentering the circulation thus influences their subsequent trafficking pattern. Similarly, upregulation of the FucT-VII gene product in malignant cells will induce high avidity ligands for E-selectin and may account for metastases to E-selectin rich tissues such as the skin. Finally, the observation that rising FucT-VII levels in PMA-treated Jurkat cells upregulates HECA452 expression at a faster rate than CSLEX1 suggests that the relative densities of the two epitopes depend on the level or duration of FucT-VII activity. Therefore, the differences in the ratio of these two epitopes on T-lymphoblasts from lymph nodes and memory T cells from peripheral blood may reflect a fall in the level of FucT-VII as the cells enter a resting state. The competitive RT-PCR techniques needed to test these hypotheses directly on the relatively small number of T cells regulating selectin ligands in vivo are currently under development. However, the current study provides a mechanistic foundation for these studies by identifying the synthetic pathway and control points likely to regulate E-selectin ligand synthesis in vivo.

In summary, this study provides the first evidence that modulation of fucosyltransferase activity regulates the synthesis of E-selectin ligands during T cell activation/proliferation. Both the FucT-VII and FucT-IV enzymes contribute to the synthesis of E-selectin ligands on human leukocytes. However, the FucT-VII enzyme predominates in T cells while both participate in ligand production by myeloid cells. In addition to E-ligand synthesis, FucT-VII regulates expression of the CSLEX1 and HECA4452 epitopes while FucT-IV regulates CD15 and CD66s levels on leukocytes. Whether these carbohydrate epitopes form part of the endogenous E-selectin ligands on T-lymphoblasts or other leukocytes remains to be determined. Nonetheless, the findings herein suggest that inhibitors of the FucT-VII enzyme, in particular, will suppress E-selectin-dependent inflammatory and immunologic diseases. Furthermore, specific blockade of this enzyme may prevent ligand synthesis by T cells while preserving low-level, FucT-IV mediated production in neutrophils. As a consequence, such agents may inhibit selectin-dependent chronic inflammatory diseases in the skin and elsewhere without the global suppression of inflammation that limits treatment with currently available drugs.

The authors are indebted to Drs. C.W. Smith, O. Abbassi, and L.V. McIntire for their help in establishing the parallel-plate flow chamber assay.

This work was supported under the following grants from the National Institutes of Health: AI33189, AR41703, and HL51963 to L.M. Stoolman and GM47455 to J.B. Lowe.

Received for publication 20 November 1995 and in revised form 21 February 1996.

References

1. Abbassi, O., T.K. Kishimoto, L.V. McIntire, D.C. Anderson, and C.W. Smith. 1993. Selectin supports neutrophil rolling in vitro under conditions of flow. J. Clin. Invest. 92:2719–2730.

2. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Strahl. 1993. Current Protocols in Molecular Biology. Chapter 4. Greene Publishing Association and Wiley-Interscience, NY.

3. Berg, E.L., T. Yoshino, L.S. Rott, M.K. Robinson, R.A. Warnock, T.K. Kishimoto, L.J. Picker, and E.C. Burcher. 1991. The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. J. Exp. Med. 174:1461–1466.

4. Borowitz, M.J., A. Weidner, E.A. Olsen, and L.J. Picker. 1993. Abnormalities of circulating T-cell subpopulations in patients with cutaneous T-cell lymphoma: cutaneous lymphocyte-associated antigen expression on T cells correlates with extent of disease. Leukemia. 7:859–863.

5. Chang, A.E., M.J. Cameron, B.A. Fox, and S. Shu. 1993. Generation of anti-CD3/IL-2 activated, vaccine-primed lymph node cells for the adoptive immunotherapy of human malignancy (Meeting abstract). Proc. Annu. Meet. Amer. Assoc. Cancer Res. 34:A2924.

6. Chia, D., P.J. Terasaki, S. Niyama, J. Galton, M. Hirota, and D. Katz. 1985. Use of monoclonal antibodies to sialylated Lewisx and sialylated Lewisa for serological tests of cancer. Cancer Res. 45:435–437.

7. Gelb, A.B., B.R. Smoller, R.A. Warnke, and L.J. Picker. 1993. Lymphocytes infiltrating primary cutaneous neoplasms selectively express the cutaneous lymphocyte-associated antigen (CLA). Am. J. Pathol. 142:1556–1564.

8. Gersten, K.M., S. Natsuka, M. Trinchieri, B. Petryniak, R.J. Kelly, N. Hiraiwa, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, and J.B. Lowe. 1993. Molecular cloning, expression, chromosomal assignment, and tissue-specific expression of a murine n-(1,3)-fucosyltransferase locus corresponding to the human ELAM-1 ligand fucosyl transferase. J. Biol. Chem. 270(42):25047–25056.

9. Goetz, S.E., C. Hession, D. Goff, B. Griffiths, R. Tizard, B. Newman, G. Chi Rosso, and R. Lobb. 1990. ELFT: a gene that directs the expression of an ELAM-1 ligand cell-surface transmembrane. J. Biol. Chem. 270(42):25047–25056.

10. Grober, J.S., B.L. Bowen, H. Ebling, B. Athey, C.B. Thompson, D.A. Fox, and L.J. Picker. 1993. Lymphocytes infiltrating primary cutaneous neoplasms selectively express the cutaneous lymphocyte-associated antigen (CLA). Am. J. Pathol. 142:1556–1564.

11. Groves, R.W., M.H. Allen, J.N. Barker, D.O. Haskard, and D.M. MacDonald. 1991. Endothelial leukocyte adhesion molecule-1 (ELAM-1) expression in cutaneous inflammation. Br. J. Dermatol. 124:171–173.

12. Heald, P.W., S.L. Yan, R.L. Edelson, R. Tigel, and L.J. Picker. 1993. Skin-selective lymphocyte homing mechanisms in the pathogenesis of leukemic cutaneous T-cell lymphoma. J. Invest. Dermatol. 101:222–226.
