motor EPSP, cells surrounding the sensory neuron and the motor neuron were labeled with Fast Green [D. V. Buonomano and J. H. Byrne, *Science* **249**, 420 (1990)]. The ganglia were incubated in L15 culture medium (Sigma) at 15°C. The culture medium contained 430 mM NaCl, 27 mM $MgSO_4$, 11 mM $CaCl_2$, 27 mM $MgCl_2$, 10 mM KCl, streptomycin sulfate (0.10 g/liter), penicillin-G (0.12 g/liter), and Hepes (7.15 g/liter). The pH of the culture medium was adjusted to 7.65. At 24 and 48 hours, the same sensory and motor neurons were reimpaled and EPSPs were reexamined. In each test period, three EPSPs were elicited by injecting brief suprathreshold depolarizing currents into a sensory neuron at an interstimulus interval (ISI) of 10 s. The mean values at 24 and 48 hours were normalized to that of the baseline test. All experiments were performed in a blind fashion. The experimenter did not know the composition of the solutions that were applied until after the sequence of experiments was completed.

12. Human TGF-β (TGF-β1, R&D Systems) was used in the experiments. Lyophilized recombinant human TGF-β1 samples were reconstituted with BSA solution. The final concentration of BSA was adjusted to 10 μg/ml in all the experiments. In control experiments, only BSA solution was used. In pilot experiments, no effects were observed when TGF-β1 was used at 10 ng/ml. The relatively high dose of TGF-β1 required to induce long-term facilitation could be attributable to a difference in potency of the species-specific forms. However, the concentration of TGF-β1 in human serum is >30 ng/ml [I. E. Eder *et al.*, *J. Urol.* **156**, 953 (1996)]. There are currently no known data from *Aplysia* or other systems regarding the effective duration of TGF-β application. Therefore, TGF-β1 was applied for 24 hours to provide sufficient time to induce long-term facilitation.
13. Excitability of a sensory neuron (separate from the one used for the EPSP test) was measured as the number of action potentials elicited during a constant-current pulse (1 s, 2 nA). In the TGF-β1 group, the number of action potentials was 144 ± 22% of baseline at 24 hours and 124 ± 20% at 48 hours (n = 15). These values were not significantly different ($F_{1,27}$ = 0.35) from the control group (115 ± 16% at 24 hours and 127 ± 20% at 48 hours, n = 14). The input resistance of motor neurons was measured by injecting a hyperpolarizing current pulse (2 nA). There was no significant difference ($F_{1,25}$ = 0.09) between the input resistance of the control group (102 ± 9% of baseline at 24 hours and 89 ± 8% at 48 hours) and the TGF-β1 group (98 ± 6% at 24 hours and 89 ± 7% at 48 hours). The threshold of a motor neuron was measured by injecting a graded depolarizing current until an action potential was elicited. No significant difference ($F_{1,26}$ = 0.26) in the threshold was observed between the control group (99 ± 2% at 24 hours and 100 ± 2% at 48 hours) and the TGF-β1 group (101 ± 3% at 24 hours and 101 ± 3% at 48 hours).
14. N. J. Emptage and T. J. Carew, *Science* **262**, 253 (1993).
15. N. Dale, E. R. Kandel, S. Schacher, *J. Neurosci.* **7**, 2232 (1987).
16. The same sensory and motor neurons were impaled three times (baseline, after 2 hours, and after 4 hours). TGF-β1 or BSA was applied immediately after the baseline test. Then, the connections were tested three times in each period, with an ISI of 5 min. The average of the three values in each period was normalized to the baseline value.
17. F. Zhang, S. Endo, L. J. Cleary, A. Eskin, J. H. Byrne, data not shown.
18. The nonadditive effect is probably not the result of a ceiling, because more facilitation was seen at 48 hours than at 24 hours. In addition, the sensorimotor synapses can be increased to 300 to 400% of baseline [M. Stopfer and T. J. Carew, *J. Neurosci.* **16**, 4933 (1996); F. Zhang and J. H. Byrne, unpublished data], which is more than the extent of facilitation reported here. Moreover, the peak amplitudes of the EPSPs did not approach either the reversal potential of the EPSP or the spike threshold, which indicated that a simple biophysical limitation on the amplitude of the EPSP was not reached.
19. TGF-β sRII (R&D Systems) is a polypeptide containing the extracellular domain of human TGF-β receptor type II [H. Y. Lin, X. F. Wang, E. Ng-Eaton, R. A. Weinberg, H. F. Lodish, *Cell* **68**, 775 (1992)]. This is a small soluble molecule that is capable of binding TGF-β1, TGF-β3, and TGF-β5 with sufficient affinity to act as an inhibitor with a half-maximal effective dose (ED_{50}) of ~30 ng/ml [M. L.-S. Tsang *et al.*, *Cytokine* **7**, 389 (1995)]. This approach was preferred to an attempt with antibodies to TGF-β1, because immunoglobulin does not penetrate well through the neuropil of the isolated ganglion preparation.
20. F. Zhang and J. H. Byrne, data not shown. Nerves were stimulated as described [F. Zhang *et al.*, in (*11*)].
21. D. L. Glanzman *et al.*, *J. Neurosci.* **9**, 4200 (1989); S. L. Mackey, E. R. Kandel, R. D. Hawkins, *ibid.*, p. 4227; Z. S. Zhang, B. Fang, D. W. Marshak, J. H. Byrne, L. J. Cleary, *J. Comp. Neurol.* **311**, 259 (1990).
22. E. R. Kandel *et al.*, in *Synaptic Function*, G. M. Edelman, W. E. Gall, W. M. Cowan, Eds. (Wiley, New York, 1987), pp. 471–518; J. H. Byrne, R. Zwartjes, R. Homayouni, S. D. Critz, A. Eskin, *Adv. Second Messenger Phosphoprotein Res.* **27**, 47 (1993); J. H. Byrne and E. R. Kandel, *J. Neurosci.* **16**, 425 (1996).
23. Several other second messenger cascades and regulatory proteins have been implicated in mediating aspects of the cellular changes associated with long-term sensitization [see (*22*) for reviews]. These agents could be components of the TGF-β pathway or could act in parallel to it. In addition, cleavage and activation of procollagen by apTBL-1, in a manner analogous to BMP-1 [E. Kessler, K. Takahara, L. Biniaminov, M. Brusel, D. S. Greenspan, *Science* **271**, 360 (1996)], could contribute to sensitization.
24. K. Yamaguchi *et al.*, *Science* **270**, 2008 (1995); H. Shibuya *et al.*, *ibid.* **272**, 1179 (1996).
25. E. Nishida and Y. Gotoh, *Trends Biochem Sci.* **18**, 128 (1993); K. J. Blumer and G. L. Johnson, *ibid.* **19**, 236 (1994); C. J. Marshall, *Cell* **80**, 179 (1995).
26. K. C. Martin *et al.*, *Soc. Neurosci. Abstr.* **22**, 1934 (1996); D. Michael, K. C. Martin, R. Seger, E. R. Kandel, *ibid.*, p. 1934.
27. Supported by Air Force Office of Scientific Research grant F49620-92-J-0494 (A.E.) and NIH grants RO1-NS-28462 (A.E.), RO1-NS-19895 (J.H.B.), and K05 MH-00649 (J.H.B.).

27 September 1996; accepted 2 January 1997

Isolation of a Common Receptor for Coxsackie B Viruses and Adenoviruses 2 and 5

Jeffrey M. Bergelson,* Jennifer A. Cunningham, Gustavo Droguett, Evelyn A. Kurt-Jones, Anita Krithivas, Jeong S. Hong, Marshall S. Horwitz, Richard L. Crowell, Robert W. Finberg

A complementary DNA clone has been isolated that encodes a coxsackievirus and adenovirus receptor (CAR). When transfected with CAR complementary DNA, nonpermissive hamster cells became susceptible to coxsackie B virus attachment and infection. Furthermore, consistent with previous studies demonstrating that adenovirus infection depends on attachment of a viral fiber to the target cell, CAR-transfected hamster cells bound adenovirus in a fiber-dependent fashion and showed a 100-fold increase in susceptibility to virus-mediated gene transfer. Identification of CAR as a receptor for these two unrelated and structurally distinct viral pathogens is important for understanding viral pathogenesis and has implications for therapeutic gene delivery with adenovirus vectors.

Adenoviruses and coxsackieviruses are common human pathogens. Adenoviruses are nonenveloped DNA viruses that cause respiratory and gastrointestinal infections (*1*) as well as infections of the heart (*2*). Adenoviruses have also been adapted for use as vectors for vaccination and gene therapy (*3*). Coxsackie B viruses are nonenveloped RNA viruses belonging to the picornavirus family. They cause meningoencephalitis (*4*), are implicated in acute pancreatitis (*5*) and as triggering agents in childhood-onset diabetes (*6*), and are the viruses most frequently identified in acute infections of the heart (*7*).

Coxsackievirus B3 forms a detergent-stable complex with a 46-kD HeLa cell surface protein (*8*). We have used a monoclonal antibody (mAb) raised against this complex (*9*) to isolate and clone a 46-kD protein that mediates attachment and infection by coxsackie B viruses. The same receptor also functions in adenovirus attachment and adenovirus-mediated gene transfer.

The coxsackievirus and adenovirus receptor (CAR) protein was purified from HeLa cell lysates by immunoaffinity chromatography (*10*) with mAb RmcB (*9*), the

J. M. Bergelson, J. A. Cunningham, E. A. Kurt-Jones, A. Krithivas, R. W. Finberg, Division of Infectious Diseases, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.
G. Droguett and M. S. Horwitz, Departments of Microbiology-Immunology, Cell Biology, and Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461, USA.
J. S. Hong, Department of Cell Biology and Anatomy, Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham, Birmingham, AL 35294, USA.
R. L. Crowell, Department of Microbiology and Immunology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19102, USA.

*To whom correspondence should be addressed. E-mail: jeff_bergelson@macmailgw.dfci.harvard.edu

sequences of four tryptic peptides were determined (*11*), and a cDNA clone was isolated from a HeLa cell library (*12*). After transfection of CHO cells with CAR cDNA (*13*), a cell line (CHO-CAR) was selected that, as determined by indirect flow cytofluorometry, showed homogeneous expression of antigen recognized by RmcB (Fig. 1A). Untransfected CHO cells and control cells transfected with the human integrin α2 subunit (CHO-al 2) (*14*) did not express antigen detectable by RmcB. RmcB immunoprecipitated a 46-kD cell-surface protein from detergent lysates of iodinated CHO-CAR transfectants, as it did from HeLa cell lysates, but not from lysates of CHO-al 2 control cells (Fig. 1B). These results confirmed that CAR cDNA encodes the protein recognized by RmcB.

Analysis of its deduced amino acid sequence indicated that CAR is a 365–amino acid transmembrane protein with a short leader, a 222–amino acid extracellular domain, a membrane-spanning helical domain, and a 107–amino acid intracellular domain (Fig. 1C). Alignment of the CAR sequence with sequences of proteins belonging to the immunoglobulin gene superfamily suggested that its extracellular portion consists of two immunoglobulin-like domains. The deduced amino acid sequence of a murine CAR homolog was 91% identical to that of the human protein overall and 95% identical within the cytoplasmic domain (*15*). The relatively large and highly conserved cytoplasmic domain suggests the potential for interaction with other intracellular proteins, but CAR's cellular function remains to be determined.

CHO-CAR cells, but not the control transfectants, bound radioactively labeled coxsackievirus B3 and coxsackievirus B4

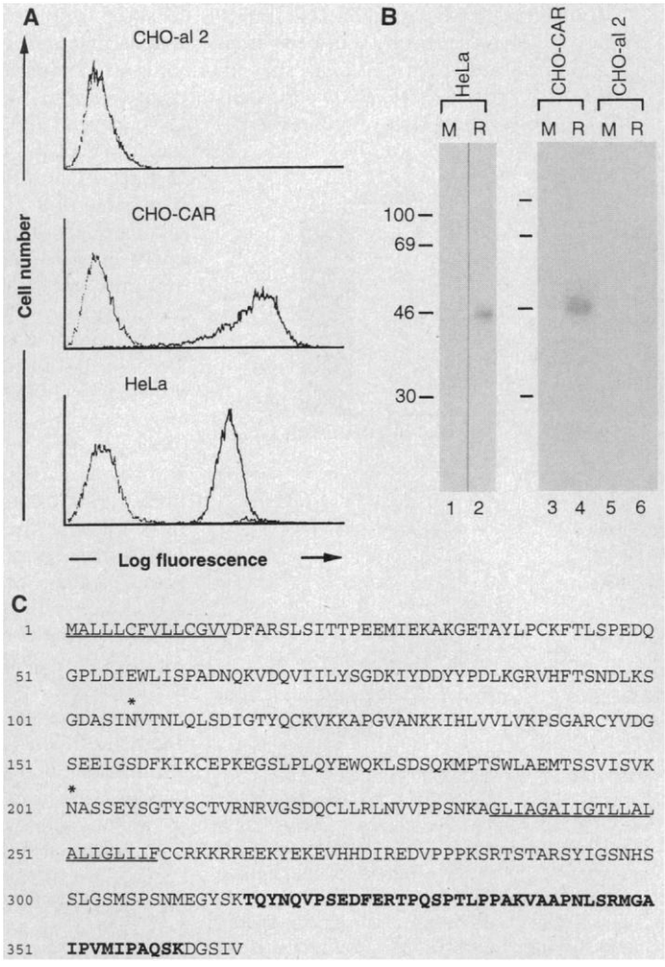

Fig. 1. CAR protein expression and sequence. (**A**) Immunofluorescence. Control CHO cells transfected with CHO-al 2, CHO cells transfected with CAR (CHO-CAR), and HeLa cells were incubated with mAb RmcB or the control antibody MOPC 195 for 1 hour on ice. Cells were then washed, incubated with fluorescein isothiocyanate–conjugated goat antibody to mouse immunoglobulin, and analyzed by flow cytometry. Control staining is seen on the left and RmcB staining on the right; the two overlap in the top panel. (**B**) Immunoprecipitation. HeLa cells, CHO-CAR cells, or control CHO-al 2 cells were iodinated by the glucose oxidase–lactoperoxidase method and extracted in buffer containing 1% Triton X-100. Immunoprecipitation was performed with the control antibody MOPC 195 (M) or with mAb RmcB (R) bound to protein G–Sepharose beads. Immunoprecipitated proteins were analyzed in 10% SDS-polyacrylamide gels under reducing conditions. The positions of marker proteins are indicated at left in kilodaltons. (**C**) Deduced CAR amino acid sequence (*29*). Amino acids experimentally determined by peptide sequencing are in bold letters. The predicted hydrophobic leader and transmembrane domains are underlined. Based on homology to members of the immunoglobulin gene superfamily, cysteines at positions 41 and 120 and at positions 162 and 212 may form intrachain disulfide bonds. Two potential sites for N-linked glycosylation in the extracellular domain are marked with an asterisk. Tyrosine 255 (KRREEKY) is a potential site for phosphorylation. The CAR cDNA sequence has been deposited in the EMBL database (accession number Y07593).

Table 1. Virus attachment to CHO-CAR transfectants. CHO-CAR or CHO-al 2 monolayers were incubated with the control antibody MOPC 195 (C) or with mAb RmcB for 1 hour at room temperature, then rinsed and incubated for 4 hours with ^{35}S-labeled coxsackievirus B3 or B4 (20,000 cpm). Coxsackievirus B3 (strain Nancy), maintained in the laboratory of R.L.C., and coxsackievirus B4 (strain J.V.B.), obtained from the American Type Culture Collection, were radiolabeled and purified, and binding assays were performed as described (*14*). Results are shown as mean virus bound ± SD for triplicate monolayers and are representative of three experiments.

Cell line	Virus bound (cpm)			
	Coxsackie B3		Coxsackie B4	
	C	RmcB	C	RmcB
CHO-CAR	3003 ± 219	217 ± 24	2026 ± 13	156 ± 11
CHO-al 2	59 ± 5	78 ± 28	44 ± 3	41 ± 2

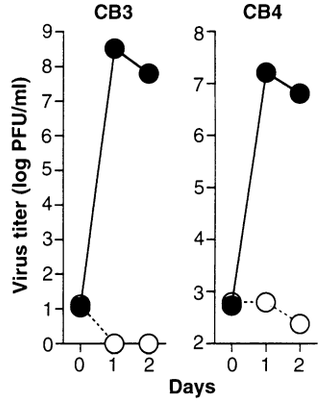

Fig. 2. Coxsackie B virus infection of CHO-CAR cells. CHO-CAR (solid circles) or CHO-al 2 (open circles) monolayers were incubated for 1 hour at room temperature with coxsackievirus B3 [CB3, 1 plaque forming unit (PFU) per cell] or coxsackievirus B4 (CB4, 4 PFU per cell), then monolayers were washed to remove unbound virus and incubated at 37°C for 1 hour (0 days), 1 day, or 2 days. Monolayers were frozen and thawed to release virus, and plaque assays were performed as described (*14*). The figure shows the mean virus titer for triplicate cultures. These experiments were performed twice.

(Table 1). Echovirus 1, a picornavirus that binds to the integrin α2 subunit (*14*, *16*), did not bind to CHO-CAR cells but did, as expected, bind to the CHO-al 2 control (*17*). CHO-CAR cells became infected by coxsackieviruses B3 and B4, as evidenced by viral cytopathic effect (*17*) and by active virus replication (Fig. 2). Pretreatment with mAb RmcB prevented virus attachment (Table 1) and protected CHO-CAR cells from infection (*17*). These results demonstrate that the protein encoded by CAR cDNA is a functional virus receptor, mediating both cell attachment and infection by coxsackieviruses B3 and B4 .

Monoclonal antibody RmcB, which recognizes CAR protein, protects cells from infection by prototype strains of all six coxsackie B serotypes (9), which is consistent with the observation that all six serotypes compete for a common cell surface attachment site (*18*) and suggests that CAR is the major coxsackievirus B receptor (*19*). Although some coxsackievirus B strains have gained the capacity to bind to an additional receptor—the complement regulatory protein decay-accelerating factor (DAF) (*20*)—virus attachment to DAF-transfected CHO cells does not lead to productive infection (*20*). Several DAF-binding strains of coxsackie B3 and B5 were examined and found to infect CHO-CAR transfectants (*17*). It is possible that DAF functions in virus attachment for some strains, but that subsequent events, such as virus internalization and initiation of uncoating, depend on interaction with CAR.

A depression on the coxsackie B3 surface is believed to be the site for receptor attachment (*21*). In contrast, adenovirus attachment to cells is mediated by fibers projecting from the adenoviral capsid (*22*), and globular knob domains at the tips of the fibers directly contact the cellular receptor (*23*). Despite these structural differences, adenovirus 2 and coxsackievirus B3 were found 20 years ago to compete for a HeLa cell attachment site (*24*). We therefore tested whether CAR might be a receptor for adenovirus as well as for coxsackievirus B. Radioactively labeled adenovirus 2 (Fig. 3A) and isolated adenovirus 2 fibers (Fig. 3B) bound specifically to CHO-CAR cells. Virus attachment was blocked both by fibers isolated from adenovirus 2 and by recombinant adenovirus 5 knob domains (Fig. 3C). Adenoviruses 2 and 5 have previously been shown to compete for a common binding site (*22*). Inhibition by isolated fibers and knob domains was specific, as determined by their failure to inhibit attachment to HeLa cells by adenovirus 35—a more distantly related virus that did not bind to CHO-CAR transfectants—or echovirus 1 (*17*). Scatchard analysis of fiber binding to CHO-CAR and HeLa cells showed parallel lines (Fig. 3D), indicating that fibers bound to both cell lines with the same affinity; there were more specific binding sites on CHO-CAR cells, which, as determined by flow cytometry with mAb RmcB (Fig. 1A), expressed more CAR protein than did HeLa cells. These results confirm that CAR is a receptor responsible for specific fiber-mediated adenovirus attachment to cells.

Adenovirus enters CHO cells to some extent, but there is a post-entry block to viral protein synthesis and assembly (*25*). We therefore measured the efficiency of adenovirus-mediated gene delivery to CHO-CAR transfectants rather than virus production, using adenovirus 5 engineered to encode β-galactosidase (β-Gal) [Ad.CMV-βgal (*26*)]. Expression of CAR greatly enhanced gene transduction by adenovi-

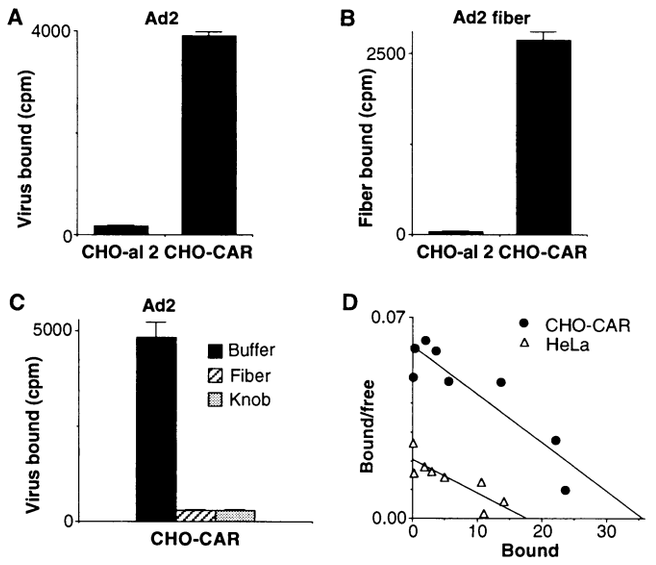

Fig. 3. Adenovirus interaction with CAR on transfected CHO cells. (**A** through **C**) show ^{35}S-labeled virus and fiber attachment to transfected cells (*30*). Each panel shows mean values for virus or fiber bound ± SD for triplicate monolayers and is representative of at least two experiments. (A) Adenovirus attachment. CHO-CAR and CHO-al 2 monolayers in 24-well plates were incubated with labeled adenovirus 2 (Ad2; 20,000 cpm) (*30*) for 1 hour at room temperature, then washed and dissolved for scintillation counting. (B) Adenovirus fiber attachment. Monolayers in six-well plates were incubated with labeled adenovirus 2 fibers (25,000 cpm). (C) Inhibition of adenovirus attachment by purified fibers and knob domains. CHO-CAR monolayers were incubated with buffer (solid bar), isolated adenovirus 2 fibers (5 μg, hatched bar), or recombinant adenovirus 5 knob domains (0.7 μg, stippled bar) before addition of labeled adenovirus 2. (**D**) Scatchard analysis of fiber binding. Duplicate CHO-CAR and HeLa monolayers were incubated with ^{125}I-labeled adenovirus 2 fibers at different specific activities for 1 hour at 4°C. Monolayers were then washed four times and bound radioactivity was determined. Nonspecific binding was determined by incubating labeled fiber in the presence of a 200-fold excess of unlabeled fiber. Specific binding is shown as nanograms bound per million cells. There was no specific binding to control CHO-al 2 monolayers.

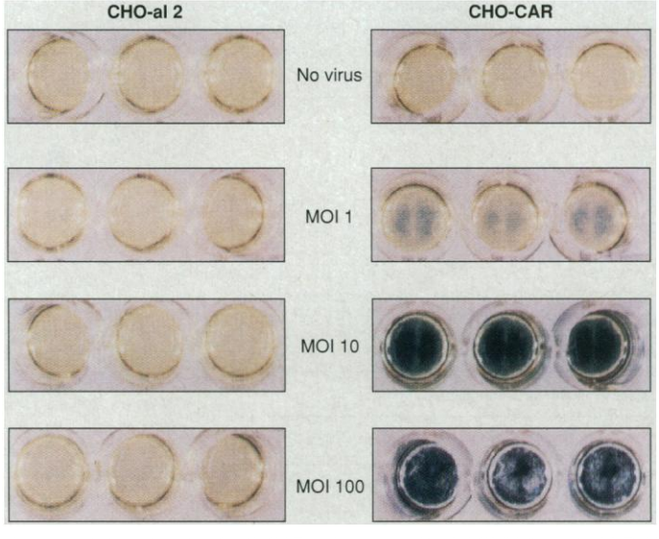

Fig. 4. Adenovirus-mediated gene transfer. CHO-al 2 and CHO-CAR cells in 24-well plates were exposed to Ad.CMV-βgal at different multiplicities of infection [MOI (in PFU per cell)] for 1 hour at room temperature, then unbound virus was removed and cells were incubated for 40 hours at 37°C. Cells were fixed with 2% paraformaldehyde and β-Gal activity was determined by incubation with phosphate-buffered saline containing 5 mM ferric and 5 mM ferrous cyanide, 1 mM $MgCl_2$, and X-Gal (1 mg/ml). Examination of monolayers before staining revealed some cytotoxicity in the CHO-CAR cells exposed to Ad.CMV-βgal at 100 PFU per cell. This experiment was performed three times.

rus 5, as demonstrated by in situ staining with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (Fig. 4), and, as expected, preincubation with isolated fibers or knob domains blocked virus-mediated β-Gal transduction (*17*). In quantitative colorimetric assays on cell lysates, enzyme activity was nearly 100-fold greater in the CAR transfectants than in the control cells at each multiplicity of infection and was also greater than in HeLa cells (*27*). These results demonstrate that attachment to CAR on the cell surface markedly enhances virus entry and adenovirus-mediated gene transfer. Adenovirus entry is also facilitated by interaction between the penton base protein and integrins on the cell surface (*28*). Our data do not exclude the possibility that, once fiber-mediated virus attachment to CAR protein has occurred, internalization may involve a secondary interaction with integrins or other molecules endogenously expressed on CHO cells.

Adenoviruses are being studied extensively as vectors for genetic therapy in humans (*3*). The defective vectors in present use, such as the Ad.CMV-βgal used in these studies, are derived from adenovirus 2 and 5 and will bind to CAR. Because our data indicate that CAR expression greatly enhances gene transfer, identification of the adenovirus receptor and an understanding of its tissue distribution should be of importance in targeting gene delivery to specific tissues. Manipulation of receptor expression should be useful in achieving efficient adenovirus-mediated transduction both in vivo and in vitro. Identification of CAR as the functional receptor for coxsackie B viruses and adenoviruses may also facilitate development of new strategies to limit disease caused by these pathogens.

Note added in proof. CAR sequences are identical to genomic and EST clones that map to human chromosome 21, which is consistent with the recent localization of the adenovirus 2 receptor to this chromosome (*31*). Other investigators (*32*) have recently used the acronym CAR1 to describe an avian leukosis virus receptor that is unrelated to the coxsackie and adenovirus receptor.

REFERENCES AND NOTES

1. M. S. Horwitz, in *Virology*, B. N. Fields, D. M. Knipe, P. M. Howley, Eds. (Lippincott-Raven, Philadelphia, 1996), vol. 2, pp. 2149–2171.
2. A. B. Martin *et al.*, *Circulation* **90**, 330 (1994).
3. E. J. Kremer and M. Perricaudet, *Br. Med. Bull.* **51**, 31 (1995).
4. J. L. Melnick, in *Virology*, B. N. Fields, D. M. Knipe, P. M. Howley, Eds. (Lippincott-Raven, Philadelphia, 1996), vol. 2, pp. 655–712.
5. C. W. Imrie, J. C. Ferguson, R. G. Sommerville, *Gut* **18**, 53 (1977).
6. J. W. Yoon, M. Austin, T. Onodera, A. L. Notkins, *N. Engl. J. Med.* **300**, 1173 (1979); G. B. Clements, D. N. Galbraith, K. W. Taylor, *Lancet* **346**, 221 (1995).
7. N. R. Grist and D. Reid, in *Viral Infections of the Heart*, J. Banatvala, Ed. (Arnold, London, 1993), pp. 23–31; M. C. Savoia and M. N. Oxman, in *Principles and Practice of Infectious Diseases*, G. M. Mandell, J. E. Bennet, R. Dolin, Eds. (Churchill Livingstone, New York, 1995), vol. 1, pp. 799–813.
8. J. E. Mapoles, D. L. Krah, R. L. Crowell, *J. Virol.* **55**, 560 (1985); K.-H. L. Hsu, S. Paglini, B. Alstein, R. L. Crowell, in *New Aspects of Positive-Strand RNA Viruses*, M. Brinton and F. Heinz, Eds. (American Society for Microbiology, Washington, DC, 1990), pp. 271–277.
9. K.-H. L. Hsu, K. Lonberg-Holm, B. Alstein, R. L. Crowell, *J. Virol.* **62**, 1647 (1988).
10. HeLa S3 cells were homogenized in 10 mM tris buffer (pH 7.6) containing 2.5 mM EDTA, 2.5 mM EGTA, and protease inhibitors. After centrifugation of the homogenate at 145,000*g* for 90 min, the pellet was homogenized in the same buffer containing 150 mM NaCl. Triton X-100 was added to a final concentration of 1%, and proteins were extracted for 3 hours. Insoluble material was removed by recentrifugation, then the extract was pre-cleared with protein G–Sepharose to which irrelevant antibodies had been covalently coupled. Affinity chromatography was performed by pumping the pre-cleared extract through a column containing mAb RmcB coupled to protein G–Sepharose. The column was washed extensively, then bound protein was eluted in 50 mM diethylamine (pH 11.5).
11. Fractions containing the 46-kD protein were repurified by electrophoresis in a 10% SDS-polyacrylamide gel and stained as described [J. Rosenfeld, J. Capdevielle, J. C. Guillemot, P. Ferrara, *Anal. Biochem.* **203**, 173 (1992)]. The protein bands were excised. After in-gel digestion with trypsin, the sequences of four peptides were determined at the Harvard Microchemistry Facility by collisionally activated dissociation on a Finnigan TSQ 7000 triple quadrupole mass spectrometer.
12. A corresponding expressed sequence tag (EST) cDNA (emb F05145; IMAGE Consortium Clone ID 25001) isolated from an infant brain library [G. G. Lennon, C. Auffray, M. Polymeropoulos, M. B. Soares, *Genomics* **33**, 151 (1996)] was identified in the dbEST database and used to screen a pcDNAI HeLa cell library (Invitrogen, San Diego, CA). The longest cDNA insert [2437 base pairs (bp)] was sequenced and contained a short upstream untranslated region, a consensus sequence for translation initiation [M. Kozak, *Nucleic Acids Res.* **15**, 8125 (1987)], a 1095-bp open reading frame, a 1259-bp downstream untranslated region, and a polyadenylate tail.
13. Electroporation of *dhfr*$^-$ CHO cells with CAR cDNA and a plasmid encoding dihydrofolate reductase, selection of transfectants in nucleoside-free medium, and fluorescence-activated cell sorting with RmcB were performed as described for transfection with the integrin α2 subunit (*14*).
14. J. M. Bergelson *et al.*, *J. Virol.* **67**, 6847 (1993).
15. Murine CAR cDNA [European Molecular Biology Laboratory (EMBL) accession number Y10320] was obtained from a mouse liver cDNA library (Gibco-BRL).
16. J. M. Bergelson, M. P. Shepley, B. M. C. Chan, M. E. Hemler, R. W. Finberg, *Science* **255**, 1718 (1992).
17. J. M. Bergelson, unpublished results.
18. R. L. Crowell, in *Ninth Miles International Symposium: Cell Membrane Receptors for Viruses, Antigens and Antibodies, Polypeptide Hormones and Small Molecules*, R. F. Beers and E. G. Bassett, Eds. (Raven, New York, 1976), pp. 179–202; ——— and R. P. Tomko, in *Cellular Receptors for Animal Viruses*, E. Wimmer, Ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1994), pp. 75–99.
19. CAR cDNA sequences are represented in ESTs derived from human brain and pancreatic islet cells. When used to probe a human tissue Northern (RNA) blot (Clontech), CAR cDNA hybridized most strongly to RNA from the heart, brain, and pancreas, which is consistent with the known tropism of coxsackie B viruses for these tissues. Faint hybridization to liver and lung RNA was also detected, but none to RNA from the kidney, placenta, or skeletal muscle.
20. J. M. Bergelson *et al.*, *J. Virol.* **69**, 1903 (1995); D. R. Shafren *et al.*, *ibid.*, p. 3873.
21. J. K. Muckelbauer *et al.*, *Structure* **3**, 653 (1995).
22. L. Philipson, K. Lonberg-Holm, U. Pettersson, *J. Virol.* **2**, 1064 (1968); C. Defer, M.-T. Belin, M. L. Caillet-Boudin, P. Boulanger, *ibid.* **64**, 3661 (1990).
23. L. J. Henry, D. Xia, M. E. Wilke, J. Deisenhofer, R. D. Gerard, *ibid.* **68**, 5239 (1994); N. Louis, P. Fender, A. Barge, P. Kitts, J. Chroboczek, *ibid.*, p.4104; S. C. Stevenson, M. Rollence, B. White, L. Weaver, A. McClelland, *ibid.* **69**, 2850 (1995).
24. K. Lonberg-Holm, R. L. Crowell, L. Philipson, *Nature* **259**, 679 (1976).
25. M. Longiaru and M. S. Horwitz, *Mol. Cell. Biol.* **1**, 208 (1981); R. L. Radna, B. Foellmer, L. A. Feldman, U. Francke, H. L. Ozer, *Virus Res.* **8**, 277 (1987).
26. Y. Manome *et al.*, *Nature Med.* **2**, 567 (1996).
27. Lysates were prepared and assayed with the use of reagents provided in the Promega β-Gal enzyme assay system. After addition of development buffer containing o-nitrophenyl β-D-galactopyranoside, absorbance at 405 nm was measured at 20-s intervals in a kinetic microplate reader (Molecular Devices) and maximum *V* was calculated for each sample. Cells infected at 1 plaque-forming unit (PFU) per cell gave the following values (milli–optical density units per minute ± SD for triplicate samples): CHO-al 2, 0.01 ± 0.01; HeLa, 0.16 ± 0.05; and CHO-CAR, 1.29 ± 0.28. At 10 PFU per cell, values were CHO-al 2, 0.25 ± 0.04; HeLa, 2.82 ± 0.22; and CHO-CAR, 20.64 ± 1.52. At 100 PFU per cell, values were CHO-al 2, 3.13 ± 0.41; HeLa, 53.57 ± 4.10; and CHO-CAR, 210.41 ± 16.38.
28. T. J. Wickham, P. Mathias, D. A. Cheresh, G. R. Nemerow, *Cell* **73**, 309 (1993); M. Bai, L. Campisi, P. Freimuth, *J. Virol.* **68**, 5925 (1994); M. J. Goldman and J. M. Wilson, *ibid.* **69**, 5951 (1995); S. Huang, T. Kamata, Y. Takada, Z. M. Ruggeri, G. R. Nemerow, *ibid.* **70**, 4502 (1996).
29. 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.
30. Adenovirus 2, labeled by growth in ^{35}S-methionine, was purified by centrifugation in CsCl as described [J. V. Maizel Jr., D. O. White, M. D. Scharff, *Virology* **36**, 115 (1968)]. Adenovirus fibers were purified from the top 10 ml of the CsCl gradients by chromatography on Mono Q and Mono S ion exchange columns (Pharmacia). Iodinated fibers were prepared with Iodogen. To prepare recombinant knob domains, the Hpa I–Sma I fragment of the adenovirus 5 fiber gene [J. Chroboczek and B. Jacrot, *Virology* **161**, 549 (1987)] was cloned into the pTrcHisC vector (Invitrogen). A recombinant protein including 5 of 22 fiber-shaft repeating units and the COOH-terminal knob was expressed in *Escherichia coli* and purified by adsorption to Ni^{2+}NTA-Agarose (Qiagen).
31. G. A. Mayr and P. Freimuth, *J. Virol.* **71**, 412 (1997).
32. J. Brojatsch, J. Naughton, M. M. Rolls, K. Zingler, J. A. T. Young, *Cell* **87**, 845 (1996).

33. We thank W. Lane and T. Addona of the Harvard Microchemistry Facility for expert peptide sequencing; K. Solomon, J. Fingeroth, and D. Sage for advice; J. Kang, M. Chan, and N. Egea for technical assistance; T. Tanaka for providing Ad5.CMV-βgal; and S. King, M. Schneider, A. Goldfeld, W. Marshall, and L. Finkelstein for helpful comments on the manuscript. Large-scale culture of HeLa cells was performed by the Cell Culture Center (Cellex Biosciences, Minneapolis, MN), and DNA sequencing and cell sorting were performed by members of the Dana-Farber Molecular Biology and Flow Cytometry Core Facilities. Supported by grants from NIH (AI35667, AI31628, and CA69703), the American Heart Association (95012650), the Barr Program, the Cystic Fibrosis Foundation (R464), and the Juvenile Diabetes Foundation. J.M.B. is an Established Investigator of the American Heart Association.

1 October 1996; accepted 18 December 1996