JCI The Journal of Clinical Investigation

Leukocyte Adhesion Deficiency Type II is a generalized defect of de novo GDP-fucose biosynthesis. Endothelial cell fucosylation is not required for neutrophil rolling on human nonlymphoid endothelium.

A Karsan, … , H D Ochs, J M Harlan

J Clin Invest. 1998;101(11):2438-2445. https://doi.org/10.1172/JCI905.

Research Article

Leukocyte Adhesion Deficiency Type II (LAD II) is a recently described syndrome and the two patients with this defect lack fucosylated glycoconjugates. These glycoconjugates include the selectin ligand, sialyl LewisX, and various fucosylated blood group antigens. To date, the molecular anomaly in these patients has not been identified. We localized the defect in LAD II to the de novo pathway of GDP-fucose biosynthesis, by inducing cell-surface expression of fucosylated glycoconjugates after exposure of lymphoblastoid cell lines from the LAD II patients to exogenous fucose. This defect is not restricted to hematopoietic cells, since similar findings were elicited in both human umbilical vein endothelial cells (HUVEC) and fibroblasts derived from an affected abortus. We have used these LAD II endothelial cells to examine the consequence of fucosylation of endothelial cells on the rolling of normal neutrophils in an in vitro assay. Neutrophil rolling on LPS-treated normal and LAD II HUVEC was inhibited by an E-selectin monoclonal antibody at both high and low shear rates. LAD II HUVEC lacking fucosylated glycoproteins supported leukocyte rolling to a similar degree as normal HUVEC or LAD II cells that were fucose-fed. At low shear rates, an L-selectin antibody inhibited neutrophil rolling to a similar degree whether the LAD II cells had been fucose-fed or not. These findings suggest that fucosylation of nonlymphoid endothelial cells does […]

Leukocyte Adhesion Deficiency Type II Is a Generalized Defect of De Novo GDP–Fucose Biosynthesis

Endothelial Cell Fucosylation Is Not Required for Neutrophil Rolling on Human Nonlymphoid Endothelium

Aly Karsan,* Carol J. Cornejo,[§¶] Robert K. Winn,[§¶] Barbara R. Schwartz,[‖] William Way, Naomi Lannir,[‡‡] Ruth Gershoni-Baruch,[‡‡] Amos Etzioni,[‡‡] Hans D. Ochs,[‡] and John M. Harlan[‖]**

**Department of Pathology and Laboratory Medicine, University of British Columbia and St. Paul's Hospital, Vancouver, British Columbia, Canada V6Z 1Y6; [‡]Department of Pediatrics, [§]Department of Surgery, [‖]Division of Hematology, University of Washington, Seattle, Washington 98195; [¶]Department of Surgery, Harborview Medical Center, Seattle, Washington 98195; **Cytel Corporation, San Diego, California 92121; [‡‡]Department of Pediatrics, Rambam Medical Center, Haifa, Israel 31096*

Abstract

Leukocyte Adhesion Deficiency Type II (LAD II) is a recently described syndrome and the two patients with this defect lack fucosylated glycoconjugates. These glycoconjugates include the selectin ligand, sialyl Lewis^X, and various fucosylated blood group antigens. To date, the molecular anomaly in these patients has not been identified. We localized the defect in LAD II to the de novo pathway of GDP-fucose biosynthesis, by inducing cell-surface expression of fucosylated glycoconjugates after exposure of lymphoblastoid cell lines from the LAD II patients to exogenous fucose. This defect is not restricted to hematopoietic cells, since similar findings were elicited in both human umbilical vein endothelial cells (HUVEC) and fibroblasts derived from an affected abortus. We have used these LAD II endothelial cells to examine the consequence of fucosylation of endothelial cells on the rolling of normal neutrophils in an in vitro assay. Neutrophil rolling on LPS-treated normal and LAD II HUVEC was inhibited by an E-selectin monoclonal antibody at both high and low shear rates. LAD II HUVEC lacking fucosylated glycoproteins supported leukocyte rolling to a similar degree as normal HUVEC or LAD II cells that were fucose-fed. At low shear rates, an L-selectin antibody inhibited neutrophil rolling to a similar degree whether the LAD II cells had been fucose-fed or not. These findings suggest that fucosylation of nonlymphoid endothelial cells does not play a major role in neutrophil rolling and that fucose is not a critical moiety on the L-selectin ligand(s) on endothelial cells of the systemic vasculature. (*J. Clin. Invest.* 1998. 101:2438–2445.) Key words: selectin • inflammation • sialyl Lewis^X • fucose

Introduction

Leukocyte Adhesion Deficiency Type II (LAD II)[1] is a recently described syndrome in which the neutrophils of two unrelated patients do not express sialyl Lewis X (sLe^X) (1, 2), the fucose-containing carbohydrate reported to be the ligand for adhesion molecules of the selectin family (3, 4). Neutrophils from both patients fail to bind to E-selectin on IL-1β–activated endothelial cells (1). The neutrophils from one patient were evaluated and found to be unable to bind P-selectin as well (5). However, LAD II is not a syndrome confined to a leukocyte adhesion defect. Both patients described also suffer severe psychomotor and growth retardation and have dysmorphic features, hypotonia, seizures, and strabismus (2). Of interest is the fact that serology of both patients demonstrates that the red cells are of the Bombay phenotype (lack the H antigen) and Lewis a^-b^-, and that both patients are nonsecretors (2). All three phenotypes result from the lack of fucosylation of glycoconjugates (6).

Previous studies have concentrated on the adhesion deficiency and leukocyte kinetics (1, 5, 7, 8), but the defect that results in this syndrome has not been characterized. At least four different fucosyltransferases would have to be affected (sLe^X, H, Lewis, and secretor) if they were the cause of the syndrome, but these genes are not physically linked in the human genome (loci include chromosomes 11, 19p, and 19q; references 9, 10), thus it is unlikely that LAD II is the result of abnormal fucosyltransferase(s). Moreover, in one patient levels of the H and secretor transferases were found to be normal (11). Hence, we directed our attention to events necessary for fucosylation, that are proximal to terminal fucosyltransferase activity. In this report, we extend the preliminary findings of Etzioni at al. (12) to localize the defect in LAD II to the de novo pathway of GDP-fucose biosynthesis and also demonstrate that this defect is not limited to hematopoietic cells, because, at the very least, endothelial cells and fibroblasts show the same defect.

Recently, it has been proposed that fucosylation of the L-, E-, and P-selectin ligands is essential for leukocyte adhesion and trafficking (13, 14). The α(1,3) fucosyltransferase, FucT–VII, has been shown to be the critical enzyme for synthesizing the sLe^X determinant and fucosylation of selectin ligands in mice (13–15). Studies in FucT-VII–null mice implicate specific α(1,3)-linked fucose residues for the expression of L-selectin ligand activity in high endothelial venules of lymph node and Peyer's patches (14). Lymphocyte homing was also noted to be defective in these mice (14). The demonstration that endothelial cells of LAD II patients also exhibit a lack of

Address correspondence to Aly Karsan, M.D., Department of Pathology and Laboratory Medicine, St. Paul's Hospital, 1081 Burrard St., Vancouver, BC, Canada V6Z 1Y6. Phone: 604-631-5346; FAX: 604-631-5351; E-mail: akarsan@prl.pulmonary.ubc.ca

Received for publication 12 June 1997 and accepted in revised form 1 April 1998.

J. Clin. Invest.

0021-9738/98/06/2438/08 $2.00
Volume 101, Number 11, June 1998, 2438–2445
http://www.jci.org

1. *Abbreviations used in this paper:* HUVEC, human umbilical vein endothelial cells; LAD II, Leukocyte Adhesion Deficiency Type II; LTA, Lotus tetragonolobus agglutinin; sLe^X, sialyl Lewis^X; UEA, Ulex europaeus I agglutinin.

fucosylated proteins that can be corrected by fucose-feeding, allowed us to investigate the role of endothelial fucosylation in neutrophil rolling. Our studies indicate that fucosylation of human umbilical vein endothelial cells (HUVEC) is not required for neutrophil rolling.

Methods

Reagents. Fluorescein isothiocyanate-conjugated lectins, Ulex europaeus I agglutinin (UEA) and Lotus tetragonolobus agglutinin (LTA), were purchased from Sigma Chemical Co. (St. Louis, MO) and Anguilla anguilla agglutinin was obtained from E.Y. Laboratories, Inc. (San Mateo, CA). Fluorescein isothiocyanate–conjugated rat anti–mouse IgM was purchased from Biosource International (Camarillo, CA). The CSLEX1 (anti-sLe^X, IgM) hybridoma was acquired from the American Type Culture Collection (ATCC, Rockville, MD) and ascites was prepared by standard techniques (16). Briefly, BALB/c mice were primed with 0.5 ml of pristane (Sigma Chemical Co.) 1 wk before intraperitoneal injection of CSLEX1 hybridoma cells. Ascites was harvested after 2 wk and used at 1:100 dilution. All lectins were used at 25 μg/ml and rat anti–mouse IgM was used at 5 μg/ml for flow cytometry experiments. The CD18 mAb, 60.3, was prepared as previously described (17) and added to all neutrophil suspensions for rolling experiments. The anti–L-selectin mAb, LAM 1-3, was a gift from T.F. Tedder (Duke University, Durham, NC; reference 18). The anti–E-selectin mAb used was CY1787 (gift of J. Paulson, Cytel Corp., San Diego, CA). All mAbs were added at a concentration of 25 μg/ml and the concentration was maintained throughout the rolling experiments.

Cell culture and generation of LAD II lymphoblastoid cell lines. Lymphoblastoid cell-lines were generated from one of the children with LAD II as previously described (19). Mononuclear cells were isolated over a Ficoll-Hypaque density gradient. Separated leukocytes were incubated with Epstein-Barr virus supernatant for 24 h, then cyclosporin A (2 μg/ml) was added. Thereafter, cells were split 1:1 on a weekly basis until the cultures were established. Several cell lines were generated, and two (R5 and H8) were used in this study. These lymphoblastoid cell lines were cultured in RPMI 1640 medium supplemented with 15% FCS, sodium pyruvate, penicillin, and streptomycin. The final concentration of glucose in this medium was 12 mM.

Besides the two patients with LAD II who have been described, an abortus from one of the pairs of consanguineous parents was also found to be of the Bombay phenotype. HUVEC from this abortus as well as from normal umbilical cords were obtained by collagenase treatment of these umbilical cord veins as previously described (20). Cultures were propagated in RPMI 1640 medium supplemented with 20% BCS, 90 μg/ml heparin (Sigma Chemical Co.), endothelial cell growth factor (50 μg/ml) prepared from bovine hypothalamus, penicillin, streptomycin, and fungizone. Fibroblasts from this cord were also harvested and passaged in DME supplemented with 10% FCS. Fibroblasts and HUVEC were used up to passage 8 and split at confluence. Normal and LAD II HUVEC were used at comparable passage numbers in all experiments. All cells were maintained in 5% CO_2 at 37°C.

Feeding with sugars was accomplished by adding either L-fucose or D-glucose (Sigma Chemical Co.) to a final concentration that was 10 mM above that in the basal medium. Exposure to the monosaccharides was continued for at least 5 d with medium changes every second or third day.

Flow cytometry. Fibroblasts and HUVEC were detached with EDTA before staining. All cells were washed once with PBS containing 0.1% BSA and 0.02% NaN_3 and resuspended in 50 μl. For adherent cells, one well of a 6-well plate was used for each staining reaction. Lymphoblastoid cells were resuspended at a concentration of 2×10^5 cells per 50 μl. Fibroblasts and HUVEC were preincubated with 50 μl of heat-inactivated adult bovine serum to reduce nonspecific binding. Lectin or primary antibody was added to the cells and allowed to incubate on ice for 45 min. Where required, secondary antibody was added and allowed to incubate an additional 45 min on ice. Cells were washed twice with cold PBS after incubation with each lectin or antibody. Cells were fixed in 1% paraformaldehyde after staining. Samples were run on a FACScan® flow cytometer (Becton Dickinson, Mountain View, CA). Data were acquired using Lysis II software and analyzed with Repromac (Truefacts Software, Seattle, WA) software.

Isolation of neutrophils. Neutrophils were purified from heparin anticoagulated venous blood samples from healthy adult volunteers using a Polymorphoprep (Nycomed Pharmaceuticals, Oslo, Norway) density gradient. Isolated neutrophils were washed once with HBSS (Bio-Whittaker, Walkersville, MD) and then resuspended in HBSS. Neutrophils were diluted to a concentration of 125,000/ml immediately before each flow chamber run. Neutrophils solutions were incubated with mAb for 5–10 min at 37°C before each flow chamber run.

Flow chamber. A parallel plate flow chamber was constructed using a design similar to those described previously (21). Briefly, a Plexiglas base was constructed with an inlet and outlet port, a silastic membrane with a slit cut ~ 28 mm long and 2 mm wide was placed on the base and secured with springs. The slit in the membrane created a channel between the base and the monolayers through which the neutrophils flow. Endothelial cells collected from the umbilical cord of a LAD II abortus or normal HUVEC were grown to confluency on gelatin-coated chamber slides (Nunc, Inc., Naperville, IL), stimulated with LPS (100 ng/ml) for 4 h, and mounted in parallel plate flow chambers. The monolayers were perfused with HBSS for 5–10 min. Neutrophils were diluted to a concentration of 125,000/ml and incubated with mAb where indicated at 37°C for 5–10 min. The concentration of mAb was maintained throughout the experiment. The neutrophil solutions were passed through the flow chamber at shear stresses of 1.16 or 2.33 dyn/cm^2 using an infusion pump (Harvard Apparatus, Inc., South Natick, MA). The interaction of the neutrophils with the HUVEC was observed using phase contrast microscopy (Diaphot 200; Nikon Inc., Melville, NY) and recorded on videotape for 10 min. The recorded images were analyzed using the computer program NIH Image 1.55. Images were digitized and frames captured every 4 s. Then the first frame of the analysis was subtracted from the later frame. The initial images were captured on a gray scale between 0 (black) and 255 (white). The image was scaled after subtraction to again result in a gray scale between 0 and 255. Thus, a rolling neutrophil would appear as a black spot at its original position and as a white spot at its final location after 4 s. If the neutrophil did not move, it would appear as a gray spot. The number of neutrophils rolling per frame were counted at three different times for each experiment (at 7, 8.5, and 10 min of the 10 min neutrophil perfusion). The mean number of rolling neutrophils for each experiment was then calculated and converted to neutrophils/mm^2. Only those neutrophils that rolled in the 4 s interval were counted. Using the same computer program, the distance that the neutrophil traveled in 4 s was measured in pixels, converted to micrometers, and the velocity was calculated. The velocities of 50 neutrophils in the last 3 min of neutrophil perfusion (7–10 min) were measured and the mean neutrophil velocity for the experiment was calculated. The temperature for all experiments was maintained at 37°C using an incubator around the microscope and an incubator warmer (Nikon Inc.). The temperature of the perfusate was measured with a thermistor-tipped catheter threaded into the outflow tract of the flow chamber and the incubator temperature adjusted to maintain the perfusate temperature at 37°C.

Results

Fucosylation of glycoproteins can be induced in lymphoblastoid cell-lines from a patient with LAD II. Leukocytes and red cells of patients with LAD II previously have been shown to lack several fucosylated glycoproteins (1, 2). For instance the neu-

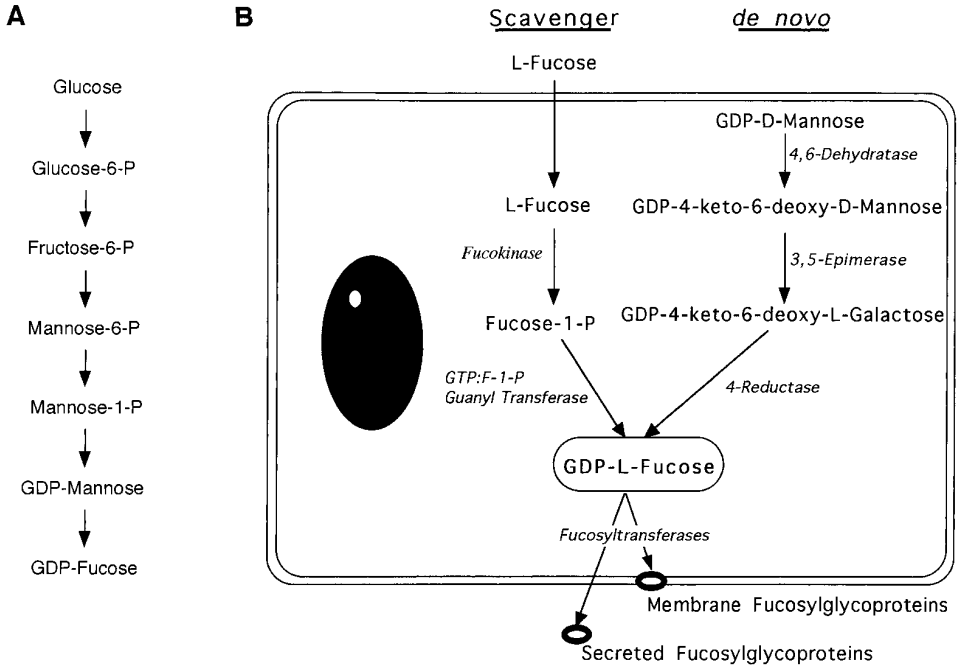

Figure 1. (*A*) The proposed pathway for the conversion of glucose to GDP-fucose in the de novo pathway. (*B*) Schema for the generation of GDP-L-fucose via the scavenger and de novo pathways.

trophils of these patients lack sLe^X as determined by lack of staining with the antibody CSLEX-1 (1, 5, 7). The incorporation of sugars into glycoproteins by glycosyltransferases requires a high-energy sugar nucleotide substrate (22). In the case of fucose, fucosyltransferases require GDP-L-fucose (23). Two pathways for the generation of GDP-fucose have been delineated (Fig. 1; references 23–36). The de novo pathway synthesizes GDP-fucose from GDP-mannose, which itself originates from glucose (Fig. 1 *A*; references 24–29, 31, 34, 35). The salvage or scavenger pathway converts exogenous fucose to GDP-fucose (23, 30, 32, 33, 36). To determine whether a defect in either of these pathways could explain a lack of fucosylated glycoproteins, two lymphoblastoid lines were created from one of the LAD II patients. As with neutrophils of these patients, both cell-lines were found to lack sLe^X (12), whereas a subset of normal lymphoid cells do express sLe^X (37). If the defect in fucose biosynthesis were in the de novo pathway, provision of exogenous fucose should induce the expression of fucosylated glycoproteins by synthesis of GDP-fucose through the scavenger pathway. This would not be true if the defect lies in the scavenger pathway.

As shown in Fig. 2 *A*, feeding the R5 cell line 10 mM fucose for 5 d resulted in the expression of sLe^X in a subset of cells (12), whereas addition of 10 mM glucose did not induce the antigen. However, attempts at blocking the binding of CSLEX-1 with fucose were unsuccessful (data not shown). Hence, to demonstrate the specificity of fucose feeding in the induction of fucosylated glycoproteins, several fucose-specific lectins were tested (UEA I, Anguilla anguilla agglutinin, and LTA). One of these, LTA, was found to bind to fucose-fed H8 cells, but not to glucose-fed cells (Fig. 2 *B*). Ligands for the other two lectins could not be induced. Furthermore, the binding of LTA to the H8 cell line could be blocked by 2.5 mM fucose but not by the same concentration of glucose (Fig. 2 *C*). These results indicate that the scavenger pathway of GDP-fucose biosynthesis must be intact, but that the de novo pathway is compromised.

The defect in GDP-fucose biosynthesis is not restricted to hematopoietic cells. An abortus from one of the pairs of consanguineous parents was also found to be of the Bombay phenotype. Endothelial cells from this umbilical cord were also tested with the three lectins above. As would be expected for HUVEC (38–40), receptors for two of these lectins—UEA and LTA—were found to be induced by fucose-feeding (Fig. 3, *A* and *B*). Again, specificity of the lectins for fucose was confirmed by competition of binding by fucose (125 mM in the case of UEA and 2.5 mM for LTA), but not by the same concentration of glucose (Fig. 3, *C* and *D*). The requirement of a higher concentration of fucose to inhibit UEA than LTA has previously been described (41). Normal HUVEC also showed a slight induction of UEA with fucose-feeding (data not shown), but this was minimal with a mean channel fluorescence increase of 1.4-fold compared with a 160-fold increase with the LAD II HUVEC. This would suggest that the limiting factor of UEA receptor expression on HUVEC is the provision of GDP-fucose rather than fucosyltransferase activity.

In similar experiments, fibroblasts from this umbilical cord could also be induced to express UEA and LTA receptors (Fig. 4, *A* and *C*). On the other hand, normal skin fibroblasts, which expressed these lectin receptors at baseline, were not able to further induce these receptors when exposed to exogenous fucose (Fig. 4, *B* and *D*), demonstrating that these findings are not a nonspecific effect of feeding cells fucose. The results presented here indicate that the defect in de novo GDP-fucose biosynthesis is not restricted to hematopoietic cells.

Fucosylglycoconjugates on HUVEC are not required for the rolling of normal neutrophils. It has been proposed that a major mechanism of neutrophil rolling is via L-selectin and PSGL-1 interactions with E- and P-selectin (42). The L-selectin interaction has been proposed to result mainly from its pre-

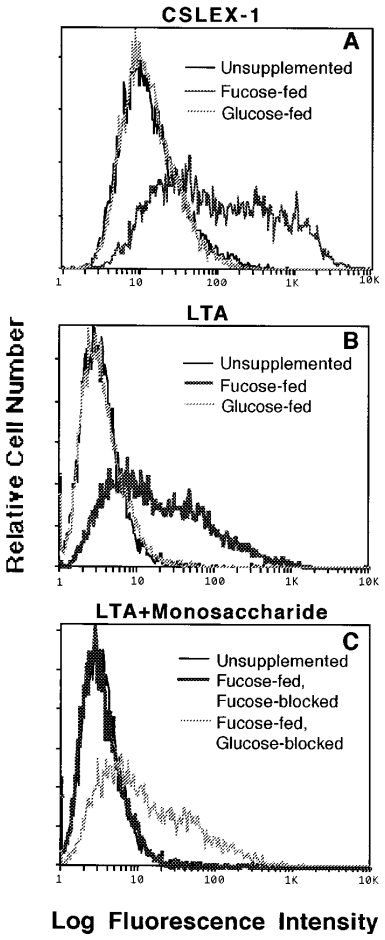

Figure 2. Induction of fucosylated glycoconjugates by fucose-feeding of LAD II lymphoblastoid cells. (*A*) Surface expression of sLe^X on R5 cells after 5 d in unsupplemented medium, addition of 10 mM fucose, or addition of 10 mM glucose. (*B*) Surface expression of LTA receptors after 5 d incubation in unsupplemented medium, addition of 10 mM fucose, or 10 mM glucose. (*C*) Surface expression of LTA receptors after 5 d incubation in unsupplemented medium or fucose-containing medium. Staining of fucose-fed cells was carried out with LTA incubated with 2.5 mM fucose, or 2.5 mM glucose. Figure shows one representative experiment of at least three separate experiments.

sentation of sLe^X to E-selectin (43, 44). However, L-selectin has also been reported to mediate endothelial interactions by its lectin domain through recognition of endothelial carbohydrates (45, 46). It is not clear whether fucose is an important moiety in this latter interaction. Since we were able to show that expression of fucosyl moieties could be manipulated on the cell surface, we used the LAD II HUVEC to determine if HUVEC fucosylation was critical for the rolling of normal neutrophils.

Neutrophil rolling experiments were performed at a shear stress of 2.33 dyn/cm^2, as well as a lower shear stress of 1.16 dyn/cm^2 after treatment of HUVEC for 4 h with LPS to induce E-selectin. No rolling was detected without LPS pretreatment (data not shown). Neutrophil adhesion and transmigration were prevented with a CD18 blocking mAb (60.3) and the CD18 mAb was used in all experiments. Normal HUVEC, LAD II HUVEC, and fucose-fed HUVEC supported similar numbers of rolling neutrophils (Fig. 5 *A*), with similar rolling velocities (Table I) at the higher shear stress. All rolling could be abolished by treating the HUVEC with a blocking anti–E-selectin mAb (CY1787), but there was only minimal blocking when neutrophils were treated with a blocking anti–L-selectin mAb (LAM 1–3; data not shown) at the higher shear stress.

Unfortunately, because of the scarcity of the LAD II HUVEC, only two separate experiments could be performed at the lower shear stress. Similar results were noted for the rolling velocities of LAD II HUVEC, whether they were fucose-fed or not (Table I). Anti–E-selectin mAb treatment completely abrogated all rolling (data not shown). On the other hand, at the lower shear stress, L-selectin provided a significant component of the rolling, as determined by attenuation with anti–L-selectin mAb LAM 1–3 (Fig. 5 *B*). The

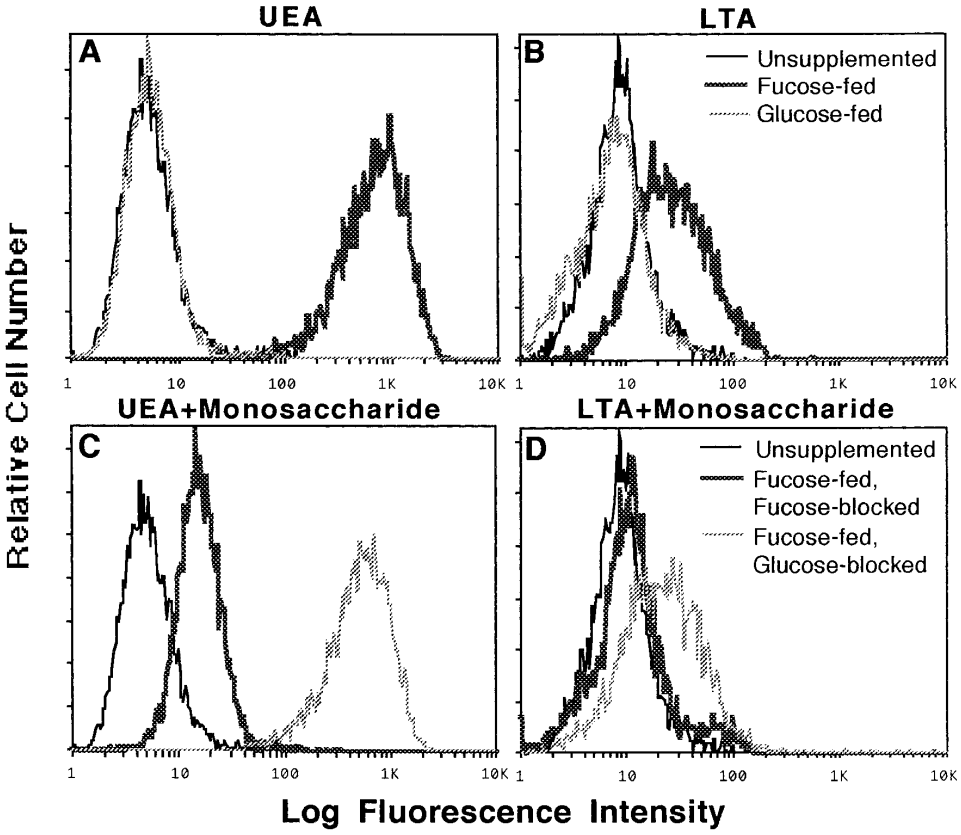

Figure 3. Induction of fucosylated glycoconjugates after fucose-feeding of LAD II HUVEC. Surface expression of (*A*) UEA receptors or (*B*) LTA receptors after a 5-d culture of LAD II HUVEC with unsupplemented medium or medium supplemented with 10 mM fucose or 10 mM glucose. The legend in *B* applies to *A* and *B*. (*C*) UEA staining of fucose-fed cells was carried out after incubation of lectin with fucose (125 mM) or glucose (125 mM). (*D*) LTA staining of fucose-fed cells was carried out after incubation of lectin with fucose (2.5 mM) or glucose (2.5 mM). Legend in *D* applies to panels *C* and *D*. Figure shows one representative experiment of at least three separate experiments.

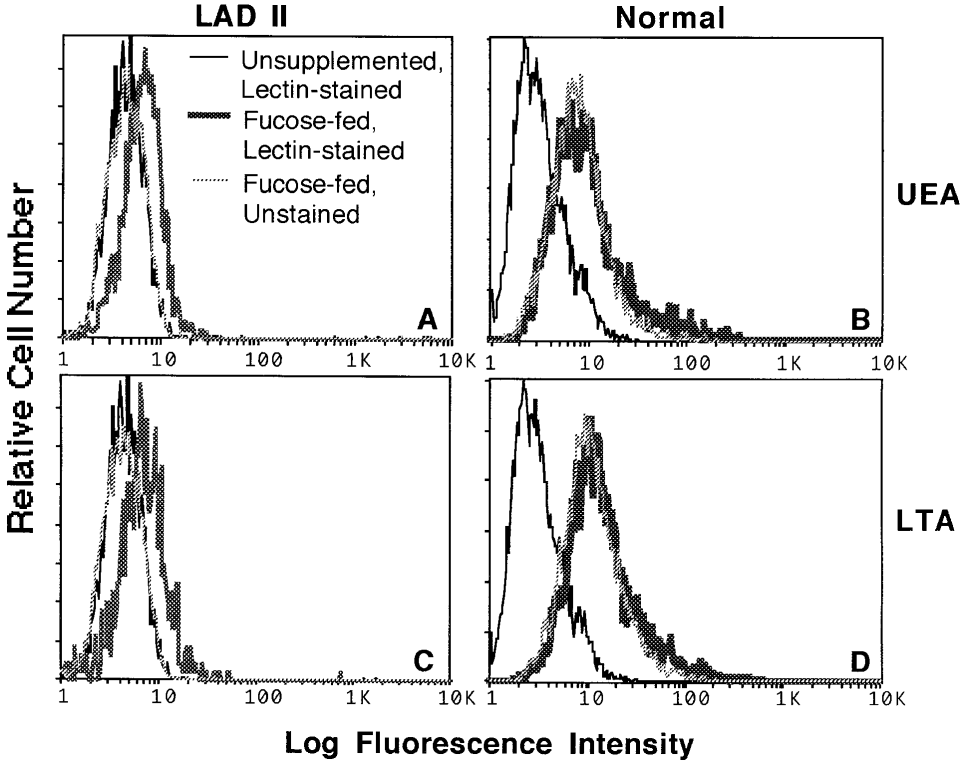

Figure 4. Induction of fucosylated glycoconjugates after fucose-feeding of LAD II umbilical cord fibroblasts or normal skin fibroblasts. Surface expression of LTA receptors on fucose-fed (*A*) LAD II fibroblasts or (*B*) normal skin fibroblasts. Surface expression of UEA receptors on fucose-fed (*C*) LAD II fibroblasts or (*D*) normal skin fibroblasts. Legend in *A* applies to all panels. Figure shows one representative experiment of two separate experiments.

epitope of mAb LAM 1–3 has been mapped to the lectin domain of L-selectin (18) and has been shown to block L-selectin–mediated rolling while a second mAb (LAM 1–11) which maps to an epitope within the lectin domain not involved in ligand binding does not affect rolling (46). Our laboratory has shown previously that mAb LAM 1–3 attenuates reperfusion injury in the rabbit ear, but the nonfunction blocking mAb, LAM 1–14, does not (47). Hence, the similar degree of blocking by LAM 1–3 mAb whether the LAD II HUVEC were fucose-fed or not suggests that fucosylation of HUVEC is not critical for L-selectin–mediated rolling of neutrophils.

With each of the rolling experiments, the LAD II HUVEC were analyzed by flow-cytometry to show that fucose-feeding did result in expression of UEA receptors and that LPS-induced E-selectin levels were similar for all the cells. Fig. 5 *C* shows one representative experiment.

Discussion

Post-translational modification of proteins by the addition of carbohydrate residues is a complex process requiring multiple steps (22, 48). One of the key requirements for the addition of saccharides to proteins or lipids is the presence of specific glycosyltransferases which act on their corresponding high-energy sugar nucleotide (22, 48). In the case of fucose this nucleotide-saccharide substrate is GDP-L-fucose (22). In LAD II, several fucosylated glycoconjugates have been noted to be absent (1, 2, 22.) Since the fucose-deficient molecules identified thus far require different fucosyltransferases to catalyze the addition of fucose, it was presumed that the defect lay in the generation of GDP-L-fucose.

Two major pathways have been described for the synthesis of GDP-L-fucose (23–35; Fig. 1). In a study on HeLa cells, it was estimated that ~ 90% of the GDP-L-fucose generated in a cell arises from the de novo pathway (31). Previous studies on mouse lymphoma and Chinese hamster ovary glycosylation mutants demonstrated that defects in the de novo pathway of GDP-fucose synthesis could be bypassed with the scavenger pathway, by feeding the cells 10 mM fucose (49, 50). In this paper, we show that fucose-feeding results in the expression of sLe^X as well as fucose-specific lectin receptors on lymphoblastoid cells, HUVEC, and fibroblasts of LAD II patients. This finding indicates that the scavenger pathway of GDP-L-fucose biosynthesis must be intact and also confirms that the fucosyltransferases in this syndrome are active (11), but lack their substrate.

It has been demonstrated in bacteria and in man that glucose is converted to fucose with the carbon chain intact (24–28). A pathway for this conversion has been proposed by Foster and Ginsburg (reference 28; Fig. 1 *A*). If LAD II cells were able to use this pathway, the presence of glucose in the me-

Table I. Leukocyte Rolling Velocities on Normal or LAD II HUVEC

	Velocity (μm/s)	
	High shear* (2.33 dyn/cm²)	Low shear‡ (1.16 dyn/cm²)
Normal	8.18±5.68	—
LAD II	8.64±3.29	6.87±2.02
LAD II/fucose	9.45±3.38	6.83±0.21

*Mean±SD of four independent experiments; ‡Mean±SD of two independent experiments.

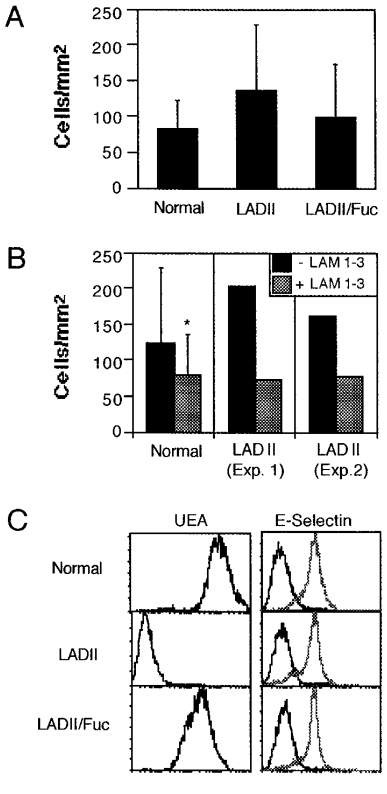

Figure 5. Neutrophil rolling on LPS-stimulated HUVEC at (*A*) high shear stress or (*B*) low shear stress. Neutrophils from normal donors were allowed to roll on normal HUVEC, LAD II HUVEC cultured in unsupplemented medium (*LAD II*) or LAD II HUVEC cultured in medium supplemented with 10 mM fucose for at least 5 d (LAD II/Fuc). All HUVEC were stimulated with LPS (100 ng/ml) for 4 h before rolling experiments to induce E-selectin expression. All rolling experiments were performed in the presence of a CD18 neutralizing mAb (60.3) to prevent firm adhesion and neutrophil transmigration. An L-selectin lectin domain-blocking mAb (LAM 1–3) was used in some experiments at low shear (*B*). (*C*) Representative flow-cytometry experiment showing the relative surface expression of UEA receptors and E-selectin after LPS-stimulation. *A* shows the mean±SD of four independent experiments. There is no difference in the number of rolling neutrophils as determined by ANOVA. *B* shows the mean±SD of nine independent experiments for normal HUVEC and the two individual experiments for LAD II HUVEC. A paired *t* test shows that neutrophil rolling was inhibited in the normal HUVEC treated with LAM 1–3 ($P < 0.05$).

dium should induce membrane fucosylglycoproteins as demonstrated with fucose. The finding that provision of fucose but not of glucose results in the surface expression of normally expressed fucosylated antigens, indicates that the defect in LAD II must lie in the de novo pathway of GDP-L-fucose biosynthesis. It can be inferred that the synthesis of GDP-mannose is not affected, since this would result in a major defect of NH_2-linked glycosylation with additional clinical findings consequent to, among other things, disruption of lysosomal targeting of enzymes (51, 52). Hence, the LAD II defect must involve one of the enzymes involved in the conversion of GDP-mannose to GDP-fucose (references 34 and 35; Fig 1 *B*). The epimerase and reductase activities have been demonstrated to reside on a single protein (35). Thus, we predict that the molecular defect in LAD II involves either GDP-D-mannose-4,6-dehydratase or GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase-4-reductase (34, 35). The epimerase-reductase function has been attributed to the FX protein, the cDNA for which has recently been cloned (53). It will be interesting to determine whether transfection of this cDNA can correct the defect in LAD II cells.

The wide ranging clinical findings demonstrated by these patients and the fact that they are nonsecretors suggests that the molecular defect in these patients must be generalized (2). However, previously there was no experimental evidence to verify this hypothesis. By showing that both HUVEC and fibroblasts of an abortus with the Bombay phenotype from one set of parents can be induced to express fucose-specific lectin receptors, we prove that the LAD II defect is not isolated to the hematopoietic system. This finding may provide an explanation for the widespread clinical manifestations of patients with this syndrome.

The demonstration that we could manipulate fucose expression on LAD II HUVEC allowed us to investigate the role of endothelial fucosylation on normal neutrophil rolling in an in vitro assay. Although, one major role of L-selectin appears to be the displaying of sLe^X to endothelial E-selectin (43, 44), it has also been proposed that L-selectin can recognize carbohydrates on endothelial cells via its lectin domain (45, 46). The carbohydrates involved in this latter interaction have not been identified. However, it is thought that sulfation is important for high affinity binding to L-selectin (54–58), and, specifically, that heparan sulfate proteoglycans serve as ligands for monocyte L-selectin (59). Our study suggests that fucose is not an important saccharide for L-selectin–mediated rolling on systemic endothelium. This finding is consistent with the observation that L-selectin can bind 3′-sulfated oligosaccharides lacking fucose, but not to fuco-oligosaccharide analogues lacking sulfate or sialic acid (56). Although the findings reported here appear to contradict those seen in the FucT-VII–deficient mice (14), several explanations may be offered. The first is that in the previous study (14) L-selectin binding was only examined in the high endothelial venules of lymphoid tissue which may exhibit different L-selectin ligands from systemic endothelium (60). Further, the fucosylation defect in these mice is present in both the leukocyte and the endothelium as opposed to only the endothelial cells in this study. Another difference between this study and the study by Maly et al. (14) is that only lymphocyte trafficking was examined in that paper. It has been shown that rolling of T cells on cytokine-stimulated HUVEC in vitro is not affected by mAb to L-selectin, in contrast to rolling of both normal neutrophils and monocytes (60). Hence, the defect in lymphocyte homing in FucT-VII–null mice may not necessarily extend to the rolling of normal neutrophils or monocytes on nonfucosylated endothelium. Finally, as noted by Varki (61), the differences in evolutionary pressures on the carbohydrate components of selectin ligands mean that it may not always be valid to extrapolate data from mice to humans.

The above discussion is predicated on the fact that LPS-stimulated HUVEC express functional L-selectin ligands. Although, this has been demonstrated in other studies, because of the limited availability of LAD II HUVEC, we were unable to show that in our experiments a functional L-selectin ligand was present (45, 46). However, it has been shown that L-selectin from human, but not mouse, neutrophils binds directly to E-selectin (62). Taken together with our findings that an E-selectin neutralizing mAb blocks neutrophil rolling (see below), it seems likely that neutrophil L-selectin engages counterstructures on HUVEC E-selectin for rolling. Nevertheless, an alternative explanation for our findings may be that neutrophil rolling on LPS-stimulated LAD II HUVEC is independent of L-selectin and its ligand. If this were the case, one would have to assume that inhibition of rolling by anti–L-selectin mAb LAM 1-3 was due to inhibition of neutrophil–neutrophil

interactions and not neutrophil–endothelial interactions (63). Regardless of the actual interacting molecules between neutrophils and endothelial cells, it appears that fucose is not a critical carbohydrate for neutrophil rolling on LPS-stimulated HUVEC.

The presumed lack of requirement for fucose in L-selectin binding contrasts with the importance of fucose for E- and P-selectin binding (58). The fucose requirement of E- and P-selectin has been confirmed by studies showing that neutrophils from a LAD II patient express normal levels of L-selectin, but bind minimally to recombinant E-selectin and purified platelet P-selectin (5) and roll poorly on inflamed mesenteric venules of rabbits (7). We speculate that the relatively mild phenotype of the LAD II patients with respect to infectious complications (Etzioni, A., unpublished observations) may reflect residual L-selectin function as well as an intact β2 integrin pathway. It would be interesting to recapitulate the LAD II defect in vitro and study the rolling of LAD II neutrophils on LAD II HUVEC.

In this study, neutrophil rolling on normal or LAD II HUVEC was completely blocked by an E-selectin mAb (data not shown). These results for normal HUVEC differ somewhat from a previous study in which E-selectin Abs did not block all interactions (rolling, adherence, and transmigration; reference 64). The differences might be explained by the presence of CD18 interactions in the previous study (64), which were blocked in this study. Furthermore, in the culture conditions used here, HUVEC cannot be induced to express P-selectin once the cells have been passaged (our unpublished observations). The requirement of E-selectin for neutrophil rolling is present at both high and low shear stresses. In contrast to E-selectin, we find that L-selectin blocking prevents neutrophil rolling only partially and only at the lower shear stress. That an L-selectin component of rolling is only noted at lower rates was recently described by Finger et al. (65). In that study, a threshold shear was required for rolling through L-selectin, peaking at a shear stress of ~ 1 dyn/cm^2. Virtually no rolling was seen at shear stresses > 2 dyn/cm^2. In this study, the fact that at the lower shear stress, an L-selectin Ab could partially block rolling and an E-selectin Ab completely blocked rolling suggests that glycoconjugates on E-selectin may be required for L-selectin rolling as previously suggested (42, 44, 62, 64). Alternatively, L-selectin may recognize a nonfucosylated ligand on HUVEC distinct from E-selectin, but rolling also requires E-selectin interaction with a neutrophil glycoconjugate. Patel et al. (42) have proposed that flowing neutrophils require the cooperative function of L-selectin and PSGL-1 to attach optimally to E-selectin. Further studies are required to distinguish between these possibilities.

Acknowledgments

The authors gratefully acknowledge J. Paulson and L. Phillips' (Cytel Corp., San Diego, CA) insights into the defect in fucose metabolism in LAD II that first suggested these studies. The authors thank T.F. Tedder for providing LAM 1–3 and T. Eunson for assistance with tissue culture.

This work was supported by a grant from Cytel Corporation (J.M. Harlan), and USPHS grants HL18645 (J.M. Harlan), GM 42686 (R.K. Winn), and HL30541 (R.K. Winn and J.M. Harlan). A. Karsan is supported in part by a Clinician-Scientist Award from the Medical Research Council of Canada.

References

1. Etzioni, A., M. Frydman, S. Pollack, I. Avidor, M.L. Phillips, J.C. Paulson, and R. Gershoni-Baruch. 1992. Recurrent severe infections caused by a novel leukocyte adhesion deficiency. *N. Engl. J. Med.* 327:1789–1792.

2. Frydman, M., A. Etzioni, T. Eidlitz-Markus, I. Avidor, I. Varsano, Y. Shechter, J.B. Orlin, and R. Gershoni-Baruch. 1992. Rambam-Hasharon syndrome of psychomotor retardation, short stature, defective neutrophil motility, and Bombay phenotype. *Am. J. Med. Genet.* 44:297–302.

3. Tamatani, T., M. Suematsu, K. Tezuka, N. Hanzawa, T. Tsuji, Y. Ishimura, R. Kannnagi, S. Toyoshima, and M. Homma. 1995. Recognition of consensus CHO structure in ligands for selectins by novel antibody against sialyl Lewis X. *Am. J. Physiol.* 269:H1282–1287.

4. Tedder, T.F., D.A. Steeber, A. Chen, and P. Engel. 1995. The selectins: vascular adhesion molecules. *FASEB J.* 9:866–873.

5. Phillips, M.L., B.R. Schwartz, A. Etzioni, R. Bayer, H.D. Ochs, J.C. Paulson, and J.M. Harlan. 1995. Neutrophil adhesion in leukocyte adhesion deficiency syndrome type 2. *J. Clin. Invest.* 96:2898–2906.

6. Petz, L.D., and S.N. Swisher. 1989. Clinical Practice of Transfusion Medicine. Churchill Livingstone, New York. 790 pp.

7. von Andrian, U.H., E.M. Berger, L. Ramezani, J.D. Chambers, H.D. Ochs, J.M. Harlan, J.C. Paulson, A. Etzioni, and K.-E. Arfors. 1993. In vivo behavior of neutrophils from two patients with distinct inherited leukocyte adhesion deficiency syndromes. *J. Clin. Invest.* 91:2893–2897.

8. Price, T.H., H.D. Ochs, R. Gershoni-Baruch, J.M. Harlan, and A. Etzioni. 1994. In vivo neutrophil and lymphocyte function studies in a patient with leukocyte adhesion deficiency type II. *Blood*. 84:1635–1639.

9. Ropers, H.H., and M.A. Pericak-Vance. 1991. Report of the committee on the constitution of chromosome 19. *Cytogenet. Cell Genet.* 58:751–784.

10. Easton, E.W., W.E.C.M. Schiphorst, E. van Drunen, C.E. van der Schoot, and D.H. van den Eijnden. 1993. Human myeloid α3-fucosyltransferase is involved in the expression of the sialyl-Lewis^x determinant, a ligand for E- and P-selectin. *Blood*. 81:2978–2986.

11. Shechter, Y., A. Etzioni, C. Levene, and P. Greenwell. 1995. A Bombay individual lacking H and Le antigens but expressing normal levels of α-2– and α-4–fucosyltransferases. *Transfusion (Bethesda)*. 35:773–776.

12. Etzioni, A., J.M. Harlan, M.L. Phillips, S. Pollack, R. Gershoni-Baruch, and J.C. Paulson. 1993. Leukocyte adhesion deficiency (LAD II)—deficiency in sialyl Lewis X, a ligand for selectin—due to general fucose deficiency. *J. Cell. Biochem.* 17A(Suppl.):C009 (Abstr.).

13. Wagers, A.J., J.B. Lowe, and G.S. Kansas. 1996. An important role for the α1,3 fucosyltransferase, FucT-VII, in leukocyte adhesion to E-selectin. *Blood*. 88:2125–2132.

14. Maly, P., A.D. Thall, B. Petryniak, S.E. Rogers, P.L. Smith, R.M. Marks, R.J. Kelly, K.M. Gersten, G. Cheng, T.L. Saunders, et al. 1996. The α(1,3)fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell*. 86:643–653.

15. Natsuka, S., K.M. Gersten, K. Zenita, R. Kannagi, and J.B. Lowe. 1994. Molecular cloning of a cDNA encoding a novel human leukocyte α-1,3-fucosyltransferase capable of synthesizing the sialyl Lewis x determinant. *J. Biol. Chem.* 269:16789–16794.

16. Hoogenraad, N.J., and C.J. Wraight. 1986. The effect of pristane on ascites tumor formation and monoclonal antibody production. *Methods Enzymol.* 121:375–381.

17. Beatty, P.G., J.A. Ledbetter, P.J. Martin, T.H. Price, and J.A. Hansen. 1983. Definition of a common leukocyte cell-surface antigen (Lp95-150) associated with diverse cell-mediated immune functions. *J. Immunol.* 131:2913–2918.

18. Spertini, O., G.S. Kansas, K.A. Reimann, C.R. Mackay, and T.F. Tedder. 1991. Function and evolutionary conservation of distinct epitopes on the leukocyte adhesion molecule-1 (TQ-1, Leu-8) that regulate leukocyte migration. *J. Immunol.* 147:942–949.

19. Neitzel, H. 1986. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum. Genet.* 73:320–326.

20. Jaffe, E.A., R.L. Nachman, C.G. Becker, and C.R. Minick. 1973. Culture of human endothelial cells derived from umbilical veins. *J. Clin. Invest.* 52: 2745–2756.

21. Lawrence, M.B., L.V. McIntire, and S.G. Eskin. 1987. Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. *Blood*. 70:1284–1290.

22. Stryer, L. 1981. Biochemistry. W.H. Freeman and Co., San Francisco. 949 pp.

23. Ishihara, H., and E.C. Heath. 1968. The metabolism of L-fucose: the biosynthesis of guanosine diphosphate L-fucose in porcine liver. *J. Biol. Chem.* 243:1110–1115.

24. Wilkinson, J.F. 1957. Pathway of the synthesis of fucose from glucose in *Klebsiella aerogenes*. *Nature*. 180:995.

25. Segal, S., and Y.J. Topper. 1957. On the biosynthesis of L-fucose by *Aerobacter aerogenes*. *Biochim. Biophys. Acta*. 25:419–420.

26. Heath, E.C., and S. Roseman. 1958. The conversion of D-glucose to L-fucose by Aerobacter cloacae. *J. Biol. Chem.* 230:511–519.

27. Segal, S., and Y.J. Topper. 1960. On the biosynthesis of L-fucose and L-fucose metabolism in man. *Biochim. Biophys. Acta*. 42:147–151.

28. Foster, D.W., and V. Ginsburg. 1961. Biosynthesis of L-fucose by mam-

malian tissue. *Biochim. Biophys. Acta*. 54:376–378.

29. Ginsburg, V. 1961. Studies on the biosynthesis of guanosine diphosphate L-fucose. *J. Biol. Chem.* 236:2389–2393.

30. Ishihara, H., D.J. Massaro, and E.C. Heath. 1968. The metabolism of L-fucose: the enzymatic synthesis of β–L-fucose 1-phosphate. *J. Biol. Chem.* 243:1103–1109.

31. Yurchenco, P.D., and P.H. Atkinson. 1977. Equilibration of fucosyl glycoprotein pools in HeLa cells. *Biochemistry*. 16:944–953.

32. Kilker, R.D., D.K. Shuey, and G.S. Serif. 1979. Isolation and properties of porcine thyroid fucokinase. *Biochim. Biophys. Acta*. 570:271–283.

33. Butler, W., and G.S. Serif. 1985. Fucokinase, its anomeric specificity and mechanism of phosphate group transfer. *Biochim. Biophys. Acta*. 829:238–243.

34. Broschat, K.O., S. Chang, and G. Serif. 1985. Purification and characterization of GDP-D-mannose 4,6-dehydratase from porcine thyroid. *Eur. J. Biochem.* 153:397–405.

35. Chang, S., B. Duerr, and G. Serif. 1988. An epimerase-reductase in L-fucose synthesis. *J. Biol. Chem.* 263:1693–1697.

36. Ricken, J., M. Herting, and P. Vischer. 1990. Investigation of the metabolism of L-fucose in aortic tissue and cultured arterial wall cells. *Biochem. Soc. Trans*. 18:963–964.

37. Ohmori, K., A. Takada, T. Yoneda, Y. Buma, K. Hirashima, K. Tsuyuoka, A. Hasegawa, and R. Kannagi. 1993. Differentiation-dependent expression of sialyl stage-specific embryonic antigen-1 and I-antigens on human lymphoid cells and its implications for carbohydrate-mediated adhesion to vascular endothelium. *Blood*. 81:101–111.

38. Kirkeby, S., D. Moe, and T.C. Bog-Hansen. 1993. Fucose expression in skeletal muscle: a lectin immunohistochemical study. *Histochem. J.* 25:619–627.

39. Holthofer, H., I. Virtanen, A.-L. Kariniemi, M. Hormia, E. Linder, and A. Miettinen. 1982. *Ulex europaeus* I lectin as a marker for vascular endothelium in human tissues. *Lab. Invest.* 47:60–66.

40. Schwachula, A., D. Riemann, A. Kehlen, and J. Langner. 1994. Characterization of the immunophenotype and functional properties of fibroblast-like synoviocytes in comparison to skin fibroblasts and umbilical vein endothelial cells. *Immunobiology.* 190:67–92.

41. Allen, H.J., E.A.Z. Johnson, and K.L. Matta. 1977. A comparison of the binding specificities of lectins from Ulex europaeus and Lotus tetragonolobus. *Immunol. Commun.* 6:585–602.

42. Patel, K.D., K.L. Moore, M.U. Nollert, and R.P. McEver. 1995. Neutrophils use both shared and distinct mechanisms to adhere to selectins under static and flow conditions. *J. Clin. Invest.* 96:1887–1896.

43. Picker, L.J., R.A. Warnock, A.R. Burns, C.M. Doerschuk, E.L. Berg, and E.C. Butcher. 1991. The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. *Cell*. 66:921–933.

44. Lawrence, M.B., D.F. Bainton, and T.A. Springer. 1994. Neutrophil tethering to and rolling on E-selectin are separable by requirement for L-selectin. *Immunity*. 1:137–145.

45. von Andrian, U.H., J.D. Chambers, E.L. Berg, S.A. Michie, D.A. Brown, D. Karolak, L. Ramezani, E.M. Berger, K.-E. Arfors, and E.C. Butcher. 1993. L-selectin mediates neutrophil rolling in inflamed venules through sialyl Lewis^x-dependent and -independent recognition pathways. *Blood*. 82:182–191.

46. Ley, K., T.F. Tedder, and G.S. Kansas. 1993. L-selectin can mediate leukocyte rolling in untreated mesenteric venules in vivo independent of E- or P-selectin. *Blood*. 82:1632–1638.

47. Mihelcic, D., B. Schleiffenbaum, T.F. Tedder, S.R. Sharar, J.M. Harlan, and R.K. Winn. 1994. Inhibition of leukocyte L-selectin function with a monoclonal antibody attenuates reperfusion injury to the rabbit ear. *Blood*. 84:2322–2328.

48. Varki, A., and H.H. Freeze. 1994. The major glycosylation pathways of mammalian membranes. *In* Membrane Biogenesis. Vol. 22. A.H. Maddy and J.R. Harris, editors. Plenum Publishing Corp., New York. 71–100.

49. Reitman, M.L., I.S. Trowbridge, and S. Kornfeld. 1980. Mouse lymphoma cell lines resistant to pea lectin are defective in fucose metabolism. *J. Biol. Chem.* 255:9900–9906.

50. Ripka, J., A. Adamany, and P. Stanley. 1986. Two Chinese hamster ovary glycosylation mutants affected in the conversion of GDP-mannose to GDP-fucose. *Arch. Biochem. Biophys.* 249:533–545.

51. Paulson, J.C. 1989. Glycoproteins: what are the sugar chains for? *TIBS (Trends Biochem. Sci.).* 14:272–276.

52. Fiedler, K., and K. Simons. 1995. The role of N-glycans in the secretory pathway. *Cell*. 81:309–312.

53. Tonetti, M., L. Sturla, A. Bisso, U. Benatti, and A. De Flora. 1996. Synthesis of GDP-L-fucose by the human FX protein. *J. Biol. Chem.* 271:27274–27279.

54. Imai, Y., D.D. True, M.S. Singer, and S.D. Rosen. 1990. Direct demonstration of the lectin activity of gp90^{MEL}, a lymphocyte homing receptor. *J. Cell Biol.* 111:1225–1232.

55. Imai, Y., L.A. Lasky, and S.D. Rosen. 1993. Sulphation requirement for GlyCAM-1, an endothelial ligand for L-selectin. *Nature*. 361:555–557.

56. Green, P.J., T. Tamatani, T. Watanabe, M. Miyasaka, A. Hasegawa, M. Kiso, C.-T. Yuen, M.S. Stoll, and T. Feizi. 1992. High affinity binding of the leucocyte adhesion molecule L-selectin to 3′-sulphated-Le^a and -Le^x oligosaccharides and the predominance of sulphate in this interaction demonstrated by binding studies with a series of lipid-linked oligosaccharides. *Biochem. Biophys. Res. Commun.* 188:244–251.

57. Norgard-Sumnicht, K.E., E. Varki, and A. Varki. 1993. Calcium-dependent heparin-like ligands for L-selectin in nonlymphoid endothelial cells. *Science*. 261:480–483.

58. Bevilacqua, M.P., and R.M. Nelson. 1993. Selectins. *J. Clin. Invest.* 91: 379–387.

59. Giuffre, L., A.-S. Cordey, N. Monai, Y. Tardy, M. Schapira, and O. Spertini. 1997. Monocyte adhesion to activated aortic endothelium: role of L-selectin and heparan sulfate proteoglycans. *J. Cell Biol.* 136:945–956.

60. Kansas, G.S. 1996. Selectins and their ligands: current concepts and controversies. *Blood*. 88:3259–3287.

61. Varki, A. 1997. Selectin ligands: will the real ones please stand up? *J. Clin. Invest.* 99:158–162.

62. Zollner, O., M.C. Lenter, J.E. Blanks, E. Borges, M. Steegmaier, H.-G. Zerwes, and D. Vestweber. 1997. L-selectin from human, but not from mouse neutrophils binds directly to E-selectin. *J. Cell Biol.* 136:707–716.

63. Walcheck, B., K.L. Moore, R.P. McEver, and T.K. Kishimoto. 1996. Neutrophil-neutrophil interactions under hydrodynamic shear stress involve L-selectin and PSGL-1. A mechanism that amplifies initial leukocyte accumulation of P-selectin in vitro. *J. Clin. Invest.* 98:1081–1087.

64. 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.

65. Finger, E.B., K.D. Puri, R. Alon, M.B. Lawrence, U.H. von Andrian, and T.A. Springer. 1996. Adhesion through L-selectin requires a threshold hydrodynamic shear. *Nature*. 379:266–269.