23. J. Vilcek and T. H. Lee, *J. Biol. Chem.* **266**, 7313 (1991).
24. H. Loetscher *et al.*, *Cell* **61**, 351 (1990); T. Schall *et al.*, *ibid.*, p. 361; C. A. Smith *et al.*, *Science* **248**, 1019 (1990); Z. Dembic *et al.*, *Cytokine* **2**, 231 (1990).
25. M. Brockhaus *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3127 (1990).
26. B. Thoma, M. Grell, K. Pfizenmaier, P. Scheurich, *J. Exp. Med.* **172**, 1019 (1990).
27. H. P. Hohmann, R. Remy, M. Brockhaus, A. P. G. M. van Loon, *J. Biol. Chem.* **264**, 14927 (1989); T. Espevik, M. Brockhaus, H. R. Loetscher, U. Nonstad, R. Shalaby, *J. Exp. Med.* **171**, 415 (1990); H. Engelmann *et al.*, *J. Biol. Chem.* **265**, 13397 (1990); B. Ryffel, M. Brockhaus, U. Durmuller, F. Gudat, *Am. J. Pathol.* **139**, 7 (1991).
28. K. A. Dressler, C.-C. Kan, R. N. Kolesnick, *J. Biol. Chem.* **266**, 11522 (1991); R. N. Kolesnick and S. Clegg, *ibid.* **263**, 6534 (1988).
29. J. Preiss *et al.*, *ibid.* **261**, 8597 (1986).
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Identification of the Integrin VLA-2 as a Receptor for Echovirus 1

JEFFREY M. BERGELSON,* MICHAEL P. SHEPLEY,† BOSCO M. C. CHAN,‡ MARTIN E. HEMLER, ROBERT W. FINBERG

Cell surface receptors for echovirus, a common human pathogen, were identified with monoclonal antibodies that protected susceptible cells from infection with echovirus 1. These monoclonal antibodies, which prevented virus attachment to specific receptor sites, recognized the α and β subunits of the integrin VLA-2 ($\alpha_2\beta_1$), a receptor for collagen and laminin. RD rhabdomyosarcoma cells expressed little VLA-2, did not bind to ^{35}S-labeled virus, and resisted infection until transfected with complementary DNA encoding the α_2 subunit of VLA-2. Thus, integrins, adhesion receptors important in interactions between cells and with the extracellular matrix, can mediate virus attachment and infection.

SPECIFIC RECEPTORS HAVE BEEN identified for only a few of the viruses that cause human disease (*1*). Echoviruses, nonenveloped RNA viruses belonging to the picornavirus family, are frequently responsible for febrile illness and viral meningitis (*2*). In newborn infants echoviruses can cause fatal disseminated infections (*2*). The integrin family of adhesion receptors is composed of at least 7 different β subunits and 14 different α subunits that associate to form at least 16 different heterodimers (*3*). Integrins mediate cell-to-cell interactions as well as cell interactions with the extracellular matrix and are involved in diverse functions such as cell migration, inflammation, and thrombus formation (*3–5*). VLA-2 ($\alpha_2\beta_1$) mediates cell attachment to collagen and laminin (*6*) and participates in tumor metastasis in vivo (*7*).

To identify cell surface proteins involved in echovirus attachment, we immunized mice with HeLa cells to obtain monoclonal antibodies (MAbs) capable of protecting cells from echovirus infection (*8*). Two protective MAbs, DE9 [immunoglobulin G1 (IgG1)] and AA10 (IgM), were obtained from independent hybridoma fusions. Binding of [^{35}S]methionine echovirus 1 was reduced by 90% when HeLa cell monolayers were first incubated with AA10, but this MAb did not inhibit binding of poliovirus 2 (Fig. 1A). DE9 reduced the binding of radiolabeled echovirus 1 by about 50%, but only when rabbit antibody to mouse immunoglobulin was added (Fig. 1B) (*9*).

Although both MAbs protected HeLa cells from the cytopathic effect of echovirus 1, they did not prevent cytopathic effects caused by other picornaviruses. AA10 protected cells from infection even at high multiplicity [100 plaque-forming units (PFU) per cell]. In addition, plaque formation by echovirus 1 was inhibited 100% when target monolayers were preincubated with AA10 (20 μg/ml): no plaques were seen when more than 100,000 PFU were added to AA10-treated monolayers. There was no comparable inhibition of plaque formation by other picornaviruses (Fig. 2). Incubation of echovirus directly with AA10 (20 μg/ml) did not reduce the viral titer, which indicates that the protective effect of AA10 resulted from its interaction with cells, not the virus.

Each MAb immunoprecipitated a pair of proteins (125 and 145 kD) from surface-radioiodinated HeLa cells under two detergent conditions (Fig. 3A). AA10 immunoprecipitated more of the larger protein. The molecular weights of the precipitated proteins resembled those reported for β_1 integrins (VLA proteins), which consist of a common 130-kD β_1 subunit in association with unique α subunits of 120 to 210 kD (*5*). Immunoprecipitation with DE9 and AA10 revealed the same protein patterns seen with Abs to the β_1 and α_2 integrin subunits of VLA-2 (Fig. 3B). Immunodepletion with a MAb to the β_1 subunit eliminated almost all material precipitable by DE9 (Fig. 3D). Depletion with AA10 itself

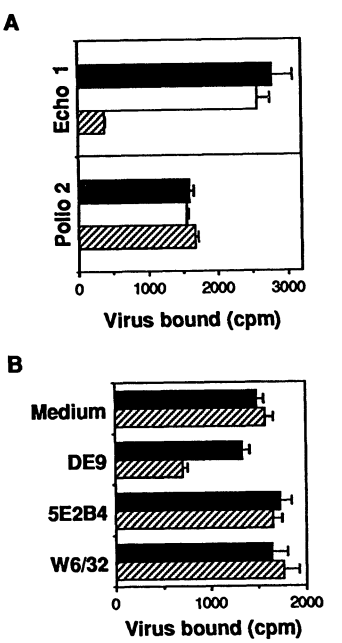

Fig. 1. Attachment of echovirus 1 to HeLa cell monolayers. Echovirus 1 and poliovirus 2w2 were labeled with [^{35}S]methionine and purified (*8*). Purity of the labeled virus was confirmed by electrophoresis in 12.5% polyacrylamide gels. (**A**) HeLa monolayers in 24-well tissue culture plates were first incubated for 30 min with MAbs (20 μg/ml) at room temperature, then washed and incubated for 30 min with the labeled virus (~20,000 cpm) in Hanks balanced salt solution containing 10 mM Hepes (pH 7.0), 20 mM $MgCl_2$, and 4% fetal calf serum. Dark bars, preincubated with medium; white bars, preincubated with control T9 (Coulter, Hialeah, Florida), an isotype-matched MAb to a HeLa cell surface antigen; hatched bars, preincubated with AA10. Radiolabeled virus bound per confluent monolayer (mean ± SD cpm bound for triplicate samples) is shown. Experiments were performed three times. (**B**) MAb-treated monolayers (dark bars) were washed and incubated with rabbit antibodies to mouse immunoglobulin (40 μg/ml; hatched bars) (Cappel, West Chester, Pennsylvania) for an additional 30 min before washing and addition of radiolabeled virus as in (A). 5E2B4 (*26*) recognizes the intracellular protein Tau. W6/32 (murine IgG2a, ATCC) recognizes a framework determinant of human major histocompatibility complex (MHC) class I, which is expressed on HeLa cells. Radiolabeled virus bound per confluent monolayer (mean ± SD cpm for four samples) is shown. In control experiments, DE9 did not inhibit binding of poliovirus 2, whether or not second antibody was used.

J. M. Bergelson, M. P. Shepley, R. W. Finberg, Laboratory of Infectious Disease, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.
B. M. C. Chan and M. E. Hemler, Division of Tumor Virology, Dana-Farber Cancer Institute, Boston, MA 02115.

*To whom correspondence should be addressed.
†Present address: Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, NY 10032.
‡Present address: Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario, NIG 2W1, Canada.

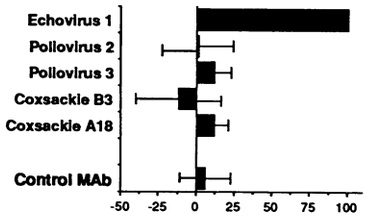

Fig. 2. Inhibition of viral plaque formation by AA10. HeLa cell monolayers in six-well tissue culture dishes (~10^6 cells per well) were incubated for 30 min at room temperature with medium, AA10 (20 μg/ml), or an isotype-matched control MAb, 23A-5-21S (anti-H-2D^b; ATCC; 20 μg/ml). Virus (~50 PFU per well) was added and incubation continued 30 to 60 min before monolayers were overlaid with agar and plaques developed as described (*8*). This experiment was performed twice. Results are expressed as mean percent inhibition = 100% × [(plaque number$_{medium}$ − plaque number$_{MAb}$)/ plaque number$_{medium}$]. For triplicate wells, 95% confidence intervals are shown.

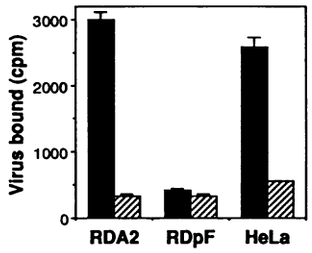

Fig. 4. Cell attachment of [^{35}S]echovirus 1 is promoted by VLA-2 expression. RD cells transfected with VLA α_2 (RDA2) or mock-transfected with the pFneo vector (RDpF) as described (*7*) were incubated with AA10 (hatched bars) or isotype-matched control MAb (solid bars), then binding of labeled echovirus 1 was determined as described (Fig. 1). Results are expressed as mean (± SD) cpm for triplicate monolayers. This experiment was performed three times with similar results. In control experiments, labeled poliovirus bound equally well (2700 to 2800 cpm) to RDA2, RDpF, and HeLa cell monolayers.

or with anti-α_2 serum (but not with nonimmune serum) eliminated all material precipitable by AA10 (Fig. 3C). These results suggest that DE9 recognized the β_1 subunit, and AA10 the α_2 subunit of VLA-2.

The RD rhabdomyosarcoma cell line expresses little VLA-2 but does express β_1 in association with other α chains (VLA-1, VLA-4, VLA-5, and VLA-6) (*7, 10*). When α_2 cDNA is transfected into RD cells, the expressed α_2 protein associates with the endogenous β_1 subunit, which results in cell surface expression of VLA-2 (*7*). To confirm the immunoprecipitation results, we used DE9, AA10, and prototype MAbs to β_1 (*11*) and α_2 (*12*) to label RD cell transfectants for analysis by indirect flow cytofluorometry. RD cells, mock-transfected with the expression vector pFneo (RDpF), bound DE9 and anti-β_1 but expressed very little antigen detectable by AA10 or the prototype anti-α_2 antibody. In contrast, RDA2 cells, transfected with α_2 cDNA, expressed VLA-2 as detected by both AA10 and anti-α_2.

RDA2 monolayers bound significantly more radiolabeled echovirus 1 than did RDpF mock transfectants and the binding was inhibited by AA10 (Fig. 4). RDA2 cells were also more susceptible to echovirus 1: infection at low multiplicity (0.5 PFU per cell) caused destruction of RDA2 monolayers within 24 hours. In contrast, RDpF cells infected at higher multiplicity (2 PFU per cell) showed no cytopathic effect after 48 hours (*13*). AA10 protected RDA2 cells from infection at a multiplicity of 100 PFU per cell.

Our results demonstrate that VLA-2 is a receptor for echovirus 1. Virus attachment and infection were inhibited by AA10 and DE9, which recognized the α_2 and β_1 subunits of VLA-2, respectively. VLA-2 expression by RD cell transfectants significantly increased their capacity to bind the virus and become infected. Other β_1 integrins (VLA-1, VLA-4, VLA-5, and VLA-6 on untransfected RD cells) do not appear to act as echovirus 1 receptors. Thus, the α_2 chain of VLA-2 probably determines the specificity of virus binding (*14*). Once the virus is bound, the receptor is likely to be involved in virus internalization and other early events in infection. These functions may depend on one or both of the subunits. Genetic manipulation of the α_2 and β_1 subunits should make it possible to identify protein domains involved in attachment and subsequent events.

Receptors for two other human picornaviruses, poliovirus (*15*) and rhinovirus (*16*), are members of the immunoglobulin superfamily and are characterized by immunoglobulin-like, disulfide-linked protein domains. It has been suggested that a single immunoglobulin domain might fit (*17*) within a depression on the picornavirus surface (*18, 19*) that is a proposed site for cell attachment (*19, 20*). Neither of the VLA-2 subunits (*21, 22*) is similar to immunoglobulin.

Other picornaviruses have been grouped in receptor families on the basis of their ability to compete for cell surface attachment sites (*23*), but the 32 echovirus serotypes have not been studied systematically. Echovirus 6 was not inhibited by AA10 and did not compete with echovirus 1 for receptor sites (*24*). Some echoviruses of different serotypes, although associated with similar clinical syndromes, probably bind receptors other than VLA-2; receptors for these (and other) viruses may include members of the integrin family. Newborn infants, but not adults, are susceptible to disseminated echovirus infection. Whether this phenomenon relates to developmental regulation of receptor expression, which has been observed for VLA-2 and other β_1 integrins (*25*), remains to be determined.

We have found that VLA-2–mediated attachment of virus and extracellular matrix ligands occur by distinct mechanisms, as determined by cation requirements, response to phorbol esters, and sensitivity to specific MAbs (*24*). The identification of VLA-2 as an echovirus receptor will broaden our understanding of integrin function as well as viral pathogenesis.

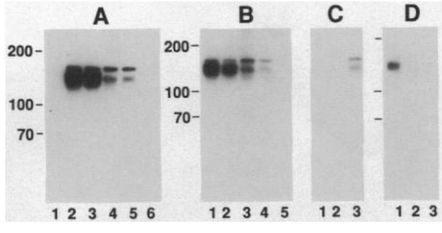

Fig. 3. Immunoprecipitation of radioiodinated HeLa cell surface proteins. HeLa cell monolayers were iodinated by the lactoperoxidase–glucose oxidase method and lysed in buffer [25 mM tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 8 mM *N*-ethylmaleimide, and 20 U/ml aprotinin] containing 1% Triton X-100. Iodination, immunoprecipitation with rabbit anti-mouse–coated protein A–Sepharose beads, and analysis by electrophoresis in 7.5% polyacrylamide gels under reducing conditions were performed as described (*27*). Molecular weight markers (kilodaltons) are shown at left. Each part of this experiment was performed two or three times with similar results. (**A**) Precipitation with DE9 and AA10. Immunoprecipitates bound to antibody-coated beads were washed extensively in lysis buffer (lanes 1, 2, 4, and 6) or in buffer containing 1% deoxycholate and 0.1% SDS in addition to 1% Triton (lanes 3 and 5). Lane 1, IgG1 control; lanes 2 and 3, DE9; lanes 4 and 5, AA10; and lane 6, IgM control. (**B**) Precipitation with DE9 and AA10 in parallel with antibodies to VLA-2. Lane 1, DE9; lane 2, anti-β_1 (A-1A5) (*11*); lane 3, AA10; lane 4, rabbit antiserum to α_2 (*20*); lane 5, control rabbit serum. (**C**) Precipitation with AA10 after immunodepletion with specific antibody-coated beads. Lane 1, precleared with AA10; lane 2, precleared with rabbit antiserum to α_2; lane 3, precleared with control rabbit serum. (**D**) Precipitation with DE9 after immunodepletion. Lane 1, precleared with IgG1 control MAb; lane 2, precleared with DE9; lane 3, precleared with anti-β_1 (A-1A5).

REFERENCES AND NOTES

1. K. L. Tyler and B. N. Fields, in *Virology*, B. N. Fields and D. M. Knipe, Eds. (Raven, New York, ed. 2, 1990), chap. 10.
2. J. Cherry, in *Infectious Disease of the Fetus and Newborn Infant*, J. Remington and J. Klein, Eds. (Saunders, New York, ed. 2, 1983), chap. 7; J. F. Modlin, *Pediatr. Infect. Dis. J.* 7, 311 (1988); J. L. Melnick, in *Virology*, B. N. Fields and D. M. Knipe, Eds. (Raven, New York, ed. 2, 1990), chap. 21.
3. S. K. Akiyama, K. Nagata, K. M. Yamada, *Biochim.*

Biophys. Acta **1031**, 91 (1991); R. O. Hynes, *Cell* **48**, 549 (1987); M. E. Hemler, in *Receptors for Extracellular Matrix*, R. P. Mecham and J. A. McDonald, Eds. (Academic Press, San Diego, 1991), pp. 255–260.
4. S. M. Albelda and C. A. Buck, *FASEB J.* **4**, 2868 (1990); R. S. Larson and T. A. Springer, *Immunol. Rev.* **114**, 181 (1990); E. F. Plow and M. H. Ginsberg, *Prog. Hemostasis Thromb.* **9**, 117 (1989).
5. M. E. Hemler, C. Huang, L. Schwarz, *J. Biol. Chem.* **262**, 3300 (1987); M. E. Hemler, *Annu. Rev. Immunol.* **8**, 365 (1990).
6. M. J. Elices and M. E. Hemler, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9906 (1989); L. R. Languino *et al.*, *J. Cell Biol.* **109**, 2455 (1989); W. Staatz, S. M. Rajpara, E. A. Wayner, W. G. Carter, S. A. Santoro, *ibid.* **108**, 1917 (1989); E. A. Wayner and W. G. Carter, *ibid.* **105**, 1873 (1987).
7. B. M. C. Chan, N. Matsuura, Y. Takada, B. R. Zetter, M. E. Hemler, *Science* **251**, 1600 (1991).
8. Echovirus 1 [American Type Culture Collection (ATCC)] was grown, purified, and assayed, and protective MAbs were generated as described for poliovirus 2 [M. P. Shepley, B. Sherry, H. L. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7743 (1988)]. DE9 (IgG1) was prepared by fusion of immune splenocytes with NS1 myeloma cells (ATCC). To generate AA10 (IgM), we fused splenocytes with P3×G3.Ag8U.1 (ATCC). Poliovirus 2w2 and poliovirus 3 (Leon) were from ATCC, coxsackie B3 was from R. Crowell, and coxsackie A18 was from R. Colonno.
9. Competition with excess unlabeled virus (1.5×10^8 PFU) confirmed that radiolabeled echovirus 1 and poliovirus 2 bound to specific, distinct, cell surface receptors on HeLa cells. Other cell lines susceptible to infection with echovirus 1 (HEL human primary fibroblasts, HEp-2 human carcinoma cells, and fibroblasts from rhesus monkey and African green monkey kidneys) all expressed the proteins identified by AA10 and DE9 (determined by cellular enzyme-linked immunosorbent assay) and bound echovirus 1 (from 27 to 36% of input radioactivity; in two experiments, results for duplicate samples did not differ more than 10%). In one experiment, duplicate monolayers were incubated with AA10 before addition of virus: binding to each susceptible cell line was reduced 92 to 96%, which indicates that the receptor identified by AA10 was responsible for echovirus attachment to a variety of susceptible cells. Nonprimate cell lines that did not bind AA10 or DE9 (murine L cells, Chinese hamster ovary cells, and canine MDCK cells) bound 1% or less of the input [^{35}S]echovirus 1.
10. M. E. Hemler *et al.*, *J. Biol. Chem.* **262**, 11478 (1987).
11. M. E. Hemler, C. F. Ware, J. L. Strominger, *J. Immunol.* **131**, 334 (1983).
12. K. D. Pischel, M. E. Hemler, C. Huang, H. G. Bluestein, V. L. Woods, *ibid.* **138**, 226 (1987).
13. However, RDpF cells did show cytopathic effect after longer incubation when infected at high multiplicity (20 to 100 PFU per cell), possibly a result of virus binding to VLA-2 present at very low levels on the parental RD cell (*7, 24*). AA10-treated RDpF cells did not show any cytopathic effect.
14. Two IgG MAbs to the α_2 subunit block virus binding as effectively as AA10, and two other β_1 MAbs, like DE9, block partially. Thus, α_2 MAbs block more effectively regardless of isotype.
15. C. L. Mendelsohn, E. Wimmer, V. R. Racaniello, *Cell* **56**, 855 (1989); S. Koike *et al.*, *EMBO J.* **9**, 3217 (1990).
16. J. M. Greve *et al.*, *Cell* **56**, 839 (1989); D. E. Staunton *et al.*, *ibid.*, p. 849; J. E. Tomassini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4907 (1989).
17. V. L. Giranda, M. S. Chapman, M. G. Rossmann, *Proteins* **7**, 227 (1990).
18. J. M. Hogle, M. Chow, D. J. Filman, *Science* **229**, 1358 (1985).
19. M. G. Rossmann *et al.*, *Nature* **317**, 145 (1985).
20. R. J. Colonno *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5449 (1988).
21. Y. Takada and M. E. Hemler, *J. Cell Biol.* **109**, 397 (1989).
22. W. S. Argraves *et al.*, *ibid.* **105**, 1183 (1987); J. W. Tamkun *et al.*, *Cell* **46**, 271 (1986).
23. R. L. Crowell and M. A. Landau, in *Comprehensive Virology*, H. Fraenkel-Conrat and R. R. Wagner, Eds. (Plenum, New York, 1983), vol. 18, pp. 1–42; G. Abraham and R. J. Colonno, *J. Virol.* **51**, 340 (1984).
24. J. M. Bergelson, M. P. Shepley, B. M. C. Chan, M. E. Hemler, R. W. Finberg, unpublished results.
25. M. Korhonen, J. Ylänne, L. Laitinen, I. Virtanen, *J. Cell Biol.* **111**, 1245 (1990).
26. Murine IgG1 from K. Kosik.
27. A. Haregewoin, G. Soman, R. C. Hom, R. W. Finberg, *Nature* **340**, 309 (1989).
28. We thank A. Haregewoin, G. Soman, and J. Fingeroth for technical advice and helpful discussions and B. Benacerraf, B. Fields, and V. Racaniello for review of the manuscript. Supported by NIH grants AI 20382 (R.W.F.) and GM 38903 (M.E.H.), the Jimmy Fund and the Dana-Farber Cancer Institute (M.P.S.), a centennial fellowship from the Medical Research Council of Canada (B.M.C.C.), and a fellowship from the Leukemia Society of America (J.M.B.).

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