

10. R. A. Williamson *et al.*, *Hum. Mol. Genet.* **6**, 831 (1997).
11. Y. Sunada, S. M. Bernier, C. A. Kozak, Y. Yamada, K. P. Campbell, *J. Biol. Chem.* **269**, 13729 (1994); H. Xu, X. R. Wu, U. M. Wewer, E. Engvall, *Nature Genet.* **8**, 297 (1994); Y. Sunada, S. M. Bernier, A. Utani, Y. Yamada, K. P. Campbell, *Hum. Mol. Genet.* **4**, 1055 (1995).
12. V. Straub and K. P. Campbell, *Curr. Opin. Neurol.* **10**, 168 (1997).
13. A. Rambukkana, J. L. Salzer, P. D. Yurchenco, E. I. Tuomanen, *Cell* **88**, 811 (1997).
14. I. Leivo and E. Engvall, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1544 (1988); R. E. Burgeson *et al.*, *Matrix Biol.* **14**, 209 (1994); R. Vuolteenaho *et al.*, *J. Cell Biol.* **124**, 381 (1994).
15. A. M. Mercurio, *Trends Cell Biol.* **5**, 419 (1995).
16. H. Yamada *et al.*, *J. Biol. Chem.* **271**, 23418 (1996).
17. *Mycobacterium leprae* was purified from armadillos and provided by P. J. Brennan (Colorado State University, Fort Collins, CO). Each isolate was tested for acid-fast labeling and *M. leprae*-specific phenolic glycolipid-1 (PGL-1) reactivity with a auramine-rhodamine Bacto TB Fluorescent Stain Kit (Difco, Detroit, MI) and mAb to native PGL-1, respectively, before the assays.
18. The bovine peripheral nerve  $\alpha$ -DG was purified as described [(16); H. Yamada, T. Shimizu, T. Tanaka, K. P. Campbell, K. Matsumura, *FEBS Lett.* **352**, 49 (1994); J. M. Ervasti, S. D. Kahl, K. P. Campbell, *J. Biol. Chem.* **266**, 9161 (1991)]. The rabbit skeletal muscle  $\alpha$ -DG was purified by using the same method but with KCl-washed heavy microsomes of rabbit skeletal muscle as a starting material.  $\alpha$ -DG fusion proteins B and D (FP-B and FP-D) were prepared as described (6).
19. Recombinant (r) LN- $\alpha$ 2G, rLN- $\alpha$ 2(VI-IVb)', and rLN- $\alpha$ 1G fragments were prepared with a baculovirus expression system as previously described [(13); P. D. Yurchenco, U. Sung, M. D. Ward, Y. Yamada, J. J. O'Rear, *J. Biol. Chem.* **268**, 8356 (1993)]. Human merosin (laminin-2 and laminin-4) was a gift from M. Paulsson. The purity of DG preparations and recombinant fragments of laminins were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with antibodies specific for each fraction as described (13, 18, 19).
20. *Mycobacterium leprae* binding to  $\alpha$ -DG was determined with a solid-phase bacterial adherence assay (13) by using immobilized native  $\alpha$ -DG purified from peripheral nerves or skeletal muscles, or recombinant  $\alpha$ -DG. Terasaki plates were coated overnight with  $\alpha$ -DGs (50  $\mu$ g/ml, 0.5  $\mu$ g per well) or bovine serum albumin (BSA) as a negative control. *Mycobacterium leprae* ( $5 \times 10^8$  bacteria/ml) suspension was preincubated with rLN $\alpha$ 2G or LN $\alpha$ 2(VI-IVb)' (10  $\mu$ g/ml, 0.1  $\mu$ g per well) or BSA for 1 hour at 37°C. After blocking the nonspecific binding with BSA, 10  $\mu$ l of the *M. leprae* mixture was added to each well and incubated for 1 hour at 37°C. Unbound bacteria were removed by washing with DPBS and wells were fixed with 2.5% glutaraldehyde (Sigma). Adherent *M. leprae* was detected by acid-fast labeling, counted, and expressed as described (13). The effect of heparin and EDTA on rLN $\alpha$ 2G-mediated *M. leprae* binding to  $\alpha$ -DG was determined similarly by incubating the bacterial mixture with 10 mM EDTA or heparin (1 mg/ml). The effect of periodate treatment was evaluated by preincubation of increasing concentrations of sodium periodate with native  $\alpha$ -DG before the addition of *M. leprae*+rLN $\alpha$ 2G. Periodate and EDTA treatment did not detach the  $\alpha$ -DG from wells because no difference was found in antibody activity to  $\alpha$ -DG before and after treatment as detected by enzyme-linked immunosorbent assay with polyclonal antibodies to  $\alpha$ -DG.
21. A. Rambukkana *et al.*, data not shown.
22. J. M. Ervasti, A. L. Burwell, A. L. Geissler, *J. Biol. Chem.* **272**, 22315 (1997).
23. O. Ibraghimov-Beskrovnya *et al.*, *Hum. Mol. Genet.* **2**, 1651 (1993); H. Yamada and K. Matsumura, unpublished data.
24. A. Chiba *et al.*, *J. Biol. Chem.* **272**, 2156 (1997); T. Sasaki, H. Yamada, K. P. Campbell, and T. Endo, unpublished data.
25. Schwann cells were isolated from neonatal rat sciatic

nerve, purified, and amplified as described [S. Einheber, T. A. Milner, F. Giancotti, J. L. Salzer, *J. Cell Biol.* **123**, 1223 (1993)]. Human Schwann cells were purified and immortalized as described [J. L. Rutkowski, J. S. Rhim, K. W. C. Peden, G. I. Tennekoon, in *Neoplastic Transformation in Human Cell Systems in Vitro*, J. S. Rhim and A. Dripschillo, Eds. (Humana, Totowa, NJ, 1991), pp. 343–346]. Schwann cells were plated onto poly-L-lysine-coated eight-well Lab-Tek chamber slides (Nunc) or 12-mm cover slips and cultured without forskolin to prevent the deposition of laminin-2. These primary rat Schwann cells and human Schwann cells were found to be 100% pure as determined by antibody to S-100 antigen.

26. Primary rat Schwann cells and immortalized human Schwann cells were used for both adherence and invasion assays because they are devoid of LN $\alpha$ -2G. The *M. leprae* adherence assay to Schwann cells was previously described (13).

27. For competitive inhibition assays, rLN $\alpha$ 2G-coated *M. leprae* were preincubated with increasing concentrations of native  $\alpha$ -DG for 3 hours at 37°C, after which the mixture was added onto Schwann cells, and the adherence assays were performed as described (13). The number of acid-fast-labeled bacteria were quantified, and values were presented as the mean percent binding of controls. The net rLN $\alpha$ 2G-mediated *M. leprae* adherence to Schwann cells was considered as 100%.

28. Light microscopy and immunofluorescence of Schwann cells were performed as described (13). Characterization of mAb IIH6C4 to  $\alpha$ -DG and affinity-purified rabbit polyclonal antibody (pAb) to human rLN- $\alpha$ 2G were described previously (7, 13). The mAb F47-21 to native PGL-1 was a gift from A. H. J.

Kolk (Royal Tropical Institute, Amsterdam). The pAb to S-100 was from Sigma.  $\alpha$ -DG detection and clustering studies were performed as previously reported [M. W. Cohen, C. Jacobson, P. D. Yurchenco, G. E. Morris, S. Carbonetto, *J. Cell Biol.* **136**, 1047 (1997)]. For bacterial-induced  $\alpha$ -DG clustering, *M. leprae* was preincubated with rLN- $\alpha$ 2G for 1 hour at 37°C, and the mixture was centrifuged and the pellet was resuspended in phosphate-buffered saline (PBS) to avoid the contact of free rLN- $\alpha$ 2G with Schwann cells. These rLN- $\alpha$ 2G-coated *M. leprae* were added onto primary Schwann cells as described in adhesion assays. Cultures were then stained live and fixed with 2.5% glutaraldehyde before processing for fluorescence microscopy. In live Schwann cells,  $\alpha$ -DG labeling is restricted to the dorsal surface because IIH6C4 immunoglobulin M mAb is unable to reach the ventral cell surface due to its large size. Colocalization of  $\alpha$ -DG and *M. leprae* was performed by double immunofluorescence with mAb IIH6 and mAb to *M. leprae* PGL-1.

29. We thank P. J. Brennan for providing *M. leprae* through a National Institute of Allergy and Infectious Disease/NIH contract, M. Zsack for graphics, and S. Terlow for *M. leprae* preparations. We also thank E. Tuomanen for the initial support and encouragement for this study. Supported by grants from the United Nations Development Programme/World Bank/World Health Organization Special Program for Research in Tropical Diseases and NIH (A.R., V.A.F., J.L.S., and P.Y.). H.Y. was supported by an American Heart Association fellowship and by the Mizutani Foundation. K.P.C. is an HHMI investigator.

18 August 1998; accepted 4 November 1998

## Identification of $\alpha$ -Dystroglycan as a Receptor for Lymphocytic Choriomeningitis Virus and Lassa Fever Virus

Wei Cao, Michael D. Henry, Persephone Borrow, Hiroki Yamada, John H. Elder, Eugene V. Ravkov, Stuart T. Nichol, Richard W. Compans, Kevin P. Campbell, Michael B. A. Oldstone\*

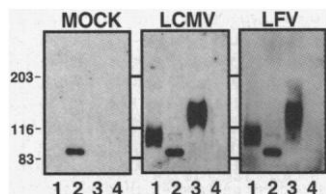
A peripheral membrane protein that is interactive with lymphocytic choriomeningitis virus (LCMV) was purified from cells permissive to infection. Tryptic peptides from this protein were determined to be  $\alpha$ -dystroglycan ( $\alpha$ -DG). Several strains of LCMV and other arenaviruses, including Lassa fever virus (LFV), Oliveros, and Mobala, bound to purified  $\alpha$ -DG protein. Soluble  $\alpha$ -DG blocked both LCMV and LFV infection. Cells bearing a null mutation of the gene encoding DG were resistant to LCMV infection, and reconstitution of DG expression in null mutant cells restored susceptibility to LCMV infection. Thus,  $\alpha$ -DG is a cellular receptor for both LCMV and LFV.

Arenaviruses consist of several causative agents of fatal human hemorrhagic fevers (1, 2). Among these pathogens, LFV causes an estimated 250,000 cases and more than 5000 deaths annually (1, 3). LCMV, the prototype arenavirus, has been studied primarily in its natural rodent host as a model of viral immunology and pathogenesis (4).

To initiate infection, the LCMV glycoprotein GP-1 anchors the virus to the cell surface through a proteinaceous receptor (5, 6),

which by a virus overlay protein blot assay (VOPBA) (7) was identified as a single high molecular weight glycoprotein (5). The presence of the receptor protein correlated directly with a cell's susceptibility to LCMV attachment and infection (Fig. 1). Its broad migration pattern on SDS-polyacrylamide gels is likely to reflect the heterogeneity in cell type-specific posttranslational modifications (5). In addition to murine cells, a broad range of rodent and primate cells express the same protein (5) (Fig.

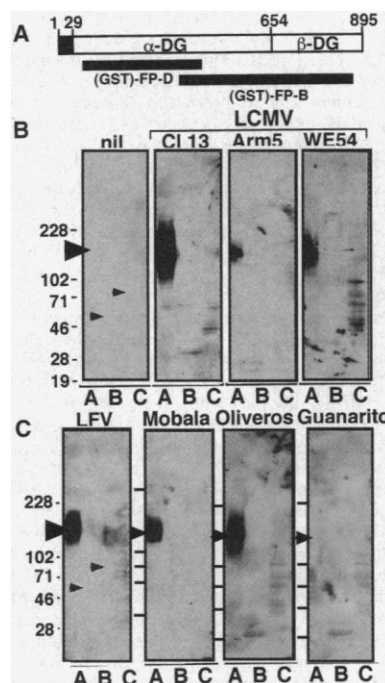
## REPORTS



**Fig. 1.** Binding of LCMV and LFV to cell membrane proteins. VOPBA was performed with either enriched uninfected baby hamster kidney cell culture fluid (Mock), LCMV (strain CI 13), or LFV on identical blots. Each lane on the blot contained 50 µg of purified cell membrane proteins prepared from two cell lines susceptible to LCMV infection: monkey kidney line Vero E6 (lane 1) and mouse fibroblast line MC57 (lane 3); and two cell lines resistant to LCMV infection and binding, human T lymphocyte line Jurkat (lane 2) and mouse T lymphocyte line RMA (lane 4). Molecular size markers are indicated at the sides (in kilodaltons).

1). LFV bound to what appears to be the same glycoprotein (Fig. 1), suggesting that both viruses may share a common cellular receptor.

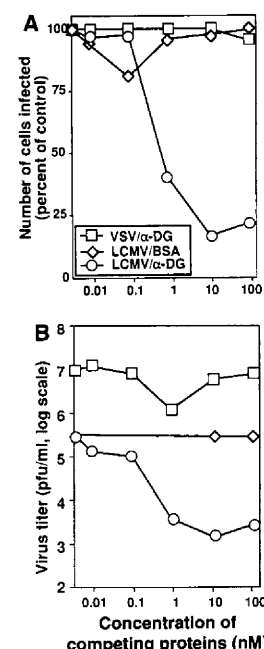
This putative receptor protein was purified from a LCMV permissive cell line by sequential column chromatography (8), and a total of five tryptic peptides were sequenced (9). Peptides GT384, GT441, and GT417 showed complete homology to dystroglycan (DG) precursor protein at the regions of amino acids 610 to 623, 571 to 585, 516 to 533, respectively. DG is encoded by a single gene and processed into two mature proteins,  $\alpha$ - and  $\beta$ -DG, which form a complex spanning the plasma membrane (10). The sequences corresponding to the three peptides are near the COOH-terminus of  $\alpha$ -DG.  $\alpha$ -DG is an extracellular peripheral membrane protein that binds to the extracellular matrix and is noncovalently associated with  $\beta$ -DG, which is a transmembrane protein linked to the cytoskeleton (11). DG complex is expressed in a wide variety of tissues and cells, and plays an important role in mediating cell-extracellular matrix interactions (12, 13). The LCMV receptor was preferentially expressed on the basolateral surface of polarized Madin-Darby canine kidney (MDCK) cells, facilitating a basolateral route of LCMV entry (14). This parallels a



**Fig. 2.** Binding of LCMV, LFV, and other arenaviruses to  $\alpha$ -DG. (A) Diagram showing DG precursor and GST-fusion proteins. The signal sequence (residues 1 to 28) is shown as a solid box. The sequence corresponding to  $\alpha$ -DG (residues 29 to 653) and  $\beta$ -DG (residues 654 to 895) are shown as open boxes. Fusion protein GST-FP-D contains residues 62 to 438 of the DG precursor. Fusion protein GST-FP-B contains residues 367 to 863 of the DG precursor. (B) Four identical blots containing purified DG were used in VOPBAs with either no virus (nil) or LCMV strains CI 13, Arm5, and WE54. Each blot contained protein samples from three different sources. Lane A contained 10 µg of purified 156-kD  $\alpha$ -DG protein from rabbit skeletal muscle; lane B contained 40 µg of recombinant 68-kD GST-FP-D; and lane C contained 40 µg of recombinant 82-kD GST-FP-B. Arrows indicate the expected full-length proteins. (C) Blots as described in (B) were used in VOPBA with arenaviruses LFV, Mobala, Oliveros, and Guanarito.

similar localization of DG expression (12).

The interaction between  $\alpha$ -DG and LCMV was demonstrated in VOPBAs with several strains of LCMV [CI 13, Armstrong 5 (Arm5), and WE54] on blots containing purified  $\alpha$ -DG proteins (Fig. 2B). All these LCMV strains bound to purified native  $\alpha$ -DG protein (15) (Fig. 2B). In contrast, none of these viruses recognized the *Escherichia coli*-expressed glutathione S-transferase (GST)-fusion proteins with either FP-D or FP-B (Fig. 2B), which encode different regions of DG precursor sequence (10) (Fig. 2A), suggesting that the extreme NH<sub>2</sub>-terminus of  $\alpha$ -DG and possibly posttranslational modifications on this protein are crucial for LCMV recognition. A similarly purified glycoprotein, the  $\alpha$ 2 subunit of the dihydropyridine receptor complex (16), also negatively charged through glycosylation, did not bind to LCMV (17). Like LCMV, the arenaviruses



**Fig. 3.** Blocking of infection by LCMV with soluble  $\alpha$ -DG protein. (A) 3T6 mouse fibroblast cells were infected with LCMV strain CI 13 or VSV in the presence of increasing concentrations of  $\alpha$ -DG. As a control, LCMV CI 13 infection was also performed in the presence of increasing concentrations of bovine serum albumin. Sixteen hours later, cells were immunostained with specific antibodies to detect LCMV nucleoprotein or VSV glycoprotein (18), and the number of infected cells was observed under fluorescence microscopy. The results were quantitated as an average of at least four fluorescent areas and plotted as percentage of control where no competing protein was added. (B) Virus titers in the 3T6 culture supernatants 16 hours after infection were determined by plaque assay. Virus titers [log (plaque-forming units) per milliliter] are plotted against the concentration of competing proteins added during absorption.

LFV, Mobala, and Oliveros bound to purified native  $\alpha$ -DG protein, but not to the recombinant GST-FP-D or GST-FP-B proteins (Fig. 2C). In contrast, the arenavirus Guanarito failed to recognize  $\alpha$ -DG protein (Fig. 2C).

Purified soluble  $\alpha$ -DG competed with the cell surface virus receptor during LCMV infections in a dose-dependent manner (Fig. 3).  $\alpha$ -DG at 0.9 nM concentration effectively blocked LCMV infectivity (18) (Fig. 3A) and significantly reduced production of progeny LCMV (Fig. 3B). Soluble  $\alpha$ -DG blocked infection by LFV at similar concentrations, but roughly 100-fold higher amounts were required to block LCMV Arm5 infectivity (17). In contrast,  $\alpha$ -DG had no effect on the infection by an unrelated RNA virus vesicular stomatitis virus (VSV) (Fig. 3B).

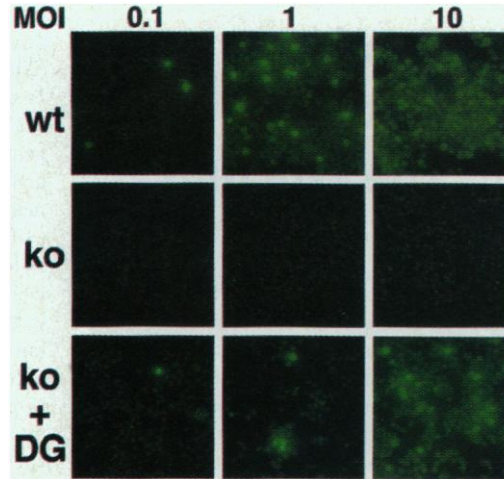
A mouse embryonic stem (ES) cell line expressing DG [wild type (wt)] was readily infected by LCMV (Fig. 4; at a MOI of 10, >90% of cells were infected). However, LCMV replication did not occur in DG null ES

W. Cao and M. B. A. Oldstone, Division of Virology, Department of Neuropharmacology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. M. D. Henry, H. Yamada, K. P. Campbell, Howard Hughes Medical Institute, Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242, USA. P. Borrow, Edward Jenner Institute for Vaccine Research, Compton, Newbury, Berkshire, RG20 7NN, UK. J. H. Elder, Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. E. V. Ravkov and R. W. Compans, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA. S. T. Nichol, Center for Disease Control, 1600 Clifton Road, NE, MSG14, Atlanta, GA 30333, USA.

\*To whom correspondence should be addressed. E-mail address: mbaobo@scripps.edu

## REPORTS

**Fig. 4.** Requirement of  $\alpha$ -DG expression by LCMV infection. Parental wt ES cells, DG null ko cells, and ko cells reconstituted with dystroglycan (ko+DG) were infected with LCMV strain CI 13 at a multiplicity of infection (MOI) of 0.1, 1, and 10, respectively. Sixteen hours later, cells were stained with mAb 1-1-3 to detect LCMV nucleoprotein by immunofluorescence. DG reconstitution was achieved by infecting ko cells with an adenovirus vector carrying rabbit DG cDNA.



(ko) cells (19) (Fig. 4; at a MOI of 10, <0.1% of cells were infected), yet these cells were viable, maintained a growth rate similar to the parental cells, and were equally infectable by VSV (17). When the  $\alpha$ -DG protein was restored on the surface of ko cells by infection with an adenovirus vector carrying DG cDNA (19), these cells again became susceptible to LCMV infection (20) (Fig. 4; at a MOI of 10, ~75% of cells were infected). However, when the same adenovirus vector expressing green fluorescent protein or LacZ was used for infection, less than 0.1% of ko cells were infectable by LCMV (17).

Old World arenaviruses including LCMV, LFV, and Mobala, which are phylogenetically and serologically distinct from the New World arenaviruses (1, 21), specifically recognized the  $\alpha$ -DG receptor protein. Oliveros virus, a group C New World arenavirus (1, 22), also bound to  $\alpha$ -DG in VOPBA, but Guanarito, another New World arenavirus, failed to do so. Use of ES ko cells alone and reconstituted with DG may prove valuable to further group the arenaviruses.

Cellular receptors are key elements in determining the tropism and pathogenesis of virus infection. The presence of  $\alpha$ -DG in all the tissues and organs examined to date correlates with the tropism of LCMV (5, 23). High sequence conservation in the gene encoding DG (11) supports the broad host range for LCMV and arenavirus infections of animals and humans (1). Characterization of the  $\alpha$ -DG-arenavirus interaction should elucidate the early events of arenavirus infection and facilitate the development of strategies to intervene and prevent this crucial interaction.

### References and Notes

- C. Peters, M. Buchmeier, P. Rollin, T. Ksiazek, in *Fields Virology*, B. Fields, Ed. (Lippincott-Raven, Philadelphia, 1996), vol. 2, pp. 1521-1551.
- A. Fabiyi, *Bull. Pan Am. Health Organ.* **10**, 335 (1976); R. Salas, et al., *Lancet* **338**, 1033 (1991).
- J. Frame, J. J. Baldwin, D. Gocke, J. Troup, *Am. J. Trop. Med. Hyg.* **19**, 670 (1970); J. McCormick, P. Webb, J. Krebs, K. Johnson, E. Smith, *J. Infect. Dis.* **155**, 437 (1987).
- P. Borrow and M. B. A. Oldstone, in *Viral Pathogenesis*, N. Nathanson, Ed. (Lippincott-Raven, Philadelphia, 1997), pp. 593-627; R. M. Zinkmagel, *Science* **271**, 173 (1996); *Scand. J. Immunol.* **46**, 421 (1997); B. A. Oldstone, R. S. Fujinami, P. W. Lampert, *Prog. Med. Virol.* **26**, 45 (1980); W. P. Rowe, *Nav. Med. Res. Inst. Rep.* **12**, 167 (1954).
- P. Borrow and M. Oldstone, *J. Virol.* **66**, 7270 (1992).
- J. Burns and M. Buchmeier, *Virology* **183**, 620 (1991).
- Modified from (5). A goat antibody to mouse immunoglobulin G (anti-mouse IgG) conjugated with horseradish peroxidase (Pierce) [1:5000 dilution in phosphate-buffered saline (PBS)] was used to substitute rabbit anti-mouse IgG and 1125-protein A. Afterward, an enhanced chemiluminescence assay was performed with the SuperSignal Chemiluminescent substrate (Pierce).
- Monolayers of MC57 cells were dissociated with PBS and 5 mM EDTA. Cells were solubilized in a buffer containing 20 mM tris (pH 7.4), 0.15 M NaCl, 1.5% octyl-glucoside, 0.5 mM phenylmethylsulfonyl fluoride, and 1 $\times$  Complete protease inhibitor cocktail (Boehringer Mannheim) for 60 min on ice. The sample was then spun for 15 min at 2500g, and the supernatant was centrifuged again for 60 min at 100,000g at 4°C. The cleared sample was loaded onto a Mono Q HR5/5 column (Pharmacia) and eluted with 1 M NaCl. Aliquots of the protein fractions were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 6% polyacrylamide gels for VOPBA analysis to locate the fractions enriched for LCMV-binding activity. The peak fractions were combined and passed through a 10-ml lentil-Sepharose column that did not retain the putative receptor of LCMV. Flow-through material was then loaded directly onto a 10-ml wheat germ agglutinin-Sepharose column, from which the protein was eluted with 0.5 M N-acetylglucosamine. Subsequently, VOPBA-positive fractions were combined and put through a 5-ml jacalin-Sepharose column. After the column was washed with 0.2 M melibiose, 1 M NaCl, 3 M KSCN, and 1% SDS to remove contaminating proteins, the whole column was disrupted and the Sepharose was boiled in SDS sample buffer with 2.5%  $\beta$ -mercaptoethanol for 5 min. The denatured sample was then concentrated fivefold by UltraFree-MC centricons (Millipore) before resolved on a 6% SDS-polyacrylamide gel.
- The Coomassie blue-stained protein band with VOPBA binding activity was excised from the SDS-polyacrylamide gel and submitted for peptide sequencing analysis. After in-gel digestion with trypsin, the sequences of five peptides were determined at the Harvard Microchemistry Facility by collisionally activated dissociation on a Finnigan TSQ7000 Triple Quadrupole Mass Spectrometer. GT384 (AGDPAPV-VNDIHK) (24), GT441 (GGLSAVDAFEIHVHK), and GT417 (IPSDTFYDNEDTTTDLK) were homologous to regions of DG precursor. GT429 (AFDDGAFTGIR) was homologous to amino acids 3 to 13 of jacalin and GT348 (KLERKLEK) to amino acids 31 to 39 of U1 small nuclear ribonucleoprotein.
- O. Ibraghimov-Beskrovnya et al., *Nature* **355**, 696 (1992).
- M. Henry and K. Campbell, *Curr. Opin. Cell Biol.* **8**, 625 (1996).
- M. Durbecq, M. Henry, M. Ferletta, K. Campbell, P. Ekblom, *J. Histochem. Cytochem.* **46**, 449 (1998).
- R. Williamson et al., *Hum. Mol. Genet.* **6**, 831 (1997).
- P. Borrow, E. V. Ravkov, C. J. Peters, M. B. A. Oldstone, S. T. Nichol, in preparation.
- H. Yamada et al., *J. Neurochem.* **66**, 1518 (1996).
- The  $\alpha$ 2 subunit of the dihydropyridine receptor complex was purified from rabbit skeletal muscle as described in S. D. Jay et al., *J. Biol. Chem.* **266**, 3287 (1991).
- W. Cao et al., unpublished results.
- We plated  $1 \times 10^5$  of 3T6 cells per well into a 24-well plate the day before the experiment. Aliquots of  $1.6 \times 10^5$  plaque-forming units of LCMV strain CI 13 and VSV Indiana strain were incubated for 20 min on ice with either bovine serum albumin (BSA) or purified  $\alpha$ -DG protein from rabbit skeletal muscle at concentrations of 0, 9 pM, 90 pM, 0.9 nM, 9 nM, and 90 nM in a final volume of 150  $\mu$ l. One aliquot of treated virus was then incubated with cells for 30 min at 37°C before being replaced with fresh growth medium. Sixteen hours later, virus titers from culture supernatant were determined by plaque assay on Vero cells. Cells were fixed with acetone and analyzed by immunofluorescence staining with a monoclonal antibody (mAb) 1-1-3 to detect LCMV nucleoprotein or mAb 11 to detect VSV glycoprotein. A fluorescein isothiocyanate-conjugated goat anti-mouse IgG diluted 1:20 with PBS was used as the secondary antibody in the staining.
- M. D. Henry and K. P. Campbell, *Cell*, in press. Briefly, DG<sup>-/-</sup> ES cells were isolated from two independently derived parental DG<sup>-/-</sup> ES cell clones TD354 and TD556 generated during the initial DG gene targeting effort by selection with active G418 (1.0 mg/ml). Surviving clones were analyzed by Southern (DNA) blot with a Hind III-Pst I fragment from intron 1 of the mouse DG gene as a probe. The DG adenovirus was constructed by subcloning a rabbit cDNA into the pAdRSVPa shuttle vector and then incorporated into an adenovirus vector through standard methods of homologous recombination. The loss of DG protein expression in clones exhibiting homozygous loss of the wild-type DG allele and expression of DG in DG null cells as a result of adenovirus infection were confirmed by protein immunoblot and immunofluorescence analysis.
- We plated  $2 \times 10^5$  of ES cells per well into a 24-well plate the day before  $2 \times 10^9$  adenovirus particles carrying rabbit DG cDNA (estimated MOI of 5) was added in the culture medium. Two days later, aliquots of LCMV CI 13 virus in 200  $\mu$ l were added to each well. After incubation for 45 min at 37°C, the inoculum was replaced by 1 ml of fresh ES growth medium. Sixteen hours later, cells were fixed with acetone and immunostained with mAb 1-1-3 to detect the LCMV nucleoprotein.
- J. Clegg, in *The Arenaviridae*, M. Salvato, Ed. (Plenum, New York, 1993), pp. 175-185.
- M. Bowen, C. Peters, S. Nichol, *Virology* **219**, 285 (1996).
- J. Fazakerley, P. Southern, F. Bloom, M. Buchmeier, *J. Gen. Virol.* **72**, 1611 (1991).
- Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
- We thank W. Lane at Harvard Microchemistry facility for peptide sequencing; Y. Lu and H. Lewicki for technical assistance; J. C. de la Torre, M. Manchester, and J. Paulson for experimental advice and consultation; and J. C. de la Torre, M. Manchester, D. Naniche, I. Novella, S. Huang, C. Evans, and C. Lebakken for helpful discussions and comments on the manuscript. Supported by a research grant AI 09484 for M.B.A.O. and a training grant AG 00080 for W.C. from the NIH. M.D.H. was supported by a National Research Service Award (DK09712) and by the Iowa Cardiovascular Research Fellowship (HL071221). H.Y. was supported by American Heart Association Fellowship (IA-97-FW-25) and by the Mizutani Foundation. K.P.C. is an investigator of the Howard Hughes Medical Institute.

25 August 1998; accepted 4 November 1998