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

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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-

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Post-translational modification of α-dystroglycan is not critical for lymphocytic choriomeningitis virus receptor function *in vivo*

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α-Dystroglycan (α-DG) is a ubiquitously expressed molecule that has been identified as a cellular receptor for lymphocytic choriomeningitis virus (LCMV) and other arenaviruses. Recently, it was demonstrated that LCMV receptor function is critically dependent on post-translational modifications, namely glycosylation. In particular, it was shown that *O*-mannosylation, a rare type of mammalian *O*-linked glycosylation, is important in determining the binding of LCMV to its cellular receptor. All studies carried out so far showed a dependence on glycosylation in LCMV receptor function *in vitro*. This work extended these studies to two *in vivo* models of α-DG hypoglycosylation. The results confirm earlier findings on the *in vitro* dependence of carbohydrate modifications in LCMV receptor function. However, experiments in animal models showed that this dependence was only very weak *in vivo*. It is likely that alternative receptors or alternative entry pathways may account for this attenuated *in vivo* phenotype.

INTRODUCTION

Lymphocytic choriomeningitis virus (LCMV) is the prototypic member of the family *Arenaviridae*. Arenaviruses are enveloped and are characterized by their bi-segmented, ambisense genome (Buchmeier *et al.*, 1980; Riviere *et al.*, 1985; Salvato & Shimomaye, 1989; Singh *et al.*, 1987). The two single-stranded RNA segments encode four proteins. Whereas the short segment encodes the nucleoprotein (NP) and the glycoprotein (GP) precursor (Riviere *et al.*, 1985; Salvato & Shimomaye, 1989), the long segment encodes the viral RNA-dependent RNA polymerase and a small zinc-binding protein (Z) (Salvato *et al.*, 1989, 1992). The GP precursor is cleaved post-translationally into the peripheral GP1 and the transmembrane GP2 proteins by the protease SKI-1/S1P (Beyer *et al.*, 2003; Buchmeier & Oldstone, 1979; Kunz *et al.*, 2003). Upon GP-1-mediated receptor binding, arenaviruses are internalized in endosomal compartments via uncoated vesicles.

α-Dystroglycan (α-DG) has been described as a cellular receptor for LCMV and other arenaviruses, as well as for *Mycobacterium leprae* (Cao *et al.*, 1998; Rambukkana *et al.*, 1998; Spiropoulou *et al.*, 2002). Initially expressed as a propeptide, DG is processed into the peripheral α-DG subunit and the membrane-spanning β-DG subunit, which remain non-covalently associated (Ibraghimov-Beskrovnaya *et al.*, 1992). α-DG is responsible for the binding of extracellular matrix molecules such as laminin, whereas the β-DG subunit represents the transmembrane-spanning protein that connects to the actin-based cytoskeleton, for example via dystrophin (Ervasti & Campbell, 1991, 1993; Holt *et al.*, 2000; Ibraghimov-Beskrovnaya *et al.*, 1992).

α-DG consists of N- and C-terminal globular domains that are connected via a highly glycosylated, mucin-like region rich in prolines, serines and threonines (Brancaccio *et al.*, 1995; Wilson *et al.*, 1991). The α-DG molecule is subject to extensive post-translational modifications, especially glycosylation. Notably, more than 50 % of the apparent molecular mass of α-DG originates from *O*-linked sugars, including a rare type of *O*-mannosyl-linked carbohydrate; these are mainly concentrated within the mucin-like region (Chiba *et al.*, 1997). The *O*-mannosylation pathway is initiated in the endoplasmic reticulum, where a mannose is transferred to serine/threonine residues, a process that is catalysed by protein *O*-mannosyltransferase-1 and -2 (POMT-1 and POMT-2). The protein *O*-linked mannose β-1,2-*N*-acetylglucosaminyltransferase 1 (POMGnT1) then catalyses the transfer of *N*-acetylglucosamine to *O*-linked mannose in a pathway that continues in the Golgi apparatus and involves a series of other glycosyltransferases that catalyse the transfer of galactose and sialic acid. This gives rise to a tetrasaccharide forming the core structure of *O*-mannosyl-linked sugars. Although not directly implicated in this *O*-mannosylation pathway, the putative glycosyltransferase Large seems to play a critical role in

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