Factors in the Emergence and Control of Rodent-borne Viral Diseases (Hantaviral and Arenal Diseases)
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Isolation of a cellular receptor for lymphocytic choriomeningitis virus and Lassa fever virus

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Summary – Previously, a 120–140-kDa glycoprotein was characterized as a putative receptor for lymphocytic choriomeningitis virus (LCMV). We purified this peripheral membrane protein by ion-exchange and lectin chromatography. Sequences of tryptic peptides from purified protein were identified as α-dystroglycan (α-DG). α-DG, a glycoprotein, functions as an important cell-surface component linking the extracellular matrix and cytoskeleton on the surface of a wide variety of cells. α-DG was specifically recognized by several strains of LCMV and other arenaviruses – Lassa fever (LFV), Oliveros and Mobala viruses. Purified soluble α-DG significantly blocked LCMV infection of permissive cells. DG gene-deficient cells were resistant to LCMV infection. Furthermore, reconstitution of α-DG on DG-null cells restored their susceptibility to LCMV. Thus, we have found that α-DG is a common cellular receptor for a group of arenaviruses including LCMV and LFV.

lymphocytic choriomeningitis virus (LCMV) / Lassa fever virus (LFV) / α-dystroglycan / receptor / virus overlay protein-blot assay (VOPBA) / hemorrhagic fever

Arenaviruses are involved in emerging virus infections and several members of this virus family can cause fatal human hemorrhagic fevers [1–3]. Among these pathogens, Lassa fever (LFV), Junin, Guanarito, Sabia and Machupo viruses produce high morbidity and mortality worldwide. For example, LFV infection occurs throughout West Africa with an estimated 250,000 cases and > 5000 deaths per year [1, 4, 5]. Lymphocytic choriomeningitis virus (LCMV) has been studied for > 60 years as the prototype of arenaviruses and is the classical model of viral immunology and pathogenesis [1, 6–12]. Though arenaviruses usually cause asymptomatic persistent infections of their rodent hosts, disease may occur when humans come in contact with rodent-excreted arenaviruses. Changes in ecology and farming practices in regions of Africa, South America and Asia have contributed to emerging arenavirus

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Table I. Peptide sequences and matching proteins.

Peptides	*Amino-acid sequence*	*Matching proteins*	*Amino-acid region*
GT429	AFDDGAFTGIR	Jacalin	3–13
GT384	LAGDPAPVVNDIHK	Dystroglycan precursor	610–623
GT441	GGLSAVDAFEIHVHK	Dystroglycan precursor	571–585
GT417	IPSDTFYDNEDTTTDKLK	Dystroglycan precursor	516–533
GT348	KLERKLREK	U1 small nuclear ribonucleoprotein	31–39

diseases [3, 13–16]. Increasing international travel has also facilitated the spread of these infections to the rest of the world [14, 17].

The initiation of LCMV and arenavirus infection involves the interaction between the glycoprotein GP-1 on the surface of the virion and proteinaceous receptor(s) on the cell surface [18–19]. Previously, a virus overlay protein blot assay (VOPBA) detected a 120–140 kDa glycoprotein that specifically bound to purified LCMV [18]. This molecule was characterized as a glycoprotein sensitive to protease, glycosidase and tunicamycin treatments [18]. The expression of this protein correlated directly with a wide range of murine and primate cells' susceptibilities to LCMV attachment and infection [18, 20]. Similarly, LFV bound to the same glycoprotein from susceptible cell membranes in VOPBA [20]. This observation suggested that both viruses might share a common cellular receptor.

Purification of this putative receptor protein from a mouse fibroblast cell line with sequential chromatographies combining an anion–exchange column followed by lentil–Sepharose, wheat germ agglutinin–Sepharose and jacalin–Sepharose affinity-columns, which recognize different carbohydrate structures on the molecule, and trypsin digestion yielded five peptides that were sequenced *(table I)*. Three peptides, GT384, GT441 and GT417, corresponded to separate regions of a single protein named dystroglycan (DG) precursor. The other two peptides, homologous to jacalin and U1 small nuclear ribonucleoprotein, were likely contaminating components in the final protein preparation.

DG is encoded by a single gene, and is processed into two mature proteins, α- and β-DG, which form a complex spanning the plasma membrane [21] *(figure 1)*. α-DG is an extracellular peripheral membrane protein that binds to the extracellular matrix and interacts noncovelantly with β-DG, a transmembrane protein that is linked to the cytoskeleton [22]. The sequences corresponding to GT384, GT441 and GT417 are found near the carboxy terminus of α-DG *(figure 1)*. Although the DG complex has been studied primarily in skeletal muscle because of its close association with dystrophin [21, 23], this well-conserved *DG* gene is expressed in a wide range of non-muscle tissues and cells [24, 25]. Mice lacking the *DG* gene displayed gross abnormalities at a very early stage of development [25]. It is thought that DG plays a critical role in mediating cell–extracellular matrix interactions [24, 25].

We demonstrated the interaction between α-DG and LCMV by performing VOPBA using blots containing purified α-DG protein with several strains of LCMV [20]. All the LCMV strains tested bound to α-DG protein purified from rabbit skeletal muscle [26]. In contrast, none of these viruses recognized the *Escherichia coli*-expressed glutathion *S*-transferase (GST)-fusion proteins, which encoded different regions of the DG precursor sequence [21] (Cao et al., unpublished observations). It should be noted that the mature α-DG protein is heavily glycosylated, with 40–50% of its molecular mass comprised of carbohydrates [22], while none of the bacterially expressed GST–α-DG proteins contained any appropriate

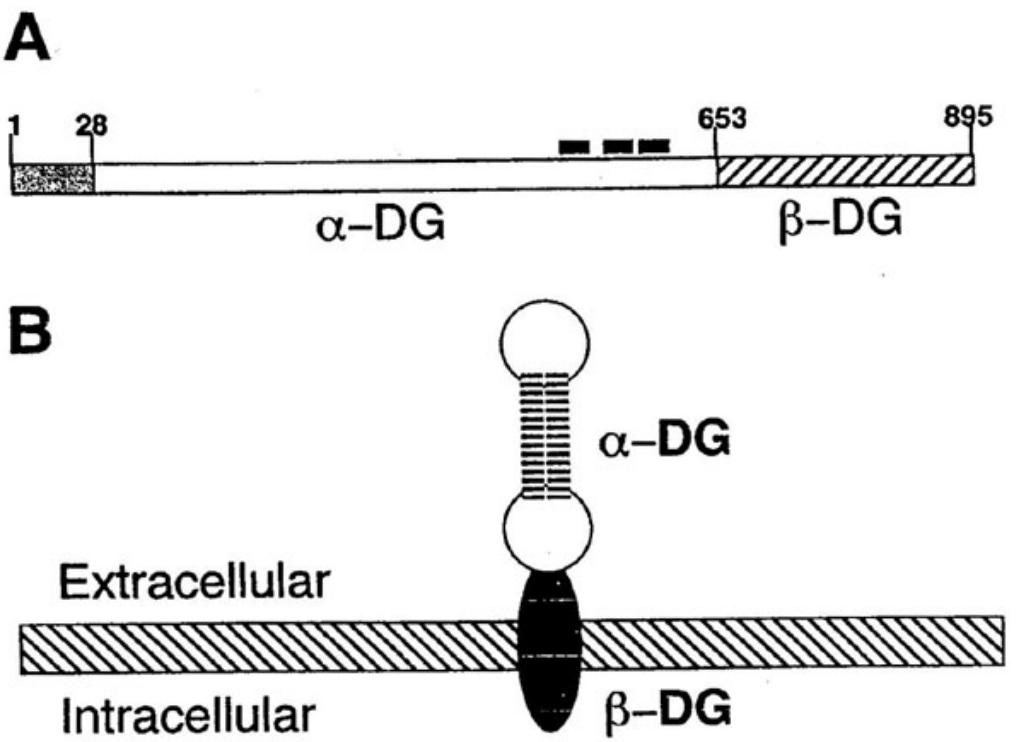

Figure 1. Schematic diagram of the DG precursor and complex. **A.** DG is synthesized as a large precursor of 895 residues which is eventually processed into α-DG (amino acids [aa] 29–653, white box) and β-DG (aa 654–895, cross-hatched box). The signal peptide (aa 1–28, grey box) is removed from the mature protein. The black bars on top of α-DG represent the schematic alignment of peptides GT384, GT441 and GT417. **B.** DG complex is assembled on the cell surface by non-covalent interaction. β-DG is a transmembrane protein, while α-DG is a peripheral membrane protein. The mature α-DG protein probably adopts a dumbbell-like shape with two globular domains (white circles) at its N′ and C′ termini, linked by an extended mucin-like region in the middle (striped region).

glycosylations. It is likely that glycosylation or other forms of post-translational modifications on native α-DG are crucial for LCMV recognition. Nevertheless, LCMV recognizes specific structures on α-DG, for another glycoprotein purified from rabbit skeletal muscle, the α2 subunit of the dihydropyridine-receptor complex [27], which is also highly negatively charged through glycosylation, did not bind to LCMV. In addition, arenaviruses LFV, Mobala and Oliveros were shown to bind to purified native α-DG protein, but not to the recombinant GST–α-DG fusion proteins [20].

Next, we showed that purified soluble α-DG competed with the cell-surface virus receptor during LCMV infections in a dose-dependent manner (*figure 2*). A mouse fibroblast line, 3T6, was infected with LCMV strain Cl 13 in the presence of increasing concentrations of either purified α-DG protein or bovine serum albumin (BSA). α-DG at about 1 n*M* concentration significantly reduced the number of cells infected by LCMV (*figure 2*). When another LCMV strain Armstrong 5 (Arm5) was tested in a similar assay, the infectivity was also diminished by the presence of soluble α-DG during virus absorption, though the inhibitory dosage for α-DG was about 100-fold higher (*figure 2*). However, α-DG had no effect on the infection of the same cells by another enveloped RNA virus, vesicular stomatitis virus (VSV) (*figure 2*).

Lastly, we found that the expression of α-DG protein was necessary for LCMV entry into cells. Two lines of genetically related mouse embryonic stem (ES) cells were analyzed:

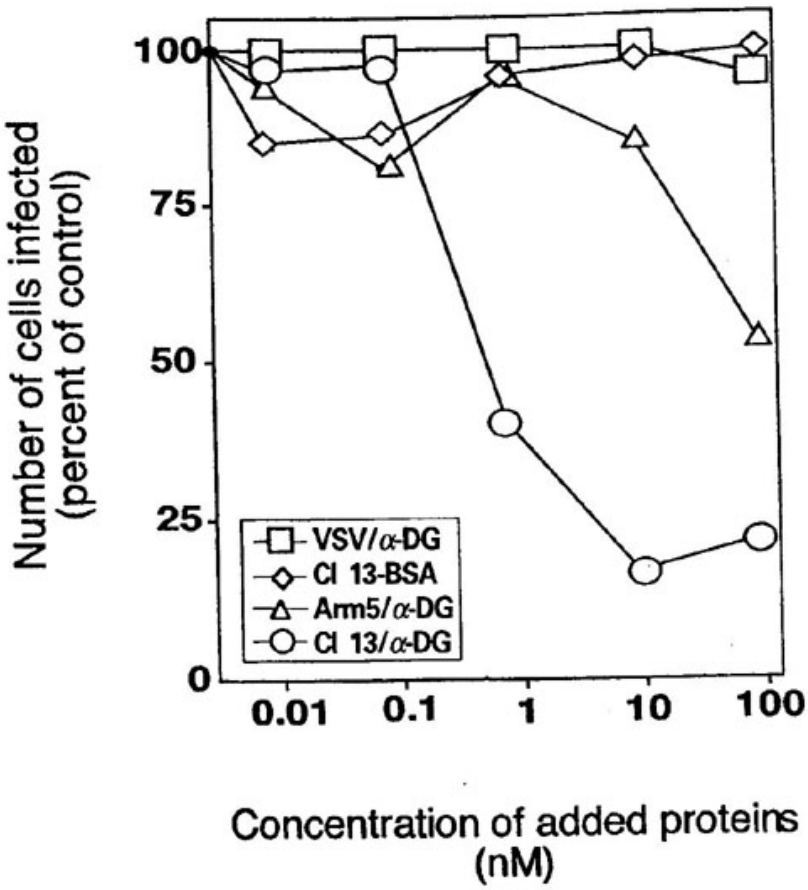

Figure 2. Soluble α-DG protein blocks infection by LCMV; 3T6 mouse fibroblast cells were infected with LCMV strain Cl 13, Arm5 or vesicular stomatitis virus (VSV) in the presence of increasing concentrations of soluble α-DG. As a control, LCMV Cl 13 infection was also performed in the presence of increasing concentrations of BSA; 16 h later, cells were immunolabeled with mAb 1-1-3 to detect LCMV nucleoprotein or with mAb I1 to detect VSV glycoprotein, and infected cells were counted under the fluorescence microscope. The results are means of at least 4 fluorescent areas and plotted as percentages of control values obtained in the absence of competing protein.

parental ES ($DG^{+/+}$) and DG-null ES ($DG^{-/-}$) cells [28]. Both lines were equally infectable by VSV *(figure 3)*. However, $DG^{-/-}$ cells were completely resistant to LCMV Cl 13 infection when examined at different multiplicities of infection (MOI) *(figure 3)*. After the $DG^{-/-}$ cells were infected by an adenovirus vector carrying rabbit DG cDNA (Adeno-DG) [28], these cells then became susceptible to LCMV infection *(table II)*. $DG^{-/-}$ cells infected by another adenovirus vector carrying green fluorescent protein cDNA (Adeno-GFP) failed to become susceptible to LCMV. Analysis by Western blot with an antibody specific to α-DG and VOPBA with LCMV confirmed the presence of α-DG protein in ES cells susceptible to LCMV infection *(table II)*.

Diseases caused by arenavirus infection are important public health and medical problems. The extensive sequence conservation in virus-encoded proteins across the *Arenaviridae* is consistent with the possibility that these viruses may use the same cellular receptor for infection [1, 29]. Interestingly, all the Old World arenaviruses tested including LCMV, LFV and Mobala virus, have been shown to specifically recognize native α-DG protein [20]. The fact that α-DG is well conserved and ubiquitously expressed is in complete agreement with the

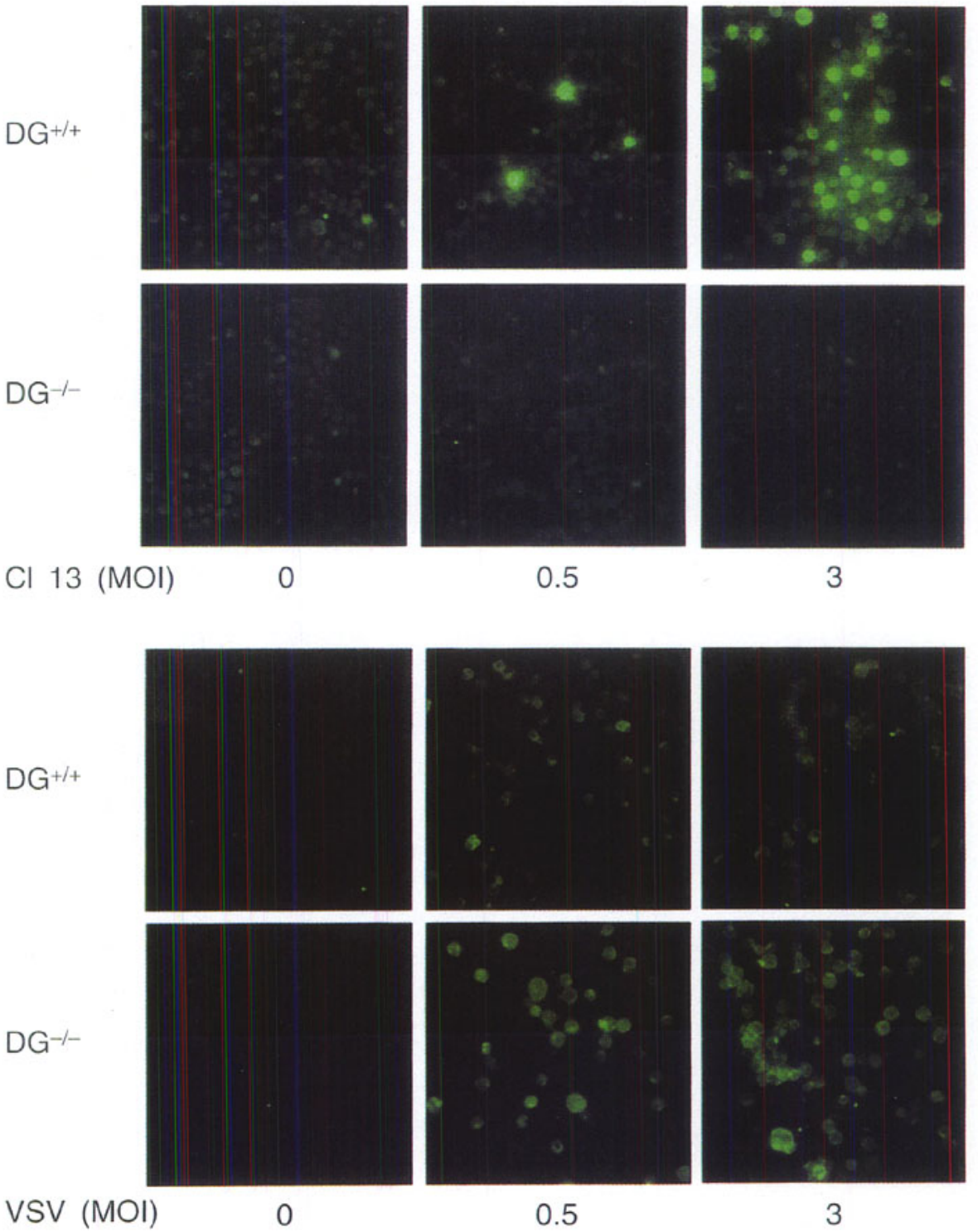

Figure 3. α-DG expression is necessary for LCMV infection. $DG^{+/+}$ and $DG^{-/-}$ ES cells were infected with LCMV strain Cl 13 and VSV at MOI of 0, 0.5 and 3; 16 h later, cells were labeled to detect LCMV nucleoprotein and VSV glycoprotein by immunofluorescence.

Table II. α-DG expression is required for LCMV infection.

ES cells	*α-DG expression*	*VOPBA*	*LCMV susceptibility*
$DG^{+/+}$	+++	+++	+++
$DG^{-/-}$	–	–	–
$DG^{-/-}$ (Adeno-DG)	++	++	++
$DG^{-/-}$ (Adeno-GFP)	–	–	–

observation that LCMV infects a wide variety of cell types from many different mammalian hosts both in vitro and in vivo [18, 30].

The initial step of receptor-mediated virus attachment largely determines the tropism and consequently the pathogenesis of infection. We have noticed that different LCMV strains interact with α-DG protein with distinct characteristics: Cl 13 bound to α-DG better than Arm5 in VOPBAs, and was blocked by soluble α-DG at a lower concentration *(figure 2)* [20]. Particularly, only a single amino-acid difference at position 260 was found in the GP-1 protein sequence between these two strains [31–33]. Previous work showed that Cl 13, which preferentially infected the interdigitating dendritic follicular cells (IDFC) in the white pulp of the spleen, caused immunosuppression in adult immunocompetent mice [33]. In contrast, Arm5 virus tropism was primarily for $F480^+$ macrophages in the red pulp of the spleen and was not associated with immunosuppression in adult mice [33]. Whether amino-acid 260 of GP-1 protein is directly involved in receptor binding is not clear but is currently under evaluation, as are studies on the binding of several GP-1 mutant viruses, differences of α-DG expression and co-receptor usage by IDFC and $F480^+$ macrophages. Finally, plans to evaluate the binding to and entry of Old World and New World arenaviruses for $DG^{+/+}$ and $DG^{-/-}$ cells and the ability of soluble α-DG to block LFV infections are currently underway.

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