O Mannosylation of α -Dystroglycan Is Essential for Lymphocytic Choriomeningitis Virus Receptor Function

Mauro Imperiali,¹ Claudio Thoma,¹ Ernesto Pavoni,² Andrea Brancaccio,² Nico Callewaert,¹
and Annette Oxenius^{1*}

*Institute for Microbiology, ETH Zurich, 8093 Zürich, Switzerland,¹ and CNR, Istituto di Chimica del
Riconoscimento Molecolare c/o Istituto di Biochimica e Biochimica Clinica,
Università Cattolica del Sacro Cuore, 00168 Rome, Italy²*

Received 25 April 2005/Accepted 23 August 2005

α -Dystroglycan (α -DG) was identified as a common receptor for lymphocytic choriomeningitis virus (LCMV) and several other arenaviruses including the human pathogenic Lassa fever virus. Initial work postulated that interactions between arenavirus glycoproteins and α -DG are based on protein-protein interactions. We found, however, that susceptibility toward LCMV infection differed in various cell lines despite them expressing comparable levels of DG, suggesting that posttranslational modifications of α -DG would be involved in viral receptor function. Here, we demonstrate that glycosylation of α -DG, and in particular, O mannosylation, which is a rare type of O-linked glycosylation in mammals, is essential for LCMV receptor function. Cells that are defective in components of the O-mannosylation pathway showed strikingly reduced LCMV infectibility. As defective O mannosylation is associated with severe clinical symptoms in mammals such as congenital muscular dystrophies, it is likely that LCMV and potentially other arenaviruses may have selected this conserved and crucial posttranslational modification as the primary target structure for cell entry and infection.

Lymphocytic choriomeningitis virus (LCMV) is a prototypic member of the arenavirus family which includes important human pathogens such as Lassa fever virus. Arenaviruses are enveloped, single-stranded RNA viruses with a bisegmented ambisense genome (9, 43, 44, 48). The S RNA encodes two structural proteins, the nucleoprotein (NP) and the glycoprotein (GP) precursor protein GPC (43, 44), which is cleaved posttranslationally into GP1 and GP2. The viral surface protein GP1 is implicated for receptor binding, and it is the target for virus-neutralizing antibodies (7, 39). GP2 contains a transmembrane region and thus anchors the GP1 in the viral envelope (8).

α -Dystroglycan (α -DG) was identified as a cellular receptor for LCMV and several other Old World arenaviruses as well as clade C New World arenaviruses (11). Different LCMV strains were shown to vary in their affinity toward α -DG and could be largely grouped into high- and low-affinity binders (49). High-affinity binders (LCMV clone 13, WE54, and Traub) were dependent on cellular α -DG for infection and include viral strains that lead to persistent infection in vivo by causing exhaustion of CD8⁺ T-cell responses (46). In contrast, low-affinity binders (LCMV Armstrong, E350) were only partially dependent on cellular α -DG expression and do not establish persistent infection in vivo (32, 49). More recently, it was shown that alternative receptors (proteins or protein-bound entities) can be used for entry and infection, in particular by the low-affinity α -DG binding strains (33).

DG is ubiquitously expressed and is an essential component

of the dystrophin-glycoprotein complex, where it constitutes a link between the cytoskeleton and the extracellular matrix (ECM) (21, 22, 25). In vertebrates, DG is encoded by a single gene (*DAG1*) comprising two exons which code for a single polypeptide chain that is posttranslationally processed into the extracellular α -DG and the transmembrane-spanning β -DG (28). DG is heavily glycosylated, particularly in the α -DG subunit (28, 29). α -DG isolated from different tissues shows marked heterogeneity in molecular mass due to varying degrees of glycosylation (17, 20, 21, 24). α -DG has a dumbbell shape in which the N- and C-terminal globular domains are connected by a central rod-like domain containing a highly glycosylated mucin-like region rich in prolines, serines, and threonines (6, 54). This mucin-like region shows extensive O-linked glycosylation, and about 50% of the O-linked glycans are O-mannosyl-linked carbohydrates, a rare type of mammalian glycosylation (12) which was previously thought to be restricted to yeast (18). In fact, to date, α -DG is the only mammalian protein that has been shown to contain O-mannosyl glycans (12). In O mannosylation, a mannose is added to the Ser/Thr residue, followed by the additions of N-acetylglucosamine, galactose, and sialic acid, which are catalyzed by a series of specific glycosyltransferases (18, 38, 52). This core chain can be further elongated, giving rise to complex sugar structures.

The LCMV viral binding site on α -DG was mapped between amino acids 169 and 408 containing the C-terminal part of the N-terminal globular domain and the N-terminal part of the mucin-like region (32). The viral binding site overlaps, at least partially, with the binding site of laminin-1, one of the natural ECM ligands of α -DG (32). The interaction between laminin-1 and α -DG is dependent on divalent cations, typical for lectin-like binding (32). In contrast, LCMV binding was not depen-

* Corresponding author. Mailing address: ETH-Hönggerberg HCI 4 G401, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland. Phone: 41 1 632 3317. Fax: 41 1 632 10 98. E-mail: oxenius@micro.biol.ethz.ch.

dent on divalent cations and could not be blocked by heparin or sialic acid or by neuraminidase treatment. Thus, the binding mechanism between LCMV GP and α -DG was proposed to consist of protein-protein interactions (4, 32).

Here, we show that posttranslational modifications of α -DG are actually required for LCMV receptor function. Specifically, O-mannosylation of α -DG plays an important role for LCMV receptor function since cells harboring genetic defects in the O-mannosylation pathway showed strikingly reduced infectibility by LCMV compared to control cells despite similar levels of DG protein expression. The fact that defective O-mannosylation is associated with severe clinical symptoms in mammals (congenital muscular dystrophies in humans, lethality in mice) (51, 53) suggests that LCMV and likely other arenaviruses have selected this conserved and crucial posttranslational modification as the primary target structure for cell entry and infection.

MATERIALS AND METHODS

Viruses and cell lines. The LCMV isolates Armstrong, Clone 13, Docile, and WE were provided by R. M. Zinkernagel (University Hospital, Zurich, Switzerland) and were propagated on BHK-21 (Armstrong and Clone 13), MDCK (Docile), or L929 fibroblast (WE) cells at a low multiplicity of infection.

The cell lines used in this study were MC57 and L929 (mouse fibroblasts), VE8 and 5A1 (CD4⁺ T-cell hybridomas), RMA and EL4 (thymomas), HEK 293 (human epithelial cells), Jurkat (T lymphoblasts), and THP-1 (monocytes). Primary skin fibroblasts were from muscle-eye-brain (MEB) patients (homozygous for the 1538 + 1G→A mutation at the splice donor site of intron 17 [15]) and from healthy controls. The 1538 + 1G→A mutation was previously reported to result in a loss of function of protein O-linked mannose β 1,2-*N*-acetylglucosaminyltransferase 1 (POMGNT1) (55).

Wild-type and mutant CHO cell lines Lec 15.1, Lec 15.2, and Lec 35.1 were generously provided by M. A. Lehrman (University of Texas Southwestern Medical Center, Dallas) and by S. S. Krag (Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland).

Antibodies. The following antibodies and antisera were used in this study: anti- α -dystroglycan (clone VIA4-1; Upstate, Luzern, Switzerland), anti- β -dystroglycan (clone 43DAG1/8D5; Novocastra, Newcastle upon Tyne, United Kingdom), anti-actin (Sigma-Aldrich, Buchs, Switzerland), mouse myeloma immunoglobulin IgG2a (Zymed, Basel, Switzerland), fluorescein isothiocyanate (FITC)-conjugated anti-LCMV NP (VL-4 [3]), phycoerythrin (PE)-conjugated donkey anti-mouse IgG (Milian Analytica SA, La Roche, Switzerland), horseradish peroxidase (HRPO)-conjugated goat anti-mouse IgG (Bio-Rad, Reinach, Switzerland), and HRPO-conjugated goat anti-rabbit IgG (Milian Analytica SA, La Roche, Switzerland).

Flow cytometric analysis of cell surface or intracellular protein expression. For cell surface staining, adherent cells were harvested using phosphate-buffered saline (PBS)–5 mM EDTA, stained with VIA4-1 for 20 min at 4°C, washed, and stained with PE-conjugated goat anti-mouse secondary antibody for 20 min at 4°C.

For intracellular staining, cells were permeabilized for 10 min at room temperature using 500 μ l of FIX/perme solution (FACSlyse; BD Biosciences, Allschwil, Switzerland) diluted to a 2 $\times$ concentration with H₂O and 0.05% Tween 20 (Sigma-Aldrich, Buchs, Switzerland). Cells were washed and incubated with FITC-labeled anti-LCMV NP-specific VL4 antibody for 20 min at 4°C, washed, and fixed in PBS–1% paraformaldehyde.

Flow cytometric analysis was performed on a FACSCalibur flow cytometer (BD Biosciences, Allschwil, Switzerland) with CELLQuest software (BD Biosciences). List mode data were analyzed using Flowjo software (Treestar, San Carlos, California).

Immunofluorescence. MEB or human control fibroblasts (1×10^5) were grown on glass coverslips and infected with LCMV WE (multiplicity of infection [MOI] = 0.1) in a 24-well plate for 30 min at 37°C. Cells were washed and incubated for 2 days at 37°C in RPMI 1640–10% fetal calf serum (FCS). Fibroblasts were washed with PBS and fixed using 4% formalin in PBS (15 min), followed by an incubation with 0.1% Triton X-100 (Sigma-Aldrich, Buchs, Switzerland) for 20 min. Blocking was done by incubation in PBS–10% FCS for 1 h at room temperature, followed by an incubation with FITC-conjugated VL4 for

30 min at room temperature. Cells were washed and analyzed by fluorescence microscopy (Axiovert 200 microscope; Zeiss AG, Feldbach, Switzerland).

Membrane protein extraction and Western blotting. The indicated cells (10^7) were harvested with PBS–5 mM EDTA. Cell membranes were solubilized in 20 mM Tris (pH 7.4)–0.15 M NaCl–1.5% *N*-octyl- β -glucopyranoside–0.5 mM phenylmethylsulfonyl fluoride–1 $\times$ complete protease inhibitor cocktail (Roche Diagnostic, Basel, Switzerland) for 60 min on ice. The sample was spun for 15 min at 2,500 $\times$ g, and the supernatant was centrifuged again for 60 min at 100,000 $\times$ g at 4°C. The clear sample was dialyzed overnight against PBS–1 mM dithiothreitol (Sigma-Aldrich, Buchs, Switzerland).

For Western blot analysis, proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 12% in the case of β -DG or actin and 6% in case of α -DG), followed by blotting onto a nitrocellulose membrane. The membrane was blocked with 3% milk powder in Tris-buffered saline (TBS) overnight, incubated with the primary monoclonal antibody (MAb; diluted 1:50 for anti- β -DG, 1:100 for anti-actin, or 1:300 for anti- α -DG) in TBS–0.1% Tween 20 (T-TBS)–3% milk powder for 1 h at room temperature. The membrane was washed several times in T-TBS and incubated for 30 min at room temperature in T-TBS–3% milk powder, followed by incubation for 1 h with HRPO-conjugated goat anti-mouse IgG (Bio-Rad, Reinach, Switzerland) diluted 1:1,000 (α - and β -DG Western blots) or HRPO-conjugated goat anti-rabbit IgG (Milian Analytica SA, La Roche, Switzerland) diluted 1:4,000 (actin Western blot) in the same buffer. After several washes in T-TBS and 30 min in T-TBS–3% milk powder, the membranes were incubated with the detection solution of the luminol-based enhanced chemiluminescence (ECL) system (Amersham, Otelfingen, Switzerland). Fluorography was carried out at room temperature by exposing the membrane sheets to Kodak films (X-omat Blue XB-1; PerkinElmer, Schwerzenbach, Switzerland).

Cell surface protein biotinylation and immunoprecipitation of the α / β -DG complex. Cell surface protein biotinylation and immunoprecipitation was described elsewhere (40). Briefly, 1.2×10^7 cells were incubated in PBS, pH 8, containing 1 mg/ml EZ-linked *N*-hydroxysulfosuccinimide (NHS)-long chain (LC)-biotin (Pierce, Lausanne, Switzerland) for 30 min at room temperature. Cells were resuspended in 400 μ l lysis buffer (1.5% Triton X-100, 140 mM NaCl, 10 mM Tris [pH 7.4], 1 $\times$ complete EDTA-free protease inhibitor cocktail [Roche Diagnostic, Basel, Switzerland]), vortexed, and incubated on ice for 10 min. After centrifugation, the supernatant was preincubated for 1 h at 4°C with 50 μ l protein A/G agarose beads (Lab Force, Nunningen, Switzerland). After bead removal, the cleared lysate was preabsorbed with 10 μ g mouse IgG2a isotype control (Zymed, Basel, Switzerland) and precipitated with protein A/G agarose beads. After removal of the beads, the samples were incubated with 60 μ l anti- β -dystroglycan MAb (clone 43DAG1/8D5; Novocastra, Newcastle upon Tyne, United Kingdom) corresponding to 1.3 μ g IgG2a for 3 h at 4°C, followed by an overnight incubation with protein A/G agarose beads. The samples (immunoprecipitations with isotype control or anti- β -dystroglycan MAb) were washed three times in T-TBS and once in T-TBS–325 mM NaCl and boiled in loading buffer for subsequent electrophoresis using an 8% polyacrylamide gel. Transfer onto a polyvinylidene difluoride (PVDF) membrane was performed overnight, followed by blocking in T-TBS–5% milk powder and incubation with streptavidin-HRPO (Amersham, Otelfingen, Switzerland) diluted 1:5,000 in blocking buffer for 1 h at room temperature. After several washes in T-TBS, the membrane was developed with the enhanced luminol-based ECL system (Amersham, Otelfingen, Switzerland).

Production, purification, and transfection of α -DG-Fc fusion protein. For the amplification of nucleotides 1 through 1455 of dystroglycan, we used the following primers: 5'-GGGGAGATCTGAGGATGTCTGTGGACAAATG-3' (forward) and 5'-GGGCTAGCCCCACTCCACTGGTGGTAGTACG-3' (reverse). Five nanograms of pSPORT6-DG (<http://www.rzpd.de>) was used as template. The PCR conditions were as follows: 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 2 min and 30 s, with a final extension of 10 min at 72°C. PCR products were cloned into pCep-Fc-C (provided by Cytos AG, Schlieren, Switzerland). HEK 293 cells were transfected with 10 μ g plasmid and selected with puromycin (1 μ g/ml; Sigma-Aldrich, Buchs, Switzerland) in the absence of serum. Supernatant was collected, concentrated, and purified by protein-A-Sepharose chromatography (Amersham, Otelfingen, Switzerland). The α -DG peptide from amino acids 170 through 315 was recombinantly produced in *Escherichia coli* as previously described (5).

Purification of α -dystroglycan from tissues. α -Dystroglycan was isolated from chicken and mouse skeletal muscle or rat brain as previously described (6). Ten grams of freshly frozen tissue was homogenized in 40 ml of lysis buffer (0.2 M NaCl, 50 mM Tris-HCl [pH 7.4], 2.5 mM *N*-ethylmaleimide [NEM], 1 mM phenylmethanesulfonyl fluoride [PMSF], 1 μ g/ml each of leupeptin and benzamide). After centrifugation at 15,000 rpm, the supernatant was filtered using a

Whatman filter paper. The solution was incubated overnight with 5 ml of DEAE-Sepharose (Amersham Pharmacia Biotech, Sweden), previously equilibrated with lysis buffer at 4°C. After extensive washing, the protein was eluted with 30 ml of the same buffer containing 0.5 M NaCl. The eluted solution was applied directly to 5 ml wheat germ lectin-Sepharose 6MB (Amersham Bioscience, Sweden) previously equilibrated with the 0.5 M NaCl containing buffer. After washing, elution was carried out with the same buffer containing 0.25 M *N*-acetylglucosamine. Upon extensive dialysis against water, the protein solution was lyophilized and a white granular powder was directly used or stored at -80°C. Lyophilized α -DG was resuspended in TBS, pH 7.4.

Infection and inhibition experiments. Cells (10^5) of the indicated type were incubated with the indicated LCMV viral strain at different MOIs for 30 min at 37°C. Cells were washed with PBS and plated in a 24-well plate in a volume of 1 ml minimal essential medium-5% FCS. Forty-eighth hours later, cell infection was assessed by flow cytometry. For the infection inhibition experiments, 1.6×10^5 or 2×10^4 PFU of the indicated LCMV strain were incubated for 20 min on ice with either the Fc-DG fusion protein, the Fc control protein, or purified α -DG in a final volume of 150 μ l. The virus-protein mixture was then incubated with MC57G cells (2×10^5 per well in a 24-well plate) for 30 min at 37°C, removed by aspiration, and replaced with fresh medium. Sixteen to twenty hours later, cell infection was assessed by flow cytometry or immunofluorescence.

Protein deglycosylation. Enzymatic CarboRelease kit (Novatec Analytical, Basel, Switzerland) was used to remove all N-linked oligosaccharides and most O-linked carbohydrates. FcDG (80 μ g) was incubated overnight at 37°C with a cocktail of glycosidases (PNGaseF, *O*-glycosidase, sialidase, β -galactosidase, and glucosaminidase) in nondenaturing conditions. Deglycosylation was assessed by SDS-PAGE and silver nitrate staining by comparing the deglycosylated protein with untreated protein control incubated overnight at 37°C. To exclude that the enzymatic activities of the glycosidase cocktail could affect the viral infection process, aliquots of viruses were incubated for 20 min on ice in the presence of these enzymes and used for infection. No interference with viral infection was observed (not shown). A control protein (fetuin) was deglycosylated under the same conditions. Deglycosylation was assessed by SDS-PAGE and silver nitrate staining.

RESULTS

Dystroglycan expression does not necessarily correlate with LCMV infectibility. To investigate whether α -DG expression correlated with the infectibility of cells by LCMV, cell surface expression of α -DG was analyzed in different cell lines by flow cytometry using an antibody which specifically recognizes a carbohydrate moiety of α -DG (VIA4-1) (21, 28) (Fig. 1A). α -DG expression was detected in fibroblasts (MC57G), in human epithelial cells (HEK 293), and in the RMA thymoma cell line. No α -DG staining was observed in CD4⁺ T-cell hybridomas (VE8, 5A1), in the EL4 thymoma, in Jurkat T-lymphoblasts, or in the monocyte cell line THP-1. Since DG glycosylation varies between tissues and cell types, it is likely that the relevant carbohydrate epitope which is recognized by the VIA4-1 antibody is present only on certain cell lines and hence does not necessarily correlate with α -DG expression.

When these cell lines were analyzed for β -DG expression using Western blot analysis (Fig. 1B), a band of 43 kDa, corresponding to the fully glycosylated β -DG molecule, was observed in all tested cell lines. Given the fact that the DG molecule is synthesized as a propeptide and subsequently cleaved in its α and β subunits (28), the presence of β -DG on Western blots suggests that α -DG is also expressed in these cells. In order to specifically test for cell surface DG expression, we performed cell surface protein biotinylation, followed by protein extraction and immunoprecipitation of the α/β -DG complex. Prior to the pull-down of the DG complex, one round of immunoprecipitation using an isotype control antibody was performed to remove unspecifically binding proteins. Western blots for biotinylated proteins revealed a prominent band cor-

responding to β -DG with an approximate molecular mass of 43 kDa. This band was absent in the samples treated with the isotype control antibody (Fig. 1C). However, cell surface biotinylation gave rise to several unspecific bands in the molecular mass range expected for α -DG, as evident in samples immunoprecipitated with isotype control antibody, which precluded an unambiguous identification of biotinylated cell surface α -DG in many cell lines (not shown). This marked discrepancy in the efficiency of cell surface biotinylation between α - and β -DG is most likely due to the fact that α -DG, in contrast to β -DG, is heavily glycosylated and hence only inefficiently modified with primary amine-reactive biotinylation reagents. However, since we could clearly demonstrate the cell surface expression of β -DG in all cell lines and since we could also demonstrate cell surface α -DG expression with VIA-4 antibody staining in some cell lines (Fig. 1A), we propose that α -DG is efficiently expressed on the cell surface of the analyzed cell lines (47).

We then infected these different cell lines with LCMV clone 13 (Cl 13) (Fig. 1D), an LCMV strain that was shown to have a high affinity for α -DG and predominantly uses α -DG as a cellular receptor (49). The percentage of infected cells was assessed by intracellular staining for the nucleoprotein of LCMV. L929 and MC57 fibroblasts as well as HEK 293 epithelial cells were readily infected by LCMV, whereas T-cell hybridomas and thymoma and monocyte cell lines were resistant to LCMV infection, except for EL4, at high multiplicities of infection. We repeated the same experiment using different viral strains that differ in their binding affinity toward the dystroglycan molecule (data not shown). The results were comparable to Cl 13 infection, with the exception of EL-4 cells, which were better infectible with the LCMV strain WE.

These results suggest that the epitope on DG recognized by the VIA4-1 antibody as well as DG protein expression per se are not sufficient for prediction of LCMV infectivity.

Inhibition of infection with purified α -DG from different tissues. The α -DG molecule has been described previously as a receptor for LCMV (11); consequently, inhibition of infection should be achieved by purified α -DG in infection inhibition experiments. We purified α -DG from chicken and mouse skeletal muscle and from rat brain. Efficient inhibition of LCMV infection was achieved with purified α -DG from skeletal muscle (at 10 nM for chicken α -DG [Fig. 2A] and at 20 nM for mouse [not shown]). In contrast, rat brain α -DG was not able to inhibit LCMV infection. Further, no infection inhibition was achieved, even at concentrations up to 8 μ M, with a bacterially produced recombinant peptide (amino acids 170 through 315) containing the essential viral binding site of DG (32) (Fig. 2A).

Next, we compared the molecular mass of purified DG from chicken skeletal muscle and rat brain (Fig. 2B). In line with previous reports, we observed marked tissue-specific differences in glycosylation: α -DG from chicken skeletal muscle migrated as a broad smear, whereas α -DG from rat brain showed a more defined band of smaller size, indicating significantly reduced levels of glycosylation. These differences in glycosylation could potentially explain their different inhibitory capacity.

Inhibition of infection with Fc-DG fusion protein. Since α -DG purification from skeletal muscle and brain was based on

FIG. 1. Dystroglycan expression does not necessarily correlate with LCMV infectivity. (A) Flow cytometric analysis of surface α -dystroglycan expression in different cells by staining with α -DG-specific antibody (VIA4-1). Dotted lines depict background staining with the secondary antibody alone. (B) Western blot analysis of β -dystroglycan expression. A total of 50 μ g of membrane protein preparations was separated by SDS-PAGE and immobilized on a nitrocellulose membrane, followed by staining for β -DG. (C) Immunoprecipitation of cell surface α/β -DG complex. Cell surface proteins were biotinylated, and the α/β -DG complex was immunoprecipitated using an anti- β -DG antibody. Biotinylated proteins were detected in a Western blot with streptavidin-HRP. Blots representing the β -DG (C) are shown. For each cell lysate, a preincubation with an isotype control antibody (iso.) was always performed before the α/β -DG complex was precipitated with the anti- β -DG antibody (β -DG). (D) Flow cytometric analysis of infection with LCMV CI13. MOIs ranging from 0.1 to 1 were tested. Infection was assessed by intracellular staining for the LCMV nucleoprotein (VL-4-FITC) after 2 days of incubation at 37°C.

lectin binding, we had to assume contaminations of other glycosylated proteins in our preparations. In order to specifically test the infection inhibition capacity of α -DG, an Fc-DG fusion protein containing the essential virus binding site of DG

(amino acids 1 through 485) (32) was produced in HEK 293 cells. As control, the Fc region of human IgG1 (human Fc) was used. Control and fusion proteins were purified with protein A-Sepharose and analyzed by SDS-PAGE (Fig. 3A). The pre-

FIG. 2. Blocking of LCMV CI 13 infection by soluble α -DG. (A) Flow cytometric analysis of infection inhibition. MC57G mouse fibroblast cells were infected with LCMV CI 13 (MOI = 0.8) in the presence of different concentrations of α -DG purified from different sources, as indicated. Sixteen hours later, intracellular staining for LCMV nucleoprotein (VL-4 FITC) was performed. (B) Immunoblot analysis of wheat germ lectin-enriched skeletal muscle (chicken) or brain (rat) glycoproteins. The differences in the α -dystroglycan staining are mainly due to tissue-specific glycosylation, resulting in a broad smear of different molecular masses.

dicted molecular mass for the reduced fusion protein was 74 kDa; however, the protein migrated as a broad smear due to extensive glycosylation. The purified human Fc migrated on an SDS-PAGE gel as a 30-kDa band, as expected.

MC57G cells which are highly susceptible to LCMV infection were infected with different LCMV strains that had been preincubated with serial dilutions of FcDG or control human Fc. The purified fusion protein neutralized LCMV in a dose-dependent manner, whereas no virus neutralization was achieved with the control human Fc (Fig. 3B). The analyzed LCMV strains showed different susceptibilities to neutralization by FcDG: CI13 and Docile were most sensitive to infection inhibition by FcDG, indicating that they exhibit the highest affinity for FcDG. Around a 0.5 μ M concentration of the fusion protein was necessary to obtain an inhibition of about 50%. For LCMV Armstrong, 50% inhibition was achieved at only 2.5 μ M. This decreased sensitivity is compatible with previous reports showing lower α -DG affinities for LCMV Armstrong than for LCMV CI13 (49). LCMV WE was the least susceptible to FcDG inhibition: less than 40% inhibition was observed at 2.5 μ M, reaching about 70% inhibition at 5 μ M.

Role of FcDG glycosylation in inhibition of LCMV infection.

To analyze the role of α -DG glycosylation in LCMV receptor function, we treated the recombinant Fc-DG fusion protein with a cocktail of glycosidases, including PNGaseF, *O*-glycosidase, sialidase, β -galactosidase, and glucosaminidase. Such treatment removes all N-linked and most of the O-linked sugars. In order to assess the extent of deglycosylation, the fusion protein was analyzed by SDS-PAGE and silver nitrate staining. A control protein (fetuin) with an expected molecular mass of 48 kDa was deglycosylated using equivalent conditions. We observed a clear reduction in molecular mass of the deglycosylated protein in comparison to the untreated FcDG (Fig. 4A). However, the treatment with the enzyme cocktail did not lead to complete deglycosylation, as indicated by the fact that the protein still migrated as a smear, albeit less pronounced and with a reduced molecular mass. We then used this partially deglycosylated Fc-DG fusion protein in LCMV infection inhibition experiments. Compared with the fully glycosylated FcDG, the partially deglycosylated protein clearly showed a reduced ability in inhibiting LCMV infection (Fig. 4B).

Role of α -DG O mannosylation for LCMV infection. The mucin-like region of α -DG shows extensive O-linked glycosylation. In particular, about 50% of the O-linked glycans in this region are *O*-mannosyl-linked carbohydrates, a rare type of mammalian glycosylation (12). The addition of *O*-mannosyl-linked carbohydrate moieties is catalyzed by a series of specific glycosyltransferases, and it has recently become apparent that genetic defects in those glycosyltransferases lead to severe congenital muscular dystrophies (1, 36). Muscle-eye-brain (MEB) disease is a severe congenital autosomal recessive disorder characterized by muscular dystrophy, ocular abnormalities, and brain malformation (14). MEB disease is caused by a defective POMGnT1, which catalyzes the transfer of GlcNAc to O-linked mannose of the glycoprotein. Due to this mutation, the α -DG molecule is hypoglycosylated but nevertheless expressed to normal amounts on the cell surface (37).

Taking advantage of this selective defect in O mannosylation, we investigated whether this particular glycosylation of

α -DG was involved in LCMV receptor function. We used primary fibroblasts from MEB patients or healthy controls for LCMV infection experiments. Cell surface staining with VIA-4 confirmed that the sugar epitope recognized by the VIA-4 antibody was absent in fibroblasts from MEB patients (Fig. 5A). Equivalent DG protein expression in MEB and control fibroblasts (37) was confirmed by β -DG Western blot analysis

of MEB or human control fibroblast membrane preparations (Fig. 5B). Interestingly, when MEB fibroblasts were infected with LCMV WE, a dramatic decrease in infectibility was observed in comparison to the two healthy controls (Fig. 6A). Similarly, when primary fibroblasts from MEB patients were infected with LCMV Cl13, Armstrong, or Docile, a clear reduction in infectibility was observed compared to the healthy control fibroblasts (Fig. 6B).

Furthermore, infection of MEB and control fibroblasts was assessed by fluorescence microscopy. Fibroblasts were infected for 2 days with LCMV WE and stained intracellularly with the LCMV NP-specific VL-4 antibody. A clear perinuclear staining was visible only in healthy donor fibroblasts (Fig. 7).

To confirm that α -DG O-mannosylation is crucial for LCMV receptor function, we investigated other cell lines with defects in O-mannosylation. Several mutant Chinese hamster ovary cell lines (CHO K1) harboring defects in dolichol-phosphate-mannose (Dol-P-Man) synthesis, the primary donor of mannose in O-mannosylation, are described. Dol-P-Man is synthesized by dolichol phosphate mannosyltransferase (DPM), an enzyme that consists of three subunits (34) and catalyzes the transfer of a mannose from guanosine-5'-diphosphate (GDP)-Man to dolichol-phosphate at the cytoplasmic side of the endoplasmic reticulum (ER) membrane. Mannose-charged dolichol-phosphate is then flipped into the ER lumen, where it acts as mannose donor. Mutations in Dol-P-Man synthesis affect glycosyl-phosphatidyl-inositol (GPI)-anchored protein expression as well as C and O-mannosylation (16, 19, 26, 27, 30). The CHO Lec15.1 (41) and CHO Lec15.2 mutants (35) harbor

FIG. 3. Blocking of LCMV infection by soluble Fc-DG fusion protein. (A) Coomassie staining of reduced FcDG and silver nitrate staining for control Fc proteins. A total of 16 μ g of purified proteins was separated on an SDS-PAGE gel. The expected mass of the reduced nonglycosylated Fc-DG fusion protein is 74 kDa. The reduced Fc fragment showed the expected band of 30 kDa. (B) Infection of MC57G cells is inhibited by preincubation with the Fc-DG fusion protein. Mouse fibroblast cells were infected with LCMV strains (MOI = 0.1) which had been preincubated with 0 to 5 μ M fusion protein. Percentages of infected cells were determined 16 h later by quantitating the percentage of cells expressing the LCMV NP.

FIG. 4. Deglycosylation of Fc-DG fusion protein. (A) The Fc-DG fusion protein and the fetuin control protein were deglycosylated with a cocktail of glycosidases. A total of 500 ng of proteins was separated on an SDS-PAGE gel, followed by silver nitrate staining. A clear difference in molecular mass between the deglycosylated and the control fusion protein as well as between the glycosylated and deglycosylated fetuin protein was apparent. (B) Infection inhibition by preincubation of LCMV with deglycosylated or control Fc-DG fusion proteins. MC57G cells were infected with LCMV CI13 which had been preincubated with 2.5 μ M deglycosylated or control fusion protein. Percentages of infected cells were determined 16 h later by quantification of LCMV-NP-expressing cells.

mutations within the catalytic subunit dpm2. The CHO Lec35.1 mutant most likely has a defect in the mannose-dolichol-phosphate ER flipping process (10).

When the VIA-4 surface staining for α -DG of wild-type (WT) CHO cells (CHO K1^K and CHO K1^L) was compared with the respective mutant cell lines (CHO Lec 15.1^K, CHO Lec 15.2^L, and CHO Lec 35.1^L), we observed, similar to the MEB fibroblasts, that the sugar epitope recognized by VIA4-1 was largely absent in mutant CHO cells (Fig. 8A). Western blot analysis for β -DG using membrane proteins extracted from the different CHO cell lines revealed comparable DG protein expression in all samples (Fig. 8B). Furthermore, surface protein biotinylation experiments confirmed cell surface β -DG expression in WT and mutant CHO cell lines (Fig. 8C). In addition to that, cell surface α -DG biotinylation was readily detected in Lec15.2 mutants but barely detectable in WT CHO cells or in Lec35.1 mutants (in which mannosylation is less compromised than in Lec15 mutants [10]), indicating that extensive α -DG glycosylation interfered with efficient biotinylation with primary amine reactive biotinylation reagents.

Next, these different CHO mutants were tested for their infectibility by LCMV. Both Lec 15.1 and Lec 15.2 cells showed a significantly decreased infectibility by LCMV CI13 in comparison to the corresponding WT CHO cells (CHO K1^K for Lec 15.1 and CHO K1^L for Lec 15.2) (Fig. 9A through C). In contrast, only marginal reduction of infection was observed in Lec35.1 cells, which is likely due to the less compromised mannosylation in these cells (10).

Taken together, these results suggest that α -DG glycosylation, and in particular, O mannosylation, plays a crucial role during the viral recognition process since hypoglycosylated α -DG on target cells resulted in decreased infectibility by LCMV.

DISCUSSION

In this study, we demonstrated that α -DG expression per se is not an absolute predictor for LCMV binding and subsequent

infection. We observed that LCMV infection is strongly dependent on α -DG glycosylation; in particular, we could demonstrate that a rare type of mammalian protein glycosylation, namely, O mannosylation, is involved in the infection process.

When different cell lines were infected with LCMV CI13, an LCMV strain that was shown to have high affinity for α -DG (49), we found that fibroblasts (L929, MC57G, or human fibroblasts) as well as epithelial cells (HEK-293) were readily infected by LCMV. All CD4⁺ T-cell hybridomas (VE8, 5A1) and lymphoma (Jurkat), thymoma (RMA), or monocyte (THP-1) cell lines were not susceptible to infection despite equivalent surface DG protein expression. Comparable results were also obtained using viral strains that differ in their binding affinity toward DG.

Although the glyco-epitope-specific VIA4-1 antibody did not stain α -DG on all cell lines, β -DG was always detectable to similar amounts in Western blots and in cell surface biotinylation experiments. Efficient cell surface biotinylation of α -DG proved difficult, apart from cell lines with hypoglycosylated α -DG, indicating that extensive α -DG glycosylation interfered with efficient primary amine reactive biotinylation reactions and confirming that the extent of glycosylation does not affect DG surface localization (47). Since we observed no correlation between α -DG expression and the infectibility of a given cell line, we assumed that posttranslational modifications of α -DG are crucial for LCMV receptor function.

LCMV infection could be inhibited by purified skeletal muscle α -DG preparations, corroborating earlier findings (11). In contrast, α -DG purified from rat brain was not able to interfere with LCMV infection. This dichotomy is likely due to different levels of glycosylation of the respective molecules. Skeletal muscle chicken α -DG is extensively glycosylated, whereas rat brain α -DG is less extensively glycosylated. Furthermore, no inhibition of infection was achieved with a recombinant peptide expressed in *E. coli* containing the essential viral binding site of α -DG (amino acids 170 through 315) (5, 32). The failure of the infection inhibition by the recombinant peptide could be

FIG. 6. Infection of MEB and human healthy donor fibroblasts. (A) Fibroblasts were infected with different MOIs of LCMV WE. Percentage of infected cells was determined after 2 days of infection by intracellular staining for LCMV-NP. (B) Comparison of LCMV-WE, CI13, Armstrong, and Docile infection. Fibroblasts were infected using an MOI of 0.1 of either LCMV-WE, CI13, Armstrong (ARM), or Docile (DOC). Two days after the infection, cells were analyzed for LCMV NP expression.

explained either by absent glycosylation or by an overall difference in the protein structure (11).

In dose-response experiments, the purified α -DG from skeletal muscle was more effective in LCMV infection inhibition than the recombinant FcDG molecule. This may be due to the more extensive glycosylation of purified α -DG from skeletal muscle compared to the overexpressed recombinant fusion protein or due to the presence of other glycosylated molecules in the α -DG preparation which contribute to interference with LCMV infection. However, the FcDG molecule was expressed in HEK 293 cells which were shown to have high endogenous protein *O*-mannosyltransferase 1 (POMT) activity (1). Between the different viral strains that we tested (Docile, Arm-

FIG. 5. Dystroglycan expression in primary fibroblasts from MEB patients and healthy donor controls. (A) Flow cytometric staining for cell surface α -DG. Primary fibroblasts from MEB (M and B) and healthy donor control (1 and 2) individuals were stained for α -DG using the VIA4-1 antibody. Numbers in the boxes depict the mean fluorescence intensity (MFI). Dotted lines depict background staining with the secondary antibody alone. (B) Western blot analysis of β -dystroglycan expression. A total of 50 μ g of protein membrane preparations was separated by SDS-PAGE, immobilized on a nitrocellulose membrane, and stained with anti- β -dystroglycan antibody (upper panels). As internal control, the same amount of protein was loaded and stained with an actin-specific antibody (lower panels).

FIG. 7. Immunofluorescence analysis of MEB or healthy donor fibroblast infection. Healthy donor or MEB fibroblasts were mock or LCMV WE infected for 2 days. Fluorescence microscopy was performed for intracellular LCMV NP expression (left panels). The right panels show phase contrast pictures of the same cells. Scale bars, 25 μ M.

strong, WE and CI13), there were differences in their susceptibilities for FcDG inhibition of infection. Both LCMV CI13 and Docile infection was inhibited at lower concentrations of FcDG compared to LCMV Armstrong and WE infection, which is mostly in agreement with the different α -DG binding affinities reported for these viral strains (49).

In mammals, the most abundant type of O-linked glycosylation is mucin-type O glycosylation, which starts with the addition of a GalNAc to a Ser/Thr residue. Recently, O mannosylation, a very rare form of mammalian glycosylation, was described (45). In O mannosylation, clusters of Sia α 2-3Gal β 1-4 GlcNAc β 1-2Man1-Ser/Thr build the core carbohydrate structure on the protein. This core structure can be further elongated, e.g., with sialic acid, giving rise to complex sugar structures. Up to now, the only known mammalian protein exhibiting O mannosylation is dystroglycan (12). Up to 50% of the O-linked sugars in the mucin-like region are O-

mannosyl carbohydrates (12). The LCMV binding site on α -DG has been mapped between the N-terminal globular domain and part of the mucin-like region (32). Previously, it was suggested that the receptor-virus interactions are protein-protein based interactions, based on several experimental approaches that aimed at excluding carbohydrate involvement. These included the treatment of cell membrane preparations with endoglycosidase H, which removes N-linked high mannose structures, treatment with PNGaseF, which removes N-linked sugars regardless of their complexity, and treatment with neuraminidase, which removes terminal sialic acid from complex glycoproteins (4, 32). Also, tunicamycin-mediated suppression of N glycosylation or treatment with O-glycanase led to no significant decrease in viral binding (4). All these results led to the conclusion that the virus recognizes the protein itself. At present, no endoglycosidases, which remove O-mannose linked sugars, are available. However, by using an

FIG. 8. Dystroglycan expression in CHO cells. (A) Flow cytometric staining for cell surface α -DG. WT or mutant CHO cells harboring a defect in Dol-P-Man synthesis were stained for α -DG using the VIA4-1 antibody. Numbers in the boxes depict the mean fluorescence intensity (MFI). Dotted lines depict background staining with the secondary antibody alone. (B) Western blot analysis of β -dystroglycan expression. A total of 50 μ g of protein membrane preparations was separated by SDS-PAGE, immobilized on a nitrocellulose membrane, and stained with anti- β -dystroglycan antibody (upper panels). As internal control, the same amount of protein was loaded and stained with an actin-specific antibody (lower panels). (C) Cell surface proteins from different CHO cells were biotinylated, and the α/β -DG complex was immunoprecipitated as described in the legend to Fig. 1C. Upper panels show biotinylated β -DG, and lower panels show bands corresponding to α -DG (arrows).

enzyme combination that strongly reduced the complexity of O-linked sugars on the Fc-DG fusion protein (including O-mannose-type glycans), we observed a reduction in its capacity to inhibit viral infection. Furthermore, since α -DG contains a significant proportion of O-mannosyl-linked carbohydrates, we assessed human fibroblasts harboring a genetic defect in the O-mannosylation pathway for their infectibility by LCMV. These fibroblasts were deficient for POMGnT1, which is responsible for the addition of the second carbohydrate, GlcNAc, to

the O-mannosylated protein. LCMV infection of POMGnT1-deficient fibroblasts was dramatically reduced compared to POMGnT1-proficient fibroblasts, despite equivalent DG protein expression (37). This finding supports the notion that glycosylation, especially O mannosylation, plays a crucial role

FIG. 9. LCMV Cl13 infection of CHO cells. CHO cells were infected using different MOIs of LCMV Cl13. After 1 day of infection, the percentage of infected cells was determined by quantification of LCMV-NP-expressing cells. (A) Mutant Lec 15.1 CHO cells (dpm2⁻) where infected (open bars) and compared with the WT control (black bars, CHO K1^K). (B) Similarly, Lec 15.2 CHO cells (dpm2⁻, open bars) were compared for LCMV Cl13 infectibility with the corresponding WT control cells (CHO K1^L, black bars). (C) LCMV CL13 infection was compared between Lec35.1 cells (defect in a putative flippase, empty bars) and WT controls (black bars, CHO K1^L).

in LCMV receptor function, which is further strengthened by the observation that cell lines harboring defects in Dol-P-Man synthesis, the primary donor of mannose in O-mannosylation, are less susceptible to LCMV infection.

Furthermore, an important role of posttranslational α -DG modification for LCMV binding was independently found by the dependence of LCMV binding on LARGE-mediated α -DG modification (31). LARGE is a putative glycosyltransferase which is crucially involved in the α -DG O-mannosylation pathway (2).

Several viruses are known to recognize carbohydrate residues on their receptors. For example, influenza virus requires host cell N-linked glycoproteins in order to infect its target cells (13) and Newcastle disease virus requires different sialic acid-containing compounds for cell entry (23). Sugar residues on target cells can also modulate viral infection; recently, it has been shown that N-linked glycosylation of the HIV CXCR4 coreceptor can inhibit viral entry (50). Glycosylation of α -DG is important for its ability to interact with constituents of the extracellular matrix. The binding of laminin-1, in particular, overlaps with the LCMV binding site (32). Thus, competition between laminin-1 and LCMV GP on α -DG occurs during LCMV target cell interaction, at least for LCMV isolates which predominantly use α -DG for target cell infection (32). Besides LCMV, *Mycobacterium leprae*, the causative organism of leprosy, also interacts with α -DG: α -DG, in conjunction with the ECM constituent laminin-2, was shown to serve as a Schwann cell receptor for *Mycobacterium leprae* (42).

In conclusion, we showed evidence that α -DG glycosylation, in particular, O-mannosylation, is important for viral recognition and the subsequent infection of target cells. α -DG glycosylation may influence the viral binding in several ways: the virus might recognize carbohydrate structures per se or in conjunction with the protein. In addition, the complexity of the carbohydrate chains could influence viral binding. Alternatively, reduction of LCMV infection in cells with defective O-mannosylation could be explained by an altered protein structure due to hypoglycosylation. In our experiments, we could not distinguish between these possibilities but we could clearly demonstrate that glycosylation is an absolute prerequisite for viral binding and infection.

ACKNOWLEDGMENTS

We thank Anna-Elina Lehesjoki (University of Helsinki, Helsinki, Finland) for providing the primary fibroblasts of MEB patients and healthy controls. We thank GlycoINIT for constructive advice and M. A. Lehrman and S. S. Krag for providing the mutant CHO cell lines.

This work was supported by the Roche Research Fund for Biology, the Swiss National Science Foundation, and the VonTobel Foundation.

REFERENCES

1. Akasaka-Manyá, K., H. Manyá, and T. Endo. 2004. Mutations of the POMT1 gene found in patients with Walker-Warburg syndrome lead to a defect of protein O-mannosylation. *Biochem. Biophys. Res. Commun.* **325**: 75–79.
2. Barresi, R., D. E. Michele, M. Kanagawa, H. A. Harper, S. A. Dovico, J. S. Satz, S. A. Moore, W. Zhang, H. Schachter, J. P. Dumanski, R. D. Cohn, I. Nishino, and K. P. Campbell. 2004. LARGE can functionally bypass alpha-dystroglycan glycosylation defects in distinct congenital muscular dystrophies. *Nat. Med.* **10**:696–703.
3. Battegay, M., S. Cooper, A. Althage, J. Banziger, H. Hengartner, and R. M. Zinkernagel. 1991. Quantification of lymphocytic choriomeningitis virus with an immunological focus assay in 24- or 96-well plates. *J. Virol. Methods* **33**:191–198.
4. Borrow, P., and M. B. Oldstone. 1992. Characterization of lymphocytic choriomeningitis virus-binding protein(s): a candidate cellular receptor for the virus. *J. Virol.* **66**:7270–7281.
5. Bozic, D., F. Sciandra, D. Lamba, and A. Brancaccio. 2004. The structure of the N-terminal region of murine skeletal muscle alpha-dystroglycan discloses a modular architecture. *J. Biol. Chem.* **279**:44812–44816.
6. Brancaccio, A., T. Schulthess, M. Gesemann, and J. Engel. 1995. Electron microscopic evidence for a mucin-like region in chick muscle alpha-dystroglycan. *FEBS Lett.* **368**:139–142.
7. Bruns, M., J. Cihak, G. Muller, and F. Lehmann-Grube. 1983. Lymphocytic choriomeningitis virus. VI. Isolation of a glycoprotein mediating neutralization. *Virology* **130**:247–251.
8. Buchmeier, M. J., and M. B. Oldstone. 1979. Protein structure of lymphocytic choriomeningitis virus: evidence for a cell-associated precursor of the virion glycoproteins. *Virology* **99**:111–120.
9. Buchmeier, M. J., R. M. Welsh, F. J. Dutko, and M. B. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* **30**:275–331.
10. Camp, L. A., P. Chauhan, J. D. Farrar, and M. A. Lehrman. 1993. Defective mannosylation of glycosylphosphatidylinositol in Lec35 Chinese hamster ovary cells. *J. Biol. Chem.* **268**:6721–6728.
11. Cao, W., M. D. Henry, P. Borrow, H. Yamada, J. H. Elder, E. V. Ravkov, S. T. Nichol, R. W. Compans, K. P. Campbell, and M. B. Oldstone. 1998. Identification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus. *Science* **282**:2079–2081.
12. Chiba, A., K. Matsumura, H. Yamada, T. Inazu, T. Shimizu, S. Kusunoki, I. Kanazawa, A. Kobata, and T. Endo. 1997. Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve alpha-dystroglycan. The role of a novel O-mannosyl-type oligosaccharide in the binding of alpha-dystroglycan with laminin. *J. Biol. Chem.* **272**:2156–2162.
13. Chu, V. C., and G. R. Whittaker. 2004. Influenza virus entry and infection require host cell N-linked glycoprotein. *Proc. Natl. Acad. Sci. USA* **101**: 18153–18158.
14. Cormand, B., H. Pihko, M. Bayes, L. Valanne, P. Santavuori, B. Talim, R. Gershoni-Baruch, A. Ahmad, H. van Bokhoven, H. G. Brunner, T. Voit, H. Topaloglu, W. B. Dobyns, and A. E. Lehesjoki. 2001. Clinical and genetic distinction between Walker-Warburg syndrome and muscle-eye-brain disease. *Neurology* **56**:1059–1069.
15. Diesien, C., A. Saarinen, H. Pihko, C. Rosenlew, B. Cormand, W. B. Dobyns, J. Dieguez, L. Valanne, T. Joensuu, and A. E. Lehesjoki. 2004. POMGNT1 mutation and phenotypic spectrum in muscle-eye-brain disease. *J. Med. Genet.* **41**:e115.
16. Doucey, M. A., D. Hess, R. Cacan, and J. Hofsteenge. 1998. Protein C-mannosylation is enzyme-catalysed and uses dolichyl-phosphate-mannose as a precursor. *Mol. Biol. Cell* **9**:291–300.
17. Durbecq, M., and K. P. Campbell. 1999. Biochemical characterization of the epithelial dystroglycan complex. *J. Biol. Chem.* **274**:26609–26616.
18. Endo, T. 1999. O-mannosyl glycans in mammals. *Biochim. Biophys. Acta* **1473**:237–246.
19. Englund, P. T. 1993. The structure and biosynthesis of glycosyl phosphatidylinositol protein anchors. *Annu. Rev. Biochem.* **62**:121–138.
20. Ervasti, J. M., A. L. Burwell, and A. L. Geissler. 1997. Tissue-specific heterogeneity in alpha-dystroglycan sialoglycosylation. Skeletal muscle alpha-dystroglycan is a latent receptor for Vicia villosa agglutinin b4 masked by sialic acid modification. *J. Biol. Chem.* **272**:22315–22321.
21. Ervasti, J. M., and K. P. Campbell. 1991. Membrane organization of the dystrophin-glycoprotein complex. *Cell* **66**:1121–1131.
22. Ervasti, J. M., and K. P. Campbell. 1993. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *J. Cell Biol.* **122**:809–823.
23. Ferreira, L., E. Villar, and I. Munoz-Barroso. 2004. Gangliosides and N-glycoproteins function as Newcastle disease virus receptors. *Int. J. Biochem. Cell Biol.* **36**:2344–2356.
24. Gee, S. H., R. W. Blacher, P. J. Douville, P. R. Provost, P. D. Yurchenco, and S. Carbonetto. 1993. Laminin-binding protein 120 from brain is closely related to the dystrophin-associated glycoprotein, dystroglycan, and binds with high affinity to the major heparin binding domain of laminin. *J. Biol. Chem.* **268**:14972–14980.
25. Henry, M. D., and K. P. Campbell. 1999. Dystroglycan inside and out. *Curr. Opin. Cell Biol.* **11**:602–607.
26. Herscovics, A., and P. Orlean. 1993. Glycoprotein biosynthesis in yeast. *FASEB J.* **7**:540–550.
27. Hirschberg, C. B., and M. D. Snider. 1987. Topography of glycosylation in the rough endoplasmic reticulum and Golgi apparatus. *Annu. Rev. Biochem.* **56**:63–87.
28. Ibraghimov-Beskrovnaya, O., J. M. Ervasti, C. J. Leveille, C. A. Slaughter, S. W. Sernett, and K. P. Campbell. 1992. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature* **355**:696–702.
29. Ibraghimov-Beskrovnaya, O., A. Milatovich, T. Ozelik, B. Yang, K. Ko-

- epnick, U. Francke, and K. P. Campbell. 1993. Human dystroglycan: skeletal muscle cDNA, genomic structure, origin of tissue specific isoforms and chromosomal localization. *Hum. Mol. Genet.* **2**:1651–1657.
30. Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **54**:631–664.
  31. Kunz, S., J. M. Rojek, M. Kanagawa, C. F. Spiropoulou, R. Barresi, K. P. Campbell, and M. B. A. Oldstone. 2005. Posttranslational modification of α -dystroglycan, the cellular receptor for arenaviruses, by the glycosyltransferase LARGE is critical for virus binding. *J. Virol.* **79**:14282–14296.
  32. Kunz, S., N. Sevilla, D. B. McGavern, K. P. Campbell, and M. B. Oldstone. 2001. Molecular analysis of the interaction of LCMV with its cellular receptor α -dystroglycan. *J. Cell Biol.* **155**:301–310.
  33. Kunz, S., N. Sevilla, J. M. Rojek, and M. B. Oldstone. 2004. Use of alternative receptors different than alpha-dystroglycan by selected isolates of lymphocytic choriomeningitis virus. *Virology* **325**:432–445.
  34. Maeda, Y., S. Tanaka, J. Hino, K. Kangawa, and T. Kinoshita. 2000. Human dolichol-phosphate-mannose synthase consists of three subunits, DPM1, DPM2 and DPM3. *EMBO J.* **19**:2475–2482.
  35. Maeda, Y., S. Tomita, R. Watanabe, K. Ohishi, and T. Kinoshita. 1998. DPM2 regulates biosynthesis of dolichol phosphate-mannose in mammalian cells: correct subcellular localization and stabilization of DPM1, and binding of dolichol phosphate. *EMBO J.* **17**:4920–4929.
  36. Manya, H., K. Sakai, K. Kobayashi, K. Taniguchi, M. Kawakita, T. Toda, and T. Endo. 2003. Loss-of-function of an N-acetylglucosaminyltransferase, POMGnT1, in muscle-eye-brain disease. *Biochem. Biophys. Res. Commun.* **306**:93–97.
  37. Michele, D. E., R. Barresi, M. Kanagawa, F. Saito, R. D. Cohn, J. S. Satz, J. Dollar, I. Nishino, R. I. Kelley, H. Somer, V. Straub, K. D. Mathews, S. A. Moore, and K. P. Campbell. 2002. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature* **418**:417–422.
  38. Michele, D. E., and K. P. Campbell. 2003. Dystrophin-glycoprotein complex: post-translational processing and dystroglycan function. *J. Biol. Chem.* **278**:15457–15460.
  39. Parekh, B. S., and M. J. Buchmeier. 1986. Proteins of lymphocytic choriomeningitis virus: antigenic topography of the viral glycoproteins. *Virology* **153**:168–178.
  40. Patnaik, S. K., and P. Stanley. 2005. Mouse large can modify complex N- and mucin O-glycans on alpha-dystroglycan to induce laminin binding. *J. Biol. Chem.* **280**:20851–20859.
  41. Pu, L., J. R. Scoeca, B. K. Walker, and S. S. Krag. 2003. A single point mutation resulting in an adversely reduced expression of DPM2 in the Lec15.1 cells. *Biochem. Biophys. Res. Commun.* **312**:555–561.
  42. Rambukkana, A., H. Yamada, G. Zanazzi, T. Mathus, J. L. Salzer, P. D. Yurchenco, K. P. Campbell, and V. A. Fischetti. 1998. Role of alpha-dystroglycan as a Schwann cell receptor for *Mycobacterium leprae*. *Science* **282**:2076–2079.
  43. Riviere, Y., R. Ahmed, P. J. Southern, M. J. Buchmeier, F. J. Dutko, and M. B. Oldstone. 1985. The S RNA segment of lymphocytic choriomeningitis virus codes for the nucleoprotein and glycoproteins 1 and 2. *J. Virol.* **53**:966–968.
  44. Salvato, M. S., and E. M. Shimomaye. 1989. The completed sequence of lymphocytic choriomeningitis virus reveals a unique RNA structure and a gene for a zinc finger protein. *Virology* **173**:1–10.
  45. Sasaki, T., H. Yamada, K. Matsumura, T. Shimizu, A. Kobata, and T. Endo. 1998. Detection of O-mannosyl glycans in rabbit skeletal muscle alpha-dystroglycan. *Biochim. Biophys. Acta* **1425**:599–606.
  46. Sevilla, N., S. Kunz, A. Holz, H. Lewicki, D. Homann, H. Yamada, K. P. Campbell, J. C. de La Torre, and M. B. Oldstone. 2000. Immunosuppression and resultant viral persistence by specific viral targeting of dendritic cells. *J. Exp. Med.* **192**:1249–1260.
  47. Singh, J., Y. Itahana, S. Knight-Krajewski, M. Kanagawa, K. P. Campbell, M. J. Bissell, and J. Muschler. 2004. Proteolytic enzymes and altered glycosylation modulate dystroglycan function in carcinoma cells. *Cancer Res.* **64**:6152–6159.
  48. Singh, M. K., F. V. Fuller-Pace, M. J. Buchmeier, and P. J. Southern. 1987. Analysis of the genomic L RNA segment from lymphocytic choriomeningitis virus. *Virology* **161**:448–456.
  49. Smelt, S. C., P. Borrow, S. Kunz, W. Cao, A. Tishon, H. Lewicki, K. P. Campbell, and M. B. Oldstone. 2001. Differences in affinity of binding of lymphocytic choriomeningitis virus strains to the cellular receptor α -dystroglycan correlate with viral tropism and disease kinetics. *J. Virol.* **75**:448–457.
  50. Wang, J., G. J. Babcock, H. Choe, M. Farzan, J. Sodroski, and D. Gabuzda. 2004. N-linked glycosylation in the CXCR4 N-terminus inhibits binding to HIV-1 envelope glycoproteins. *Virology* **324**:140–150.
  51. Willer, T., B. Prados, J. M. Falcon-Perez, I. Renner-Muller, G. K. Przemeck, M. Lommel, A. Coloma, M. C. Valero, M. H. de Angelis, W. Tanner, E. Wolf, S. Strahl, and J. Cruces. 2004. Targeted disruption of the Walker-Warburg syndrome gene *Pomt1* in mouse results in embryonic lethality. *Proc. Natl. Acad. Sci. USA* **101**:14126–14131.
  52. Willer, T., M. C. Valero, W. Tanner, J. Cruces, and S. Strahl. 2003. O-mannosyl glycans: from yeast to novel associations with human disease. *Curr. Opin. Struct. Biol.* **13**:621–630.
  53. Williamson, R. A., M. D. Henry, K. J. Daniels, R. F. Hrstka, J. C. Lee, Y. Sunada, O. Ibraghimov-Beskrovnaia, and K. P. Campbell. 1997. Dystroglycan is essential for early embryonic development: disruption of Reichert's membrane in *Dag1*-null mice. *Hum. Mol. Genet.* **6**:831–841.
  54. Wilson, I. B., Y. Gavel, and G. von Heijne. 1991. Amino acid distributions around O-linked glycosylation sites. *Biochem. J.* **275**:529–534.
  55. Yoshida, A., K. Kobayashi, H. Manya, K. Taniguchi, H. Kano, M. Mizuno, T. Inazu, H. Mitsuhashi, S. Takahashi, M. Takeuchi, R. Herrmann, V. Straub, B. Talim, T. Voit, H. Topaloglu, T. Toda, and T. Endo. 2001. Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev. Cell* **1**:717–724.