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Adenovirus Interaction with Distinct Integrins Mediates Separate Events in Cell Entry and Gene Delivery to Hematopoietic Cells

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A major impediment to the effective use of adenovirus vectors for gene therapy is a lack of knowledge of how these vectors interact with diverse cell types in vivo. Adenovirus attachment to most human cell types is mediated by the fiber protein, which binds to an as yet unidentified cell receptor. In contrast to this, we report that adenovirus type 2 (Ad2) attachment to hematopoietic cells is facilitated by interaction of the penton base protein with members of the β2 integrin family. Adenovirus particles were capable of binding to human monocytic cells, which lack fiber receptors, and virus binding could be blocked by a soluble penton base or by a function-blocking monoclonal antibody to integrin $\alpha_M\beta2$. To confirm the role of $\alpha_M\beta2$ integrins in Ad2 binding to hematopoietic cells, we analyzed virus attachment and gene delivery to CHO cells expressing recombinant β2 integrins. $\alpha_M\beta2$-expressing CHO cells supported 3- to 5-fold-higher levels of Ad2 binding and 5- to 10-fold-larger amounts of gene delivery than did nontransfected CHO cells, indicating that $\alpha_M\beta2$ facilitates adenovirus attachment to and infection of hematopoietic cells. While β2 integrins promote Ad2 attachment to hematopoietic cells, further studies demonstrated that αv integrins were required for the next step in infection, virus internalization into cell endosomes. These studies reveal a novel pathway of Ad2 infection of hematopoietic cells mediated by distinct integrins which facilitate separate events in virus entry. They also suggest a possible strategy for selective adenovirus-mediated gene delivery to hematopoietic cells.

Cells of hematopoietic origin are thought to play an important role in adenovirus (Ad) pathogenesis, since they frequently infiltrate the primary site of virus infection, a process associated with immune system-mediated inflammatory responses (10). Although Ad does not usually replicate in hematopoietic cells (2, 6), latent (persistent) infection of these cell types may promote virus dissemination in the host. Reactivation of Ad in latently infected cells may also lead to fatal disseminated disease in immunocompromised individuals (12). Lymphocytes and/or monocytes are also believed to play a major role in reducing the efficiency of Ad-mediated gene therapy by eliminating transduced cells expressing foreign gene products (7, 8).

Despite the involvement of hematopoietic cells in Ad pathogenesis and gene delivery, little is known of the specific interactions of Ad with these cell types. Previous studies have demonstrated that the fiber capsid protein is responsible for Ad attachment to the majority of human cell types (19), although the cell receptor that mediates the fiber binding has not yet been identified. It is also unknown as to whether fiber receptors are expressed on all cell types including hematopoietic cells. Ad entry into cells following virus attachment is mediated by the penton base (4, 27), which binds to cell integrins $\alpha_v\beta3$ and $\alpha_v\beta5$. Penton base is composed of five identical subunits, each containing an RGD peptide sequence (17) that is also found in a number of cell matrix and adhesion proteins, such as fibronectin and vitronectin, which bind to a family of cell surface receptors termed integrins (21). Integrins are heterodimers composed of noncovalently associated α and β subunits (14). There are 14 known α and 8 known β subunits, forming at least 21 different heterodimers, which recognize distinct ligands (14). Integrins that recognize the RGD sequence include $\alpha_5\beta1$, $\alpha_{IIb}\beta3$, and the α_v integrins. Several lines of evidence suggest that α_v integrins are important secondary receptors for Ad infection in vitro and in vivo. Recombinant Ads containing a mutation in the penton base RGD sequence have decreased infectivity (4). Human monocytes (13) and fully differentiated airway epithelial cells (11), which do not express α_v integrins, are also relatively resistant to Ad-mediated gene delivery. In contrast, upregulation of α_v integrins on monocytes/macrophages (13) and expression of $\alpha_v\beta5$ on less differentiated airway epithelial cells promotes Ad-mediated gene delivery (11). Penton base interaction with integrin $\alpha_v\beta5$ is also involved in Ad-mediated cell membrane permeabilization (26).

While there is substantial knowledge about the virus structural proteins and host cell receptors that mediate Ad internalization, relatively little is known of the receptor(s) involved in virus attachment to hematopoietic cells. In previous studies, we reported that growth factor-stimulated monocytes expressed decreased levels of the fiber receptor even though they had shown increased susceptibility to Ad infection (13). This finding suggested that Ad entry into monocytic cells involved a pathway distinct from that of virus entry into epithelial cells. In the studies reported here, we examined Ad attachment and entry into fully differentiated monocytes (macrophages) as well as a monocytic cell line, THP-1 cells. These studies revealed a novel pathway of virus entry that is mediated entirely by the interaction of the penton base with distinct cell integrins.

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MATERIALS AND METHODS

Ads, recombinant proteins, and MAbs. Ad type 2 (Ad2) was purchased from the American Type Culture Collection, Rockville, Md. Ad2 containing a deletion

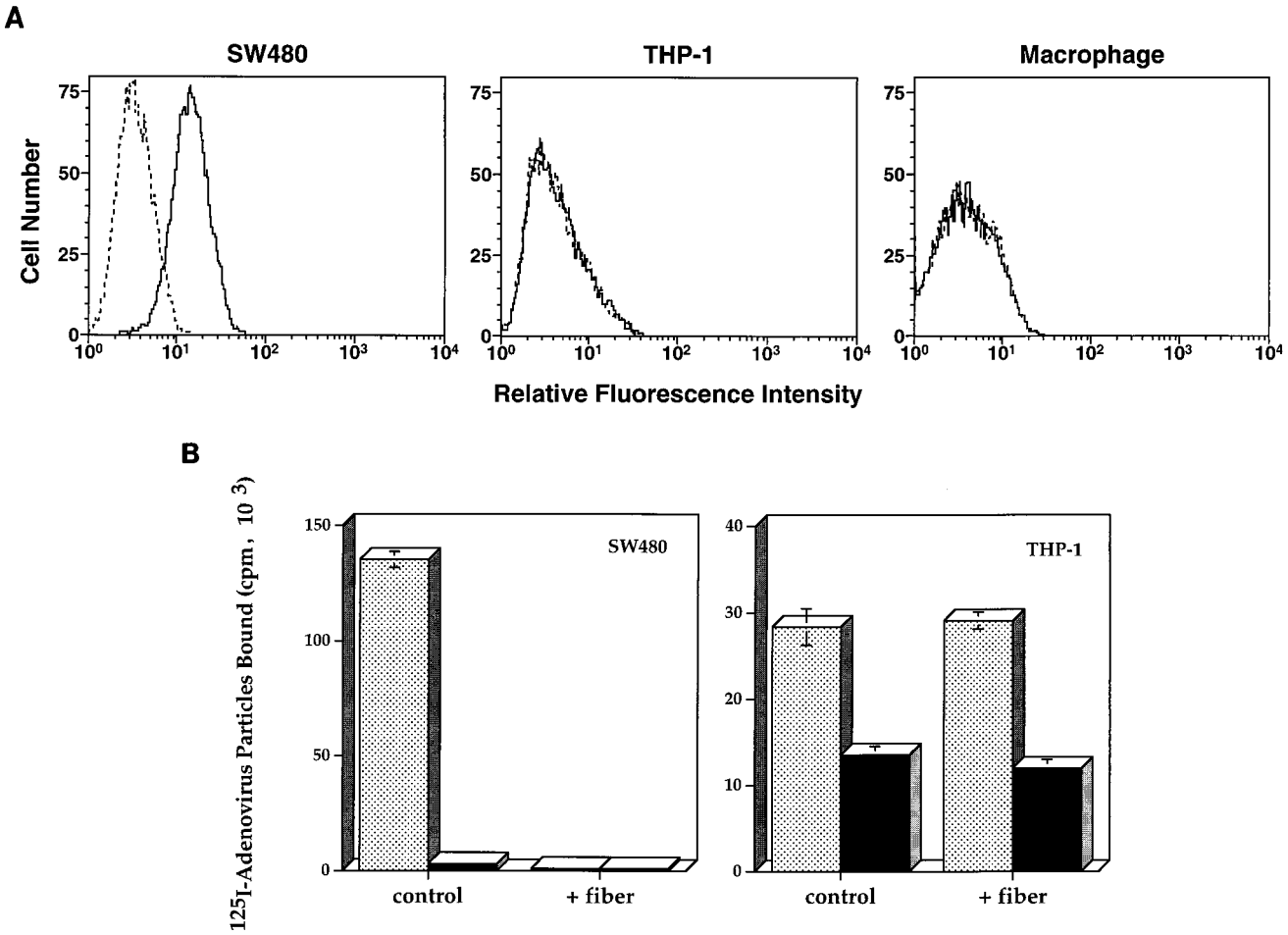


FIG. 1. (A) Flow-cytometric analysis of Ad2 fiber binding to cells. SW480 epithelial cells, THP-1 monocytic cells, and terminally differentiated monocytes/macrophages were incubated with saturating amounts of recombinant fiber, then with an anti-fiber polyclonal antibody, and then with FITC-IgG. Control cell samples were incubated with FITC-IgG alone prior to flow-cytometric analysis. The solid and dashed curves represent cells incubated with and without fiber, respectively. (B) Ad attachment to SW480 and THP-1 cells. Binding of ^{125}I-labeled Ad2 (stippled bars) or a fiberless mutant Ad2 (solid bars) was assayed in the presence or absence of recombinant Ad2 fiber. Nonspecific binding was determined by incubating the cells in the presence of an excess of unlabeled Ad2. The data represent the mean and standard deviation of duplicate samples.

in the fiber gene, H2dl807 (5), was kindly provided by Gary Ketner, Johns Hopkins University. Ad.RSVβgal, a replication-defective Ad type 5 (Ad5), was provided by Michel Perricaudet, Institute Gustave Roussy. Ad2 was purified by centrifugation on 16 to 40% cesium chloride gradients as previously described (27). H2dl807 virus was isolated free (<95%) of contaminating helper virus by three cycles of ultracentrifugation on 20 to 35% cesium chloride gradients. For cell-binding studies, purified virions were radiolabeled with Na^{125}I (Iodo-beads; Pierce). Recombinant Ad2 fiber and penton base proteins were produced in *Trichoplusia ni* insect cells as previously described (27). A function-blocking murine monoclonal antibody (MAb) directed against the penton base RGD sequence (DAV-1) (unpublished data) was purified (Hi-Trap G; Pharmacia) from ascites fluids. Fab fragments of the DAV-1 antibody were generated by papain digestion at pH 8.0 and then purified on a Resource Q (Pharmacia) fast protein liquid chromatography FPLC column. Purified function-blocking MAbs to integrins $\alpha_v\beta3$ (LM609), $\alpha_v\beta5$ (P1F6), and β1 (P4C10) were kindly provided by David Cheresh, The Scripps Research Institute. The 69-6-5 function-blocking MAb to α_v was generously provided by Maxime Lehmann, Université d'Aix-Marseille. The M1/70 MAb directed against the α_M subunit of Mac-1) (23) was provided by Ted Fan, The Scripps Research Institute. A hybridoma cell line producing the OKM1 MAb to integrin $\alpha_M\beta2$ (CD18b/CD11c; Mac-1) was purchased from the American Type Culture Collection. The CP3 MAb was originally raised against integrin $\alpha_{IIb}\beta3$ (18); however, sequential immunoprecipitation experiments indicated that it also recognizes integrin $\alpha_M\beta2$ (unpublished observation), and a similar observation has been reported by others (9).

Cell lines, peripheral blood mononuclear cells, and flow cytometry. The human epithelial cell lines SW480 and A549 and the monocytic cell line THP-1 (American Type Culture Collection) were maintained in Dulbecco modified Eagle medium with 10% fetal calf serum. Chinese hamster ovary (CHO) cells expressing wild-type $\alpha_M\beta2$, mutant D242A (Asp-242 to Ala in α_M cDNA), or $\alpha_L\beta2$ (15) were generated as described previously (15). Briefly, wild-type or mutant α_M or α_L cDNA in the pBJ expression vector were used to transfect CHO cells, together with β2 cDNA. Cells stably expressing wild-type or mutant $\alpha_M\beta2$ or $\alpha_L\beta2$ were cloned by cell sorting to obtain high expressors. The transfected cells were maintained in complete Dulbecco modified Eagle medium containing 350 μg of G418 (Geneticin; GIBCO BRL) per ml. Human peripheral blood mononuclear cells were isolated from the blood of healthy adult donors by Ficoll-Hypaque centrifugation as previously described (13). The adherent-cell population, consisting of greater than 80% monocytes, was cultured for 14 to 18 days in RPMI 1640 supplemented with 20% fetal calf serum to promote terminal differentiation of monocytes to macrophages. For analysis of integrin expression, different cell types were incubated in the presence of 10 to 20 μg of the anti-α_v or anti-β2 integrin MAb per ml for 60 min at 4°C. After being washed, the cells were incubated for 30 min at 4°C with goat anti-mouse immunoglobulin G (IgG) coupled to fluorescein isothiocyanate (FITC) (FITC-IgG; KPL Laboratories, Gaithersburg, Md.). Following additional washes in phosphate-buffered saline (PBS), the cells were resuspended in 500 μl and analyzed by flow cytometry (FACScan; Becton Dickinson) with the Lysis II program.

Ad, fiber, and penton base attachment experiments and gene delivery assays. Binding of recombinant Ad2 fiber protein to cells was assayed by flow cytometry. Epithelial or monocytic cells at 10^6 ml were incubated for 60 min at 4°C with 50 μg of recombinant Ad2 fiber per ml in PBS containing 0.5% bovine serum albumin (BSA). After being washed three times in PBS-BSA, the cells were incubated with a 1:4,000 dilution of a rabbit polyclonal antibody to the fiber protein for 45 min at 4°C and then with a 1:1,000 dilution of goat FITC-anti-rabbit IgG (KPL). After final washes, the cells were subjected to flow-cytometric analysis as described above. To measure binding to cells, ^{125}I-labeled Ad2 or

penton base was incubated for 2 h at 4°C with 10^7 cells per ml in serum-free Dulbecco modified Eagle medium containing 0.5% BSA and 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES). Unattached virus particles or penton base was removed by centrifugation at 13,000 × *g* on a cushion of 86% silicon oil–14% mineral oil. Nonspecific binding was determined by incubating the cells in the presence of a 50-fold excess of unlabeled virions or a 200-fold excess of penton base. For competition studies, cells were preincubated with 200 μg of recombinant fiber protein or function-blocking anti-integrin MAbs per ml for 30 min at 4°C prior to the addition of radiolabeled virus. In certain experiments, labeled penton base or Ad2 was preincubated with 200 μg of Fab fragments of the DAV-1 MAb per ml prior to addition to epithelial or monocytic cells. Ad-mediated gene delivery to different cell types was measured as previously described (13) with a recombinant Ad containing the *lacZ* gene (Ad.RSVβgal) (24). β-Galactosidase activity was quantitated 48 h postinfection by incubating the cells for 60 min at 37°C with a buffer containing 0.5% Nonidet P-40 and 3.5 mM *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a chromagenic substrate. The A_{415}, which was linear from 15 to 90 min at 37°C, was measured in a multiwell plate reader (Titertek; Flow Labs, MacLean, Va.).

Affinity purification of integrin $\alpha_M\beta 2$ on a penton base column. THP-1 cells (10^8) were washed three times in PBS and then solubilized at 4°C with 200 mM β-D-octylglucoside in 10 mM Tris-HCl (pH 8.0) containing 150 mM NaCl, 1% BSA, 2 mM $MgCl_2$, 2 μg of aprotinin (Sigma Chemical Co., St. Louis, Mo.) per ml, 20 μg of leupeptin per ml, and 5 mM phenylmethylsulfonyl fluoride. Nuclei and cell debris were removed by centrifugation for 15 min at 10,000 × *g*, and the soluble fraction was applied to a 2-ml column of Affi-Gel-15 (Bio-Rad, Richmond, Calif.) containing 2.8 mg of immobilized penton base. In a separate experiment, an equivalent amount of cell lysate was applied to a penton base affinity column in the presence of 20 mM EDTA. The penton base columns were then washed extensively with solubilization buffer, and the bound proteins were eluted with 50 mM diethylamine (pH 11). After neutralization with 1.0 M glycine-HCl (pH 2.0), the column fractions were pooled and concentrated 10-fold (Centricon 10; Millipore Corp., Bedford, Mass.). Samples were then electrophoresed on a sodium dodecyl sulfate–7% polyacrylamide gel under reducing conditions and transferred to a polyvinylidene difluoride membrane (Immobilon P; Millipore) by Western blotting (immunoblotting). The protein blot was incubated with 5.0% nonfat dry milk (Blotto) to block nonspecific binding sites and then incubated with 10 μg of the M1/70 anti-α_M MAb per ml in PBS-Blotto. After further washes, the blot was incubated with anti-mouse IgG conjugated to alkaline phosphatase, washed, and then incubated with a substrate solution as recommended by the manufacturer (ECL Detection System; Amersham Corp.).

RESULTS

Ad attachment and gene delivery to monocytic cells are independent of the fiber receptor. To analyze Ad2 entry into cells of the myeloid lineage, we initially tested whether monocytes/macrophages expressed Ad2 fiber receptors. A human monocytic cell line, THP-1, as well as cultured human macrophages, failed to bind the fiber protein, whereas SW480 epithelial cells, as expected, supported fiber binding (Fig. 1A). Despite lacking fiber receptors, THP-1 cells bound Ad2 particles as well as mutant Ad2 virions (Fib^-; H2dl807) which lack the fiber protein (5) (Fig. 1B). Monocytic cells exhibited a lower level of virus binding than did SW480 cells; however, this difference is probably due to the three- to fourfold-greater size of epithelial cells. Preincubation of SW480 cells with soluble fiber abrogated Ad2 attachment, and these cells also bound much smaller amounts of the fib^- Ad2. In contrast, preincubation of THP-1 cells with the fiber protein did not inhibit the binding of either the wild-type or mutant virus.

Further studies were performed to compare the susceptibility of different cell types to Ad-mediated gene delivery (Fig. 2). Monocytic cells and epithelial cells were both susceptible to Ad-mediated gene delivery; however, Ad-mediated gene delivery to SW480 cells was blocked by preincubation with the fiber protein, whereas this treatment had no effect on gene delivery to monocytic cells, suggesting that Ad-mediated gene delivery to epithelial SW480 cells but not to monocytic cells is dependent on receptor interaction via the fiber. Incubation of SW480 cells with the penton base prevented Ad-mediated gene delivery. This is clearly due to inhibition of α_v integrin-mediated Ad internalization, since function-blocking MAb to α_v integrins abrogated Ad-mediated gene delivery (data not shown). Incubation of monocytic cells with penton base also

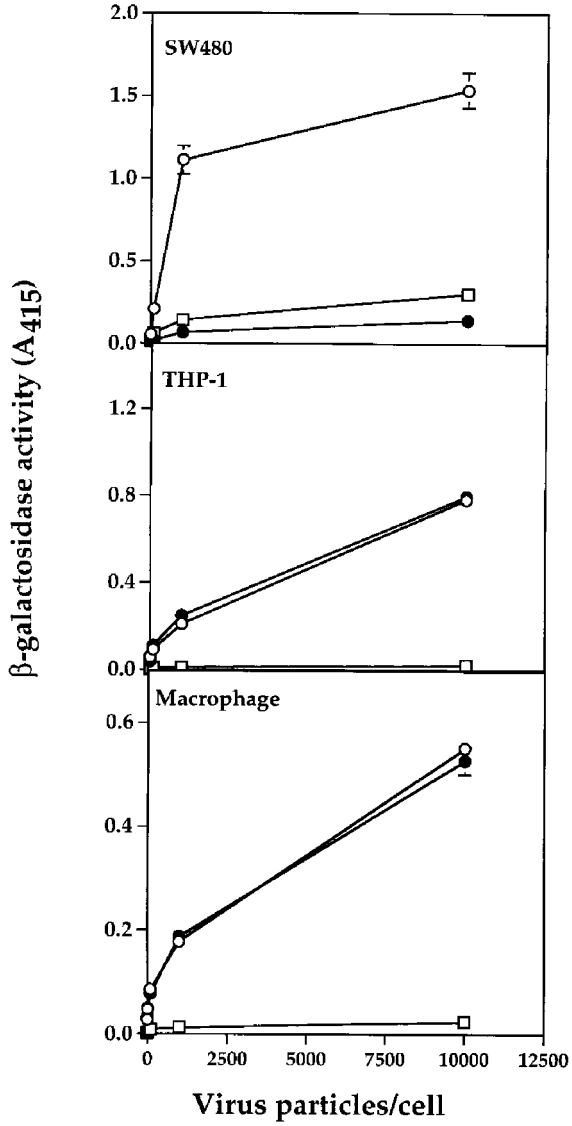


FIG. 2. Ad-mediated gene delivery to SW480 epithelial cells and to monocytic cells. Cells were incubated with various amounts of Ad.RSVβgal, and β-galactosidase activity was measured 48 h postinfection. Expression of the Ad-transduced *lacZ* gene in SW480 epithelial cells, THP-1 cells, and macrophages was quantitated as described in Materials and Methods. In competition experiments, cells were preincubated with recombinant penton base (open squares) or fiber (solid circles) or medium (open circles) prior to the addition of various amounts of Ad.RSVβgal. The data represent the mean of three experiments, with standard deviation equivalent to 5 to 10% of the mean.

prevented Ad-mediated gene delivery to these cell types. These studies indicated that both Ad attachment and infection of monocytic cells are independent of the fiber receptor and that the penton base protein plays the major role in virus interactions with these cells.

β2 integrins promote Ad attachment to and infection of monocytic cells. We next investigated whether the penton base was responsible for Ad binding to THP-1 cells. Virus attachment to THP-1 cells was blocked by chelation of divalent metal cations with EDTA, by penton base, and also by a function-blocking MAb (DAV-1) directed against the penton base RGD sequence (Fig. 3). These studies suggested that the penton base mediates virus attachment to monocytic cells via in-

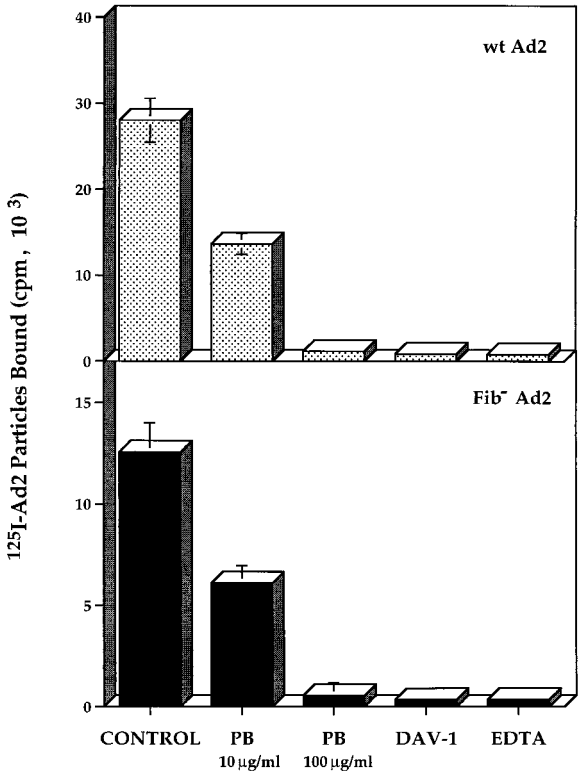


FIG. 3. The penton base facilitates Ad2 attachment to THP-1 monocytic cells. Binding of ^{125}I-labeled wild-type (wt) Ad2 or a fiberless mutant Ad2 to THP-1 cells was measured in the presence of saturating amounts of the penton base (PB) protein, 20 mM EDTA, or Fab fragments of the DAV-1 anti-penton base MAb. The data represent the mean and standard deviation of duplicate experiments.

teraction of its RGD sequence with a cell integrin. To identify the integrin involved in Ad2 attachment to THP-1 cells, we examined a panel of function-blocking MAbs to different integrins for their ability to inhibit penton base binding to THP-1 cells (Fig. 4). Somewhat surprisingly, function-blocking MAbs to integrins $\alpha_v\beta3$ and $\alpha_v\beta5$ had only minor effects on penton

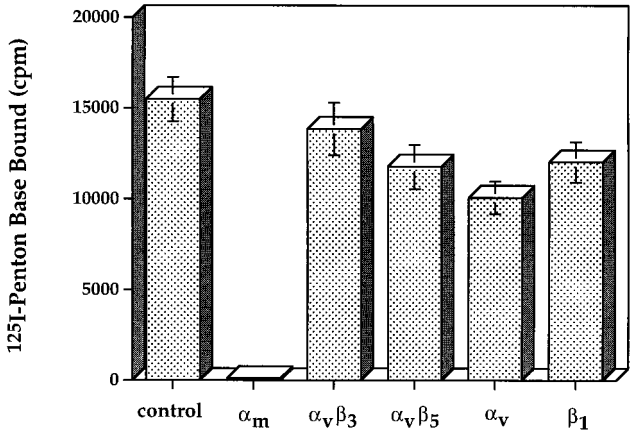


FIG. 4. Effect of anti-integrin MAbs on penton base binding to monocytic cells. Binding of ^{125}I-labeled penton base to THP-1 cells was measured in the presence of 200 μg of function-blocking MAbs to integrins $\alpha_M\beta2$ (CP3), $\alpha_v\beta3$ (LM609), α_v (69-6-5), and β1 (P4C10) per ml. The data represent the mean and standard deviation of three experiments.

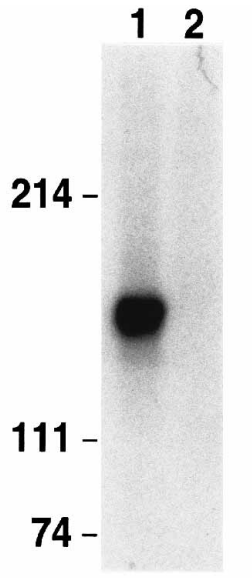
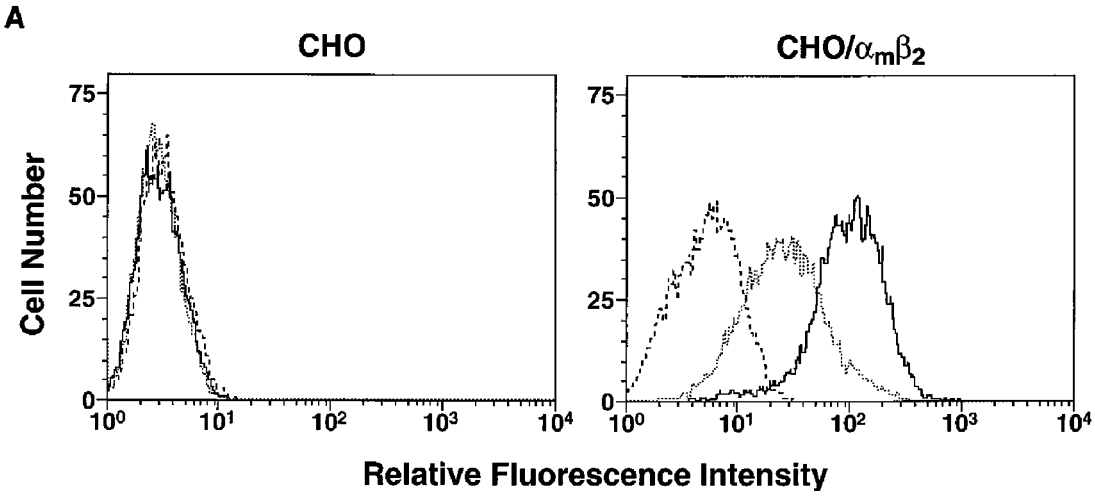


FIG. 5. Detection of penton base protein interaction with integrin $\alpha_M\beta2$ by immunoblotting. Detergent-solubilized THP-1 cell membrane proteins were affinity purified in the absence (lane 1) or presence (lane 2) of 20 mM EDTA on a Affi-Gel-15 column containing immobilized penton base. Bound proteins were eluted with low-pH buffer and then neutralized and concentrated. Fractions were then analyzed on an immunoblot for the presence of integrin $\alpha_M\beta2$ by using the M1/70 MAb as described in Materials and Methods.

base binding. In contrast, the CP3 MAb (18), which recognizes integrin $\alpha_M\beta2$, completely blocked penton base binding to THP-1 cells (Fig. 4). This result suggested that Ad interaction with monocytic cells is facilitated by the interaction of the penton base with integrin $\alpha_M\beta2$.

To ascertain whether the penton base protein binds directly to integrin $\alpha_M\beta2$, THP-1 cell membrane proteins were affinity purified on a penton base column in the presence or absence of divalent metal cations. Specifically bound proteins were eluted from the column with low-pH buffer and then analyzed on an immunoblot for the presence of integrin $\alpha_M\beta2$ (Fig. 5). A protein band of approximately 160 kDa, consistent with the size of the α_M subunit of $\alpha_M\beta2$, was detected by a non-function-blocking MAb to α_M (lane 1). This protein was not detected in membrane fractions that had been affinity purified in the presence of 20 mM EDTA (lane 2). These results indicated that the interaction of integrin $\alpha_M\beta2$ with penton base requires divalent metal cations, a property of most if not all integrin-ligand interactions.

Ad interaction with CHO cells lacking or expressing recombinant β2 integrins. To confirm that β2 integrins facilitate Ad attachment to cells, we first established a CHO cell line stably expressing $\alpha_M\beta2$. When analyzed by flow cytometry, CHO/$\alpha_M\beta2$ cells exhibited significant reactivity with the anti-α_M CP3 and OKM1 MAb but CHO cells did not (Fig. 6A). We then compared virus binding to nontransfected CHO cells which lack β2 integrins or to β2-integrin-expressing CHO cells (Fig. 6B). Since CHO cells also do not express Ad2 fiber receptors (data not shown), this allowed us to analyze Ad attachment independent of the fiber receptor. $\alpha_M\beta2$-expressing CHO cells bound approximately fivefold-higher levels of Ad2 than did nontransfected CHO cells. The majority of Ad2 attachment to $\alpha_M\beta2$-expressing CHO cells was mediated by the penton base protein interaction with integrin $\alpha_M\beta2$, since it could be inhibited by preincubating cells with the CP3 MAb or with the penton base (Fig. 7). In contrast, Ad2 fiber or function-block-



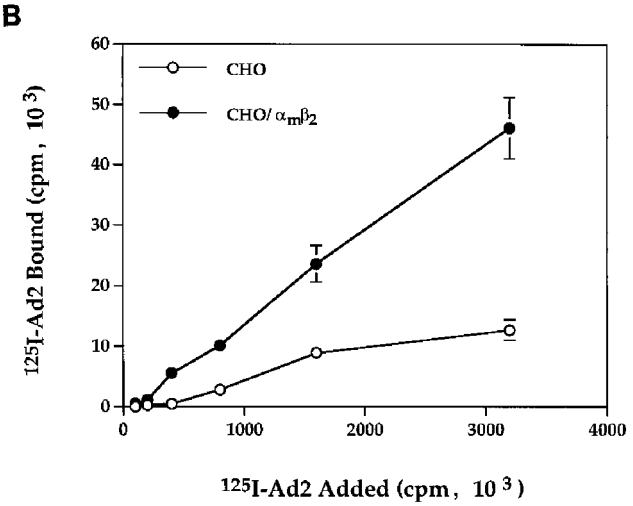


FIG. 6. (A) Flow-cytometric analysis of integrin $\alpha_M\beta2$ expression on CHO cells. Nontransfected CHO cells or CHO cells transfected with cDNAs encoding $\alpha_M\beta2$ (15) were evaluated for $\alpha_M\beta2$ expression by flow cytometry with the CP3 MAb (dashed line) or another MAb to $\alpha_M\beta2$, OKM1 (solid line), followed by incubation with FITC-IgG. Control samples were incubated with FITC-IgG alone prior to analysis by flow cytometry. (B) Ad attachment to CHO cells lacking integrin $\alpha_M\beta2$ or to CHO cells expressing recombinant $\alpha_M\beta2$. Binding of increasing amounts of ^{125}I-labeled Ad2 was measured in the presence or absence of unlabeled virus to determine the level of nonspecific binding. The data represent the mean of three experiments, with the standard deviation being equivalent to 5 to 10% of the mean.

ing MAbs directed against integrins $\alpha_v\beta5$ and $\beta1$ had no effect on virus attachment. $\alpha_M\beta2$-expressing CHO cells were also approximately 5- to 10-fold more susceptible to Ad-mediated gene delivery than were nontransfected CHO cells (Fig. 8), indicating that integrin $\alpha_M\beta2$ promotes not only Ad2 attachment but also virus infection. Interestingly, CHO cells with a point mutation (Asp-242 to Ala) in the A domain of the α_M subunit of $\alpha_M\beta2$ also showed enhanced susceptibility to Ad-mediated gene delivery (Fig. 8). Since this mutation prevents binding of the complement fragment of iC3b to $\alpha_M\beta2$ (16), these results suggest that Ad2 penton base interacts with a region in $\alpha_M\beta2$ distinct from that used for binding to iC3b. In further studies, we found that CHO cells expressing another member of the $\beta2$ integrin family, $\alpha_L\beta2$ (LFA-1), a receptor found on human lymphocytes (3), also enhanced (threefold) Ad2 attachment, as well as Ad-mediated gene delivery (fivefold) (data not shown). Thus, these studies demonstrate that two distinct members of the $\beta2$ integrin family promote Ad interaction with CHO cells.

Ad internalization into hematopoietic cells is mediated by α_v integrins. While the studies reported here demonstrated that $\beta2$ integrins promote Ad attachment to hematopoietic cells, we hypothesized that α_v integrins were, nonetheless, required for virus internalization. In support of this hypothesis, we found that Ad2 uptake into both THP-1 monocytic cells and $\alpha_M\beta2$-expressing CHO cells could be inhibited by function-blocking MAbs to α_v integrins (Fig. 9), although these antibodies had no effect on virus attachment (Fig. 4). These results indicate that α_v integrins rather than $\beta2$ integrins mediate Ad2 internalization into hematopoietic cells. Thus, Ad interaction with distinct integrins is required for virus attachment and entry into hematopoietic cells.

DISCUSSION

The identity of cell receptors that mediate Ad2 attachment to different cell types in vivo has yet to be firmly established. Previous studies had indicated that the cell receptor for the Ad2 fiber protein was probably responsible for virus attachment to the majority of different cells (19); however, there is relatively little information on whether this receptor is present on hematopoietic cells. The studies reported here demonstrate that cells of the myeloid lineage do not express Ad2 fiber receptors, although they permit virus attachment via interaction of the penton base protein with integrin $\alpha_M\beta2$. A Scatchard analysis (unpublished observation) indicates that monocytic cells, which express both $\alpha_M\beta2$ and α_v integrins, possess both high- and low-affinity binding sites for the penton base protein, whereas only low-affinity penton base-binding sites have been observed on epithelial cells, which express α_v but not $\beta2$ integrins (27). This finding is consistent with the current studies that demonstrate that β_2 integrins serve as alternative Ad2 attachment receptors on certain hematopoietic cell types which lack fiber receptors.

While $\beta2$ integrins were shown to promote Ad attachment to monocytic cells, the precise sequence in the penton base that governs virus interaction with $\beta2$ integrins remains to be determined. Penton base and Ad2 binding to monocytic cells is inhibited by an GRGDSP synthetic peptide (results not shown) and by a MAb that recognizes the penton base RGD sequence

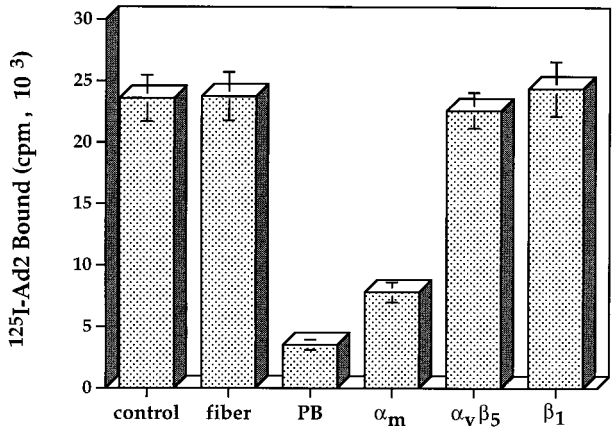
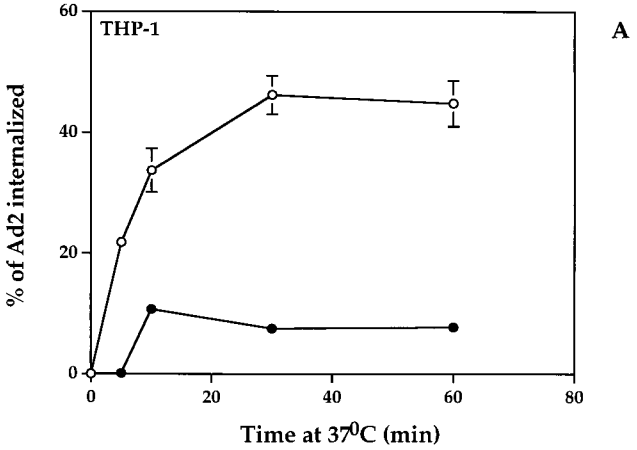


FIG. 7. Effect of anti-integrin MAbs on Ad attachment to $\alpha_M\beta2$-expressing CHO cells. $\alpha_M\beta2$-expressing CHO cells were incubated with recombinant fiber, penton base (PB), or function-blocking MAbs to $\alpha_M\beta2$ (CP3), $\alpha_v\beta5$ (P1F6), or $\beta1$ (P4C10), and then the binding of ^{125}I-labeled Ad2 was measured.

(Fig. 3), suggesting that the penton base RGD motif is involved in interactions with β2 integrins. Interestingly, the CP3 MAb, which blocks Ad and penton base interaction with β2 integrins (Fig. 7), contains an RYD sequence in its antigen-combining site (25). Since the RYD sequence in this MAb is believed to mimic the RGD motif involved in integrin binding, this finding further supports the notion that the penton base RGD sequence mediates interaction with β2 integrins. Further penton base mutagenesis studies, however, are necessary to confirm this possibility. The fact that two different β2 integrins which have different α subunits facilitated Ad2 attachment and gene delivery also suggests that the β2 subunit may play the major role in penton base interactions.

Although β2 integrins promote Ad attachment, these receptors do not appear to play a direct role in Ad2 internalization (Fig. 9). Consistent with the present studies, freshly isolated monocytes express integrin $\alpha_M\beta2$ and also support virus attachment; however, they are not susceptible to Ad infection and gene delivery (13). In contrast, upregulation of α_v integrin expression on terminally differentiated monocytes/macrophages or expression of recombinant β2 integrins in CHO cells that also have endogenous α_v integrins (Fig. 9) renders these cells competent for virus internalization and Ad-mediated gene delivery. Thus, penton base interaction with two distinct integrins is required for efficient Ad entry into these cells. Further studies are required to evaluate the overall contribution of this fiber-independent pathway to Ad infection of other hematopoietic cell types, including B and T lymphocytes and NK cells. The interaction of other microorganisms such as *Bordetella pertussis*, a bacterial pathogen, with different cell integrins has been reported previously (20); however, these interactions are thought to facilitate the same stage (attachment) in infection.



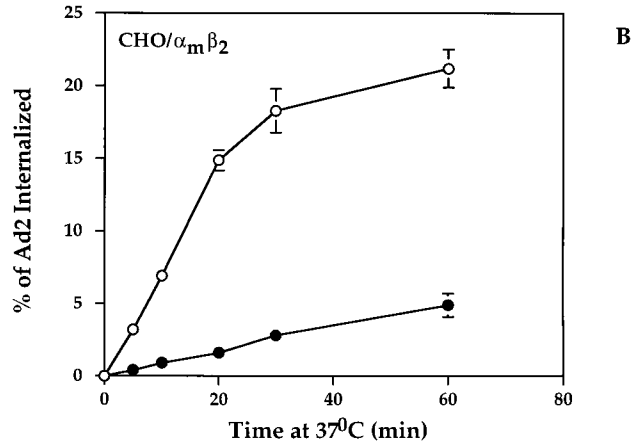


FIG. 9. Ad internalization is mediated by α_v integrins. Internalization of ^{125}I-labeled Ad2 into THP-1 cells (A) or $\alpha_M\beta2$-expressing CHO cells (B) was assayed at various times after incubation at 37°C by resistance to trypsin digestion (27) (open circles). In parallel studies, cells were preincubated with a function-blocking MAb to α_v (69-6-5) (A) or $\alpha_v\beta5$ (P1F6) (B) (solid circles) before measurement of virus internalization. The data represent the mean of three experiments, with the standard deviation being 5 to 10% of the mean.

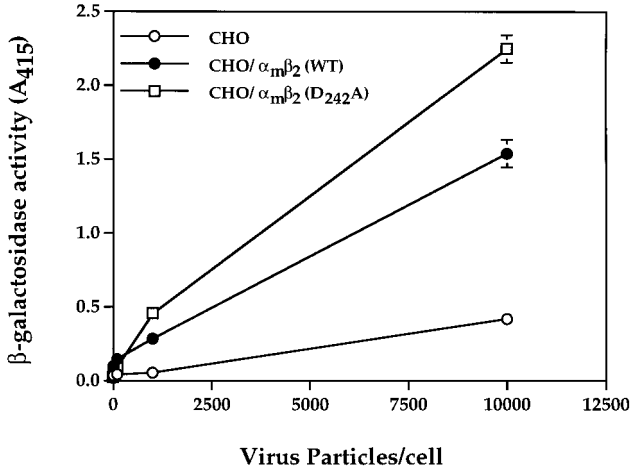


FIG. 8. Ad-mediated gene delivery to CHO cells lacking or expressing integrin $\alpha_M\beta2$. Expression of the Ad-transduced *lacZ* gene in nontransfected CHO cells lacking $\alpha_M\beta2$, CHO cells expressing wild-type (WT) $\alpha_M\beta2$, and in CHO cells expressing a point mutation (D242A) in the α_M subunit of $\alpha_M\beta2$ is shown. β-Galactosidase expression was assayed as described in Materials and Methods. The data represent the mean of three experiments, with the standard deviation being equivalent to 5 to 10% of the mean.

The findings reported here also suggest that a modified Ad vector lacking the fiber protein could be used to selectively deliver genes to hematopoietic cells in vivo because of its limited capacity to interact with the majority of other cell types that express the fiber receptor. Host immune responses di-

rected against Ad structural proteins limit the usefulness of Ad vectors (8, 28); therefore, a vector lacking a major structural protein (fiber) might also be less immunogenic, thereby providing longer-term expression of a therapeutic gene. Previous studies have demonstrated that fiberless Ad particles can be properly assembled (5); however, further development of packaging cell lines is needed to produce significant amounts of such particles in the absence of contaminating helper virus. Since monocytes/macrophages represent a site of persistent or latent virus infection by a number of important human pathogens including cytomegalovirus and human immunodeficiency virus, the ability to selectively deliver antiviral agents (e.g., ribozymes) into these cells via Ad vectors may have therapeutic value. Ad-mediated targeting of cytokine genes to monocytes/macrophages may also prove beneficial for anticancer therapy, given the well-established role of these cells in tumor cell killing (1, 22). In addition to potential practical applications, the current studies provide fundamental knowledge of virus-host cell interactions and also contribute new insights into the function of cell integrins.

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