PLENARY PAPER

Correction of Leukocyte Adhesion Deficiency Type II With Oral Fucose

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We describe a simple, noninvasive, and effective therapy for leukocyte adhesion deficiency type II (LAD II), a rare inherited disorder of fucose metabolism. This disorder leads to an immunodeficiency caused by the absence of carbohydrate-based selectin ligands on the surface of neutrophils as well as to severe psychomotor and mental retardation. The fucosylation defect in LAD II fibroblasts can be corrected by addition of L-fucose to the culture medium. This prompted us to initiate dietary fucose therapy on a patient with LAD II. Oral supplementation of fucose in this patient induced the expression of fucosylated selectin ligands on neutrophils and core fucosylation of serum glycoproteins. During 9 months of treatment, infections and fever disappeared, elevated neutrophil counts returned to normal, and psychomotor capabilities improved.

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EUKOCYTES ENTER inflamed tissue through a cascade of molecular interactions that mediate leukocyte adhesion to the endothelium as well as leukocyte activation.1 The first step, rolling of leukocytes on the endothelial surface, is mediated by members of the selectin family. These include E-selectin (CD62E) and P-selectin (CD62P), which are expressed on the surface of activated endothelial cells, and L-selectin (CD62L), which is constitutively expressed on most leukocytes.2 Subsequent stimulation by chemotrafficators leads to the activation of leukocyte integrins that allow the leukocytes to firmly adhere and transmigrate through the blood vessel wall. It is well established that the selectins as well as leukocyte integrins are essential for the entry of leukocytes into sites of infection.

The importance of adhesion molecules in normal host defense is illustrated by inherited leukocyte adhesion deficiency (LAD) syndromes in which these molecules are defective. Patients with LAD suffer from life-threatening, recurrent, nonsuppurative bacterial infections in association with elevated peripheral leukocyte counts. LAD I is characterized by mutation in the gene of the β2-integrin subunit (CD18) of leukocyte integrins,3 whereas patients with LAD II lack the fucosylated ligands for selectins4 and have a marked decrease in neutrophil rolling in postcapillary venules.5,6

LAD II was first described in 2 Arab boys by Ezioni et al.47 Besides elevated leukocyte counts and recurrent episodes of bacterial infections, both children suffered from severe mental retardation and had a short stature and a distinctive facial appearance. Both lacked the carbohydrate epitopes Lewisα (Leα) and sialyl-Lewisα (sLeα) [NeuAcα2,3Galβ1,4(Fucα1,3) GlcNAc]. Selectin ligands are carbohydrates that share structural features with sLeα. The presence of fucose at the α1,3-position is essential for selectin ligand function.5 They are generated by α1,3-fucosyltransferases that catalyze the addition of GDP-fucose to N-acetylglycosamine. In addition to the lack of α1,3-fucosylated glycoconjugates, LAD II patients lack the red blood cell H-antigen that is an intermediate in the production of the A, B, and O blood group antigens. Individuals with the Bombay phenotype lack the H-antigen and have anti-H antibodies in their serum. The H gene product is an α1,2-fucosyltransferase. Because sLeα and the H-antigen are made by different fucosyltransferases, it was postulated that the genetic defect in LAD II was a general lesion in fucose metabolism rather than a deficiency in multiple fucosyltransferases.4 Indeed, it was suggested that LAD II is caused by defect in de novo GDP-fucose biosynthesis.9

We have recently described the clinical phenotype and biochemical characterization of a third patient with LAD II.10 As described in the first 2 cases, postnatal weight gain of the patient was severely impaired. At 15 months of age, the boy had a severe neurodevelopmental delay. He showed a prominent muscular hypotonia and was unable to sit without support. In contrast to the first 2 described patients who were born normally and had no signs of intrauterine growth retardation,11 the new patient already showed signs of severely retarded growth of fetal limb bones at 28 weeks of gestation. As described for the other LAD II cases, Leα and sLeα were absent from leukocytes and no H-antigen was expressed on erythrocytes. In addition, α1,6-core fucosylated N-glycans were absent from fibroblasts of the patient. As tested with selectin-IgG fusion proteins for E- and P-selectin, no selectin ligands were detectable on the patient’s leukocytes. This probably accounted for the immunodeficiency. A dramatic increase in peripheral neutrophil counts occurred within a few days after birth and correlated with recurrent episodes of high fever. In the absence of infection, total peripheral leukocyte counts were approximately 20,000/μL, but during febrile episodes they increased to 70,000/μL. In addition to neutrophils, total lymphocyte counts were also elevated. When the patient developed high fever, his clinical condition was severely impaired. No specific sites of infection could be identified by clinical investigation. Vomiting and reduced fluid intake necessitated intravenous fluid supplementation, and C-reactive protein as an indicator for systemic infection usually reached high levels of more than 10 mg/dL (normal, <1 mg/dL). Serial blood cultures as well as urine and...
cerebrospinal fluid (CSF) cultures were always sterile and did not identify any bacterial origin of the infection. Coronavirus was isolated from stool samples on several occasions and was the only infectious agent ever isolated from the patient. Systemic antibiotic treatment with cefixime or clindamycin was initiated on several occasions and normalized body temperature within 2 days. Even in the intervals between the febrile episodes, low-dose cephalosporins were administered in an attempt to reduce the frequency of these events.

We report here on the successful treatment of this patient by oral supplementation of fucose. In addition to the re-expression of selectin ligands on neutrophils, the drastically elevated peripheral neutrophil counts could be reduced to normal levels, no episodes of fever occurred after beginning therapy, and psychomotor capabilities and growth of the patient improved. Although expression of selectin ligands was rescued, fortunately, fucose therapy did not lead to the re-expression of the α1,2-fucosylated H-antigen, avoiding complications due to the patient’s low-titer anti-H antibodies.

MATERIALS AND METHODS

Materials. L-Fucose (fucose) from Kaden Biochemicals (Hamburg, Germany) and from Pfleischtei Laboratories (Waukegan, IL) was manufactured by crystallization of acid hydrolyzed fucoidan. Anti–P-selectin glycoprotein ligand-1 (PSGL-1) monoclonal antibody (MoAb) PL1,12 was from Coulter-Immunotech (Hamburg, Germany). Anti-αLe4 mouse IgM (CSLEX-1) was obtained from ATCC (Manassas, VA). Anti–H-antigen was from Mast Diagnostica (Reinfeld, Germany). Lens culinaris agglutinin (LCA) and Histoplate 1077 and 1119 were purchased from Sigma (Deisenhofen, Germany). Purified human serum IgM, antihuman IgM-alkaline phosphatase conjugate, and goat antimouse IgG-peroxidase conjugate (mouse-specific) were from Sigma. Man-L-fucosylated H-antigen, avoiding complications due to the patient’s low-titer anti-H antibodies.

Measurement of serum IgM levels. Serum samples obtained before and after therapy, normal human control sera, and purified human IgM standard at different concentrations were individually diluted in PBS and coated on the wells of a microtiter plate. The wells were blocked with 3% BSA in PBS overnight at 4°C, washed, and incubated with antihuman IgM alkaline phosphatase conjugate for 2 hours at room temperature. This was followed by a color reaction with p-nitrophenyl phosphate substrate. Serum IgM levels were quantitated using a microplate reader and expressed as a percentage of standard human IgM.

Fucose corrects defective fucosylation in cultured LAD II fibroblasts. To determine whether defective fucosylation in LAD II cells could be corrected by exogenous fucose, we cultured fibroblasts from a healthy control and from the patient in Dulbecco’s modified Eagle’s medium (DMEM) containing 1 or 10 mmol/L fucose. After 5 to 7 days in culture, cells were analyzed by flow cytometry for binding of LCA, a lectin that binds with high affinity to core α1,6-fucosylated N-glycans with low affinity to α-mannose and α-glucose. Specific binding is inhibited in the presence of 200 mmol/L α-methyl-D-mannopyranoside. Binding was detected with 10 µg/mL R-phycocerythrin-conjugated streptavidin. After washing the cells, data acquisition and analysis was performed on a FACSCalibur using CellQuest software (Becton Dickinson, San Jose, CA).

For determination of the H-antigen, erythrocytes from a healthy control and the LADII patient were isolated as described.10 Erythrocytes of Bombay phenotype were obtained from Biotest AG (Dreieich, Germany). Cells were incubated with an anti–H-antigen MoAb (Mast Diagnostica, Reinfeld, Germany) or with an irrelevant IgM control antibody. After washing, the detection was performed using an FITC-conjugated rabbit anti-mouse IgM antibody. All antibodies were used in saturating concentrations.

Cell adhesion assay. Polymorphonuclear cells (PMN) from human blood were isolated on density gradients of Histoplate 1077 and 1119 according to the manufacturer’s instructions. Adhesion assays in rotating 96-well microtiter plates coated with selectin-IgG or VE-cadherin-IgG fusion proteins were performed as described,17 except that assays were performed at room temperature.

Immunobots. Fifteen micrograms of total serum proteins was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 12.5% polyacrylamide gels under reducing conditions and transferred to nitrocellulose membranes. The membranes were blocked overnight with 10% skimmed milk in PBS, washed with PBS containing 0.05% Tween 20, and incubated with MoAb CAB4 at 20 ng IgG/mL. This was followed by reaction with peroxidase-conjugated goat anti-mouse IgG. Bound proteins were visualized by incubating with chemiluminescence detection reagents and exposing the membrane to an x-ray film.

RESULTS

Fucose corrects defective fucosylation in cultured LAD II fibroblasts. To determine whether defective fucosylation in LAD II cells could be corrected by exogenous fucose, we cultured fibroblasts from a healthy control and from the patient in Dulbecco’s modified Eagle’s medium (DMEM) containing 1 or 10 mmol/L fucose. After 5 to 7 days in culture, cells were analyzed by flow cytometry for binding of LCA, a lectin that recognizes α-glucose and α-mannose with low affinity and α1,6-core fucosylated N-linked oligosaccharides with high affinity. LAD II cells incubated with 1 mmol/L fucose showed increased LCA binding (Fig 1, lower panel, thin, green line), and incubation with 10 mmol/L fucose resulted in LCA binding even greater than that seen in control cells (Fig 1, lower panel, bold, blue line). This is in agreement with fucose supplementation studies of cultured fibroblasts of the first identified LAD II patients, analyzed for binding to Lotus tetragonolobus agglutinin (LTA), another fucose-dependent lectin.9 Fucose supplementation did not change LCA binding in control cells (Fig 1, upper panel). Although GDP-mannose can be converted into GDP-fucose, the addition of 1 mmol/L mannose to the culture
medium had no effect on the synthesis of fucosylated molecules of either cell line (data not shown). We conclude that exogenous fucose can rescue the LAD II phenotype in fibroblasts and therefore decided to treat the patient with a fucose-containing diet.

**Fucose treatment of the LAD II patient.** At the time we considered fucose therapy, there were no data on the kinetics of fucose absorption in humans. We determined the free fucose concentration in the serum of healthy children and adults by GC/MS and found it to be less than the detection level of 5 µmol/L (data not shown). Oral administration of fucose to healthy volunteers (50 to 100 mg/kg body weight [BW]) increased serum fucose concentrations, which reached a maximum of 110 to 210 µmol/L after 60 minutes (Fig 2). Fucose was
cleared from the serum with a half time of 100 minutes. After 24 hours, fucose in the serum was still greater than basal levels.

The LAD II patient was treated with 5 doses of fucose per day, starting at 25 mg/kg BW. A single dose of fucose was dissolved in 10 mL water and administered by gastrointestinal tube 20 to 30 minutes before feeding the child. Single doses were slowly increased to a maximal dose of 492 mg/kg BW after 277 days. Peak fucose concentrations in the blood were monitored once weekly 60 to 90 minutes after ingestion and ranged from 39 to 358 µmol/L during the course of therapy. Fucose doses and measured serum fucose concentrations over the full course of therapy are given in Table 1.

Fucose therapy restores selectin binding. The effect of fucose therapy on selectin ligand expression in neutrophils was determined by FACS analysis using the MoAb CSLEX-1 against sLeα and 2 selectin-IgG fusion proteins. These proteins contained the N-terminal C-type lectin domain, the EGF-like domain, and 2 consensus repeats of E- or P-selectin fused to the Fc part of human IgG1. Before fucose therapy, neutrophils did not bind to either P- or E-selectin (Fig 3/I). After 40 days of therapy and gradually increasing the dosage to 5 doses/day of 140 mg fucose/kg BW, P-selectin–IgG chimera binding reached approximately 50% of that of control cells (Fig 3/II).

Because selectins are Ca2+-dependent cell adhesion molecules, we tested the specificity of the observed binding by incubating the cells with P-selectin–IgG in the presence of EDTA. As shown in Fig 3B/II, EDTA blocked binding demonstrating selectin specificity of the FACS signal. As an additional control, we preincubated the cells with the MoAb PL-12 against PSGL-1, the major P-selectin ligand on human leuko-

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<th>Day of Treatment</th>
<th>mg Fucose/kg BW (single dose)</th>
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Blood samples were taken 60 to 90 minutes after ingestion. Treatment was started with 25 mg/kg BW (single dose); the first determination of serum fucose concentration was performed at day 10.
Fig 3.

The salvage pathway and the de novo pathway of GDP-fucose biosynthesis. GDP-fucose can either be synthesized from fucose (salvage pathway) or from GDP-mannose (de novo pathway). GDP-fucose that is metabolized by fucosyltransferases needs to be transported into the Golgi from the cytosol.
LAD II THERAPY

To verify that fucose therapy also affected other glycoproteins, we analyzed α1,6-fucosylation of N-linked oligosaccharides on serum glycoproteins using an MoAb (CAB4) that is highly specific for fucose linkage in this structure.13 Ig heavy chains (γ and μ) are the major core fucosylated serum glycoproteins. Western blots of sera from 4 different normal individuals detected a major protein of approximately 72 kD that corresponds to purified human IgM (μ chains). This band was absent from the pretherapy sample of the patient (Fig 5, lane 1). However, within 1 week of starting fucose therapy, core fucosylation of IgM μ chains was detectable and reached 35% of normal by 32 days (Fig 5). Serum IgM levels, on the other hand, showed little variation at all time points, as was determined by enzyme-linked immunosorbent assay (ELISA) using antihuman IgM antibodies that recognize IgM independently of its glycosylation. Thus, the increase in core fucosylation was not due to a corresponding increase in IgM. A band at 53 kD (not shown) corresponds to IgG γ chains and also showed improved core fucosylation during therapy. These results also demonstrate that fucose linked in a different way than in selectin ligand structures was restored by fucose therapy. In addition, the fucose detected on IgM was incorporated in N-linked carbohydrates, in contrast to the O-linked carbohydrates preferentially found on most selectin ligands.

Fig 5. Core fucosylation of serum IgM. Immunoblots of serum samples of the LAD II patient taken before (0 days) and at 6 different time points during fucose therapy (as indicated) and from a healthy control (control). Samples were analyzed with the mouse MoAb CAB4 that recognizes Fuc-α1,6-GlcNAcβ in the core of N-linked glycans. First antibody was detected by binding with a secondary antibody that did not cross-react with human Igs. The same serum samples were analyzed for the amount of IgM protein present by ELISA using antihuman IgM antibodies as described in Materials and Methods. Serum IgM levels were quantitated by regression analysis from the reactivity of purified human IgM. Amounts given on the left refer to the amounts that were loaded on the immunoblot gel.

Fig 3. Selectin binding and sLeα expression before and during fucose therapy. Isolated neutrophils of a healthy control or of the LAD II patient before or during therapy (I through IV, as indicated) were analyzed by flow cytometry using the following reagents: (A and B) E-selectin–IgG (E-Sel-IgG) or P-selectin–IgG (P-Sel-IgG) in the presence of Ca2+ (bold, red line) or in the presence of EDTA (thin, green line), VE-cadherin–IgG (dashed, blue line); (C) P-selectin–IgG (bold, red line), preincubation with anti-PSGL-1 MoAb PL-1 before incubation with P-selectin–IgG (thin, orange line), VE-cadherin–IgG (dashed, blue line); (D) anti-sLeα MoAb CSLEX-1 (bold, red line), irrelevant IgM control antibody (dashed, blue line). In each case, the fluorescence-labeled secondary antibody alone (negative control) was depicted in black (dotted line). Because the measurements of healthy control cells depicted in row IV were performed in parallel with the measurements depicted in row III, arrows in II/B and II/D mark the position of means of the healthy control signals as determined in parallel with these measurements.

E-selectin–IgG binding was not detectable during the first 100 days of therapy, during which fucose doses had been increased to 5 doses/day of up to 207 mg/kg BW (not shown). Doses were further increased within the next 100 days to 5 doses/day of 333 mg/kg BW each. After a total of 200 days from the start of therapy, FACS analysis was repeated. As seen in Fig 3A/III and 3D/III, prolonged administration of higher doses of fucose resulted in restoration of E-selectin–IgG binding along with good recovery of sLeα. E-selectin–IgG binding was sensitive to EDTA treatment (Fig 3A/II) and resulted in signals that were 20% of those observed on healthy neutrophils. Similarly, sLeα recovered to 20% and P-selectin–IgG binding to 72% that of healthy cells, respectively (Fig 3B/II). Results of the FACS analysis for P- and E-selectin binding could be reproduced in nonstatic rotation adhesion assays with E- and P-selectin–IgG immobilized in 96-well microtiter plates (data not shown). Our results demonstrate that re-expression of E-selectin ligands required higher doses of fucose than the restoration of P-selectin ligands.

LAD II patients have the Bombay phenotype blood group, because they lack the α1,2-fucosylated H-antigen.4,10 Therefore, we had to consider the possibility that fucose supplementation therapy could lead to synthesis of the H-antigen, possibly causing unwanted autoimmune side effects due to anti–H-antigen antibodies. Fortunately, the H-antigen did not appear on the patient’s erythrocytes at any time during the entire 280 days of fucose therapy, as was tested by FACS analysis. Figure 4 shows that the FACS signal for the H-antigen was negative both before therapy and after 280 days of therapy at doses of 492 mg/kg BW. Similarly, no signal greater than that of the second antibody alone or a control IgM antibody was detected on erythrocytes of Bombay phenotype. For comparison, the mean fluorescence of the H-antigen signal on healthy erythrocytes was 400-fold more intense than that of the negative controls (Fig 4). While incubations with anti-H antibodies were performed in the course of FACS analysis, no sign of cell agglutination was observed with LAD II cells. Measuring
These results demonstrate that fucose therapy is sufficiently robust to partially correct impaired fucosylation of major serum glycoproteins and is not restricted to the fucosylated selectin ligands, which are quantitatively minor glycoproteins. Moreover, the results show that fucose corrects glycosylation of both N- and O-linked species.

**Fucose therapy reduces peripheral neutrophil counts to normal levels.** Before beginning fucose therapy, this patient had serious recurrent infections and high fever that required continuous low-dose antibiotic treatment. Peripheral neutrophil counts before therapy were consistently high (10,000 to >50,000/µL blood). Within 10 days of starting fucose therapy, neutrophil levels returned to the normal range of 1,500 to 8,500 cells/µL blood (Fig 6). Consistent with this effect, there were no further infections and antibiotic prophylaxis was discontinued. However, lymphocyte counts were not normalized by fucose therapy (mean before therapy, 11,632/µL; mean during therapy, 14,144/µL; normal, 4,000 to 10,000/µL).

**Psychomotor development during fucose therapy.** Psychomotor development improved during fucose therapy. The widely administered Griffiths Test measured changes in psychomotor functioning in the areas of head and body motor control, eye-hand coordination, speech and language, and social interaction. A score of 100 EQ is equivalent to the 50th percentile. Before therapy, the patient showed a severe psychomotor retardation as evidenced by a total score of 28.5 EQ. Reassessment after 3 months of fucose therapy showed a significant increase in psychomotor functioning, with a total score of 45 EQ. Equal improvement was found across all of the assessed domains. Fucose concentrations in the CSF were measured (n = 2) at approximately 25% of the serum fucose concentrations, indicating that fucose crosses the blood-brain barrier.

At present, it cannot be decided whether the marked improvement is a consequence of the generally improved clinical condition of the child without recurrent episodes of high fever or whether fucose treatment has a specific effect on the psychomotor and mental development of the child. At 2 years of age, the boy is still severely retarded. He does not speak yet, but is able to actively turn around when lying on his back, takes toys that are presented in his hands, and starts to sit briefly without support.

**DISCUSSION**

We have shown that the defect in fucose metabolism in an LAD II patient can be partially corrected by oral fucose substitution therapy. Peripheral neutrophil counts decreased to normal values, and the frequently observed recurrent episodes of infections and high fever did not occur again after the onset of therapy.

LAD II is based on an as yet undefined genetic defect in fucose metabolism. No fucosylated glycoconjugates have yet been identified in these patients. The most significant consequence of this defect is the absence of α1,3-fucosylated sLeα-like selectin ligands, which causes an immunodeficiency.
due to impaired leukocyte migration into sites of infection in these patients. Other fucosylated glycoconjugates, such as the α1,2-fucosylated H-antigen on erythrocytes and the α1,6-core fucosylated N-glycans on serum and cell surface proteins, are also missing. Because all of these glycoconjugates are synthesized by different fucosyltransferases, Etzioni et al suggested that LAD II is probably caused by a defect in the synthesis of the common donor substrate, GDP-fucose.

GDP-fucose is synthesized by 2 different pathways, a de novo and a salvage pathway (Fig 7). The de novo pathway starts from GDP-mannose, which itself originates from glucose or mannose. The 2 enzymes that catalyze the conversion of GDP-mannose to GDP-fucose are GDP-mannose-4,6-dehydratase (GMD) and the FX protein, a homodimer that has epimerase as well as reductase activity. The salvage pathway starts from fucose that is taken up into the cell or derived from degraded glycoconjugates and is converted into GDP-fucose via a fucose-kinase followed by a GDP-L-fucose-pyrophosphorylase.

In cultured mammalian cells, the flux through the salvage pathway is thought to contribute only approximately 10% to the synthesis of the total GDP-fucose; however, high concentrations of exogenous fucose were sufficient to partially rescue expression of α1,6-core fucosylated N-glycans on fibroblasts of the patient. This is in agreement with results by Karsan et al for cells of the other LAD II patients. These results lead to the conclusion that the defect in LAD II is based on an insufficient amount of GDP-fucose. This could be due to a mutation in one of the enzymes in the de novo GDP-fucose biosynthetic pathway or a mutation that affects the efficiency of the transport of GDP-fucose from the cytosol, where it is synthesized, to the Golgi, where it is used. The mammalian GDP-fucose transporter has not yet been purified or cloned.

Despite these encouraging in vitro results, there were 2 major concerns that argued against using fucose supplementation therapy for the patient. First, fucose ingestion studies had never been performed in humans. It was discouraging that 1 mmol/L fucose in the cell culture medium only partially restored α1,6-core fucosylated N-glycans on fibroblasts and that 10 mmol/L fucose was necessary for complete rescue. Second, we had to expect that re-expression of fucosylated glycoconjugates would include the expression of the α1,2-fucosylated H-antigen. Although titers of anti-H antibodies were very low and only undiluted serum of the patient could agglutinate healthy erythrocytes, we had to consider the possibility of complications due to hemolysis. For these reasons, we started the therapy with low doses of fucose that were gradually increased, while we carefully monitored for any signs of hemolysis. Fortunately, low levels of sLex expression and a 50% recovery of P-selectin binding of neutrophils was observed as early as 40 days after beginning therapy, without any sign of H-antigen re-expression on the erythrocytes. Even after 280 days of therapy using 5 doses/day of up to 492 mg fucose/kg BW, H-antigen was undetectable. This may suggest that different levels of GDP-fucose are necessary for the synthesis of α1,3-fucosylated selectin ligands and α1,2-fucosylated H-antigen. It is possible that the α1,2-fucosyltransferase that generates the H-antigen requires higher concentrations of GDP-fucose for its activity than the α1,3-fucosyltransferases that generate selectin ligands. Alternatively, erythrocyte progenitors might have a quantitatively insignificant or inefficient salvage pathway for GDP-fucose synthesis.

The differences in the expression kinetics of the P- and E-selectin ligands on neutrophils during fucose therapy were even more surprising. Using 5 daily doses of 140 mg/kg, P-selectin-binding of neutrophils was re-established to 50%, whereas no binding was seen with E-selectin. Even treatment with 5 doses/day of 207 mg/kg BW E-selectin ligands were still undetectable. Continuing the same regimen and increasing the doses to 333 mg/kg produced E-selectin binding that was 20% of the intensity of healthy control cells. We conclude that different levels of GDP-fucose are necessary to express P- and E-selectin ligands. Because 2 different α1,3-fucosyltransferases, Fuc-TIV and Fuc-TVII, are expressed in myeloid cells, it would be possible that E- and P-selectin ligands differ in their dependence on each of these enzymes and that each of the 2 enzymes differs in its Km for GDP-fucose. Alternatively, P-selectin ligands could require lower levels of fucosylation for selectin-binding than for E-selectin ligands. The latter explanation would be in agreement with studies on activated T cells, in which PSGL-1 binds well to P- and E-selectin shortly after antigen-specific activation, but only retains its ability to bind to P-selectin at later stages of activation. These results could be explained by different levels of fucosylation of PSGL-1 possibly caused by different levels of fucosyltransferase expression at different time points of activation. In the present study, different levels of fucosylation might have been caused by re-establishing different levels of GDP-fucose during therapy. Indeed, it has been shown that PSGL-1 has only few fucosylated O-linked oligosaccharides, and it is possible that only one site is sufficient for binding to P-selectin, whereas more sites might be necessary for binding to E-selectin.

Other fucosylated glycoconjugates were also partially restored during fucose therapy. The recovery of core α1,6-fucosylated N-glycans on serum IgM heavy chains is significant, because it shows that treatment is sufficiently robust to drive the fucosylation of major serum glycoproteins. The half life of IgM in serum is 5 to 10 days, which may explain why we could detect this change very early in the therapy. Much lower levels of fucose were necessary to restore core fucosylation of IgM in the patient than to normalize core fucosylation of N-linked oligosaccharides of fibroblasts in culture. As discussed above, the variable effectiveness of fucose therapy suggests that correction may be cell-type and glycoconjugate specific.

Despite the successful correction of immunodeficiency-related defects in LAD II, correcting the delayed psychomotor development was expected to be more difficult to achieve. However, the patient showed significant psychomotor improvement while on fucose therapy. Fucose crosses the blood-brain barrier, resulting in elevated free fucose levels in the CSF during therapy. It is a topic of further investigation whether fucosylation of glycoproteins produced in the central nervous system and found in the CSF is influenced by fucose therapy. A long-term follow-up of different LAD II patients will be needed to decide whether fucose treatment will have a beneficiary effect in avoiding the severe psychomotor and mental retardation in these children.
Fucose ingestion studies have not been performed in humans; therefore, we need to consider potential side effects of dietary fucose. Fucose is a potent inhibitor of myoinositol transport (Ki, 3 mmol/L). Neurolasticoma cells exposed to 1 to 30 mmol/L fucose decrease incorporation of myoinositol into phospholipids. Rats fed high amounts of fucose (10% to 20% of total weight of food) showed nerve conduction velocity that is much lower than those used on cultured cells. Third, humans can synthesize myoinositol, whereas rats and mice might not. We found that motor nerve conduction velocities in the patient were normal after 5 and 9 months of therapy (Marquardt and Kurleman, unpublished observations). Future studies need to determine whether increasing the level of fucose substitution is beneficial.

In cells, fucose is found in N- and O-linked oligosaccharides and in glycosphingolipids. Consequently, the genetic defect in LAD II leads to a multisystemic disorder. The list of diseases resulting from inherited glycosylation defects may be growing in the future. Several of these genetic defects cause multisystemic disorders. Well-studied examples are the carbohydrate-deficient glycoprotein syndromes (CDGS) that are characterized by hypoglycosylation of glycoproteins. Oral mannose supplements were successfully used to reverse clinical and biochemical symptoms in a patient with a recently identified subtype of these syndromes, CDGS type 1b. Since then, this therapy has been effectively used in other patients with the same disorder. We show here that another simple monosaccharide effectively alleviates the major symptoms caused by a glycosylation defect. Needless to say, the simplicity of an oral substitution therapy is superior to a bone marrow transplantation or some form of gene therapy. In addition, fucose substitution allowed us to successfully treat the patient without precisely knowing the gene that is affected in LAD II.

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