Golgi GDP-fucose Transporter-deficient Mice Mimic Congenital Disorder of Glycosylation IIc/Leukocyte Adhesion Deficiency II*

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Modification of glycoproteins by the attachment of fucose residues is widely distributed in nature. The importance of fucosylation has recently been underlined by identification of the monogenetic inherited human disease “congenital disorder of glycosylation IIc,” also termed “leukocyte adhesion deficiency II.” Due to defective Golgi GDP-fucose transporter (SLC35C1) activity, patients show a hypofucosylation of glycoproteins and present clinically with mental and growth retardation, persistently leukocytosis, and severe infections. To investigate effects induced by the loss of fucosylated structures in different organs, we generated a mouse model for the disease by inactivating the Golgi GDP-fucose transporter gene (Slc35c1). Lectin binding studies revealed a tremendous reduction of fucosylated glycoconjugates in tissues and isolated cells from Slc35c1-/-mice. Fucose treatment of cells from different organs led to partial normalization of the fucosylation state of glycoproteins, thereby indicating an alternative GDP-fucose transport mechanism. Slc35c1-deficient mice presented with severe growth retardation, elevated postnatal mortality rate, dilatation of lung alveoles, and hypoplastic lymph nodes. In vitro and in vivo leukocyte adhesion and rolling assays revealed a severe impairment of P-, E-, and L-selectin ligand function. The diversity of these phenotypic aspects demonstrates the broad general impact of fucosylation in the mammalian organism.

The covalent attachment of oligosaccharide moieties to newly synthesized proteins comprises one of the most frequent but also complex forms of co- and posttranslational protein modifications, which has been found in nearly all living organisms (1). Glycan structures affect the physico-chemical properties and the function of proteins in a variety of biological processes, including folding, solubility, sorting, proteolytic stability, and receptor-ligand interactions. In mammalian organisms, the biosynthesis of the oligosaccharide chains requires a broad spectrum of glycosyltransferases, glycosidases, transport proteins, and 13 different monosaccharides (2–4). Due to its variability of binding types (α-1,2-, α-1,3-, α-1,4-, and α-1,6-fucosylation have been described), the monosaccharide fucose plays an important role in the microheterogeneity of oligosaccharide structures (5). Fucose residues are predominantly linked to peripheral parts of N-, O-, and lipid-linked oligosaccharides, thereby building cap structures, which have been observed in many surface-localized and secreted proteins, but also modify the core of some N-linked glycans. Fucose covalently attached to serine and threonine residues can be elongated by further glycosyltransferases of regulatory function for signaling molecules, such as Notch (6). The fucosylation of mammalian glycoproteins is catalyzed by at least 11 different glycosyltransferases. They show high specificity for their glycan acceptor substrates and their common donor substrate GDP-fucose (7). The GDP-fucose-specific transporter SLC35C1, which is located in the Golgi membrane, translocates GDP-fucose from the cytosol into the Golgi lumen (8), where it serves as donor of fucose for fucosyltransferase-catalyzed reactions during the modification of glycans (Fig. 1).

Recently, we and others could underline the crucial importance of SLC35C1 by identification of the monogenetic autosomal recessive inherited human disease “congenital disorder of glycosylation IIc” (CDG-IIc,6 also termed “leukocyte adhesion deficiency II,” OMIM 266265), caused by defective transporter activity of SLC35C1, thereby leading to severely impaired Golgi-localized fucosylation of glycoconjugates (9–12). CDG-IIc patients suffer from mental and growth retardation, facial stigmata, and recurrent bacterial infections with persistently

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FIGURE 1. Supply mechanisms for GDP-fucose in the endoplasmic reticulum and Golgi. 90% of the cytosolic GDP-fucose is derived from the de novo pathway, and 10% is from the salvage pathway. The transport of GDP-fucose into the Golgi, where the fucosyltransferases Fut1 to -9 are located, is accomplished by SLC35C1. How the endoplasmic reticulum (ER)-resident fucosyltransferases POFUT1 and POFUT2 are supplied with their substrate GDP-fucose is still unknown.

Elevated peripheral leukocyte levels (13). Some CDG-IIc patients were partially treatable with an oral fucose therapy (14, 15).

Effects of hypofucosylation have been described in a variety of mouse models defective for fucosyltransferases as well as for the FX protein, an enzyme that is important for the de novo synthesis of GDP-fucose (Fig. 1). Mice deficient for the fucosyltransferases Fut1 and Fut2, respectively, which are expressed in the male and female reproductive tracts, show deficiencies for the respective fucosyl residues, but neither morphological abnormalities nor infertility has been reported (16). Fut9 mutant mice are deficient for stage-specific embryonic antigen 1 but also develop normally (17). The fucosyltransferases Fut4 and Fut7 catalyze the α-1,3-linked attachment of fucose in the course of the biosynthesis of sialyl Lewis X, a structural element of E-, P-, and L-selectin ligands. On the basis of single and double deficient mice, it could be shown that Fut4 and Fut7 control the selectin-dependent process of leukocyte recruitment and lymphocyte homing in a collaborative manner (18, 19). The most recently generated mouse model for a defective fucosyltransferase describes the loss of Fut8 activity. The mice exhibit a lack of α-1,6-linked core fucose and present with growth retardation, high postnatal mortality, and lung abnormalities (20). In contrast to the above mentioned fucosyltransferases, which are located in the Golgi, the O-fucosyltransferases POFUT1 and POFUT2 are localized in the endoplasmic reticulum, where they catalyze the attachment of O-linked fucose residues (e.g. of POFUT1 to the Notch receptor) (21). A deficiency for POFUT1 causes prenatal lethality at embryonic day 10 (22). Finally, a knock-out of the FX protein has been reported (23). This 3,5-epimerase/4-reductase is implicated in the conversion of GDP-mannose to GDP-fucose (Fig. 1). FX−/− mice are characterized by partial embryonic lethality, retarded development, persistent diarrhea, infertility, and leukocytosis. However, the loss of fucosylated structures and resulting symptoms can be compensated by a fucose diet, leading to GDP-fucose supply via a salvage pathway (Fig. 1).

Despite the numerous insights from these different models with hypofucosylation in confined contexts, many fucose-dependent biological events remain unknown. Here we present for the first time the generation and characterization of a knock-out mouse model for Slc35c1, which mimics the pathological situation in CDG-IIc patients and should lead to a complete loss of fucosyl residues attached to glycoconjugates generated in the Golgi.

EXPERIMENTAL PROCEDURES

Targeted Disruption of the Murine Golgi GDP-fucose Transporter in Embryonic Stem Cells—A cDNA fragment obtained by PCR, with primers designed according to a mouse expressed sequence tag homologous to the human nucleotide sequence, was used to screen a commercially available cosmid genomic library (strain 129/ola; Deutsches Ressourcenzentrum für Genomforschung, RZPD). An EcoRI fragment of 11.3 kb containing exons 1 and 2 was used to construct a targeting vector generating a total knock-out of the murine Golgi GDP-fucose transporter. The reading frame was interrupted inside the first exon by insertion of a neomycin resistance cassette into a SnaBI restriction site. Embryonic day 14.1 embryonic stem (ES) cells were cultured on mitomycin C-treated feeder layers of primary murine embryonal fibroblasts (MEF) derived from transgenic neomycin-resistant mice (Transgenic Research Facility, Stanford University, Stanford, CA) in knock-out Dulbecco’s modified Eagle’s medium supplemented with 15% fetal calf serum, minimal essential medium nonessential amino acids, 2 mM L-glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin, 100 μM β-mercaptoethanol, and 1,000 units/ml recombinant leukemia inhibitory factor (ESGRO; Chemicon, München, Germany). 25 μg of linearized targeting vector was electroporated into 107 ES cells by using a Bio-Rad Gene Pulser (230 V, 500 microfarads). ES cells were then diluted onto feeder layers and cultured with 180 μg of active form (active form)/ml G418 for 10 days. DNA derived from selected G418-resistant clones was screened by Southern blot analysis and verified by PCR and sequencing analysis for correct homologous recombination.

Generation of Slc35c1−/− Mice—Two different clones of targeted ES cells were injected into C57BL/6 blastocysts to generate chimeras, following standard protocols (24, 25). Agouti colored offspring from matings of chimeric male mice with C57BL/6 female mice indicated germ line transmission. Heterozygous mice were back-crossed for at least four generations with wild type C57BL/6 mice. All reported experiments were performed with wild type C57BL/6 mice.
performed with F2 progeny from 129Sv/C57BL/6 intercrosses because of a substantially higher recovery of Slc35c1−/− animals as compared with uniform C57BL/6 or 129Sv background (data not shown). Mice were kept under conventional conditions with food and water ad libitum. Experiments were conducted according to institutional ethical guidelines for animal experiments and safety guidelines for gene manipulation experiments.

**Genotyping of Targeted ES Cells and Mice**—DNA from ES cells and tail biopsy specimens was purified by Proteinase K digestion followed by ethanol precipitation. For Southern blot analysis, genomic DNA was HincII-digested, and a probe of 693 bp homologous to an external region in the 5′-direction of the targeting vector was generated by PCR with primers GFT-F1, GFT-R2 (5′-GGT TGT TGG TCA AGA GTG TAA CCT ATG-3′) and GFT-R3 (5′-CCA GCT CGC AGG CCG TTG C-3′). This probe was used to identify the HincII fragments of the wild-type allele of 4.25 kb and the knock-out allele of 1.85 kb, respectively. PCR analysis identified the HincII fragments of the wild-type allele of 4.25 kb and the knock-out allele of 1.85 kb, respectively. PCR analysis was done by multiplex PCR with primers GFT-F1, GFT-R2 (5′-CCG TCG ACG GTA TCG ATA AGC-3′), and GFT-R1 (5′-GTG TGT TGG TCA AGA GTG TTA AGC CTC TAG-3′), amplifying a 2.3-kb wild-type fragment and a 1.8-kb knock-out fragment, respectively.

**Preparation of Golgi-enriched Membrane Fractions from Liver**—Golgi-enriched membrane fractions from mouse liver were prepared as described (26) with some modifications. Livers were homogenized twice in 3.5 ml of 0.25 M sucrose. After centrifugation at 600 × g for 10 min at 4 °C, the postnuclear supernatant was collected, layered on 5 ml of 1 M sucrose, and centrifuged at 105,000 × g for 60 min at 4 °C. Golgi-enriched membranes were collected at the 0.25–1.3 M sucrose interface, and enrichment was quantified by galactosyltransferase activity as described (27).

**Import of GDP-[14C]Fucose and UDP-[3H]Galactose**—Transfer of GDP-[14C]fucose and UDP-[3H]galactose into membrane vesicles was carried out as described (10). For each reaction, 400 μg of protein were used.

**Primary Cell Culture**—For preparation of fibroblasts from mouse embryos, heterozygous mice were mated. Females were checked for vaginal plugs and sacrificed at 12.5 days postcoitum. Embryos were removed from the uterus and amniotic cavity under sterile conditions. After removal of heart and liver and tissue for genotyping, tissues were minced and trypsinized. Cells were prepared from cultured in Dulbecco’s modified Eagle’s medium containing D-Val (PAN Biotech, Aidenbach, Germany), supplemented with 20% fetal calf serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin. Single cell suspensions of murine spleen cells were prepared by mechanical disaggregation and lysis of erythrocytes.

Granulocytes were prepared as follows. Bone marrow cells were prepared from femur and tibia bones, passed through a 70-μm mesh, and layered on a Histopaque 1077/Histopaque 1119 double gradient (Sigma). Cells were pelleted according to the manufacturer’s protocol and taken from the interphase between the Histopaque solutions. Cells were washed twice in Hanks’ balanced salt solution (HBSS; Biochrom, Berlin, Germany) before being used in flow cytometry or adhesion assays.

**Cytochemical Staining with Aleuria aurantia Lectin (AAL)**—MEF were cultured for 24 h in medium without and supplemented with 10 mM fucose (Sigma). Histochemical staining was carried out as described (11).

**Histology and Lectin Histochemistry**—Tissues were fixed with 5 mM PBS, 4% formaldehyde and embedded in paraplast. Lungs were inflation-fixed at 25-cm H2O pressure. Sections were stained with hematoxylin/eosin. For lectin histochemistry, sections were treated with biotinylated lectins and counterstained with streptavidin-fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA).

**Flow Cytometry**—Flow cytometry was performed according to standard protocols (14). Biotinylated AAL and Maackia amurensis lectin II (Vector Laboratories) were used at 10 μg/ml and detected with phycoerythrin-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA). Streptavidin-phycocerythrin alone or AAL + 75 mM L-fucose (Sigma) served as negative controls. E-selectin-, P-selectin-, and VE-cadherin-Fc constructs were published before (30, 31). In some experiments, splenocytes were cultured with 10 mM L-fucose (Sigma) for 24 h before they were analyzed in flow cytometry.

**Cell Adhesion Assays under Static Conditions**—Adhesion assays were performed in 96-well flat bottom plates (Maxisorp, Nunc, Wiesbaden, Germany) coated with 50 μl of E-selectin-Fc, P-selectin-Fc, or VE-cadherin-Fc (10 μg/ml) diluted in HBSS. Subsequently, plates were blocked with 10% fetal calf serum in HBSS for 1 h at 37 °C. Wells were washed twice with HBSS before 2 × 105 granulocytes were added in 200 μl of HBSS. The plates were kept at 4 °C for 20 min under mild rotation (80 rpm). Wells were washed three times with the same buffer, and remaining cells were fixed with HBSS containing 2% glutaraldehyde (Sigma) at 4 °C for 30 min and evaluated by computer-aided image analysis with NIH Image 1.55 software (National Institutes of Health, Bethesda, MD). Each condition was tested in triplicates. Bound cells from four areas of each well were counted. Nonspecific binding was tested by allowing cells to bind in the presence of 5 mM EDTA.

**Peyer’s Patch Preparation and Intravital Microscopy**—The functional blocking mAb MEL-14 (rat IgG2a) against L-selectin and PS/2 (rat IgG 2b; 30 μg/mouse) against α4 integrin were purified from hybridoma supernatants (American Type Cul-
ture Collection, Manassas, VA). The P-selectin-blocking mAb RB40.34 (rat IgG1, 30 µg/mouse) was from Prof. D. Vestweber (32).

The surgical preparation of Peyer’s patch high endothelial venules (PP-HEV) for the observation of leukocyte rolling was performed as described previously (33, 34). Briefly, after opening the peritoneal cavity and locating a Peyer’s patch on the small intestine, leukocytes were stained in vivo by systemic injection of 0.15 ml of 1.5 mg/ml rhodamine 6G (Molecular Probes, Inc., Eugene, OR). Three minutes after injection, leukocyte rolling in PP-HEV was observed by intravital epifluorescence microscopy (Leica, Wetzlar, Germany) using a flash illumination system (60/s; Strobex, Chadwick Helmuth, Mountain View, CA). Each venule was observed for 60 s. Venules with diameters between 14 and 34 µm were observed and recorded via a CCD camera (CF 8/1; Kappa, Gleichen, Germany) on a Panasonic S-VHS recorder.

Data Analysis of Intravital Experiments and Statistics—Vessel diameter and vessel segment length of postcapillary venules or HEV were measured using an image processing system (35). An empirical factor of 0.625 was used to convert centerline velocities to mean blood flow velocities (36). To assess centerline blood flow velocities in HEV, 1-µm diameter fluorescent YG microspheres (Polysciences, Warrington, PA) were injected systemically, and blood flow velocity was measured by frame-to-frame displacement of the bead (three microspheres per venule). Wall shear rates (γw) were estimated as 4.9 (8vb/d), where vb is the mean blood flow velocity and d is the diameter of the vessel (37, 38).

Rolling leukocyte flux fraction was defined as the percentage of rolling leukocytes relative to all leukocytes passing through the same venule per time unit. Due to the preferential delivery of leukocytes to the terminal capillaries in microvascular networks (39, 40), leukocyte rolling flux is consistently higher than the product of the flow rate and the systemic leukocyte concentration. Therefore, we set the flux fraction in venules of Peyer’s patch from untreated control mice at 100% and expressed flux fractions of mAb-treated controls and untreated and mAb-treated Slc35c1−/− mice relative to control. Individual leukocyte rolling velocities were measured from video recordings by analyzing 5–15 leukocytes/venule and measuring frame-to-frame displacement of rolling leukocytes.

The Sigma Stat 2.0 software package (SPSS Science, Chicago, IL) was used for statistical analysis. Vessel diameters, leukocyte rolling flux fractions, leukocyte rolling velocities, and shear rates between groups and treatments were compared with one-way analysis of variance on ranks (Kruskal–Wallis) with a multiple pairwise comparison test (Dunn’s test). Leukocyte counts and differentials were compared with Student’s t test or by the Wilcoxon rank sum test as appropriate. Statistical significance was set at p < 0.05, indicated by an asterisk.

Preparation of [2-3H]Mannose-radiolabeled Glycopeptides—MEF, hepatocytes, and mesangial cells in 6-cm dishes were labeled with 125 µCi of [2-3H]mannose for 20 h in the presence or absence of fucose. Glycoproteins were extracted sequentially as described (41). The resulting glycoprotein pellet was dissolved in TBS and subjected to digestion with 400 µg/ml Pronase (Roche Applied Science) for 10 h at 50 °C.

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**Lens culinaris Affinity Chromatography—**Radiolabeled glycopeptides were subjected to lectin affinity chromatography on a column containing agarose-bound *Lens culinaris* lectin (Sigma) as described (42). After binding of the samples to the column for 1 h at 4 °C, unbound material was eluted with 20 ml of Tris-buffered saline. Elution was carried out with 100 mM methyl-α-D-mannopyranoside (Sigma), followed by collection of 20 further 1-ml fractions.

**RESULTS**

Generation of Slc35c1-deficient Mice—The murine protein Slc35c1 (NCBI accession number NP_997597) shows 87% identity (as calculated by BLAST at www.ncbi.nlm.nih.gov/blast/) with the human ortholog SLC35C1 (NCBI accession no. AAK51705). Orthologs from other species show identities of 95% (*Rattus norvegicus*, accession number XP_230292), 60% (*Caenorhabditis elegans*, accession number AAK50396), and 52% (*Drosophila melanogaster*, accession number Q9VHT4) with Slc35c1. Analysis of protein topology (with TMpred, available on the World Wide Web at www.ch.embnet.org/software/TMPRED_form.html) predicts 10 transmembrane domains with both termini exposed to the cytosol (as in the human ortholog, the highly hydrophilic transmembrane domains 4 and 9 are missed by standard algorithms). The *Slc35c1* gene consists of two exons and is expressed in all organs, with the strongest signal in liver (data not shown). Translation of the cDNA results in a protein with a molecular mass of 40 kDa, corresponding to results from Puglielli and Hirschberg (8), who have purified and characterized the rat *Slc35c1* ortholog from liver. This also had a molecular mass of 39 kDa and was present as a homodimer in the medial Golgi compartment.

For homologous recombination-mediated inactivation of the murine *Slc35c1* locus in ES cells, a targeting vector corresponding to 11.3 kb of a genomic region, including both exons, was prepared. The open reading frame was interrupted by insertion of a neomycin resistance cassette into an early region of the first exon (Fig. 2A) downstream of the sequence region encoding the third transmembrane domain. Additionally, by this cloning strategy, a stop codon inside the cassette was generated.

Two independent positive ES cell clones, identified by Southern blot analysis with an external probe and by PCR analysis followed by sequencing (data not shown), were used for microinjection into C57BL/6 blastocysts. Chimeric males crossed with wild type C57BL/6 females gave rise to heterozygous progeny (*Slc35c1+/−*). Genotype analysis was performed by PCR with two alternative reverse primers (Fig. 2B). Mice with homozygosity for the mutated allele (*Slc35c1−/−*) used in these experiments were generated by heterozygous intercrossing (after ascertainment of the same phenotype concerning physical and biochemical aspects, progeny from one of the two injected ES cell clones was used for the reported experiments). In RNA extracts from several tissues of *Slc35c1−/−* animals, no transcript was detectable (data not shown).

Loss of GDP-fucose Import Activity and Fucosylated Glycans—Import activity of nucleotide sugars into the Golgi apparatus was assayed in Golgi enriched vesicles prepared from liver homogenates. The import of GDP-[14C]fucose into vesicles
derived from livers of Slc35c1-deficient mice was reduced to 5.7% of controls. To account for variations in Golgi enrichment and integrity of vesicles in each preparation, the import of UDP-[³H]galactose was determined in parallel and used for normalization of GDP-fucose import activity by that of an unrelated Golgi nucleotide sugar transporter. The GDP-fucose/UDP-galactose import activity ratio in Slc35c1−/− preparations was 3.3% of controls (Fig. 3A). In heterozygous mice, the ratio was comparable with controls (Fig. 3A).

For a qualitative examination of a fucosylation deficiency resulting from the Slc35c1 knock-out, we prepared MEF. The fucosylation of glycoconjugates was analyzed with a cytochemical assay using biotinylated AAL. AAL is specific for α-1,2-, α-1,3-, and α-1,6-linked fucosyl residues. After reaction with streptavidin-coupled alkaline phosphatase control cells showed a distinct staining (Fig. 3B), whereas no fucosylated glycans were detectable in Slc35c1−/− MEF (Fig. 3B′).

Next, we investigated the fucose depletion in tissue sections of organs, including spleen, thymus, lymph nodes, lung, heart and skeletal muscle, brain, liver, kidney, uterus, testes, stomach, and intestine with biotinylated AAL and streptavidin-fluorescein isothiocyanate. All control organs showed pronounced and structure-specific staining patterns (e.g. in

FIGURE 2. Targeted disruption of the Slc35c1 locus and genotyping. A, structure of the wild type and mutated allele (exons 1 and 2 represented by black boxes). The targeting vector (11.3 kb) mediates the insertion of a neomycin resistance cassette (neo) into the first exon, interrupting the open reading frame. B, genotyping was performed by PCR with primers F1, R1, and R2, leading to products of 2.3 kb and/or 1.8 kb in length, according to the wild type and mutated allele, respectively.

FIGURE 3. Nucleotide sugar import activity and expression of fucosylated glycans. A, the GDP-fucose import activity was determined in Golgi-enriched vesicles from liver homogenates and normalized for UDP-galactose import activity (p < 0.005 for Slc35c1+/− versus Slc35c1−/−). B and B′, fucosylated structures in Slc35c1+/− (B) and Slc35c1−/− (B′) MEF were detected using AAL. C–E, histochemical detection of fucosylated glycans (green) with AAL in sections from different Slc35c1+/− (C–C′) and Slc35c1−/− (C′–E′) organs: peripheral lymph nodes (C and C′), cortices of cerebrum (D and D′), and cerebellum (E and E′) (nuclei stained blue with 4',6-diamidino-2-phenylindole). Bar, 100 μm.
lymph nodes, the cortex of cerebrum and cerebellum (Fig. 3, C–E), or epithelia of gastrointestinal and reproductive tracts (data not shown). In contrast, Slc35c1−/− organs showed virtually no fucosylation (Fig. 3, C–E′), whereas control lectins, such as M. amurensis lectin 1, which is specific for galactosyl-β-1,4-N-acetylglucosamine conjugates, showed normal glycosylation patterns in Slc35c1−/− organs (data not shown). Additional fucose-specific lectins, such as Lotus tetragonolobus agglutinin and Lens culinaris agglutinin, confirmed the hypofucosylation pattern seen for AAL in Slc35c1−/− organs but gave again pronounced staining in control organs (data not shown).

**Elevated Mortality Rate and Leukocytosis in Slc35c1−/− Mice—**
Heterozygous mice showed normal development and fertility. Analysis of the progeny resulting from crosses among Slc35c1−/− mice revealed a Mendelian distribution of wild type (24.8%), heterozygous (50.0%), and homozygous (25.2%) offspring, giving no evidence for prenatal lethality (Fig. 4A). Slc35c1−/− mice showed no obvious abnormalities at birth, but within the first days of life they developed profound retardation in growth and weight gain (Fig. 4B). No significant differences in weight gain were observed between wild type and heterozygous mice. At the age of 3 weeks, Slc35c1+/+ and Slc35c1−/− mice showed mean body weights of 9.8 ± 0.6 and 10.4 ± 0.3 g, respectively. In contrast, weight of Slc35c1−/− mice at this age was reduced by nearly 50% to 5.7 ± 0.3 g. No gross morphological abnormalities were observed, but one-third of the homozygous pups died during the first week of life (Fig. 4C). Thereafter, the number of surviving Slc35c1−/− mice decreased continuously, with a second distinct increase of mortality during the weaning period. The remaining mice comprised less than one-third compared with the amount of Slc35c1−/− mice at birth and presented with a ruffled coat and a bent posture. Approximately 50% of homozygous mice, male and female, were fertile. However, all pregnant female Slc35c1−/− mice aborted or had very small litters and failed to nurture their pups after birth, which excluded homozygous breeding to gain more Slc35c1−/− mice.

Since persistent leukocytosis is one of the major symptoms of CDG-IIc, peripheral blood counts were analyzed (Fig. 5A). Slc35c1−/− mice showed a prominent leukocytosis (2.6-fold increase compared with Slc35c1+/+), caused by a 5-fold increase in neutrophil counts and to a lesser extent by increases of eosinophil (3-fold), lymphocyte (2.2-fold), and monocyte (3.3-fold) counts (Fig. 5B).

**Histological Abnormalities in Lymph Nodes and Lungs of Slc35c1−/− Mice—**
Organs of Slc35c1-deficient mice were proportionally smaller as compared with age-matched controls but showed no significant abnormalities concerning cellularity and architecture in histological sections stained with hematoxylin/eosin. However, histological examination of peripheral lymph nodes from Slc35c1-deficient mice showed in contrast to Slc35c1+/+ lymph nodes (Fig. 6A) a distinct hypocellularity reminiscent of peripheral lymph nodes in α,1,3-fucosyltransferase Fut7-deficient mice (43). Primary follicles were rudimentary or even absent (Fig. 6A′).

Additionally, lungs of adult (3–6-month-old) Slc35c1−/− mice showed dilated alveoles and thin alveolar walls (Fig. 6B). Morphometric analysis of representative sections from three animals per genotype showed a 1.5-fold increase in mean linear intercepts in Slc35c1−/− mice compared with wild type littersmates (Fig. 6C).

**Fucosylation and Function of Selectin Ligands on Leukocytes—**
Since it is known from CDG-IIc patients that leukocyte rolling is severely affected due to the loss of fucose residues linked to
sialyl Lewis X, an important binding partner for selectins, we next studied fucosylation and function of these ligands.

Peripheral granulocytes were analyzed with AAL. Flow cytometry showed that Slc35c1−/− granulocytes were strongly hypofucosylated with only 1.5% of AAL staining detectable as compared with wild type granulocytes. The reduction of lectin binding was fucose-specific, since binding of the sialic acid-reactive M. amurensis lectin II was comparable with wild type granulocytes. The observation of leukocyte rolling in PP-HEV of M. amurensis lectin II was comparable with wild type levels (Fig. 7A). We expected that hypofucosylation of granulocytes leads to reduced binding to endothelial selectins. Indeed, flow cytometry analyses with soluble selectin-Fc constructs showed that binding of E- and P-selectin to Slc35c1−/− granulocytes was completely absent (Fig. 7A). These data were confirmed in static adhesion assays, in which the Slc35c1−/− granulocytes were unable to bind to immobilized E- and P-selectin-Fc constructs, respectively (Fig. 7B). We conclude that the absence of Slc35c1 causes a severe adhesion defect comparable with that seen in CDG-IIC.

**Leukocyte Rolling in HEV of Peyer’s Patches**—To analyze the effect of fucose depletion in Slc35c1−/− mice on endothelial L-selectin ligand function in vivo, we investigated leukocyte rolling in PP-HEV by means of intravital microscopy.

Leukocyte rolling was analyzed in 55 venules of seven Slc35c1−/− mice and compared with 65 venules in seven littermate control mice. Hemodynamic parameters (Fig. 8A) demonstrate no significant differences in vessel diameter, centerline velocity, and wall shear rates between Slc35c1−/− and control mice. However, there was a significant increase in systemic leukocyte counts in Slc35c1−/− mice. As reported previously, leukocyte rolling in PP-HEV is mostly dependent on L-selectin and to a lesser degree on αβ integrin and P-selectin (44). Endothelial L-selectin ligand function on HEV of secondary lymphoid organs from CDG-IIc patients has not been studied so far. The observation of leukocyte rolling in PP-HEV of Slc35c1−/− mice revealed a marked decrease in leukocyte rolling flux fraction (RFF) compared with control mice (14% versus 100%, respectively) (Fig. 8B). To investigate whether the decrease in leukocyte rolling in Slc35c1−/− mice is due to defective L-selectin ligands, we injected the L-selectin-blocking mAb MEL-14. This led to no significant changes in leukocyte rolling in Slc35c1−/− mice (RFF 23%) but caused a marked reduction in the number of rolling leukocytes in control mice (RFF 37%), suggesting that L-selectin-dependent rolling is dramatically impaired in Slc35c1−/− mice. Injection of the αβ integrin-blocking mAb PS/2 into Slc35c1−/− mice abolished rolling completely (Fig. 8B), demonstrating 1) that L-selectin-dependent rolling is absent in Slc35c1−/− mice and 2) that the residual leukocyte rolling in PP-HEV of Slc35c1−/− mice is completely dependent on αβ integrin.

Next, we analyzed leukocyte rolling velocities in Slc35c1−/− mice and control mice (Fig. 8C). In PP-HEV of control mice, average leukocyte rolling velocity was 47.0 ± 1.3 μm/s. In Slc35c1−/− mice, leukocyte rolling velocity was significantly

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**FIGURE 5. Blood leukocyte counts.** A, total and differential blood leukocyte counts (n = 12 per genotype; mean ± S.E.). B, n-fold change of total and differential blood leukocyte counts in Slc35c1−/− mice compared with respective counts in Slc35c1+/+ mice.

**FIGURE 6. Histological findings in lymph nodes and lung.** A–B′, formalin-fixed, paraffin-embedded, and hematoxylin/eosin-stained histological sections of peripheral lymph nodes and lung tissue derived from Slc35c1−/− and Slc35c1+/− mice (A and A′, B and B′, respectively). Bar, 100 μm. C, for determination of mean linear intercepts, three representative fields each from three lungs per genotype were analyzed (mean ± S.E.; Slc35c1+/+, 30.6 ± 0.9 μm; Slc35c1−/−, 46.9 ± 1.8 μm; p < 0.005).

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**TABLE**

| Cell type [μmol] | Slc35c1+/+ | Slc35c1−/− |
|-----------------|------------|------------|
| leukocytes [μmol] | 5421 ± 317 | 14206 ± 1372 |
| neutrophils [μmol] | 841 ± 169 | 4184 ± 677 |
| eosinophils [μmol] | 11 ± 8 | 33 ± 26 |
| basophils [μmol] | 4 ± 4 | 0 ± 0 |
| lymphocytes [μmol] | 4494 ± 336 | 9775 ± 1134 |
| monocytes [μmol] | 71 ± 35 | 234 ± 115 |
reduced to $32.2 \pm 1.2 \mu m/s$ ($p < 0.05$ versus control mice) and did not change after injection of L-selectin-blocking mAb MEL-14 and P-selectin-blocking mAb RB40.34 (Fig. 8C). In contrast, functional blockade of P- and L-selectin in control mice (leading to isolated $\alpha_4$ integrin-dependent leukocyte rolling) led to similar leukocyte rolling velocities ($28.3 \pm 1.1 \mu m/s$) as in untreated and anti-P- and anti-L-selectin-blocking mAb-treated $Slc35c1^{-/-}$ mice ($32.2 \pm 1.2$ and $33.2 \pm 1.4 \mu m/s$, respectively) (Fig. 8C), further suggesting that leukocyte rolling observed in $Slc35c1^{-/-}$ mice is entirely dependent on $\alpha_4$ integrin.

The Fucosylation Defect in Primary Mammalian Cells Deficient for $Slc35c1$ Can Be Corrected by Fucose Supplementation—Supplementation of fucose to culture medium of skin fibroblasts derived from CDG-IIc patients led to restoration of fucosylation (10). Moreover, therapeutic use of a high fucose diet led to a significant improvement in selectin ligand function and restored normal white blood cell counts in some of the CDG-IIc patients (14, 15). However, it has not been shown if normalization of fucose incorporation by treatment with exogenous l-fucose is dependent on a residual activity of SLC35C1 or whether it is mediated by an alternative GDP-fucose transport system.
To investigate the mechanism leading to improved Golgi-located fucosylation by fucose treatment, we performed lectin affinity chromatography with radiolabeled glycopeptides derived from MEF, hepatocytes, and mesangial cells of \(\text{Slc35c1}\)/H11002 mice. The cells were grown in culture medium in the presence or absence of \(\text{L-fucose}\). After metabolic labeling with \([2-3\text{H}]\text{mannose}\), glycoproteins were extracted, and glycopeptides were prepared by Pronase digestion and subjected to lectin affinity chromatography with agarose-bound \(\text{L. culinaris}\) agglutinin, which is characterized by an affinity to mannose-rich glycans containing \(\alpha-1,6\)-linked core fucose. As shown in the elution profiles (Fig. 9, B–D), small amounts of fucosylated glycans were detected in all cell types derived from \(\text{Slc35c1}\)/H11002 mice. By supplementation of \(\text{L-fucose}\) in increasing concentrations, the ratio of specifically bound glycopeptides compared with the total load of radioactivity increased in a dose-dependent manner from 0.8% in untreated \(\text{Slc35c1}\)/H11002 MEF to 6.3% in \(\text{Slc35c1}\)/H11002 MEF treated with 10 mM fucose (Fig. 9B), whereas it was 8.4% in wild type littermate MEF (Fig. 9A). In \(\text{Slc35c1}\)/H11002 hepatocytes (Fig. 9C) and mesangial cells (Fig. 9D), the total amount of specifically bound glycopeptides was less than in MEF, but correction with 10 mM fucose to a nearly normal level in \(\text{Slc35c1}\)/H11002 cells was also observed. Fluorescence-activated cell sorting analysis of AAL-stained splenocytes led to similar results. Fig. 9E shows the strongly reduced staining of \(\text{Slc35c1}\)-deficient splenocytes in comparison with spleen cells derived from littermate control mice. In the case where \(\text{Slc35c1}\)/H11002 splenocytes were cultured in the presence of 10 mM \(\text{L-fucose}\), a partial restoration of cellular fucosylation was observed.

Because no conceivable residual transport activity of \(\text{Slc35c1}\) is existent in \(\text{Slc35c1}\)/H11002 cells, our results indicate that restoration of protein fucosylation by supplementation with exogenous \(\text{L-fucose}\) is independent of \(\text{Slc35c1}\). Moreover, our data suggest that the therapeutic effect of \(\text{L-fucose}\) supplementation in CDG-IIc patients is dependent on an alternative GDP-fucose transport mechanism.

**DISCUSSION**

Binding of fucose residues to glycoconjugates plays a crucial role in many intra- and intercellular recognition processes and interactions as well as in signal transduction pathways (45). In recent years, several mouse models for different enzymes involved in glycoprotein fucosylation have been generated and characterized. Moreover, the importance of glycoprotein fucosylation has lately been underlined by the identification of the human disease CDG-IIc. Despite the fact that the molecular defect of CDG-IIc has been found, the general relevance of the loss of fucosylation in CDG-IIc patients remains mainly elusive. This is also true for the molecular mechanisms enabling the

**FIGURE 9.** The fucosylation defect can be corrected with exogenous fucose in different primary cell types. A, primary cells were labeled with \([2-\text{H}]\text{mannose}\) in the presence (different concentrations in MEF, 10 mM in hepatocytes and mesangial cells) or absence of fucose. Glycopeptides isolated from cell lysates were assayed for binding to fucose-specific \(\text{L. culinaris}\) agglutinin by affinity chromatography. A and B, MEF (\(\text{Slc35c1}\)/H11001 and \(\text{Slc35c1}\)/H11001, respectively); C, hepatocytes. D, mesangial cells. E, primary splenocytes derived from \(\text{Slc35c1}\)/H11002 mice were cultured without (thin line) or with (thick line) 10 mM fucose for 24 h before they were analyzed for AAL binding by flow cytometry. Splenocytes from \(\text{Slc35c1}\)/H11002 mice were used for comparison (dotted line). Negative control (shaded graph) was secondary streptavidin conjugate only.
successful treatment of patient-derived fibroblasts as well as CDG-IIc patients by fucose supplementation (10, 14, 15). To improve the understanding of the pathogenesis of CDG-IIc and get new insights into the functional role of fucosylation in mammals, we generated mice with a null mutation of the murine ortholog of the human SLC35C1 gene.

According to results from the two most recently described CDG-IIc patients of Brazilian and Pakistani origin with severely truncated transporters (15, 46), the resulting gene product of our Slc35c1−/− mice should be mislocalized and inactive even if it would be translated. This is in agreement with the finding that the GDP-fucose import activity into Golgi-enriched membrane preparations from mouse livers was severely reduced in Slc35c1−/− mice. In contrast, heterozygous mice exhibit an unchanged import activity, corresponding to the activity obtained from parents of a CDG-IIc patient (10), suggesting that one intact Slc35c1 allele is sufficient for a normal GDP-fucose transport activity.

Slc35c1-deficient mice showed a high postnatal mortality rate and suffered, like CDG-IIc patients, from a generalized growth deficiency. Further parallels include severe leukocytosis, which is mostly caused by elevated neutrophil counts and to a lesser degree by an increase in eosinophil, lymphocyte, and monocyte counts. We also identified the absence of P- and E-selectin ligand function on granulocytes. This is caused by defective posttranslational fucosylation of selectin ligands, leading to the loss of the sialyl Lewis X epitope on selectin ligands. Several studies have identified sialyl Lewis X as a crucial E-selectin ligand function on granulocytes. This is caused by defective posttranslational fucosylation of selectin ligands, leading to the loss of the sialyl Lewis X epitope on selectin ligands. Selectins and selectin ligands are required for the rolling of leukocytes on endothelial cells of postcapillary venules as a prerequisite for their extravasation through the endothelium at sites of inflammation (47). Selectins are also required for the recirculation of lymphocytes into lymph nodes (48). Corresponding to these data and to results from mice deficient for Fut7 (43), lymph nodes in Slc35c1-deficient mice show a distinct hypocellularity. Furthermore, we observed the absence of selectin-dependent leukocyte rolling in PP-HEV from Slc35c1−/− mice. This novel observation demonstrates for the first time that high endothelial L-selectin ligand function in PP-HEV is completely dependent on Slc35c1 activity. Moreover, the results indicate that the remaining rolling completely depends on fucosylation-independent α4 integrin-mediated mechanisms.

Comprehensive studies with lectins binding to terminal fucose residues on N- and O-glycans performed on histological sections of different organs from control mice showed a high expression of these epitopes on epithelial structures; in mucus layers of the respiratory, gastrointestinal, and reproductive tract; and in cerebral tissue. In contrast to other glycosylated structures, the fucosylation in Slc35c1−/− tissues is virtually lost. Surprisingly, we found no abnormalities in architecture and cellular organization in most organs by histological analysis using light microscopy, indicating an involvement of the described fucosylated structures in organ function rather than structure. Despite the fact that the CDG-IIc index patient developed a frontal cerebral atrophy at 6 months of age (13), no comparable abnormalities were found in Slc35c1−/− mice.

Nevertheless, histological analysis of lung tissue derived from Slc35c1-deficient mice exhibited alveolar dilatation, a finding that has also been described for mice deficient for Fut8 and had been attributed to a defect in transforming growth factor-β signaling as a possible reason for the emphysema-like changes in lung tissue (17). Whether this might also apply for Slc35c1-deficient mice and might moreover give an explanation for the growth defect in Slc35c1−/− mice as well as in CDG-IIc patients will be the aim of further studies.

Lectin binding studies in cultured Slc35c1−/− primary cells from different organs revealed a severe hypofucosylation of glycoconjugates. Therefore, these cells enable investigations on the mechanism of fucose treatment in a cell system with complete loss of Slc35c1 activity. Despite the fact that no other GDP-fucose transporter has been described so far, supplementation of L-fucose in high amounts to the culture medium of Slc35c1−/− primary cells led to partial normalization in the fucosylation state of glycoconjugates. The import of GDP-fucose into the Golgi of Slc35c1−/− cells might be explained by an increase in the cytosolic amount of GDP-fucose under dietary conditions which is taken up into the Golgi by an alternative transport mechanism (e.g. by another nucleotide sugar transporter with low affinity for GDP-fucose). Nevertheless, the affinity and capacity of the putative transporter for GDP-fucose must be sufficient to explain the effect seen by dietary treatment of CDG-IIc patients with L-fucose, where serum concentrations of up to 0.36 mM were reached (14). Whatever may be the nature of the mechanism, the Slc35c1−/− mouse model provides for the first time direct evidence for an alternative, Slc35c1-independent transport of GDP-fucose into the Golgi.

Taken together, our findings unequivocally demonstrate the importance of the Golgi GDP-fucose transporter for the fucosylation of glycoconjugates and also underscore the functional relevance of fucosylation for a whole variety of different processes, including growth development, leukocyte rolling during leukocyte recruitment and in lymphocyte trafficking, the homeostatic regulation of leukocyte production, and lung development. The newly developed mouse model of hypofucosylation presented here provides an interesting tool to uncover additional processes where fucosylation is intimately linked to protein function.

**REFERENCES**

1. Spiro, R. G. (2002) Glycobiochemistry 12, 43R–56R
2. Sears, P., and Wong, C. H. (1998) Cell. Mol. Life. Sci. 54, 223–252
3. Varki, A. (1993) Glycobiochemistry 3, 97–130
4. Helenius, A., and Aebi, M. (2001) Science 291, 2364–2369
5. Staudacher, E., Allmann, F., Wilson, I. B., and Marz, L. (1999) Biochim. Biophys. Acta 1473, 216–226
6. Moloney, D. I., Panin, V. M., Johnston, S. H., Chen, J., Shao, L., Wilson, R., Wang, Y., Stanley, P., Irvine, K. D., Haltiwanger, R. S., and Vogt, T. F. (2000) Nature 406, 369–375
7. Martinez-Duncker, I., Mollicone, R., Candelier, J. J., Breton, C., and Oriol, R. (2003) Glycobiochemistry 13, 1C–5C
8. Puglielli, L., and Hirscheck, C. B. (1999) J. Biol. Chem. 274, 35596–35600
9. Körner, C., Linnebank, M., Koch, H. G., Harms, E., von Figura, K., and Marquardt, T. (1999) J. Leukocyte Biol. 66, 95–98
10. Lübke, T., Marquardt, T., von Figura, K., and Körner, C. (1999) J. Biol. Chem. 274, 25986–25989
11. Lübke, T., Marquardt, T., Etzioni, A., Hartmann, E., von Figura, K., and Körner, C. (2001) Nat. Genet. 28, 73–76
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12. Lührk, K., Wild, M. K., Eckhardt, M., Gerardy-Schahn, R., and Vestweber, D. (2001) Nat. Genet. 28, 69–72
13. Marquardt, T., Brune, T., Lührk, K., Zimmer, K. P., Körner, C., Fabritz, L., van der Werff, N., Vormoor, J., Freeze, H. H., Louwen, F., Biermann, B., Harms, E., von Figura, K., Vestweber, D., and Koch, H. G. (1999) J. Pediatri. 134, 681–688
14. Marquardt, T., Lührk, K., Srikrishna, G., Freeze, H. H., Harms, E., and Vestweber, D. (1999) Blood 94, 3976–3985
15. Hidalgo, A., Ma, S., Peired, A. J., Weiss, L. A., Cunningham-Rundles, C., and Marks, R. M., Misra, A. K., Hindsgaul, O., von Andrian, U. H., and Lowe, J. B. (2002) Mol. Cell. Biol. 21, 8336–8345
16. Dominos, E. R., Zhang, L., Gillespie, P. J., Saunders, T. L., and Lowe, J. B. (2001) Mol. Cell. Biol. 21, 1705–1712
17. Kudo, T., Kaneko, M., Iwasaki, H., Togayachi, A., Nishihara, S., Abe, K., and Narimatsu, H. (2004) Mol. Cell. Biol. 24, 4221–4228
18. Weninger, W., Uffmann, L. H., Cheng, G., Souchkova, N., Quackenbush, E. J., Lowe, J. B., and von Andrian, U. H. (2000) Immunity 12, 665–676
19. Homeister, J. W., Thall, A. D., Petryniak, B., Maly, P., Rogers, C. E., Smith, P. L., Kelly, R. J., Gersten, K. M., Askari, S. W., Cheng, G., Smithson, G., Marks, R. M., Misra, A. K., Hindsgaul, O., von Andrian, U. H., and Lowe, J. B. (2001) Immunity 15, 115–126
20. Wang, Y., Zhou, W., Sakai, M., Noda, K., Li, W., Mizuno-Horikawa, Y., Nakano, M., Asahi, M., Takahashi, M., Uozumi, N., Ibara, S., Lee, S. H., Ikeda, Y., Yamaguchi, Y., Aze, Y., Tomiyama, Y., Fujii, J., Suzuki, K., Kondo, A., Shaprio, S. D., Lopez-Otin, C., Kuzaki, T., Okabe, M., Honke, K., and Taniguchi, H. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 15791–15796
21. Luo, Y., and Haltiwanger, R. S. (2005) J. Biol. Chem. 280, 11289–11294
22. Shi, S., and Stanley, P. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 5234–5239
23. Smith, M. L., Long, D. S., Damiano, E. R., and Ley, K. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10060–10065
24. Smith, M. L., Long, D. S., Damiano, E. R., and Ley, K. (2003) Biophys. J. 85, 637–645
25. Lipowsky, H. H., and Zweifach, B. W. (1978) Microvasc. Res. 15, 93–101
26. Long, D. S., Smith, M. L., Pries, A. R., Ley, K., and Damiano, E. R. (2000) Nat. Genet. 25, 1705–1712
27. Braendli, A. W., Hansson, G. C., Rodriguez-Boulan, E., and Simons, K. (1988) J. Biol. Chem. 263, 16283–16290
28. Meredith, M. J. (1988) Cell. Biol. Toxicol. 4, 405–425
29. Krakower, C. A., and Greenspon, S. A. (1951) Am. Med. Assoc. Arch. Pathol. 51, 629–639
30. Homeister, J. W., Thall, A. D., Petryniak, B., Rogers, C. E., Smith, P. L., Marks, R. M., Misra, A. K., Hindsgaul, O., von Andrian, U. H., and Lowe, J. B. (2002) Mol. Cell. Biol. 21, 8336–8345
31. Gotsch, U., Barges, E., Bosse, R., Boggemeyer, E., Simon, M., Mossman, H., and Vestweber, D. (1997) J. Cell Biol. 121, 655–664
32. Bosse, R., and Vestweber, D. (1994) Eur. J. Immunol. 24, 3019–3024
33. Baragzie, R. F., Jutila, M. A., and Butcher, E. C. (1995) Immunity 3, 99–108
34. Wagner, N., Löhrer, J., Kunkel, E. J., Ley, K., Leung, E., Krisssang, G., Rajewsksy, K., and Müller, W. (1996) Nature 382, 366–370
35. Klyscz, T., Junger, M., Jung, F., and Zeintbl, H. (1997) Biomed. Tech. (Berl.) 42, 168–175
36. Lipowsky, H. H., and Zweifach, B. W. (1978) Microvasc. Res. 15, 93–101
37. Long, D. S., Smith, M. L., Pries, A. R., Ley, K., and Damiano, E. R. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10060–10065
38. Smith, M. L., Long, D. S., Damiano, E. R., and Ley, K. (2003) Biophys. J. 85, 637–645
39. Ley, K., Pries, A. R., and Gaehltgens, P. (1988) Pfluegers Arch. 412, 93–100
40. Ley, K., Meyer, J. U., Intaglietta, M., and Artlows, K.-E. (1989) Am. J. Physiol. 256, H85–H93
41. Körner, C., Lehl, L., and von Figura, K. (1998) Glycoconjug. J. 15, 499–505
42. Cummings, R. D. (1994) Methods Enzymol. 230, 66–86
43. Maly, P., Thall, A. D., Petryniak, B., Rogers, C. E., Smith, P. L., Marks, R. M., Kelly, R. J., Gersten, K. M., Cheng, G., Saunders, T. L., Camper, S. A., Camphausen, R. T., Sullivan, F. X., Isogai, Y., Hindsgaul, O., von Andrian, U. H., and Lowe, J. B. (1996) Cell 86, 643–653
44. Sprando, M., Forlow, S. B., Thatte, J., Ellis, L. G., Marth, J. D., and Ley, K. (2001) J. Immunol. 167, 2268–2274
45. Haltiwanger, R. S., and Lowe, J. B. (2004) Annu. Rev. Biochem. 73, 491–537
46. Helmus, Y., Denecke, J., Yakubeni, S., Robinson, P., Lührk, K., Watson, D. L., McGrogan, P. J., Vestweber, D., Marquardt, T., and Wild, M. K. (2006) Blood 107, 3959–3966
47. Kansas, S. (1996) Blood 88, 3259–3287
48. Lowe, J. B. (1997) Kidney Int. 51, 1418–1426