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 Lewis^x-related (sLe^x-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 sLe^x-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 sLe^{x,a} epitopes increased the level of FucT-VII mRNA, the synthesis of sialylated-Lewis^x 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 α/β memory T cells (20, 33), a large percentage of γ/δ 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 α/β T cells occurs during the naïve 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)[1] 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, α/β 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 fucosyltransferases capable of synthesizing sialylated Lewis^x (sLe^x) 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 sLe^x 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.

1. *Abbreviations used in this paper*: CHO-E, CHO cells expressing E-selectin; CLA, cutaneous lymphocyte antigen; HUVE, human umbilical vein endothelium; FucT-III-VII, fucosyltransferases III-VII; sLe^x, sialylated Lewis^x; MDPBL, monocyte depleted peripheral blood lymphocyte; MFI, mean fluorescence intensity; PMA, phorbol myristate acetate; TNF, tumor-necrosis factor.

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 transfected 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-Lewis^x (MMA, Becton Dickinson, San Jose, CA), and isotype-matched control antibodies were used at concentrations ranging from 10–20 μg/ml. The FITC- and phycoerythrin (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 glycogen (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 6× SCC buffer, and baked for 2 h at 80°C. The blot was prehybridized for 6–20 h at 42°C in buffer consisting of 5× SCC, 5× 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 [^{32}P] using a Random Primed labeling kit (Boehringer Mannheim Biochemicals) and α-[^{32}P]dCTP (Amersham Corp.) following the manufacturer's protocol. The blot was hybridized with the ^{32}P-labeled probe at a concentration of at least 10×10^6 cpm/ml in the prehybridization buffer for 24 h at 42°C, followed by four washes for 5 min each in 2× SCC containing 0.2% SDS. The blot was then washed

twice at high stringency in buffer containing 0.2× SCC and 0.2% SDS (65°C, 30 min), wrapped in Saran and analyzed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Blots were stripped with a solution of boiling 0.2% SDS in water and examined for bound radioactivity before being probed for expression of a distinct mRNA. The blots were probed sequentially with cDNAs specific for FucT-VII, FucT-IV, a sequence common to FucT-III, V, and VI, and finally β-actin.

The PhosphorImager produced a digitized image reflecting the distribution of counts on the blot. The region of the blot containing each mRNA species was identified by a positive control run in a parallel lane. The HL60 and U937 cells served as positive controls for FucT-VII and FucT-IV mRNA (41). Colo205 cells served as positive control for the common FucT-III,V,VI probe (41). The appropriate regions were cropped and assembled into the composite figures using Photostyler (Aldus, Inc., Seattle, WA) and Stanford Graphics (Visual Numerics, Houston, TX).

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 × 10^6 cells/mL. The reaction cocktails contained the following constituents in a total volume of 20 μL: extract from 1–5 × 10^5 cells, 3 μM GDP-[^{3}H]Fucose or [^{14}C]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 Dowex1x4-400, formate form (FucT-IV) or Dowex1x8-400, phosphate 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-10 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 (Immunotech) and TS1/18 (ATCC), respectively. The remaining adhesive interactions are E-selectin dependent since addition of the E-selectin specific monoclonal antibody 7A9 (ATCC) to the antibody cocktail completely eliminated all interactions. Adhesive interactions on CHO-E were completely inhibited by treatment with streptococcal neuraminidase (Genzyme, Inc., Cambridge, MA) or blockade with the monoclonal antibody 7A9.

Data analysis was conducted with software developed in conjunction with Compix, Inc. (Mars, PA) for use on their C-Imaging 1280 Computerized Morphometry System (Compix, Inc.). This module (termed Celltrak) uses computerized morphometry to track the leukocytes in real time as they cross over the monolayer expressing the adhesion receptor of interest. Sequential images of the tracked cells were digitized and matched on the basis of their trajectories and morphometric characteristics. The distances between the centers of the paired cells were measured and the velocities calculated using the time-delay between the captured images. A time-delay of 3–4 s was used since this provided optimal resolution for cells rolling at velocities <50 μm/s. The Celltrak program acquired the digitized images, measured velocities of all the identified cells, compiled statistics from the prescribed number of fields and generated histograms of the velocity distribution. A measurement derived from a single pair of images is referred to as an "event." A given cell may contribute to one or more events since cells which remain in contact with the monolayer are sampled multiple times. Therefore, the number of events is a measure of the prevalence of adhesive interactions but not the absolute number of adherent cells. Each histogram consists of 1,000–4,000 events collected from 60–100 sequential fields covering a 2–6-min period of observation. The velocity histograms were plotted using Stanford Graphics (Visual Numerics, Houston, TX).

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® Electroporator 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 dextrose) were pulsed at 280 V, 880 μFarads in 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 mitogensis (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^x (sLe^x), sialylated Lewis^a and other yet to be defined capping structures (3). The CSLEX1 antibody reacts with glycoconjugates carrying the sLe^x tetrasaccharide primarily (6). These two epitopes will subsequently be referred to as sLe^x-related. The response varied 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

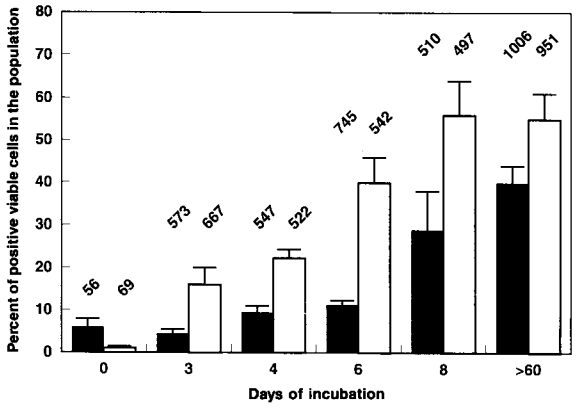

Figure 1. Time course for induction of HECA452 ■ and CSLEX1 □ epitopes on phorbol myristate acetate (PMA)–treated Jurkat cells. 5–10 nM PMA was added to the culture medium for the times indicated followed by single color flow cytometry to measure expression of the two epitopes. Each time point includes data from 3–13 independent experiments. The error bars define the standard error for each data set. The number in bold print above each column is the average of the mean fluorescence intensities (MFIs) for the pooled data.

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^x-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^x-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 exposures of the ^{32}P-labeled blot in the PhosphorImager (14 d). The FucT-IV probe readily detected FucT-IV specific transcripts in an equal amount of purified mRNA from myeloid cell lines (see Fig. 7). In addition, no increase in the mRNA levels of FucT-III, FucT-V, or FucT-VI were detected using a probe reactive with homologous regions of these three gene products. This probe reacted with mRNA from the Colo205 cell line (data not shown) which expresses high levels of the FucT-III enzyme (41).

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^x-related epitopes at the highest levels observed on peripheral blood lymphocytes (Fig. 4 *A*). The

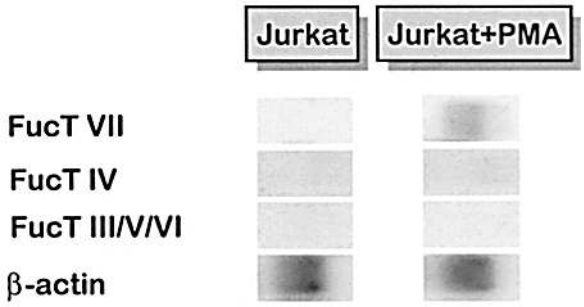

Figure 3. Induction of FucT-VII mRNA in PMA-treated Jurkat cells. The figure shows the steady-state prevalence of mRNA species in untreated (*Jurkat*) and PMA-treated (*Jurkat+PMA*) cells. Northern analysis of 7–10 μg of purified messenger RNA provided measures of the fucosyltransferase and β-actin gene products.

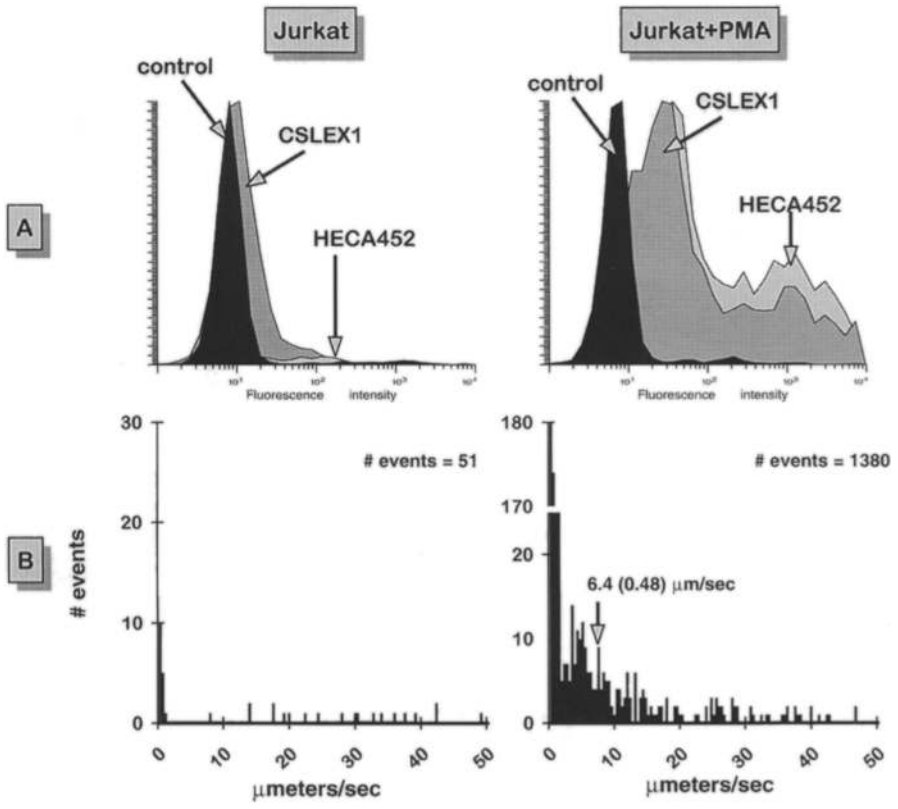

Figure 2. Comparison of carbohydrate epitopes and E-selectin ligands on PMA-treated Jurkat cells. Jurkat cells were stimulated with 10 nM PMA for 6–8 d. The figure shows the expression of carbohydrate epitopes (*A*) and functional ligands for E-selectin (*B*) on untreated (*Jurkat*) and PMA-treated (*Jurkat+PMA*) cells. The histograms in *A* show the prevalence of the epitopes CSLEX1 and HECA452 in a sample of 10^4 cells after indirect immunofluorescence staining with a phycoerythrin-tagged secondary reagent. The modes for each histogram are set to the same height so that the induction of the carbohydrate epitopes is clearly seen. The histograms in *B* show the velocity distribution of the cells adhering to tumor-necrosis factor treated human umbilical vein endothelium at 1.9 dynes/cm^2 with both the α4- and β2-integrins blocked (see text). The "# events" (*upper right corner* of histograms) are proportional to the overall prevalence of adhesive interactions during the period of observation (see Materials and Methods). The mean leukocyte rolling velocity (*above arrow*, standard deviation in parenthesis) reflects the avidity of the adhesive interactions.

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. 4 *B*). 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 sLe^x-related epitopes in the two populations. Transfection of the vector alone did not result in the expression of either the sLe^x-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. 5 *A*). These cells rolled and arrested on E-selectin transfected CHO cells at 1.9 dynes/cm^2 of shear-stress confirming the presence of functional ligands (Fig. 5 *B*). 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. 6 *A*). 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 sLe^x-related epitopes and functional E-selectin ligands correlated with the level of sLe^x synthesis by cell-free extracts (Fig. 6 *B*). The Le^x synthetic activity, in contrast, was the same regardless of the prevalence of sLe^x-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

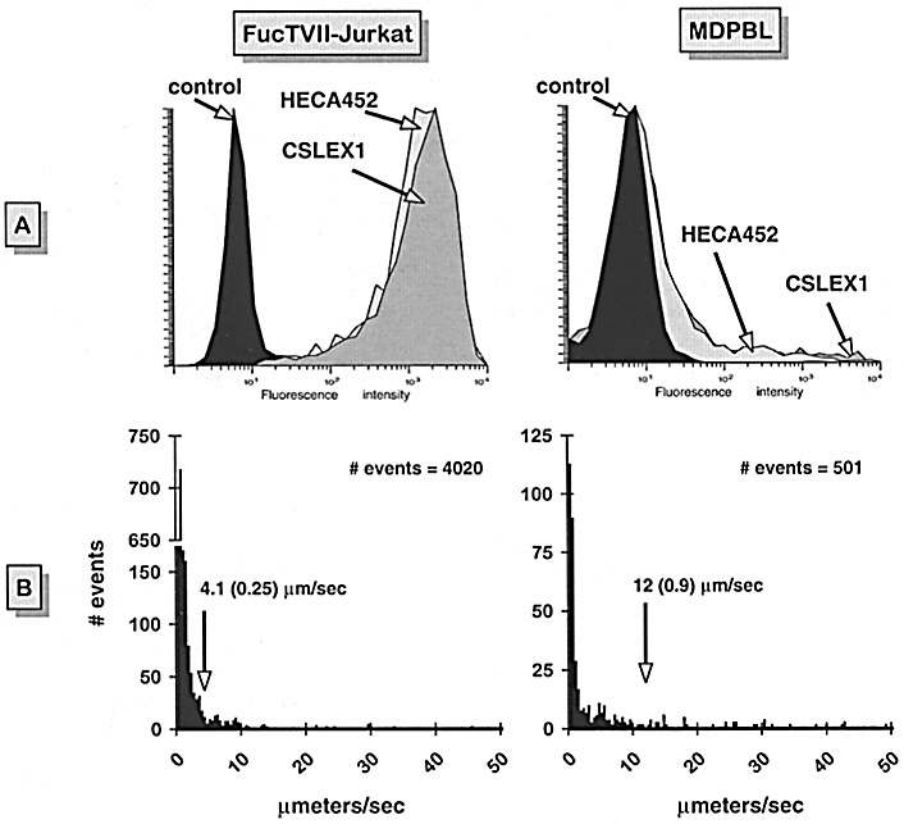

Figure 4. Induction of carbohydrate epitopes and E-selectin ligands by overexpression of the FucT-VII gene product in Jurkat cells. Jurkat cells were transfected with an expression vector containing the FucT-VII and G418 resistance genes. Transfectants were selected in G418 containing media and subcloned for expression of the CSLEX1 epitope. The figure shows the expression of carbohydrate epitopes (*A*) and functional ligands for E-selectin (*B*) on the transfectants (*FucT-VII-Jurkat*) and monocyte-depleted peripheral blood lymphocytes (*MDPBL*). See Fig. 1 for addition information on the assays.

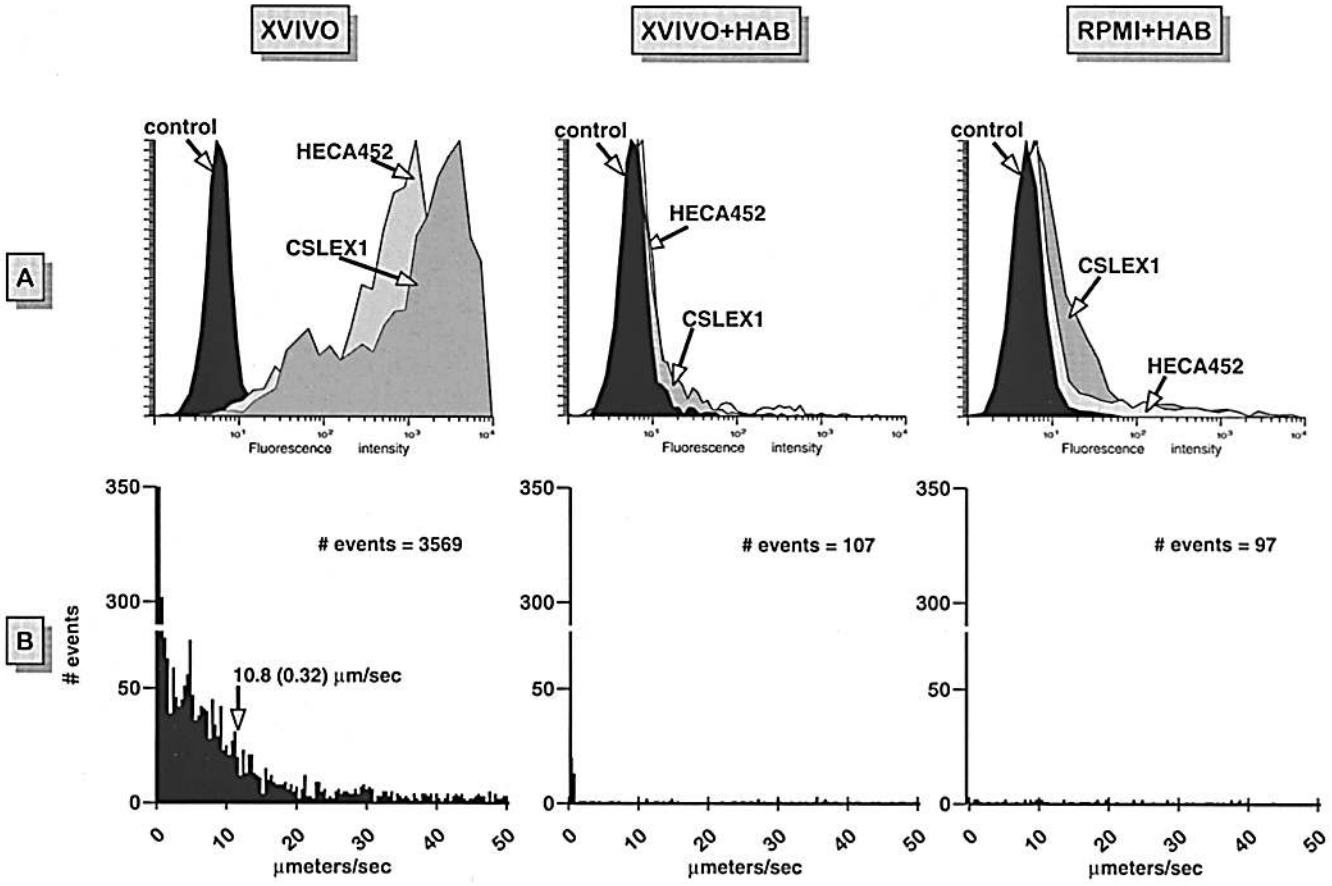

Figure 5. Coregulation of carbohydrate epitopes and E-selectin ligands during T cell proliferation in vitro. Vaccine-primed lymph node cells (VPLNs) were expanded in serum-free or serum-supplemented media. The figure shows the results of flow cytometry (*A*) and parallel-plate adhesion assays (*B*) on 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 I). The level of CD15, the Le^x 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

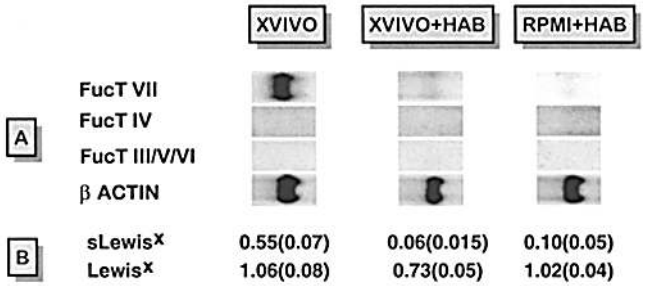

Figure 6. Induction of FucT-VII mRNA and sialyl-Lewis^x ligands during T cell proliferation in vitro. Vaccine-primed lymph node cells (VPLNs) were expanded in serum-free or serum-supplemented media. The figure shows the steady-state prevalence of mRNA species (*A*) and the fucosyltransferase activities 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 Lewis^x and Lewis^x synthesis on exogenous sialyl-LacNAc and LacNAc acceptors (in pmol/h/10^6 cells, standard deviation in parentheses) provided measures of fucosyltransferase activities.

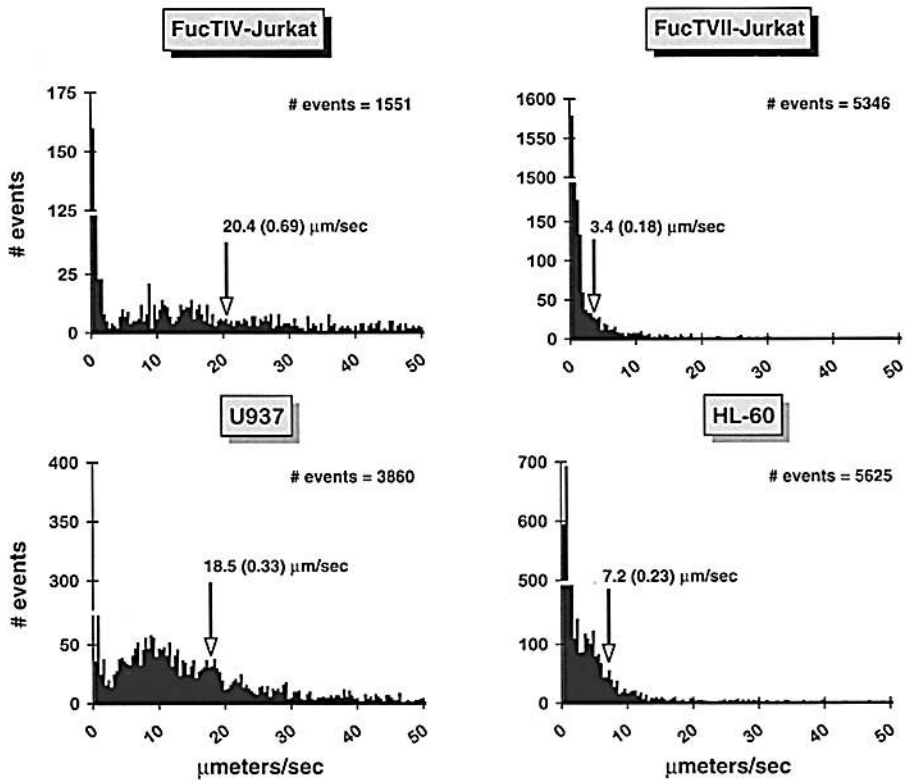

Figure 7. Velocity histograms of Jurkat transfectants and myeloid cell lines on CHO-E. Jurkat cells were transfected with expression vectors containing either the FucT-VII or FucT-IV genes as described in Fig. 2. The bulk FucT-VII transfectants expressed the CSLEX1 epitope while the FucT-IV transfectants 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.

the fucosyltransferase mRNAs in approximately equivalent amounts of twice-purified mRNA. FucT-IV transcripts were detected in both myeloid cell lines at levels approaching the FucT-VII mRNA. In contrast, only the FucT-VII transcripts were sufficiently abundant in the T-lymphoblasts to be detected on the blots. The cell-free enzyme assays provided a direct comparison of relative synthetic activities. The sLe^x synthesis was relatively high in all three populations while Le^x synthesis was 10–16-fold higher in the myeloid cell lines. Thus, the steady-state levels of FucT-VII mRNA and sLe^x synthetic activity in these cells paralleled the expression of the sLe^x-related epitopes shown in Table I. In contrast, the level of FucT-IV mRNA and Le^x synthetic activity paralleled the expression of the CD15 and CDw65 epitopes.

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 sLe^x-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 CDw65 negative) recapitulates the phenotype observed on cultured T-lymphoblasts (Table I) and reported for T-lymphoblasts synthesizing E-selectin ligands in vivo (33). FucT-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. FucT-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 sLe^x on the 2,3 sialyl-LacNAc substrate increases in conjunction with E-selectin binding activity. The synthesis of Le^x on the LacNAc sub-

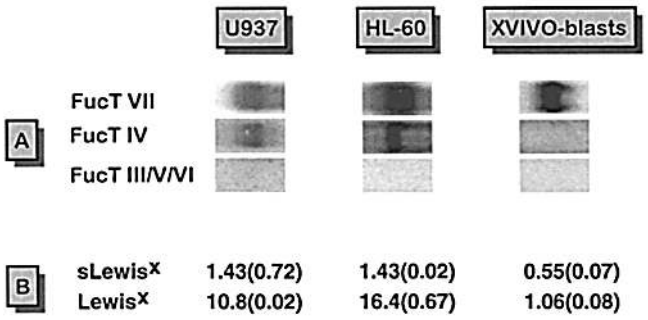

Figure 8. Comparison of fucosyltransferases in myeloid cell lines and T-lymphoblasts expressing functional ligands for E-selectin. The figure shows the steady-state prevalence of mRNA species (*A*) and the fucosyltransferase activities in cell-free extracts (*B*) of two myeloid cells lines (U937, HL-60)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

Table I. Comparison of Carbohydrate Epitopes on Leukocytic Cell Lines, Peripheral Blood Leukocytes, Jurkat Transfectants, and T-Lymphoblasts

	U937	HL60	PMNs	Monos	Lymph	Jurkat	FucT-IV Jurkat	FucT-VII Jurkat	T-blasts (XVIVO)
CD15	2148 ± 208 (97%)	3936 ± 348 (100%)	6758 ± 449 (100%)	888 ± 34 (91%)	191 ± 13 (13%)	1133 ± 36 (100%)	5516 ± 157 (100%)	370 ± 5 (92%)	437 ± 20 (95%)
CDw65	1032 ± 12 (100%)	2815 ± 109 (100%)	878 ± 20 (90%)	189 ± 7 (83%)	194 ± 33 (2%)	119 ± 6 (77%)	1095 ± 10 (100%)	– (<1%)	– (<1%)
CSLEX1	614 ± 46 (100%)	3176 ± 185 (99%)	2017 ± 79 (82%)	444 ± 13 (93%)	514 ± 59 (16%)	– (<1%)	– (0%)	1841 ± 73 (100%)	1513 ± 86 (76%)
HECA452	1258 ± 30 (100%)	2482 ± 79 (97%)	1585 ± 39 (82%)	920 ± 17 (87%)	2219 ± 71 (17%)	– (<1%)	– (0%)	921 ± 39 (100%)	2173 ± 92 (100%)

Indirect immunofluorescence and flow cytometry were conducted as described in Materials and Methods. The MFI ± SD ($N = 3$) and the mean percent of positive cells (in parentheses) is reported. The MFI for subpopulations of <1% are not statistically significant. The samples were run in a single batch to minimize variations due to culture conditions, reagents, and instrument settings. However, the values are representative of 4–20 independent experiments. PMNs (neutrophils), Monos (monocytes), and lymphs (lymphocytes) were isolated from heparinized peripheral blood over Neutrophil Isolation Medium (Cardinal Associates, Santa Fe, NM). T-blasts refers to T cells cultured for 2 wk in the XVIVO15 medium.

strate, in contrast, is not significantly different in extracts of E-selectin ligand positive and negative populations. Of the three fucosyltransferases detected by RT-PCR in normal human lymphocytes (FucT-IV, FucT-VI, and FucT-VII[41]), only the FucT-VII enzyme shows this substrate specificity in cell-free assays and transfectants (8, 17, 27, 41). We conclude that the FucT-VII enzyme catalyzes the final step in the synthesis of E-selectin ligands on T cells and that regulation of its activity during T cell proliferation controls the level of binding activity.

The CD15 (Le^x) epitope expressed on Jurkat cells and cultured T-lymphoblasts cannot be attributed to the FucT-VII enzyme. This enzyme shows a marked predilection for 2,3 sialyl-*N*-acetyllactosamine over *N*-acetyllactosamine substrates in cell-free assays (8, 27). In addition, transfection of the FucT-VII gene-product into Jurkat increases sLe^x-related epitope expression but decreases the level of the Le^x epitope (Table I). Thus, an increase in the intracellular concentration of the FucT-VII appears to divert endogenous *N*-acetyllactosamine substrates away from the pathway responsible for Le^x synthesis. This implies that a distinct, endogenous fucosyltransferase functions in this pathway. FucT-IV and VI mRNAs were not detected in Northern blots of T-lymphoblasts. However, in previous studies, the more sensitive RT-PCR analysis identified transcripts in both Jurkat cells and pooled peripheral blood lymphocytes (41). These two enzymes show markedly different synthetic activities. The FucT-IV enzyme synthesizes Le^x more efficiently than sLe^x in both cell-free assays and CHO-transfectants (8, 17, 41). The same applies to Jurkat cells since FucT-IV transfectants increase Le^x (CD15) expression more than fivefold but do not synthesize either the CSLEX1 or HECA452 epitopes (Table I). The FucT-VI gene, in contrast, produces high levels of both the Le^x and sLe^x-related epitopes on CHO-transfectants (45). However, Le^x and sLe^x-related epitopes are regulated independently on Jurkat cells and cultured T-lymphoblasts. Consequently, the FucT-IV enzyme most likely controls Le^x synthesis in these cells and relatively low steady-state levels of mRNA are sufficient for activity.

Previous studies concluded that the FucT-IV enzyme also synthesizes 2,3 sialyl-*N*-acetyllactosamine epitopes in CHO-transfectants. However, they are divided on the nature of the structures made and whether the enzyme actually synthesizes functional ligands for E-selectin in intact cells. Specifically, some strains of FucT-IV transfected CHO cells produce sLe^x epitopes (9) while others synthesize the VIM-2 (CDw65) epitope exclusively (17, 21). In these studies, only FucT-IV transfected CHO cells with sLe^x epitopes on their surface show E-selectin binding activity. These observations lead to the hypothesis that the FucT-IV enzyme regulates E-selectin binding on leukocytes through synthesis of the sLe^x structure (9).

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^x-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^x-moiety 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^x-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^x epitope but not high enough to synthesize the CDw65 epitope or E-selec-

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 the 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 HECA452 epitopes while FucT-IV regulates CD15 and CDw65 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 HL31963 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. E-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. 4.0.1–4.10.9.
3. Berg, E.L., T. Yoshino, L.S. Rott, M.K. Robinson, R.A. Warnock, T.K. Kishimoto, L.J. Picker, and E.C. Butcher. 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. Am. Assoc. Cancer Res.* 34:A2924.
6. Chia, D., P.I. Terasaki, N. Suyama, 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. Trinchera, B. Petryniak, R.J. Kelly, N. Hiraiwa, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, and J.B. Lowe. 1995. Molecular cloning, expression, chromosomal assignment, and tissue-specific expression of a murine α-(1,3)-fucosyltransferase locus corresponding to the human ELAM-1 ligand fucosyl transferase. *J. Biol. Chem.* 270 (42):25047–25056.
9. Goelz, 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.* 63:1349–1356.
10. Grober, J.S., B.L. Bowen, H. Ebling, B. Athey, C.B. Thompson, D.A. Fox, and L.M. Stoolman. 1993. Monocyte-endothelial adhesion in chronic rheumatoid arthritis. In situ detection of selectin and integrin-dependent interactions. *J. Clin. Invest.* 91:2609–2619.
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.* 124:117–123.
12. Heald, P.W., S.L. Yan, R.L. Edelson, R. Tigelaar, 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.

13. Jutila, M.A., R.F. Bargatze, S. Kurk, R.A. Warnock, N. Ehsani, S.R. Watson, and B. Walcheck. 1994. Cell surface P- and E-selectin support shear-dependent rolling of bovine gamma/delta T cells. *J. Immunol.* 152:3917–3928.
14. Kaldjian, E.P., and L.M. Stoolman. 1995. Regulation of L-selectin mRNA in Jurkat cells: opposing influences of calcium- and protein kinase C-dependent signalling pathways. *J. Immunol.* 22:4351–4362.
15. Larsen, G.R., D. Sako, T.J. Ahern, M. Shaffer, J. Erban, S.A. Sajer, R.M. Gibson, D.D. Wagner, B.C. Furie, and B. Furie. 1992. P-selectin and E-selectin. Distinct but overlapping leukocyte ligand specificities. *J. Biol. Chem.* 267:11104–11110.
16. Leung, D.Y., J.S. Pober, and R.S. Cotran. 1991. Expression of endothelial-leukocyte adhesion molecule-1 in elicited late phase allergic reactions. *J. Clin. Invest.* 87:1805–1809.
17. Lowe, J.B., J. Kukowska-Latallo, R.P. Nair, R.D. Larsen, R.M. Marks, B.A. Macher, R.J. Kelly, and L.K. Ernst. 1991. 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. *J. Biol. Chem.* 266(26):17467–17477.
18. Lowe, J.B., L.M. Stoolman, R.P. Nair, R.D. Larsen, T.L. Behrend, and R.M. Marks. 1991. A transfected human fucosyltransferase cDNA determines biosynthesis of oligosaccharide ligand(s) for endothelial-leukocyte adhesion molecule I. *Biochem. Soc. Trans.* 19:649–653.
19. Lowe, J.B., L.M. Stoolman, R.P. Nair, R.D. Larsen, T.L. Berhend, and R.M. Marks. 1990. ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell.* 63:475–484.
20. Luscinskas, F.W., H. Ding, and A.H. Lichtman. 1995. P-Selectin and vascular cell adhesion molecule 1 mediate rolling and arrest, respectively, of CD4+ T-lymphocytes on tumor necrosis factor α-activated vascular endothelium under flow. *J. Exp. Med.* 181:1179–1186.
21. Macher, B.A., J. Buehler, P. Scudder, W. Knapp, and T. Feizi. 1988. A novel carbohydrate, differentiation antigen on fucogangliosides of human myeloid cells recognized by monoclonal antibody VIM-2. *J. Biol. Chem.* 263:10186–10191.
22. Manger, B., A. Weiss, J. Imboden, T. Laing, and J.D. Stobo. 1987. The role of protein kinase C in transmembrane signaling by the T cell antigen receptor complex. Effects of stimulation with soluble or immobilized CD3 antibodies. *J. Immunol.* 139:2755–2760.
23. McEver, R.P., K.L. Moore, and R.D. Cummings. 1995. Leukocyte trafficking mediated by selectin-carbohydrate interactions. *J. Biol. Chem.* 270: 11025–11028.
24. Meier, W., D.R. Leone, K. Miatkowski, R. Lobb, and S.E. Goelz. 1993. The ELAM ligand fucosyltransferase, ELFT, directs E-selectin binding to a secreted scaffold protein: a method to produce and purify large quantities of specific carbohydrate structures. *Biochem. J.* 294(Pt 1):25–30.
25. Moore, K.L., and L.F. Thompson. 1992. P-selectin (CD62) binds to subpopulations of human memory T lymphocytes and natural killer cells. *Biochem. Biophys. Res. Commun.* 186:173–181.
26. Moore, K.L., S.F. Eaton, D.E. Lyons, H.S. Lichenstein, R.D. Cummings, and R.P. McEver. 1994. The P-selectin glycoprotein ligand from human neutrophils displays sialylated, fucosylated, O-linked poly-N-acetyllactosamine. *J. Biol. Chem.* 269:23318–23327.
27. Natsuka, S., K.M. Gersten, K. Zenita, R. Kannagi, and J.B. Lowe. 1994. Molecular cloning of a cDNA encoding a novel human leukocyte alpha-1,3-fucosyltransferase capable of synthesizing the sialyl Lewis x determinant [published erratum appears in *J. Biol. Chem.* 1994. Aug 12;269(32): 20806]. *J. Biol. Chem.* 269:16789–16794.
28. Petzelbauer, P., J.R. Bender, J. Wilson, and J.S. Pober. 1993. Heterogeneity of dermal microvascular endothelial cell antigen expression and cytokine responsiveness in situ and in cell culture. *J. Immunol.* 151:5062–5072.
29. Phillips, M.L., E. Nudelman, F.C. Gaeta, M. Perez, A.K. Singhal, S. Hakomori, and J.C. Paulson. 1990. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. *Science (Wash. DC).* 250: 1130–1132.
30. Picker, L.J., R.J. Martin, A. Trumble, L.S. Newman, P.A. Collins, P.R. Bergstresser, and D.Y. Leung. 1994. Differential expression of lymphocyte homing receptors by human memory/effector T cells in pulmonary versus cutaneous immune effector sites. *Eur. J. Immunol.* 24:1269–1277.
31. Picker, L.J., S.A. Michie, L.S. Rott, and E.C. Butcher. 1990. A unique phenotype of skin-associated lymphocytes in humans preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am. J. Pathol.* 136:1053–1068.
32. Picker, L.J., L.W. Terstappen, L.S. Rott, P.R. Streeter, H. Stein, and E.C. Butcher. 1990. Differential expression of homing-associated adhesion molecules by T cell subsets in man. *J. Immunol.* 145:3247–3255.
33. Picker, L.J., J.R. Treer, B. Ferguson-Darnell, P.A. Collins, P.R. Bergstresser, and L.W. Terstappen. 1993. Control of lymphocyte recirculation in man. II. Differential regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. *J. Immunol.* 150:1122–1136.
34. Pilewski, J.M., R.A. Panettieri, Jr., L.R. Kaiser, and S.M. Albelda. 1994. Expression of endothelial cell adhesion molecules in human bronchial xenografts. *Am. J. Respir. Crit. Care Med.* 150:795–801.
35. Postigo, A.A., R. Garcia-Vicuna, F. Diaz-Gonzalez, A.G. Arroyo, M.O. De Landazuri, G. Chi-Rosso, R.R. Lobb, A. Laffon, and F. Sanchez-Madrid. 1992. Increased binding of synovial T lymphocytes from rheumatoid arthritis to endothelial-leukocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1). *J. Clin. Invest.* 89:1445–1452.
36. Postigo, A.A., M. Marazuela, F. Sanchez-Madrid, and M.O. De Landazuri. 1994. B lymphocyte binding to E- and P-selectins is mediated through the de novo expression of carbohydrates on in vitro and in vivo activated human B cells. *J. Clin. Invest.* 94:1585–1596.
37. Rosen, S.D., and C.R. Bertozzi. 1994. The selectins and their ligands. *Curr. Opin. Cell Biol.* 6:663–673.
38. Rosen, S.D., M.S. Singer, T.A. Yednock, and L.M. Stoolman. 1985. Involvement of sialic acid on endothelial cells in organ-specific lymphocyte recirculation. *Science (Wash. DC).* 228:1005–1007.
39. Rossiter, H., F. van Reijsen, G.C. Mudde, F. Kalthoff, C.A. Bruijnzeel-Koomen, L.J. Picker, and T.S. Kupper. 1994. Skin disease-related T cells bind to endothelial selectins: expression of cutaneous lymphocyte antigen (CLA) predicts E-selectin but not P-selectin binding. *Eur. J. Immunol.* 24:205–210.
40. Sako, D., X.J. Chang, K.M. Barone, G. Vachino, H.M. White, G. Shaw, G.M. Veldman, K.M. Bean, T.J. Ahern, B. Furie et al. 1993. Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell.* 75:1179–1186.
41. Sasaki, K., K. Kurata, K. Funayama, M. Nagata, E. Watanabe, S. Ohta, N. Hanai, and T. Nishi. 1994. Expression cloning of a novel alpha 1,3-fucosyltransferase that is involved in biosynthesis of the sialyl Lewis x carbohydrate determinants in leukocytes. *J. Biol. Chem.* 269:14730–14737.
42. Stoolman, L.M. 1992. Selectins (LEC-CAMs): lectin-like receptors involved in lymphocyte recirculation and leukocyte recruitment. *In* Cell Surface Carbohydrates and Cell Development. M. Fukuda, editor. CRC Press, Inc, Boca Raton, FL. 71–97.
43. Sussman, J.J., S. Shu, V.K. Sondak, and A.E. Chang. 1994. Activation of T lymphocytes for the adoptive immunotherapy of cancer. *Ann. Surg. Oncol.* 1:296–306.
44. Walcheck, B., G. Watts, and M.A. Jutila. 1993. Bovine gamma/delta T cells bind E-selectin via a novel glycoprotein receptor: first characterization of a lymphocyte/E-selectin interaction in an animal model. *J. Exp. Med.* 178:853–863.
45. Weston, B.W., P.L. Smith, R.J. Kelly, and J.B. Lowe. 1992. Molecular cloning of a fourth member of a human alpha (1,3)fucosyltransferase gene family. Multiple homologous sequences that determine expression of the Lewis x, sialyl Lewis x, and difucosyl sialyl Lewis x epitopes [published erratum appears in *J. Biol. Chem.* 1993. Aug 25;268(24):18398]. *J. Biol. Chem.* 267:24575–24584.