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Upregulation of Integrins αvβ3 and αvβ5 on Human Monocytes and T Lymphocytes Facilitates Adenovirus-Mediated Gene Delivery

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Entry of human adenovirus into host cells involves interaction of virus particles with two distinct receptors. The initial binding event is mediated by the fiber protein, while subsequent interaction of the penton base protein with αv integrins promotes virus internalization and/or penetration. Although these interactions in epithelial and endothelial cells have been well characterized, relatively little is known as to whether these events occur during virus infection of human peripheral blood mononuclear cells. We demonstrate that freshly isolated peripheral blood monocytes and T lymphocytes express very small amounts of αv integrins and also are resistant to adenovirus infection. Exposure of monocytes to hematopoietic growth factors granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor induced expression of cell surface αv integrins, promoted the binding of penton base protein, and also rendered these cells susceptible to adenovirus-mediated gene delivery. Stimulation of T cells with a mitogen, phytohemagglutinin, or a cell-activating agent, phorbol myristate acetate, induced expression of αv integrins and also enhanced adenovirus-mediated gene delivery. These studies further indicate that αv integrins play a crucial role in adenovirus infection and also provide a useful strategy for enhancing adenovirus-mediated gene delivery into human peripheral blood mononuclear cells.

Replication-defective forms of human adenovirus have been used to deliver foreign genes into diverse cell types, including liver, muscle, nerve, and airway epithelial cells (4, 6, 14, 17, 18, 21, 24). The successful use of adenovirus as a gene delivery vector is based in part on its highly efficient mode of cell entry and its lack of requirement for host cell replication.

Entry of human adenovirus into host cells involves interactions of virus particles with two separate cell receptors. Initial binding of adenovirus to an as yet unidentified cell receptor is mediated by the fiber capsid protein (15). The subsequent event of virus entry into cells occurs via internalization into clathrin-coated vesicles (5) and is mediated by penton base protein binding to αv integrins (2, 23). Adenovirus interaction with αv integrins is mediated by an RGD sequence predicted to be located at the apex of a 10-Å (1-nm) protrusion on penton base protein (13, 20). The RGD sequence motif is conserved in multiple adenovirus serotypes, indicating that these viruses use a common pathway for entry into host cells (2, 23). Following internalization, adenovirus efficiently disrupts cell endosomes, allowing the virus genome and associated capsid proteins to be rapidly transported to the cell nucleus. Although these later events are still poorly understood, a number of minor capsid proteins, including IIIa, penton base, IX, and VI, are removed from the virus particle and/or degraded during the early phases of virus penetration (9).

In contrast to knowledge of the early events of adenovirus infection of epithelial and endothelial cells, relatively little is known of adenovirus entry into human peripheral blood cells such as lymphocytes and monocytes/macrophages. In particular, little is known of the role that αv integrins play on these cell types. Since monocytes/macrophages and lymphocytes play an important role in cell-mediated immunity and also serve as host cells for a number of important viral and bacterial pathogens, the ability to deliver genes or antiviral agents into these cells via adenovirus is of potential value. Human monocyte-derived and alveolar macrophages have been reported to be permissive for adenovirus cell entry; however, freshly isolated human monocytes are unable to support virus internalization (3, 10). Adenovirus has also been reported to have limited capacity for binding to human lymphocytes (12, 19), although these cells are clearly capable of being infected (11). Since the expression of integrins αvβ3 and αvβ5 can be differentially induced on human monocytes by the hematopoietic growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) (7), this suggested that freshly isolated human monocytes were susceptible to adenovirus infection following upregulation of these cell surface receptors. In these studies, we demonstrate that induction of αv integrin expression on human monocytes/macrophages as well as human T lymphocytes by hematopoietic growth factors or cell-activating agents facilitates adenovirus-mediated gene delivery. These studies further substantiate the role of αv integrins in adenovirus infection and also suggest a mechanism by which the host cell range of adenovirus is extended to monocytes and lymphoid cells.

MATERIALS AND METHODS

Recombinant adenovirus, MAbs, synthetic peptides, proteins, and cell-activating agents. Ad.RSVβgal, a replication-defective form of adenovirus type 5 (Ad5) which encodes the β-galactosidase gene (21), was kindly provided by Michel Perricaudet (Institut Gustave Roussy). Function-blocking monoclonal antibodies (MAbs) that recognize integrins αvβ3 (LM609), αvβ5 (P3G2), and β1 (P4C10) were obtained from David Cheresh (The Scripps Research Institute). Recombinant Ad2 penton base and fiber proteins were produced in *Trichoplusia ni* (Tn 5B1-4) insect cells (Invitrogen, San Diego, Calif.) as previously described (23). M-CSF was purchased from Genzyme (Cambridge, Mass.), and GM-CSF was obtained from Collaborative Biomedical Products (Bedford, Mass.). Phytohemagglutinin (PHA) was from Calbiochem (San Diego, Calif.), and phorbol-

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12-myristate-13-acetate (PMA) was obtained from Sigma (St. Louis, Mo.). GRGDSP and GRGESP synthetic peptides were obtained from GIBCO/BRL (Gaithersburg, Md.).

Cell lines, stimulation of peripheral blood mononuclear cells with growth factors, and flow cytometry. Human epithelial cell lines A549 and H2981 (American Type Culture Collection, Rockville, Md.) were maintained in Dulbecco modified Eagle medium with 10% fetal calf serum. Human peripheral blood mononuclear cells were isolated from healthy adult donors by centrifugation on Ficoll-Hypaque gradients ($d = 1.077$). The mononuclear cell fraction was washed by low-speed centrifugation ($400 \times g$) in Hanks' balanced salt solution to remove platelets. Mononuclear cells were resuspended in RPMI 1640 supplemented with 20% fetal calf serum and allowed to adhere to plastic tissue culture flasks for 60 min at 37°C in a 5% CO_2-humidified incubator. Nonadherent cells were collected for further isolation of the T-lymphocyte population. The adherent cell population that represented approximately 90% monocytes as determined by Giemsa-Wright staining and detection of nonspecific esterase (1) was maintained in RPMI that contained 10% fetal calf serum and antibiotics (Pen/Strep/Fungizone; GIBCO/BRL). To isolate peripheral blood T lymphocytes, nonadherent mononuclear cells were incubated at a 50:1 ratio with 2-aminoethylisothiuronium hypobromide (AET)-treated sheep erythrocytes for 1 h at 4°C. T-cell rosettes were isolated by sedimentation on Ficoll-Hypaque gradients, and erythrocytes were eliminated by hypotonic lysis.

For cell activation studies, monocytes were treated with 50 U of M-CSF per ml or 10 ng of GM-CSF per ml for 24 to 72 h in RPMI media. T lymphocytes were incubated with 1 μg of PHA per ml, 30 nM PMA, or a combination of these agents for 48 h. Control (untreated) cells were maintained in RPMI for equivalent times.

For analysis of cell surface αv integrins, monocytes and T lymphocytes, either untreated or cultured in the presence of growth factors or activating agents for 48 h, were washed with phosphate-buffered saline (PBS) at 4°C and then incubated in the presence of 25 μg of anti-αv integrin MAb, P3G2 or LM609, per ml for 30 min at 4°C. Cells were washed twice and then incubated with anti-mouse immunoglobulin G conjugated to fluorescein isothiocyanate (KPL Laboratories, Gaithersburg, Md.) for 30 min at 4°C. Control cell samples were incubated with the secondary antibody conjugate alone. Following additional washes in PBS, cells were resuspended to 500 μl and analyzed by flow cytometry (FACScan; Becton Dickinson) with the Lysis II program.

Penton base and fiber protein binding assays. Purified recombinant penton base and fiber proteins (23) were labeled with ^{125}I (Na ^{125}I; Amersham, Arlington Heights, Ill.) by using Iodobeads (Pierce Chemical Co., Rockford, Ill.). Briefly, 10 μg of penton base or fiber protein was labeled by the addition of 500 μCi of ^{125}I and Iodobeads for 30 min at 22°C. Unincorporated ^{125}I was removed by desalting on a PD-10 column (Pharmacia, Piscataway, N.J.). The specific activities of labeled penton base and fiber proteins were 1.75×10^7 cpm/μg and 1.721×10^7 cpm/μg, respectively. For cell binding studies, 2×10^6 monocytes or T lymphocytes were incubated for 2 h at 4°C with 5×10^5 cpm of ^{125}I-labeled penton base or fiber protein in 200 μl of RPMI that contained 0.5% bovine serum albumin and 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer. Unbound radiolabeled proteins were then removed by centrifugation for 3 min at $13,000 \times g$ on a 200-μl cushion of 86% silicon–16% mineral oil. Nonspecific cell-associated counts, which usually represented approximately 10 to 15% of total binding, were determined by incubation of cells in the presence of a 100-fold excess of unlabeled protein.

Detection of adenovirus-mediated gene delivery. For gene delivery experiments, monocytes were cultured in the absence (control) or presence of M-CSF or GM-CSF for 48 h while T lymphocytes were incubated with PHA, PMA, or PHA-PMA for 24 to 72 h. Cells were then infected with various multiplicities (10^0 to 10^3 virus particles per cell) of Ad.RSVβgal for 18 h at 37°C. Unbound virus was removed by washing, and cells were then reincubated for 48 to 72 h at 37°C in a 5% CO_2-humidified incubator. Uninfected control cells were maintained in medium for equivalent times. For competition studies, cells were preincubated with 500 μg of anti-αv integrin MAb P3G2 (anti-αvβ5) or LM609 (anti-αvβ3) or MAb P4C10 (anti-β1) per ml or with 1 mg of GRGDSP or GRGESP peptides per ml prior to the addition of Ad.RSVβgal. Uninternalized virus particles were removed by incubating cells with trypsin-EDTA for 5 min at 37°C and then washing them in complete RPMI medium. β-Galactosidase activity was measured by washing cells with saline at 4°C, fixing them for 5 min with 0.5% glutaraldehyde, and then incubating them with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) solution for 12 h. Adenovirus-infected cells showed intense blue-green staining indicative of β-galactosidase activity and were quantitated by light microscopy.

RESULTS

Stimulation of human monocytes with hematopoietic growth factors induces αv integrin expression and promotes adenovirus penton base binding. Flow cytometry was used to determine whether unstimulated human monocytes or monocytes cultured in the presence of GM-CSF or M-CSF express specific αv integrins on their cell surfaces (Fig. 1). Very low levels of αv integrins were detected on monocytes cultured in medium alone (controls). Approximately 5% of monocytes expressed αvβ3, and less than 2% expressed αvβ5 in the absence of growth factors. In contrast, significant amounts of αvβ3 were detected on the surfaces of monocytes (approximately 60% of cells) cultured in the presence of GM-CSF. A minor proportion of GM-CSF-treated cells (10%) also expressed integrin αvβ5. In contrast, monocytes cultured for 24 h in the presence of M-CSF expressed significant levels of integrin αvβ5 (approximately 77% of cells), while only 10% of these cells expressed integrin αvβ3. Over 90% of two human epithelial cell lines, A549 and H2981, expressed both αvβ3 and αvβ5 (data not shown). Thus, these studies substantiated previous observations that expression of integrins αvβ3 and αvβ5 on human monocytes can be differentially induced by specific growth factors (7).

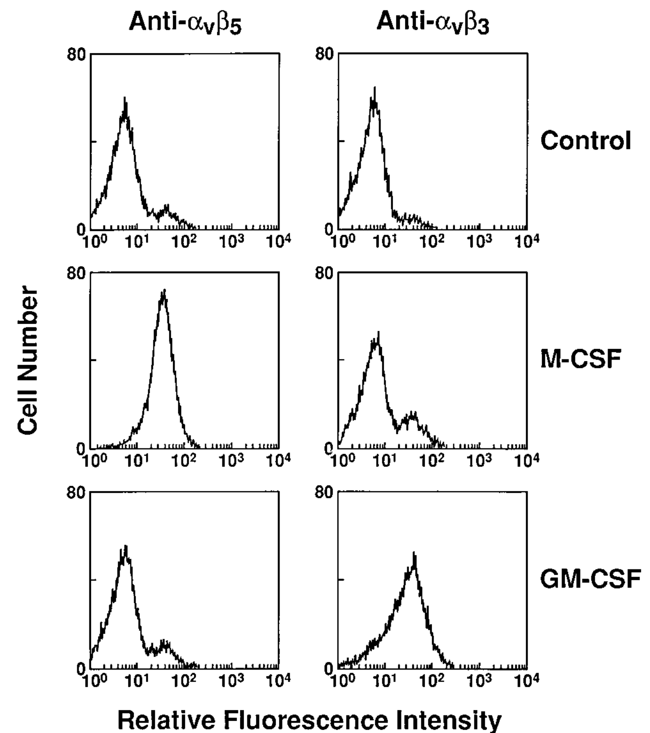


FIG. 1. Flow cytometric analysis of αv integrin expression on human monocytes. Purified human peripheral blood monocytes were cultured in the absence (control) or presence of 10 ng of GM-CSF per ml or 50 U of M-CSF per ml for 48 h. Cells were incubated with anti-αvβ3 (LM609) or anti-αvβ5 (P3G2) MAbs and then with fluorescein isothiocyanate-labeled anti-mouse immunoglobulin G and then subjected to flow cytometric analysis.

Since integrins αvβ3 and αvβ5 mediate adenovirus internalization via interaction with the penton base capsid protein (23), we next sought to determine whether expression of αv integrins on human monocytes promoted Ad2 penton base binding. Monocytes cultured in the presence of medium alone for 2 days and therefore expressing very low levels of αv integrins also showed very low levels of specific binding of ^{125}I-labeled penton base (Fig. 2). In contrast, monocytes cultured in the presence of GM-CSF or M-CSF expressed significant amounts of αvβ3 and αvβ5, respectively, and also supported dose-dependent binding of ^{125}I-labeled penton base. Kinetic studies showed that maximal binding of penton base to GM-CSF-treated monocytes or M-CSF-treated cells occurred at 48 h after growth factor addition (data not shown). In further stud-

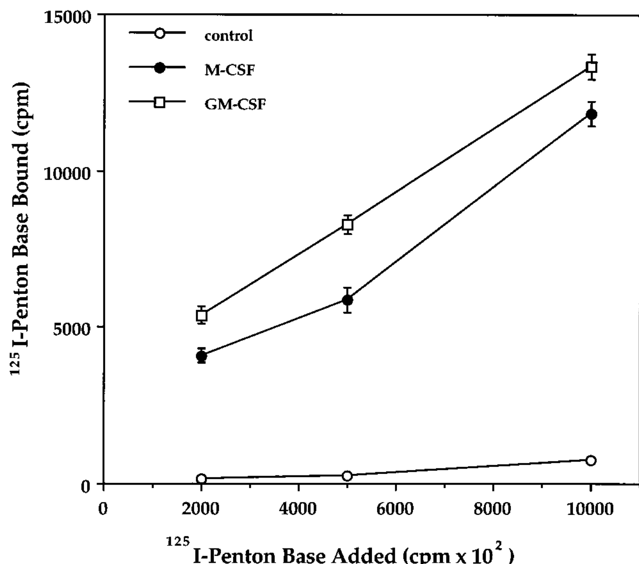


FIG. 2. Binding of adenovirus penton base to unstimulated monocytes and monocytes incubated with GM-CSF or M-CSF. Monocytes (2×10^6) were incubated in the presence of growth factors or medium (control) for 48 h prior to the addition of various amounts of ^{125}I-labeled penton base. Binding reactions were carried out in the absence or presence of a 100-fold excess of unlabeled protein to determine the amounts of nonspecific binding. Data are the means ± standard deviations of triplicate samples.

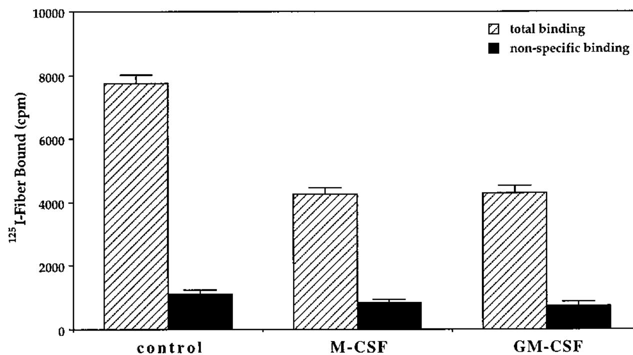
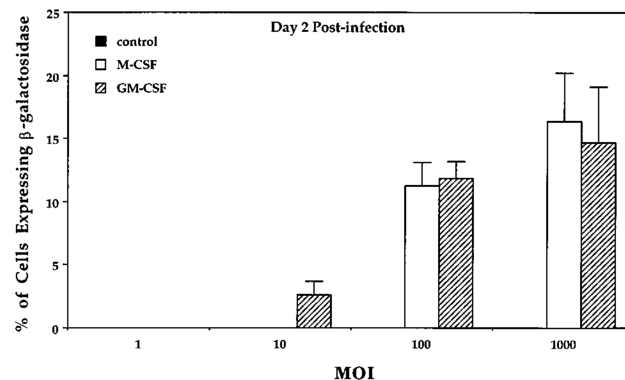


FIG. 3. Binding of adenovirus fiber to unstimulated monocytes and monocytes stimulated with hematopoietic growth factors. Monocytes (2×10^6) were cultured in the absence (control) or presence of GM-CSF or M-CSF for 48 h. Cells were incubated with 500,000 cpm of ^{125}I-labeled fiber in the absence or presence of a 100-fold excess of unlabeled fiber. Data are the means ± standard deviations of triplicate samples.

ies, we found that A549 and H2981 cells bound 60,000 and 148,000 cpm of ^{125}I-labeled penton base, respectively, when 500,000 cpm of I^{125}-labeled penton base was added, while GM-CSF- and M-CSF-treated monocytes bound 8,000 and 6,000 cpm of ^{125}I-labeled penton base (data not shown). Since a human monocyte is approximately one-fifth the size of an A549 or H2981 cell, the density of αv integrins expressed on growth factor-stimulated monocytes appears to be comparable to that of cultured epithelial cell lines. These studies indicated that upregulation of αv integrin expression on human monocytes also promotes binding of adenovirus penton base.

In contrast to upregulation of αv integrin expression on human monocytes by M-CSF and GM-CSF, these growth factors caused an approximately 50% decrease in the expression of adenovirus attachment receptor as measured by radiolabeled fiber binding (Fig. 3). Control monocytes bound 3,318 cpm of ^{125}I-labeled fiber per 10^6 cells, compared with approximately 20,000 cpm for A549 and H2981 epithelial cells, while growth factor-stimulated cells were capable of binding approximately 1,700 cpm of ^{125}I-labeled fiber.

Growth factor stimulation of αv integrin expression on human monocytes facilitates adenovirus-mediated gene delivery. The ability of GM-CSF and M-CSF to significantly upregulate αv integrin expression on human monocytes suggested that growth factor-activated monocytes were susceptible to adenovirus-mediated gene delivery. To test this hypothesis, we used a recombinant adenovirus vector, Ad.RSVβgal, that constitutively expresses β-galactosidase following targeting to the nuclei of infected cells (21). Untreated and growth factor-stimulated human monocytes were infected with various multiplicities and then assayed for β-galactosidase activity at 48 to 72 h postinfection (Fig. 4). Expression of adenovirus-encoded β-galactosidase was not detected in the cytoplasm of control monocytes cultured in medium alone for either 48 or 72 h (Fig. 4 and 5). In contrast, a significant number of monocytes treated with GM-CSF or M-CSF expressed β-galactosidase activity in the cytoplasm at both times (Fig. 4 and 5). Approximately 36% of GM-CSF-stimulated monocytes and 52% of M-CSF-stimulated monocytes expressed β-galactosidase activity at 72 h postinfection in cells infected with a multiplicity of infection (MOI) of 10^3. In parallel studies, infection of A549 and H2981 cells at an MOI of 10^3 resulted in over 80% of the cells expressing β-galactosidase (data not shown). These studies



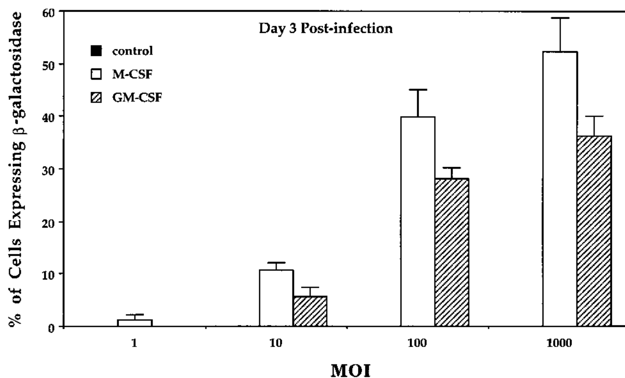


FIG. 4. Adenovirus-mediated gene delivery into unstimulated and growth factor-stimulated human monocytes. Monocytes were incubated in the absence (control) or presence of 10 ng of GM-CSF per ml or 50 U of M-CSF per ml for 48 h prior to infection with various multiplicities of Ad.RSVβgal (MOI, 1 to 1,000 virus particles per cell) for 48 to 72 h. β-Galactosidase activity was measured as described in Materials and Methods.

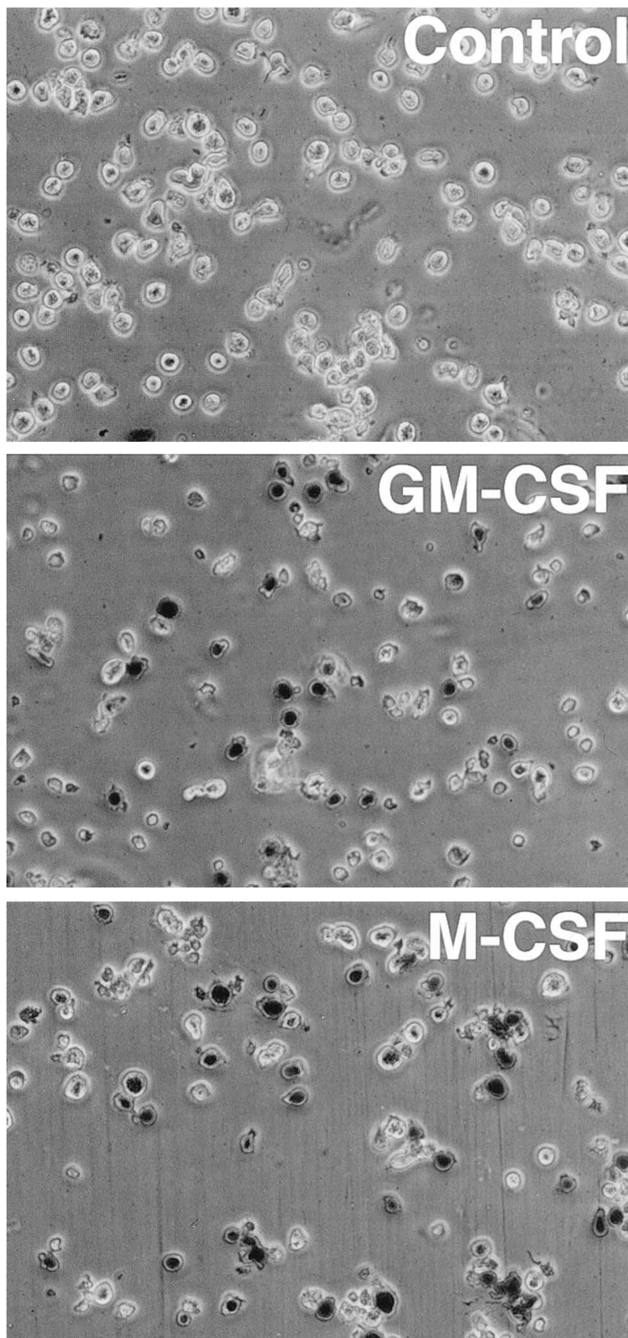


FIG. 5. Photomicroscopy of unstimulated and growth factor-activated human monocytes infected with Ad.RSVβgal. Monocytes were infected at an MOI of 10^3 and analyzed for β-galactosidase activity on day 3 postinfection. Photographs were taken on an inverted-phase light microscope equipped with a camera. Control monocytes show no evidence of β-galactosidase activity in the cytoplasm. Magnification, ×200.

indicated that upregulation of αv integrin expression by GM-CSF and M-CSF promotes adenovirus-mediated gene delivery. To substantiate this finding, competition studies were performed with function-blocking MAbs to αv integrins and also with synthetic RGD peptides (Table 1). Preincubation of GM-CSF-treated monocytes with anti-αvβ3 MAb LM609 significantly blocked adenovirus-mediated gene delivery. Similarly, preincubation of M-CSF-treated monocytes with anti-αvβ5 MAb P3G2 also abrogated adenovirus-mediated gene delivery. Control anti-β1 MAb P4C10 had little effect on cells treated with either growth factor. Synthetic GRGDSP peptides but not control GRGESP peptides also blocked adenovirus-mediated gene delivery. These results indicate that adenovirus-mediated gene delivery into monocytes is due to enhanced expression of integrins αvβ3 and αvβ5.

TABLE 1. Inhibition of adenovirus-mediated gene delivery into human monocytes by anti-αv integrin MAbs and RGD peptides[a]

Cell type	Competitor	% Expressing β-galactosidase ± SD
GM-CSF-stimulated monocytes	Control	32.4 ± 4.4
	P3G2	24.2 ± 5.6
	LM609	5.1 ± 1.1
	P4C10	30.6 ± 3.4
	GRGDSP	2.2 ± 0.8
	GRGESP	33.8 ± 6.2
M-CSF-stimulated monocytes	Control	45.2 ± 5.1
	P3G2	6.7 ± 1.2
	LM609	41.8 ± 4.3
	P4C10	42.1 ± 5.6
	GRGDSP	4.4 ± 0.2
	GRGESP	42.2 ± 6.1
A549	Control	81.9 ± 6.2
	P3G2	56.2 ± 7.4
	LM609	44.3 ± 2.9
	P4C10	78.1 ± 6.3
	P3G2 + LM609	7.4 ± 2.5
	GRGDSP	3.1 ± 0.2
	GRGESP	83.2 ± 9.3

[a] Growth factor-stimulated monocytes and A549 cells were treated with medium alone (control), with 500 μg of P3G2, LM609, or P4C10 per ml, or with 1 mg of GRGDSP or GRGESP peptides per ml for 1 h at 4°C. Cells were infected at an MOI of 10^3 with Ad.RSVβgal for 1 h at 4°C and then incubated for 45 min at 37°C to allow virus internalization. Uninternalized virus particles were removed by incubation with trypsin-EDTA for 5 min at 37°C. Cells were cultured for 3 days before being assayed for β-galactosidase activity as described in Materials and Methods.

Upregulation of αv integrin expression on T lymphocytes enhances adenovirus-mediated gene delivery. Further experiments were performed to determine whether other subpopulations of peripheral blood mononuclear cells, including T lymphocytes, express αv integrins on their cell surfaces and whether agents known to trigger cell activation modulate expression of αv integrins on T cells (Fig. 6). Freshly isolated peripheral blood T lymphocytes were found to express only very small amounts of integrins αvβ3 and αvβ5. Expression of these molecules was detected on only approximately 1% of T cells. Treatment of T cells with PHA or PMA alone resulted in a small increase in αv integrin expression. Under these conditions, approximately 10% of T cells expressed detectable amounts of αv integrins. In contrast, exposure of T cells to a combination of PHA and PMA resulted in over 80% of T cells expressing integrins αvβ3 and αvβ5. This treatment also resulted in increased binding of ^{125}I-labeled penton base to stimulated cells; in contrast, only very low levels of penton base binding was detected on unstimulated T cells (Fig. 7). Treatment of T cells with PHA or PMA alone increased penton base binding approximately three- to fourfold compared with that of control cells, while combined treatment of T cells with PHA and PMA resulted in a 12-fold increase in penton base binding. In further studies, low levels of Ad2 fiber receptors were de-

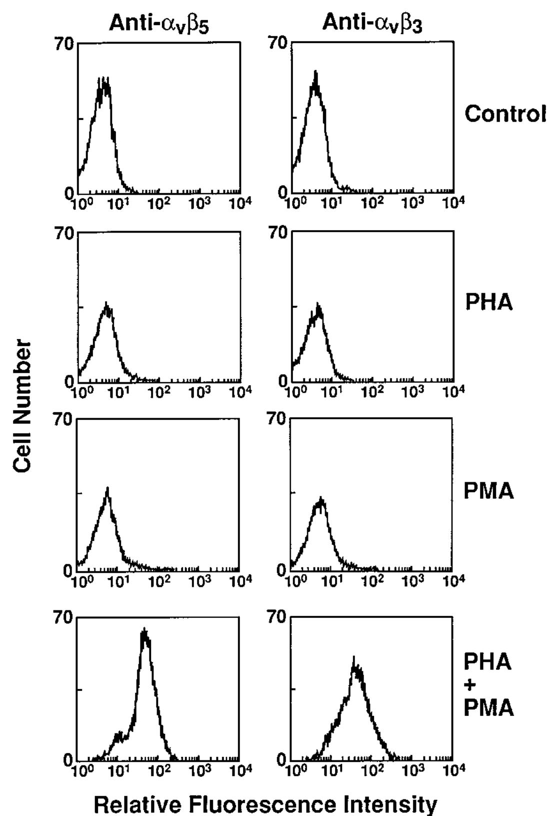


FIG. 6. Flow cytometric analysis of αv integrin expression on human T lymphocytes. Unstimulated T cells and T cells stimulated with PHA, PMA, or a combination of these activating agents for 48 h were analyzed for expression of integrins αvβ3 (anti-αvβ3 MAb LM609) and αvβ5 (anti-αvβ5 MAb P3G2) by flow cytometry.

tected on unstimulated T cells and fiber binding to stimulated T cells was increased only slightly (data not shown).

To determine whether upregulation of αv integrin expression on human peripheral blood T cells promotes adenovirus-mediated gene delivery, unactivated and PHA-, PMA-, and PHA-PMA-stimulated T cells were infected at an MOI of 10^3 with Ad.RSVβgal and then assayed for the presence of β-galactosidase activity at various times after infection (Fig. 8). Unstimulated T cells showed only low levels of β-galactosidase activity on days 2 and 3 postinfection. Approximately 4% of unstimulated T cells maximally expressed β-galactosidase activity. In contrast, T cells treated with PHA or PMA alone or with a combination of these agents showed significant increases in β-galactosidase activity. Under these conditions, 10, 12, and 17% of stimulated T cells showed β-galactosidase expression, respectively. Enhanced delivery of the *lacZ* gene into T cells was directly related to enhanced expression of αv integrins since preincubation of these cells with function-blocking MAbs to αv integrins or with soluble GRGDSP synthetic peptides abrogated gene delivery (Table 2). These results also indicate that stimulation of αv integrin expression on T lymphocytes facilitates adenovirus-mediated gene delivery into these cells.

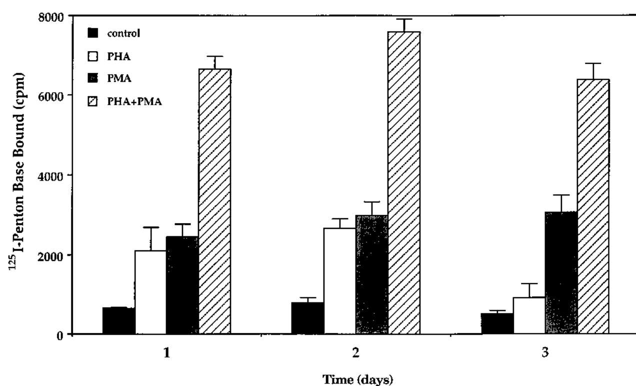


FIG. 7. Penton base binding to human peripheral blood T lymphocytes. Unstimulated and PHA-, PMA-, and PHA-PMA-stimulated T cells (2×10^6) were incubated with 500,000 cpm of ^{125}I-labeled penton base in the presence or absence of a 100-fold excess of unlabeled protein for 60 min at 4°C. Unbound radiolabeled penton base was removed by centrifugation through a cushion of mineral oil. Data are the mean specific binding data of triplicate samples ± standard deviations.

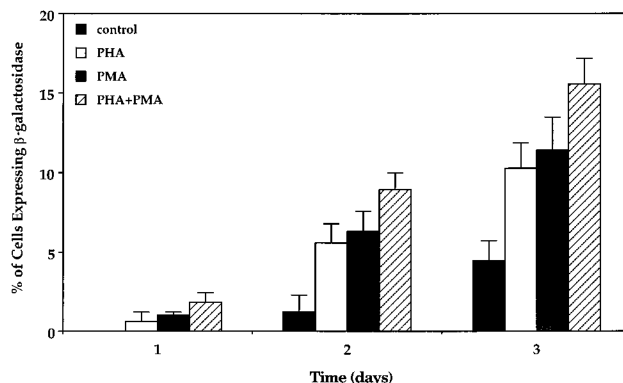


FIG. 8. Adenovirus-mediated gene delivery into human T lymphocytes. Unstimulated T cells and T cells stimulated with PHA, PMA, or a combination of these agents were infected at an MOI of 10^3 with Ad.RSVβgal for 3 days prior to analysis of the cells for β-galactosidase expression as described in Materials and Methods.

DISCUSSION

Cell surface αv integrins play a major role in adenovirus entry into cells (23). Cells deficient in these receptors are significantly less susceptible to adenovirus internalization and

TABLE 2. Inhibition of adenovirus-mediated gene delivery into T lymphocytes by anti-αv integrin MAbs and RGD peptides[a]

Competitor	% Expressing β-galactosidase ± SD
Control	17.2 ± 1.3
P3G2	4.6 ± 1.4
LM609	7.7 ± 1.1
P4C10	18.1 ± 2.6
P3G2 + LM609	1.2 ± 0.2
GRGDSP	0.0
GRGESP	16.9 ± 4.2

[a] PHA-PMA-activated T lymphocytes were treated with medium alone (control), with 500 μg of P3G2, LM609, or P4C10 per ml, or with 1 mg of GRGDSP or GRGESP peptides per ml for 1 h at 4°C. T cells were infected at an MOI of 10^3 with Ad.RSVβgal for 1 h at 4°C and then incubated for 45 min at 37°C to allow virus internalization. Uninternalized virus particles were removed by incubation with trypsin-EDTA for 5 min at 37°C. Cells were cultured for 3 days before being assayed for β-galactosidase activity as described in Materials and Methods.

infection. Moreover, mutant adenovirus particles that contain an RGE sequence instead of RGD in the penton base protein also have decreased ability to bind to αv integrins as well as reduced infectivity (2). Conservation of the penton base RGD sequence in multiple adenovirus serotypes also indicates the central role that αv integrins play in virus infection (13).

The ability of human Ad2 and Ad5 to utilize cell surface αv integrins on human airway epithelial cells has enabled recombinant adenovirus vectors to be used for gene delivery. However, previous studies have indicated that adenovirus has limited ability to infect human peripheral blood mononuclear cells, a feature that potentially restricts the use of this virus vector for gene therapy for these cells. For example, human monocytes were reported to be nonpermissive for adenovirus entry (10). Moreover, following antibody-mediated entry into cells, adenovirus fails to undergo late steps in virus replication (assembly) (3). Thus, virus entry into monocytes via interaction with components of the immune system may allow the establishment of virus persistence in these cells. In the studies reported here, we demonstrated that freshly isolated human monocytes express only very low levels of αv integrins on their cell surfaces and also were not susceptible to adenovirus-mediated gene delivery. Since the monocyte/macrophage-specific growth factors GM-CSF and M-CSF have been shown to upregulate αv integrin expression on human monocytes (7), we investigated the possibility that exposure of human monocytes to these growth factors also renders these cells susceptible to adenovirus infection. Incubation of human monocytes with GM-CSF or M-CSF differentially induced expression of αvβ3 and αvβ5 (Fig. 1), and this resulted in the ability of these cells to support binding of soluble penton base protein as well as adenovirus-mediated gene delivery. Although all growth factor-stimulated monocytes expressed αv integrins, not all cells supported adenovirus infection. This may be due to a requirement for a certain threshold level of integrin expression to permit virus entry. This is consistent with the fact that untreated monocytes express only very low levels of αv integrins and also are resistant to adenovirus infection. Upregulation of αv integrin expression was shown to be directly related to enhanced gene delivery since this was abrogated by synthetic RGD peptides and function-blocking MAbs to αv integrins. Moreover, enhanced adenovirus infection was not due to increased expression of the fiber receptor since expression of this receptor decreased following growth factor treatment (Fig. 3). Despite decreased fiber receptor expression, growth factor-stimulated monocytes were more susceptible to virus infection. These results therefore emphasize the importance of αv integrins in adenovirus cell entry.

Recent studies have indicated that integrin αvβ5 also plays an important role in adenovirus entry following internalization. Cells that expressed αvβ5 as their only αv integrin were shown to have increased susceptibility to adenovirus-mediated membrane permeabilization as well as to adenovirus-mediated gene delivery compared with that of cells that expressed only αvβ3 (22). The selective role of αvβ5 in membrane permeabilization may be related to the ability of αvβ5 to interact with penton base protein in the low-pH environment of the cell endosome. In these studies, however, we did not detect a significant difference in adenovirus-mediated gene delivery between GM-CSF-stimulated monocytes, which expressed primarily αvβ3, and M-CSF-treated monocytes, which expressed αvβ5. A likely explanation for this is that GM-CSF-treated cells also express a low level of integrin αvβ5, which may be sufficient to mediate adenovirus-mediated membrane permeabilization.

In further studies, we demonstrated that human T lymphocytes cultured in the presence of a T-cell mitogen, PHA, a nonspecific activator, PMA, or particularly a combination of these two agents significantly upregulated αv integrin expression. This resulted in increased penton base protein binding and adenovirus-mediated gene delivery, although the percentage of β-galactosidase-expressing T cells was not as high as that achieved by growth factor stimulation of human monocytes. This may be due to the very low level of fiber protein binding to T cells (data not shown) or perhaps to later events in virus infection that involve uncoating of the virus genome (12). The fact that 15 to 20% of T cells supported adenovirus-mediated gene delivery (β-galactosidase expression) was not due to contaminating monocytes since flow cytometric analysis with a monocyte-specific MAb (anti-CD14) indicated that the purified T-cell population contained less than 1% monocytes. In further studies, we also found that peripheral blood B cells were not capable of being infected by the Ad.RSVβgal virus at an MOI of 10^3. Moreover, stimulation of B cells with a mitogen, *Staphylococcus aureus* protein A, or PMA did not enhance gene delivery on day 2 postinfection. These studies have not examined the possibility that other peripheral blood mononuclear cells, such as NK cells, also are susceptible to adenovirus-mediated gene delivery.

The studies described here further demonstrate the important role of αv integrins in adenovirus infection. This is particularly the case with human monocytes which express the initial attachment receptor (fiber), lack significant levels of αv integrins, and also are resistant to infection. This situation is overcome by exposure of cells to hematopoietic growth factors. These findings suggest that the host cell range for adenovirus may be extended to peripheral blood mononuclear cells, particularly during the inflammatory processes which accompany adenovirus infection. In this situation, the elaboration of specific growth factors or cytokines following primary infection of epithelial or endothelial cells could enhance expression of αv integrins on peripheral blood cells, thus increasing their susceptibility to adenovirus infection. In vivo evidence for such a model has been obtained in previous studies with mouse and cotton rat models of adenovirus respiratory disease which have identified monocytes/macrophages as important host cell types infected by adenovirus (8, 16).

These findings also have important practical implications for the use of adenovirus as a vector for gene delivery. Since monocytes and T lymphocytes are major cell types involved in host defense against microbial agents as well as malignant cells, the ability to deliver genes that encode cytokines such as interferons and tumor necrosis factor into T cells or monocytes may be of significant value. Alternatively, it may be useful to use adenovirus-encoded genes to downregulate expression of specific cytokines, such as interleukin-1β, which contribute to inflammatory processes. Finally, delivery of antiviral agents into monocytes infected with human immunodeficiency virus or cytomegalovirus via adenovirus may also have significant benefits.

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