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Redirected infection of directly biotinylated recombinant adenovirus vectors through cell surface receptors and antigens

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ABSTRACT **The inability of adenovirus to infect primitive hematopoietic cells presents an obstacle to the use of adenovirus vectors for gene transfer to these cell types. Therefore, expanding the tropism of adenovirus vectors to unique cell surface antigens would be an important development for gene therapy protocols. In this study, we sought to redirect infection of adenovirus vectors to primitive human hematopoietic cells that universally express the c-Kit receptor on their cell surface. To accomplish this, a vector was constructed by covalently linking biotin molecules to recombinant adenovirus, followed by addition of the biotinylated ligand for the c-Kit receptor, stem cell factor (SCF), through an avidin bridge. Gene transfer was directed specifically to c-Kit-positive hematopoietic cell lines, resulting in up to a 2,440-fold increase in luciferase expression with frequencies equivalent to recombinant virus infection of permissive cells. Substitution of biotinylated antibodies directed against c-Kit, CD34 (binds L-selectin), and CD44 (hyaluronate receptor) receptors for biotinylated SCF resulted in 50-, 8-, and 260-fold increases in reporter gene expression, respectively, demonstrating that infection also could be redirected through antibody–antigen interactions and through antigens other than growth factor receptors. The versatility of this vector was demonstrated further by infection of primary T cells with vectors targeted with antibodies to CD44 (resting and activated T cells) and biotinylated IL-2 (activated T cells only). Taken together, directly biotinylated adenovirus vectors represent a versatile and efficient method for redirection of virus infection to specific cells.**

Gene transfer to pluripotential hematopoietic stem cells (PHSCs) has achieved limited success, in part, because of low frequency of infection of these cells by viral vectors, the inability to target vectors, and the need to culture hematopoietic cells *in vitro* to induce them to enter the cell cycle, which can result in the loss of PHSC function (1). Several approaches have been taken to develop gene therapy vectors to target gene transfer through antigens or receptors expressed on specific cell types. These include nonviral vectors, composed of polycation-condensed, plasmid-encoded genes, an endosomalytic agent such as replication-defective adenovirus, and streptavidin for addition of biotinylated targeting moieties. When biotinylated asialoorosomucoid, transferrin, stem cell factor (SCF), or antibodies to CD3 were linked to these vectors, increased gene transfer to liver and hematopoietic cells occurred (2–5). Viral vectors, including retroviruses and adenoviruses, have been targeted to specific cell types by using bifunctional antibodies that recognize both viral epitopes and target cell antigens (6–10) and by molecular modifications to the virus that include replacing viral sequences with antigen- or antibody-binding domains, ligand, or peptide sequences (11–14). In this regard, both the use of bifunctional antibodies and the introduction of nonviral sequences, including erythropoietin, heregulin, and epidermal growth factor, into retroviral envelope proteins have been shown to adversely affect virus infection (15–17). In comparison, targeting of adenovirus vectors has been achieved by using bispecific antibodies, antibodies linked to growth factors, and molecular modifications of capsid proteins to introduce targeting domains into capsid proteins (18–20). We sought to redirect adenovirus vector infection to PHSCs without the requirement for production of bifunctional antibody reagents or molecular modifications of capsid proteins. In this study we demonstrate that biotin can be covalently linked to recombinant adenovirus and that after attachment of biotinylated targeting moieties through an avidin bridge, viral tropism can be redirected. Because PHSCs are refractory to adenovirus infection (21) and express c-Kit (22), the receptor for SCF, we chose to redirect the host range of adenovirus with biotinylated SCF (bio-SCF).

MATERIALS AND METHODS

Biotinylation of Adenovirus Vectors. Recombinant serotype 5 adenovirus containing a cytomegalovirus-driven firefly luciferase gene (AdLuc; ref. 23) and a cytomegalovirus-driven green fluorescent protein (GFP) gene (AdGFP; Quantum, Montreal, QC) were grown on 293 cells and concentrated to 5×10^{10} and 3×10^{9} infectious particles/ml, respectively, by centrifugation on two successive, discontinuous CsCl gradients. The concentrated virus was incubated on ice with between 25 and 1,000 μg/ml photoactivatable biotin (Pierce) in HBS (5 mM Hepes, pH 7.3/150 mM NaCl) and irradiated at a wavelength of 350 nm for 5 min. Unbound biotin was removed by a Sephadex G-25M column equilibrated with HBS. No significant loss of titer on 293 cells was observed between untreated and biotinylated Ad (bio-Ad) after column separation. In titration experiments, incubation of the virus with 100 μg/ml biotin was optimal. To demonstrate that biotin had been linked directly to the Ad, a 2-μl vol (2×10^{7} particles) of bio-AdLuc or untreated AdLuc was placed on a slide and

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Abbreviations: Ad, adenovirus; AdLuc, luciferase Ad; PHSC, pluripotential hematopoietic stem cell; SCF, stem cell factor; bio-SCF, biotinylated SCF; moi, multiplicity of infection; RLU, relative light units; GFP, green fluorescent protein; PHA, phytohemagglutinin.
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treated with 25 μl of 10-nm colloidal gold-streptavidin (Sigma) diluted 1:25 in TBS (50 mM Tris, pH 7.4/0.1% BSA/125 mM NaCl) at 4°C overnight. Five microliters was placed on a 5-mm^3 agar block, and a formvar-filmed grid was floated on the drop. After 3 min, the grid was washed in TBS followed by H_2O. The sample was negatively stained in 2% phosphotungstic acid (24) and analyzed by electron microscopy.

Construction of SCF-Directed Vectors. SCF was biotinylated by adding Biotin-X-NHS (Calbiochem) dissolved in DMSO to 1 mg/ml recombinant human SCF in 0.1 M $NaHCO_3$, pH 8.4, to yield 8.5 μg biotin/100 μg SCF and incubating for 25 min at room temperature. Free biotin was removed and the buffer was replaced by PBS with a centriprep concentrator (Amicon). Comparison of SCF and bio-SCF in binding and proliferation assays demonstrated that the ability of SCF to bind receptors and induce proliferation in hematopoietic cell lines was not affected by biotinylation (data not shown). To construct the SCF-directed vector, bio-Ad was incubated with between 1 and 500 μg/ml neutravidin (Pierce) for 30 min at room temperature. Unbound avidin was removed by separation on a Sephacryl 300 column equilibrated with HBS. Addition of avidin and column separation did not cause a significant decrease in titer of bio-Ad on 293 cells. After addition of bio-SCF to the avidin-bridged biotinylated vector and infection of M-O7e cells, we determined that 5 μg/ml neutravidin was the optimal concentration. Bio-Ad linked to avidin (100 μl) then was incubated with 25 ng of SCF or bio-SCF for 30 min at room temperature. Target cells [1.5×10^6/ml in RPMI medium 1640/2% FBS and 30 ng/ml granulocyte macrophage–colony-stimulating factor (GM-CSF) for M-O7e and MB-O2 cells] were incubated with 100 μl of the SCF-directed vector or a control vector (bio-Ad linked to avidin) at a multiplicity of infection (moi) of between 70 and 175 for 2 hr at 37°C. Cells were diluted to 2 ml in RPMI medium 1640 with supplements to yield a final concentration of 10% FBS (20% FBS for KG-1a cells) and 100 ng/ml SCF, 30 ng/ml GM-CSF, and 30 ng/ml IL-3 for M-O7e and MB-O2 cells and incubated for an additional 22 hr at 37°C. Cells were washed once with PBS and lysed in 1% Triton X-100/50 mM NaCl/10 mM Tris, pH 7.6/5 mM EDTA. Luciferase activity was determined by adding 20 μl of cell lysate to 100 μl of luciferase assay reagent (Promega) and measuring relative light units (RLU) on a luminometer. The protein content of each sample was determined by the bicinchoninic acid method (Pierce). Luciferase activity was determined by dividing the average RLU by the protein content in the sample. For infection of cells with AdGFP, 1×10^5 target cells were infected at moi = 100 as described above. Expression of GFP was analyzed 24 hr after infection either by fluorescence-activated cell sorting (FACS; Coulter) or fluorescent microscopy after fixation for 10 min in 1% paraformaldehyde in PBS and staining for 10 min in 100 ng/ml 4′,6-diamidino-2-phenylindole (Sigma) in PBS.

Construction of Antibody-Directed Vectors. Bio-AdLuc linked to avidin was incubated with 20, 20, and 2.5 μg/ml biotinylated mAb directed against CD117 (57A5; Ancell, Bayport, MN), CD34 (QBEND10; Immunotech, Westbrook, ME), and CD44 (G44–26; PharMingen), respectively, for 30 min at room temperature before infection of M-O7e, KG-1a, or HL-60 cells. Luciferase activity was assayed 24 hr later. We tested concentrations of antibody ranging from 0.25 to 200 μg/ml bio-AdLuc and determined that 20 μg/ml anti-CD117, 20 μg/ml anti-CD34, and 2.5 μg/ml anti-CD44 antibodies were optimal. When biotinylated isotype control antibodies (MOPC31C; Ancell) were used in construction of the vector, luciferase expression was equivalent to that observed with control vectors.

Infection of Primary Human T Cells. Normal peripheral blood mononuclear cells were obtained by protocols approved by the Frederick Cancer Research and Development Center Review Board. Cells were separated on Ficoll-Hypaque gradients (Sigma), depleted of cells adhering to nylon and plastic, and then separated on Percoll gradients (Amersham Pharmacia). The resulting cells (90–95% T cells) were incubated for 48 hr in RPMI medium 1640 supplemented with 10% FBS in the presence or absence of 1 μg/ml phytohemagglutinin (PHA, Sigma) before infection with bio-AdLuc or bio-AdLuc linked to biorecombinant human IL-2 (25 ng/100 μl virus) or bio-anti-CD44 as described above.

RESULTS

Biotinylation of Adenovirus Vectors. To construct the redirected virus, AdLuc was labeled with photoactivatable biotin by exposure to visible light at a wavelength of 350 nm. Western blot analysis of viral lysates demonstrated that proteins the size of adenoviral capsid proteins were biotinylated (data not shown). Electron microscopy confirmed that intact viral particles were labeled efficiently with biotin (Fig. 1). Specifically,

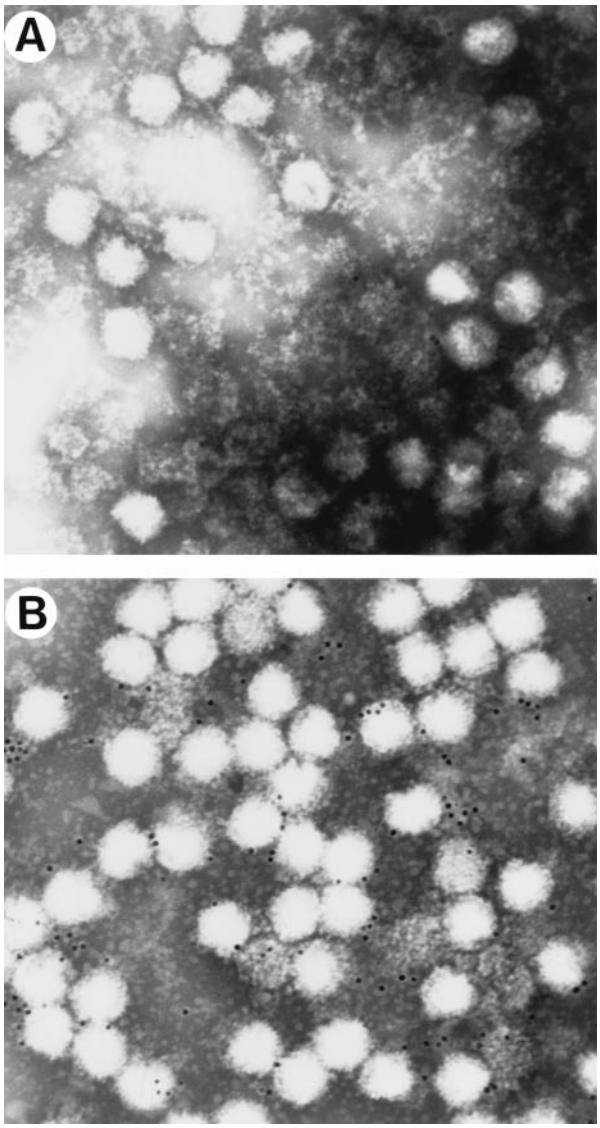

FIG. 1. Electron micrographs of control (*A*) and biotinylated adenovirus (*B*). AdLuc was incubated with 100 μg/ml photoactivatable biotin and irradiated at a wavelength of 350 nm for 5 min; control virus was treated identically except for the addition of photoactivatable biotin. After incubation with colloidal gold-streptavidin, negative-stain immunoelectron microscopy was performed and photographs were taken at ×135,000.

incubation of virus with streptavidin-linked gold particles resulted in the association of bio-AdLuc with gold (Fig. 1*B*) whereas nonbiotinylated virus did not associate with gold particles (Fig. 1*A*). Further, biotinylation of the virus did not affect significantly the titer in infectious center assays on 293 cells (data not shown).

SCF-Directed Gene Transfer. To determine whether we could redirect the viral tropism of AdLuc to c-Kit receptors, we added avidin to the biotinylated Ad followed by addition of bio-SCF as a final step in construction of the vector. This vector then was incubated with growth factor-dependent human progenitor cell lines expressing c-Kit and with cell lines negative for c-Kit. Cells were analyzed 24 hr later for luciferase expression. Incubation of c-Kit$^+$ M-O7e (25) and MB-O2 (26) cells with either biotinylated or nonbiotinylated AdLuc yielded a 3- and 10-fold increase in luciferase activity, respectively, compared with uninfected cells, confirming that these cells are infected by adenovirus vectors at a very low frequency. By varying the concentration of avidin in the vector construction, we found that 5 μg of avidin/ml of bio-Ad was optimal for vector redirection (data not shown). Incubation of the c-Kit$^+$ cells with bio-Ad linked to bio-SCF through an avidin bridge resulted in a 2,350- and 440-fold increase in luciferase activity in M-O7e and MB-O2 cells, respectively, compared with cells infected with the control vector, which was not linked to bio-SCF (Fig. 2*A*). In comparison, cell lines that do not express c-Kit, HL-60 (27) and KG-1a (28, 29), showed no increase in luciferase activity in SCF-directed infections compared with the control vector. To demonstrate that optimal concentrations of bio-SCF were used in the vector construction, increasing concentrations of bio-SCF (0–1,000 ng/ml) were added to the vector while holding other components constant. A dose-dependent increase in luciferase activity was observed, with optimal targeting of M-O7e cells achieved when 250 ng/ml bio-SCF was added (Fig. 2*B*). To confirm that the SCF-directed vector required c-Kit to enter cells, M-O7e cells, which are also positive for IL-3 receptors, were pretreated with an excess of nonbiotinylated SCF or IL-3 before addition of the vector. The addition of SCF competitively inhibited greater than 99% of the SCF-directed luciferase expression in M-O7e cells whereas pretreatment with IL-3 had no effect (Fig. 2*B*).

The kinetics of virally encoded luciferase expression were determined by assaying for luciferase activity at various times after infection of M-O7e cells with the SCF-directed vector. A 1,500-fold increase in luciferase expression was observed 12 hr after infection with the SCF-directed vector compared with the control vector (Fig. 2*C*). As predicted for infection of rapidly dividing cells with a replication-defective virus, luciferase activity was maintained at high levels in M-O7e cells over a 4-day period, with little or no activity detected after 1 week. To determine the efficiency of gene transfer, bio-AdGFP was targeted with bio-SCF to M-O7e cells. The frequency of GFP-expressing M-O7e cells and K562 cells (29), which are hematopoietic cells susceptible to infection by adenovirus, was determined by flow cytometry and fluorescent microscopy 24 hr after infection. For comparison, infection of K562 cells with control vector under conditions identical to those shown in Fig. 2*A* results in a 25,000-fold increase in luciferase expression (data not shown). Twenty-four hours after infection, 30% of M-O7e cells infected with SCF-directed bio-AdGFP were positive for GFP (Fig. 3 *g* and *h*) and 40% of K562 cells were positive for GFP (Fig. 3 *c* and *d*) by fluorescent microscopy. Infection of HeLa cells with AdGFP under identical conditions resulted in greater than 95% positive cells (data not shown). To confirm this frequency, M-O7e and K562 cells were analyzed by flow cytometry 24 hr after infection. FACS analysis showed that 17% of M-O7e cells infected with SCF-directed bio-AdGFP were positive for GFP (Fig. 3*f*) whereas uninfected cells and M-O7e cells infected with control vector were negative for GFP (Fig. 3*e*). In comparison, infection of K562

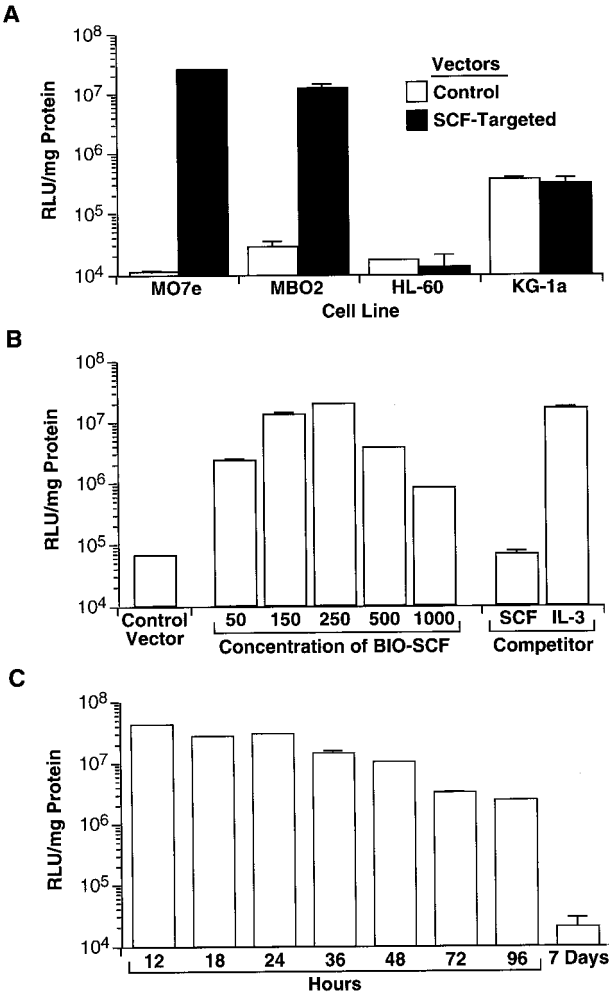

FIG. 2. (*A*) Infection of c-Kit$^+$ and c-Kit$^-$ cells by SCF-directed bio-AdLuc. Bio-AdLuc linked to avidin was incubated with 250 ng/ml SCF (control vector) or bio-SCF (SCF-directed vector) before infection of the c-Kit$^+$ cell lines M-O7e and MB-O2 and the c-Kit$^-$ cell lines HL-60 and KG-1a (moi = 165). (*B*) Specificity of SCF-directed gene transfer to M-O7e cells. SCF-targeted vectors were constructed by incubating Bio-AdLuc (moi = 100) linked to avidin with 50–1,000 ng/ml bio-SCF. In competition experiments, M-O7e cells were incubated with 500 ng/ml nonbiotinylated SCF or IL-3 before infection with 100 μl of the SCF-targeted vector constructed by incubation with 250 ng/ml bio-SCF. In *A* and *B*, luciferase activity at 24 hr after infection is reported as the mean ± SE of duplicate determinations and is representative of two experiments. (*C*) Kinetics of luciferase gene expression after infection of M-O7e cells with SCF-directed vectors. SCF-directed vectors were constructed by adding 250 ng/ml SCF to bio-AdLuc linked to avidin (moi = 120). At various times after infection, M-O7e cells were harvested and assayed for luciferase expression. At all time points, luciferase activity of cells infected with the control vector was less than 2×10^4 RLU/mg protein.

cells susceptible to infection with Ad resulted in 15% of cells positive for GFP compared with uninfected controls (Fig. 3 *a* and *b*). Thus, the SCF-directed vector targets c-Kit$^+$ cells refractive to adenovirus infection to yield a percentage of infected cells equivalent to that observed after infection of hematopoietic cells susceptible to infection with AdGFP.

Redirection of Infection Through Biotinylated Antibodies Linked to Bio-Ad. To determine whether this vector could be more broadly applied to direct infection to cells through antibody–antigen interactions in addition to ligand–receptor interactions, we substituted a biotinylated mAb that recognizes human c-Kit (CD117) for bio-SCF in the vector construction. When 0–200 μg/ml biotinylated anti-CD117 was added to the

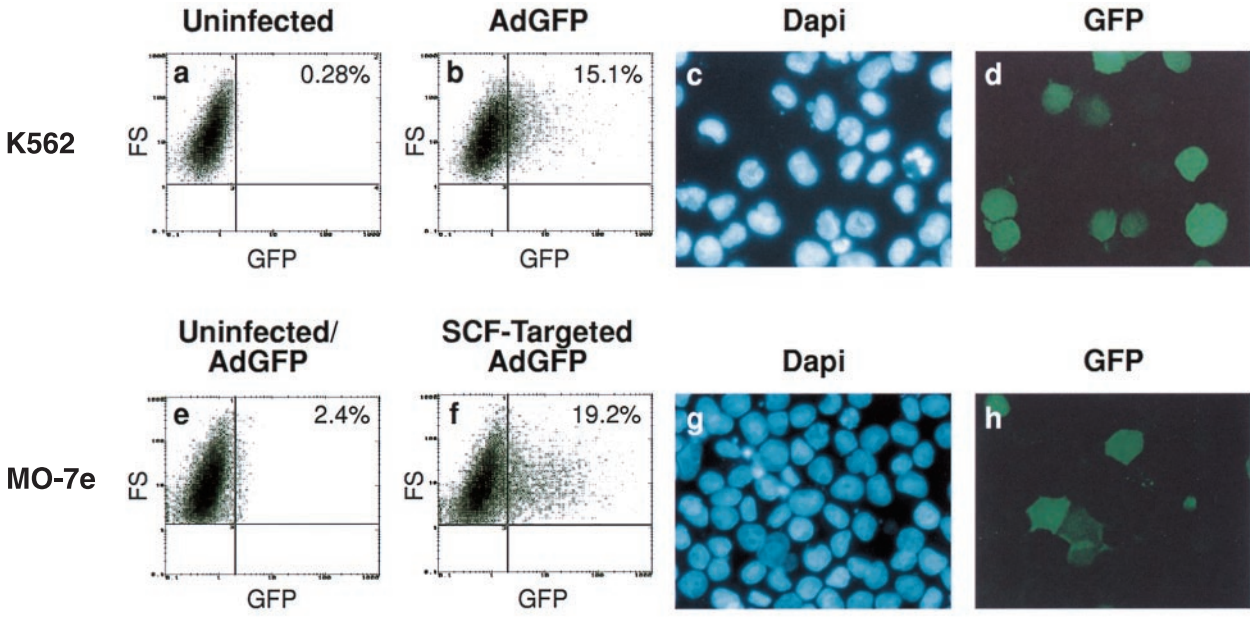

FIG. 3. Comparison of frequency of SCF-directed infection of M-O7e cells with infection of K562 cells with AdGFP. MO-7e and K562 cells were either untreated, treated with 100 μl of bio-AdGFP (moi = 100), or treated with 100 μl of the SCF-directed vector constructed with 250 ng/ml bio-SCF (moi = 100). Twenty-four hours later, K562 cells uninfected (*a*) and infected with bio-AdGFP (*b*) and MO-7e cells uninfected (*e*), infected with bio-AdGFP (*e*), and infected with SCF-targeted bio-AdGFP (*f*) were analyzed by FACS for GFP expression. K562 cells infected with AdGFP (*c* and *d*) and MO-7e cells infected with SCF-targeted bio-AdGFP (*g* and *h*) also were analyzed by photomicroscopy for 4′,6-diamidino-2-phenylindole staining and expression of GFP. Photographs were taken at ×400. Results are representative of two experiments.

vector before infection of M-O7e cells, a dose-dependent increase in luciferase activity compared with the control vector was observed. Optimal targeting by the anti-CD117-directed vector was observed at 20 μg/ml antibody, which resulted in up to a 50-fold increase in luciferase activity (data not shown and Fig. 4*A*). In comparison, HL-60 and KG-1a cell lines that do not express c-Kit showed no increase in luciferase expression after infection with the CD117-directed vector (Fig. 4 *B* and *C*). Anti-CD117-directed gene transfer was consistently less efficient than SCF-directed gene transfer, which resulted in a 2,440-fold increase in gene expression (Fig. 4*A*) in M-O7e cells.

Because anti-CD117 antibodies effectively redirected adenovirus infection to c-Kit$^+$ cells, we next examined whether antibodies that recognize proteins other than cytokine receptors could be used to direct gene transfer to specific populations of hematopoietic cells. We first chose CD34, an antigen restricted to hematopoietic progenitor cells that binds L-selectin (30). When 20 μg/ml biotinylated anti-CD34 was substituted for bio-SCF in the final step of vector construction, 5- and 8-fold increases in luciferase activity were observed in the CD34$^+$ cell lines M-O7e and KG-1a, respectively, compared with cells infected with control vector (Fig. 4 *A* and *B*). No increase in luciferase activity was seen in the CD34$^-$ HL-60 cell line (Fig. 4*C*). Second, to target an antigen expressed more broadly on hematopoietic cells, antibodies directed against CD44, an adhesion molecule that binds hyaluronate (31), were used to redirect the tropism of the recombinant adenovirus vector. When 2.5 μg/ml biotinylated anti-CD44 antibody was added to bio-Ad, an optimal increase in luciferase activity of 260-, 18-, and 12-fold was observed in the CD44$^+$ cell lines M-O7e, KG-1a, and HL-60, respectively (Fig. 4). Thus, this vector is highly versatile and biotinylated antibodies can be used to greatly broaden adenoviral vector tropism to permit transfer of genes to many cell types.

Infection of Activated Primary Human T Cells. To evaluate whether redirected infection of adenovirus vectors may be used to develop a model for targeted cell killing in graft-vs.-host disease, we compared infection of PHA-stimulated and unstimulated primary human T cells. Both stimulated and unstimulated T cells are positive for expression of CD44 whereas only PHA-stimulated cells express significant levels of the IL-2 receptor (data not shown). Infection of unstimulated T cells with nontargeted vector showed little or no increase in luciferase expression, whereas infection of PHA-stimulated T cells with the nontargeted vectors resulted in a significant increase in luciferase activity compared with uninfected T cells, suggesting that adenovirus receptors may be induced on PHA-stimulated cells (Fig. 5). Infection of PHA-stimulated T cells with IL-2-directed AdLuc vectors resulted in a 4-fold increase in luciferase expression above vector alone. As expected, no increase in luciferase expression was observed after infection of unstimulated T cells with the IL-2 receptor-targeted adenovirus vectors. In comparison, infection of unstimulated and stimulated T cells with CD44-targeted bio-AdLuc resulted in a 3- and 8-fold increase in luciferase expression, respectively, above control vector. Thus, adenovirus vectors can be targeted to both resting and stimulated T cells.

DISCUSSION

The experiments reported here demonstrate that by covalently linking biotin to recombinant adenovirus, we can produce a versatile vector for receptor-mediated gene transfer to hematopoietic progenitor cells, PHA-stimulated T cells, and, potentially, to other specific cell types. We present evidence that we can redirect infection of biotinylated adenovirus vectors to the c-Kit$^+$ hematopoietic cell line M-O7e, which we have shown to be refractive to adenovirus infection, through the growth factor SCF. In addition, the tropism of this vector can be broadened further by substituting biotinylated antibodies to a variety of cell surface antigens in the final step of vector construction. Furthermore, high frequencies of infection and expression of transferred genes were observed.

When biotinylated antibodies to c-Kit were used to direct virus infection, up to 50-fold increases in gene expression were observed. The highest level of gene expression, a 2,440-fold

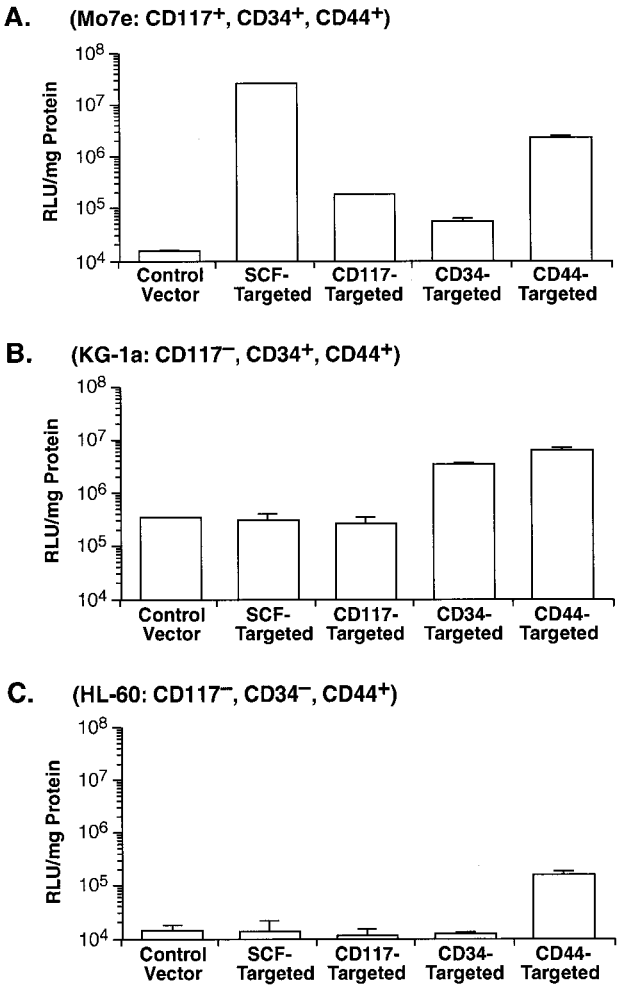

FIG. 4. Redirection of infection of hematopoietic cell lines with biotinylated AdLuc linked to antibodies. (*A*) The c-Kit$^+$, CD34$^+$, CD44$^+$ cell line M-O7e was infected with 100 μl (moi = 165) of bio-AdLuc linked to avidin (control), 250 ng/ml bio-SCF (SCF-targeted), 20 μg/ml bio-anti-CD117 (antibody to c-Kit, CD117-targeted), 20 μg/ml bio-anti-CD34 (CD34-targeted), or 2.5 μg/ml bio-anti-CD44 antibodies (CD44-targeted). The c-Kit$^-$, CD34$^+$, CD44$^+$ KG-1a cells (*B*) and c-Kit$^-$, CD34$^-$, CD44$^+$ HL-60 cells (*C*) were treated identical to M-O7e cells. Luciferase activity at 24 hr after infection is reported as the mean ± SE of duplicate determinations and is representative of two experiments.

increase compared with control vector, was observed after infection of cells with SCF-directed Ad vectors. We do not know whether these results reflect the fact that the affinity of hormone–receptor interactions normally are higher than the affinities of antibody–antigen binding or whether internalization of the vector differs between SCF- and anti-c-Kit antibody-directed vectors. Interestingly, although gene transfer can be directed through SCF and antibodies to CD117 and CD44, which are known to internalize, gene transfer also can be directed through antibodies to CD34, which is not known to internalize. This result suggests that although interactions between antibodies attached to the biotinylated vectors and antigens on the cell surface replace the normal cellular recognition of adenovirus by its receptor, the integrin-mediated internalization pathway of adenovirus may play a role in vector internalization. We are investigating the contribution of cell surface receptor internalization to increases in gene transfer observed with SCF-, CD117-, and CD44-directed vectors.

To date, approaches to target gene transfer to specific cell types have included the use of molecular conjugate vectors and retrovirus and adenovirus vectors. In molecular conjugate

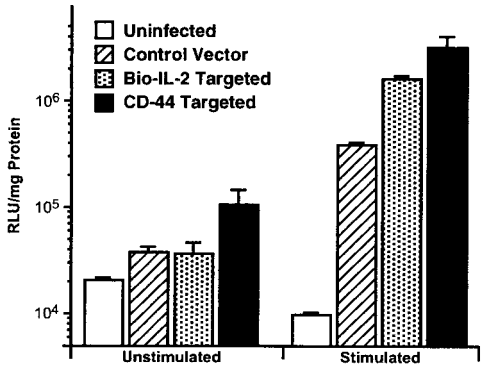

FIG. 5. Redirected infection of primary human T cells. Human T cells were cultured for 48 hr in the presence or absence of 1 μg/ml PHA before infection with 100 μl (moi = 70) of bio-AdLuc (control vector) or bio-AdLuc linked to 25 ng of bio-IL-2 (bio-IL-2-targeted) or to 2.5 μg of bio-anti-CD44 (CD-44-targeted). Luciferase activity at 24 hr after infection is reported as the mean ± SE of duplicate determinations and is representative of three experiments.

vectors, targeting moieties have been attached to reporter genes through electrostatic interactions. Targeted retrovirus and adenovirus vectors have been constructed by using bifunctional antibodies that recognize both viral epitopes and target cell antigens and molecular modifications to the virus that include replacing viral sequences with targeting domains. Because of the potential for disruption of electrostatic charges holding molecular conjugate vectors together and the loss of targeting moieties attached to vectors by bifunctional antibodies after *in vivo* administration of vectors, we chose to use high-affinity, avidin–biotin interactions to attach targeting moieties to recombinant adenovirus vectors. We have demonstrated that biochemical modification of recombinant adenoviruses through covalent linkage of biotin also can yield vectors for targeted gene transfer without the need for molecular manipulation of the viral genome. Furthermore, because targeting moieties are attached to directly biotinylated vectors through biotin–avidin interactions, these vectors may provide increased stability upon *in vivo* administration.

We have demonstrated that SCF-directed adenovirus vectors expressing GFP efficiently infect growth factor-dependent hematopoietic progenitor cell lines (M-O7e) at frequencies equivalent to adenovirus infection of K562 cells, which are infected readily with adenovirus. In other experiments using PCR to detect luciferase DNA after SCF-directed infection of M-O7e cells and infection of K562 cells with control vectors, both cell lines showed a high frequency of infection and were greater than 95% positive for luciferase (data not shown). Taken together, this suggested that either virus is not internalized efficiently, that not all viral genomes are expressed, or that there are low levels of reporter gene expression. Although we are unable to distinguish between these possibilities, we are characterizing the kinetics of internalization of vectors in hematopoietic cells and investigating the use of alternate promoters in an effort to improve the frequency of gene expression. Our results suggest that directly biotinylated recombinant adenovirus vectors may be suitable for gene transfer to produce a therapeutic effect. Because expression of transferred genes is transient, this vector is not suitable for transfer of genes to normal bone marrow cells where sustained gene expression would be required for a therapeutic effect. However, SCF- or anti-CD117-targeted vectors could be used to direct gene transfer of suicide genes to acute and chronic myelogenous leukemias, which express high levels of c-Kit (32). CD34$^+$ leukemia cell killing may be enhanced further by the use of anti-CD34-targeted vectors. Because expression of c-Kit and CD34 is low on normal PHSCs, this approach to targeted killing of leukemic cells directed through specific cell

surface antigens may represent a means for purging of bone marrow before transplantation. Furthermore, we have shown previously that transfer of thymidine kinase to T cells by retroviral vectors represents a therapeutic approach to graft-vs.-host disease (33). Here we demonstrate redirection of adenovirus infection to activated T cells through the IL-2 receptor, suggesting that this vector system may provide a means for delivery of suicide genes to control graft-vs.-host disease after allogenic bone marrow transplantation.

We also have redirected adenovirus infection to hematopoietic progenitor cell lines through the cell surface antigen CD44. The enhanced expression of CD44 in the vasculature of solid tumors (34) suggests that anti-CD44-targeted recombinant adenovirus vectors may be useful in gene therapy of cancer for disruption of tumor vascularization. However, the normal vascular endothelium is not refractory to adenovirus infection, and biotinylation of adenovirus vectors has not been shown to disrupt the interaction of adenovirus with its authentic receptor. Thus, direct biotinylation of the recently reported adenovirus vectors containing deletions of the fiber knob that ablate recognition of the virus by cellular receptors may permit application of this vector to target gene transfer to cells that are not normally refractory to adenovirus infection (35, 36).

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