Bone Marrow Extracellular Matrix Molecules Improve Gene Transfer into Human Hematopoietic Cells via Retroviral Vectors

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Abstract

Direct contact between hematopoietic cells and viral packaging cell lines or other sources of stroma has been shown to increase the efficiency of retroviral-mediated gene transfer into these target cells compared with infection with viral supernatant. We have investigated the role of defined bone marrow extracellular matrix molecules (ECM) in this phenomenon. Here we report that infection of cells adhering to the carboxy-terminal 30/35-kD fragment of the fibronectin molecule (30/35 FN), which contains the alternatively spliced CS-1 cell adhesion domain, significantly increases gene transfer into hematopoietic cells. Two retroviral vectors differing in recombinant viral titer were used. Gene transfer into committed progenitor cells and long-term culture-initiating cells, an in vitro assay for human stem cells, was significantly increased when the cells were infected while adherent to 30/35 FN–coated plates compared with cells infected on BSA-coated control plates or plates coated with other bone marrow ECM molecules. Although gene transfer into committed progenitor cells and to a lesser degree into long-term culture-initiating cells was increased on intact fibronectin as well, increased gene transfer efficiency into hematopoietic cells on 30/35 FN was dependent on CS-1 sequence since infection on a similar FN fragment lacking CS-1 (42 FN) was suboptimal. 30/35 FN has previously been shown by our laboratory and other investigators to mediate adhesion of primitive murine and human hematopoietic stem cells to the hematopoietic microenvironment. Additional studies showed that neither soluble 30/35 FN nor nonspecific binding of hematopoietic cells to poly-L-lysine–coated plates had any appreciable effect on the infection efficiency of these cells. Our findings indicate that hematopoietic stem cell adhesion to specific ECM molecules alters retroviral infection efficiency. These findings should aid in the design of gene transfer protocols using hematopoietic progenitor and stem cells for somatic gene therapy. (*J. Clin. Invest.* 1994. 93:1451–1457.) Key words: gene transfer • fibronectin • CS-1 • adhesion • adenosine deaminase

Introduction

Progress in understanding the molecular basis of many human diseases as well as improvement in gene transfer technology have led to recent attempts at developing protocols for somatic gene therapy of severe genetic diseases (1, 2). One target of such gene transfer protocols has been hematopoietic stem and progenitor cells. Successful gene transfer into the most primitive hematopoietic cells, long-term repopulating stem cells, may lead to a lifelong cure for a variety of diseases manifested in the progeny of these cells. Although gene transfer and long-term gene expression in repopulating stem cells have been achieved in murine models by a number of investigators (3–9), in vivo experiments in larger animals such as dogs and primates have met with limited success, largely because of the low efficiency of infection of primitive hematopoietic stem cells (10–13).

In both murine and large animal experiments involving bone marrow cells it has been noted that the most successful protocols use cocultivation of target cells with retroviral producer cell lines. In addition, infection of target cells while in direct contact with stromal cells, a major component of the hematopoietic microenvironment (HM),[1] improves gene transfer efficiency (13–15). The mechanism underlying this promoting effect of stroma on retroviral infection is unclear, but it has been known for some time that physiologic regulation of hematopoietic cell proliferation and differentiation occurs when these cells are in direct contact with cells of the HM (16, 17). The HM consists of an organized network of macrophages, fibroblasts, endothelial cells, adipocytes, and a complex extracellular matrix (ECM) made up of a variety of defined adhesion molecules. ECM molecules such as laminin, collagen, thrombospondin, proteoglycans, glycosaminoglycans, and fibronectin provide anchorage sites for both hematopoietic cells and growth factors (18–29).

In this study, we examined the effects of ECM molecules found in the bone marrow on retroviral-mediated gene transfer into hematopoietic cells. We report that a 30/35-kD carboxy-terminal chymotryptic fragment of fibronectin (30/35 FN) that contains the alternatively spliced CS-1 domain enhances retroviral-mediated gene transfer efficiency into committed hematopoietic progenitor cells and the more primitive long-term culture-initiating cells (LTC-IC).

Methods

Retroviral vectors and producer cell lines. The structure of N_2/ZipTK-NEO (TKNEO) vector has been previously reported (15). In this vector, neophosphotransferase sequences are expressed in the sense orien-

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1. *Abbreviations used in this paper:* BFU-E, burst-forming unit containing only erythroid elements; BMF, bone marrow fibroblasts; CBD, cell-binding domain; CDB, cell dissociation buffer; CFU-Mix, CFU containing myeloid and erythroid elements; ECM, extracellular matrix; FN, fibronectin; G418^r, G418-resistant; hADA, human adenosine deaminase; HM, hematopoietic microenvironment; IMDM, Iscove's modified Dulbecco's medium; LTC-IC, long-term culture-initiating cells; LTMC, long-term bone marrow cultures; mADA, murine ADA; PGK, phosphoglycerate kinase; P/S, penicillin/streptomycin; SCF, stem cell factor; VLA-4, very late antigen-4.

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tation (relative to the 5′ long terminal repeat) via the herpes simplex thymidine kinase promoter. The ZipPGK-mADA (PGK-mADA) vector is identical to the ZipPGK-hADA vector except the human adenosine deaminase (hADA) cDNA has been replaced with the murine ADA (mADA) cDNA as previously reported (15). In this vector, the mADA cDNA is expressed in the sense orientation relative to the 5′ long terminal repeat via the human phosphoglycerate kinase (PGK) promoter. GP + EnvAm 12 (30) producer cells containing either retroviral plasmid (TKNEO or PGK-mADA) were cultured in Iscove's modified Dulbecco's medium (IMDM) (GIBCO BRL, Gaithersburg, MD) containing 10% FCS (Hyclone Laboratories, Logan, UT) and 100 U/ml penicillin (GIBCO BRL) and 100 μg/ml streptomycin (P/S) (GIBCO BRL). Virus-containing supernatant was collected by adding 10 ml of IMDM containing 20% FCS to confluent plates overnight. Harvested medium was filtered through 0.45-μm filters (Gelman Sciences Inc., Ann Arbor, MI) and stored at −80°C until used.

Stromal cells. Human allogeneic bone marrow fibroblasts (BMF) were derived from healthy adult donors (see below) and grown in DME (GIBCO BRL) supplemented with 10% FCS and P/S. BMF were irradiated at confluence with 15 Gy (cesium-137 using a gamma cell 40, Nordion International Inc., Kanata, Ontario, Canada). NIH/3T3 fibroblasts were maintained in DME/10% calf serum supplemented with P/S.

Preparation of fibronectin fragments. Chymotryptic fragments of fibronectin (Fig. 1) were prepared as previously described (Goltry, L. K., and V. P. Patel, manuscript submitted for publication). For use in the infection protocol, fibronectin fragments were immobilized on either 35- or 100-mm petri dishes (Falcon Labware, Lincoln Park, NJ) at a concentration of 75 pmol/cm^2 as previously described (28). Control plates were coated in analogous fashion with 2% BSA (Boehringer Mannheim Corp., Indianapolis, IN).

Retroviral infection protocol. Bone marrow samples from healthy adult donors were collected in tubes containing sterile, preservative-free sodium sulfate heparin according to protocols approved by the Institutional Review Board of Indiana University School of Medicine. Low density mononuclear cells were prepared by centrifugation on Ficoll-Hypaque (*d* 1.077 g/ml) (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) for 45 min at 25°C. Plastic-adherent cells were removed from low density bone marrow cells by an additional incubation on tissue culture plates for 4–16 h at 37°C in 5% CO_2 in IMDM with 20% FCS. Adherent-negative low density mononuclear cells were prestimulated before retroviral infection as described previously (5) for 48 h at 37°C and 5% CO_2 in IMDM containing 20% FCS, 100 U/ml rhIL-6, 100 ng/ml rh stem cell factor (SCF) (both Amgen Biologicals, Thousand Oaks, CA), and P/S at a cell density of 1×10^6 cells/ml in petri dishes. Prestimulated cells were harvested by vigorous pipetting (to remove cells loosely adherent to the plastic). Subsequently, prestimulated cells (5×10^5 cells/ml) were incubated for 6 h on plates coated with BSA (control plates) or fibronectin fragments and then infected with virus supernatant in the presence of growth factors (as above) and 7.5 μg/ml polybrene (Aldrich Chemical Co., Milwaukee, WI). Virus supernatant was replaced with fresh virus (including growth factors and 5.0 μg/ml polybrene) after 2 h, and cells were incubated for an additional 12–24 h. Any nonadherent cells were readded with the fresh virus supernatant. In some experiments, the infection protocol was modified slightly as noted in Results.

Following the infection protocol, nonadherent cells were decanted, and adherent hematopoietic cells were collected from the cultures using cell dissociation buffer (CDB) (enzyme-free/PBS-based, GIBCO BRL) according to the manufacturer's instructions. The adherent cells were added to the nonadherent fraction, washed twice, and counted. Harvested cells were either plated in clonogenic methylcellulose progenitor assays or long-term bone marrow cultures (LTMC) (see below). For most experiments, infection was performed independently on three plates, and results from these experiments are given as mean±SEM of three separate infections. In some experiments, the complete removal of clonogenic hematopoietic cells from the plates after the infection protocol was documented by overlaying the plates with complete methylcellulose progenitor culture media and subsequent incubation (see clonogenic methylcellulose assay).

LTMC. LTC-IC assays were performed according to previously described methods (31) with slight modifications. Briefly, $0.5–1 \times 10^6$ infected cells were seeded in LTMC on confluent, preirradiated (as above) allogeneic human BMF in 5 ml IMDM containing 10% FCS, 10% horse serum (Sigma Chemical Co., St. Louis, MO), P/S, 1×10^{-5} M hydrocortisone (The Upjohn Co., Kalamazoo, MI), and 320 mosmol sodium chloride in 6-well tissue culture plates (Costar Corp., Cambridge, MA). LTMC were incubated at 33°C in 5% CO_2 and fed weekly by removal of 50% of the media and nonadherent cells. After 5 wk, LTC-IC cultures were killed by using CDB to remove adherent hematopoietic cells from BMF. Both nonadherent and adherent hematopoietic cells were combined and plated in methylcellulose to obtain colonies derived from LTC-IC.

Clonogenic methylcellulose assays. Methylcellulose assays were performed as previously described (32) with minor modifications. Briefly, $2–5 \times 10^4$ infected adult bone marrow cells were plated with 5 U/ml erythropoietin (Epo; Amgen Biologicals), 100 ng/ml rhSCF, 10 ng/ml rhIL-3 (Genzyme Corp., Cambridge, MA) in 1 ml of 2.4% IMDM methylcellulose (Fluka Chemical Corp., Ronkonkoma, NY) containing 25% FCS, 10% human plasma, 10^{-5} M β-mercaptoethanol (Sigma Chemical Co.), and P/S. Cultures were incubated at 37°C in 5% CO_2/95% air, and colonies (> 50 cells) were scored by viewing on an inverted microscope on day 13 as CFU-GM (containing granulocytes and macrophages), CFU-Mix (containing myeloid and erythroid elements), or BFU-E (burst-forming unit containing only erythroid elements).

Analysis of retroviral infection. Efficiency of infection with the TKNEO virus was analyzed by determining the percentage of methylcellulose colonies resistant to 1.5 mg/ml (dry powder) G418 (GIBCO BRL) on day 13. Mock infections were performed in each experiment

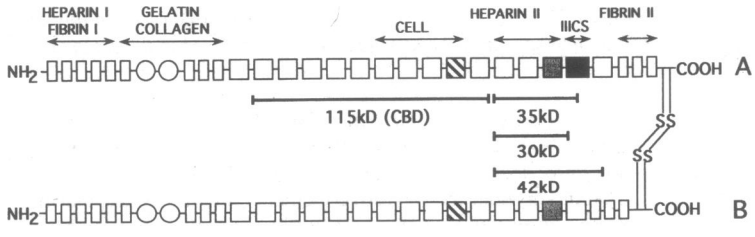

Figure 1. Schematic representation of fibronectin molecule shows A and B chains consisting of a series of type I (*rectangles*), type II (*circles*), and type III (*squares*) homologous repeats that are linked at the carboxy termini by two disulfide bonds. Some of the known functional binding domains have been indicated, including: the type III repeat containing the RGDS cell adhesion recognition sequence (*hatched squares*); the high affinity heparin-binding sequences located within the heparin II domain (*dotted squares*); and the alternatively spliced, non–type III connecting segment (*IIICS*), which contains the second cell adhesion sequence represented by the CS-1 peptide (*black square*). The location in the FN molecule of the chymotryptic fragments used in these studies along with their approximate molecular weights are represented by solid bars. Anti–CS-1 peptide antibody and fibrin-binding data indicate that the 42-kD fragment originates from the B chain and the 30- and 35-kD fragments from the A chain.

by incubating bone marrow on the GP + EnvAm 12 packaging line making no recombinant virus. Culture of these mock-infected cells with 1.5 mg/ml G418 consistently demonstrated < 1% background colonies. Efficiency of infection with the PGK-mADA vector was determined by protein analysis using ADA isoenzyme electrophoresis. Analysis of individual progenitor colonies was performed as previously described (15, 33). To analyze stringently transfer efficiency, only colonies expressing mADA at the same or a higher level than endogenous hADA were considered transduced. For analysis of pooled colonies, colonies picked out of methylcellulose culture were combined in 1.5-ml microtubes (Rainin Instrument Co. Inc., Woburn, MA), washed with warm medium and PBS, centrifuged, and stored at −20°C. In addition, in some experiments cells from the adherent and nonadherent fraction of 5-wk-old LTMC were incubated in suspension in identical conditions to progenitor cultures without methylcellulose. Total nonadherent cells from these cultures (secondary suspension cultures) were harvested at 13 d, washed, centrifuged, and stored as dry cell pellets at −20°C until used. For ADA analysis, cells were lysed in 5 μl of lysis buffer by repeated freezing-thawing cycles, and isoenzyme electrophoresis was performed.

Cell-cycle analysis of hematopoietic progenitor cells. Tritiated thymidine suicide studies were performed according to the method described by Byron (34) with some modifications. Aliquots of 10×10^6 low density mononuclear cells were preincubated on BSA or fibronectin fragment in 10 ml IMDM/20% FCS. After 6 h, either cold (nonradioactive) thymidine or 0.1 mCi/ml high specific activity (~ 80 Ci/mM) [methyl-^{3}H]thymidine (New England Nuclear, Boston, MA) was added. After a 20-min incubation period, the plates were washed three times with 10 ml ice cold IMDM/20% FCS containing excess cold thymidine (100 μg/ml), and the remaining cells were removed from the plates using CDB. Cells from all washes and CDB treatment were combined, washed twice, and placed in clonogenic methylcellulose assays as described above.

Statistical analysis. One-way ANOVA was used to compare gene transfer efficiency on 30/35 FN vs BSA (control).

Results

Gene transfer efficiency into committed progenitor cells. Gene transfer efficiency was assayed after infection with two retroviral vectors differing significantly in recombinant virus titer. Transduction efficiency was compared by infecting bone marrow cells while plated on 30/35 FN– or BSA-coated dishes. Fig. 2 shows the results of eight independent experiments and demonstrates the percentage of G418-resistant (G418^r) colonies after infection. Using the lower titer TKNEO vector (titer: 1×10^5 G418^r CFU/ml on NIH/3T3 cells), a higher percentage of G418^r colonies was noted on 30/35 FN from all types of progenitors, including those derived from lineage-restricted (BFU-E and CFU-GM) as well as multilineage (CFU-Mix) progenitor cells. Infection efficiency into all committed progenitors was increased eightfold on 30/35 FN vs BSA (18±2.4% vs 2.3±0.9% G418^r colonies, $P < 0.001$). No difference in the number of colonies obtained after infection without selection was observed between these conditions in any experiments. Similar results were obtained using the PGK-mADA vector, although, as expected with this higher titer (~ 1×10^7 virions/ml) vector, the transduction efficiency of committed progenitors was extremely high. Infection of bone marrow on 30/35 FN with PGK-mADA yielded 30/32 (94%) mADA-expressing committed progenitors, compared with 5/35 (14%) mADA-expressing progenitor colonies after infection on BSA in three separate experiments.

Gene transfer efficiency into LTC-IC. In four independent experiments performed with PGK-mADA, a significant proportion of progenitor colonies derived from 5-wk-old LTMC expressed the transferred mADA gene. Expression ranged from 2/12 (17%) to 6/6 (100%) of analyzed colonies (Table I). Expression of the introduced mADA gene exceeded or at least equaled the amount of endogenous hADA activity in all colonies considered positive. Gene transfer into LTC-IC was also assessed using the TKNEO vector. Evidence for gene transfer into LTC-IC–derived colonies was detected after infection on 30/35 FN even with this lower titer virus, while no gene transfer was noted into LTC-IC incubated on control plates (16% G418^r vs 0% G418^r colonies, 30/35 FN vs BSA).

Specificity of 30/35 FN effects on infection efficiency of hematopoietic cells. To determine the specificity of increased gene transfer efficiency seen on 30/35 FN, infection with TKNEO was performed on plates coated with BSA, 30/35 FN, intact fibronectin, a 115-kD FN fragment containing the central cell-binding domain (CBD) including the RGDS tetrapeptide sequence, and a 42-kD carboxy-terminal FN fragment (42 FN) containing the heparin II–binding domain but lacking the CS-1 sequence (Fig. 1). Fig. 3 shows results on one representative experiment. Infection on BSA yielded 2.5±0.6% G418^r BFU-E, 1.6±0.4% G418^r CFU-GM, and 0.8±0.2% G418^r CFU-Mix. No increase in the proportion of G418^r colonies was noted on CBD, while slightly higher infection of BFU-E (6.6±0.7%) was seen on 42 FN (Fig. 3). However, intact FN promoted increased gene transfer into all committed progenitors. The percentage of G418^r colonies after infection on intact FN was less than on 30/35 FN in all lineages, including BFU-E (15.7±1.2 vs 24.0±2.4%), CFU-GM (4.3±1.0 vs 15.5±4.8%),

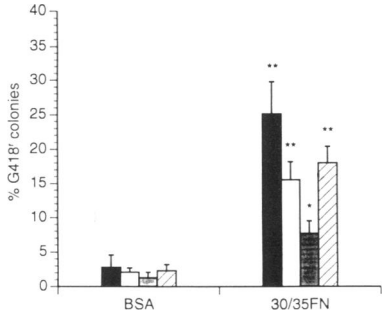

Figure 2. Infection efficiency of human committed progenitors on 30/35 FN using TKNEO vector. BFU-E, *filled bars;* CFU-GM, *open bars;* CFU-Mix, *shaded bars;* and total, *hatched bars.* Figure shows percentage of G418^r progenitors as mean±SEM. *$P < 0.01$, **$P < 0.001$; 30/35 FN vs BSA.

Table I. Infection Efficiency of Human LTC-IC Using the PGK-mADA Vector

Experiment	BSA	30/35 FN
1	0/14*	10/19
2	N/A	2/12
3	0/4	3/5
4	0/4	6/6
Total	0/22	21/42

* Number of mADA-positive colonies per total colonies analyzed. N/A, not analyzed.

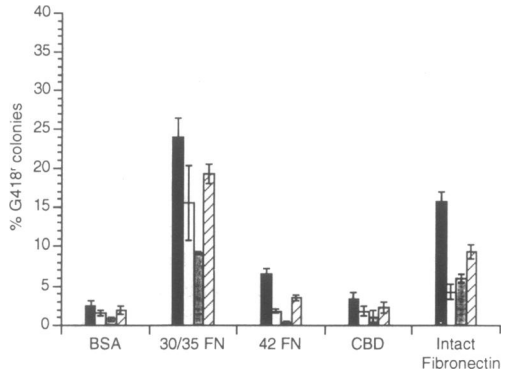

Figure 3. Comparison of infection efficiency of committed progenitors on FN fragments using TKNEO vector. BFU-E, *filled bars;* CFU-GM, *open bars;* CFU-Mix, *shaded bars;* and total, *hatched bars.* Figure shows percentage of G418^r progenitors as mean±SEM.

and CFU-Mix (6.0±1.0 vs 9.2±0.2; intact FN vs 30/35 FN, respectively). Three other experiments yielded similar results (data not shown), except that in one experiment the infection efficiency on intact FN was similar to 30/35 FN, and in two of four experiments no difference in infection efficiency on 42 FN (vs BSA) was seen.

Gene transfer efficiency into more primitive LTC-IC was increased specifically on 30/35 FN. After infection with PGK-mADA on BSA, intact fibronectin, and 42 FN, none of the LTC-IC–derived colonies (0/6, 0/4, and 0/3, respectively) demonstrated expression of mADA, whereas 3/5 LTC-IC–derived colonies infected on 30/35 FN expressed mADA. Because of the relatively small size of these secondary LTC-IC–derived colonies, the ability to perform protein analysis on single colonies was limited. To better analyze these colonies, multiple LTC-IC–derived colonies were pooled before analysis in two additional experiments. In addition, secondary suspension cultures using cells derived from 5-wk-old LTMC were analyzed. In these experiments, mADA expression was detected only after infection on 30/35 FN and to a lesser degree on intact FN, but not on 42 FN or BSA (Fig. 4). Therefore, in three different experiments, infection of LTC-IC was demonstrably better on 30/35 FN than on BSA (as shown above) as well as on intact FN and 42 FN.

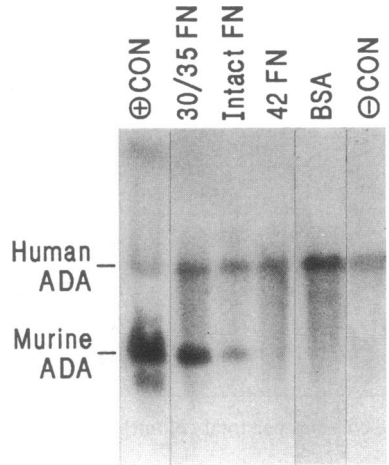

Figure 4. Expression of mADA in human hematopoietic cells derived from LTC-IC. Infection of LTC-IC was carried out on either 30/35 FN, 42 FN, intact FN, or BSA before plating in LTC-IC cultures. Cells were harvested for analysis from secondary suspension cultures 2 wk after removing from a 5-wk-old LTC-IC culture. Position of hADA and mADA are shown. Negative control: ⊖CON, mock-infected cultures. Positive control: ⊕CON, as in Fig. 3.

Role of adhesion in promoting increased gene transfer efficiency. Prestimulated bone marrow cells were allowed to adhere to 30/35 FN for 6 h and then infected using the TKNEO vector. After infection the nonadherent fraction (all cells recovered in the viral supernatant and with two consecutive mild washes with PBS) and the adherent fraction (all other cells) were analyzed separately for the presence of G418^r progenitor colonies. The number of transduced colony-forming cells was two- to threefold higher in the adherent fraction in each of four separate experiments done in triplicate (data not shown). To analyze whether this improved infection efficiency of cells exposed to 30/35 FN was dependent on continued adhesion to the fragment during the infection protocol, we incubated hematopoietic cells without virus on 30/35 FN for 6 h. After removal of hematopoietic cells from 30/35 FN, the cells were infected on BSA-coated plates at various time intervals from 0 to 24 h. The infected cells were plated in methylcellulose with or without G418 (Fig. 5 *A*). The increased efficiency of infection observed on 30/35 FN was immediately reversed after removal of cells from adhesion to this fragment, and within 2 h of removal the percentage of G418^r colonies was identical to

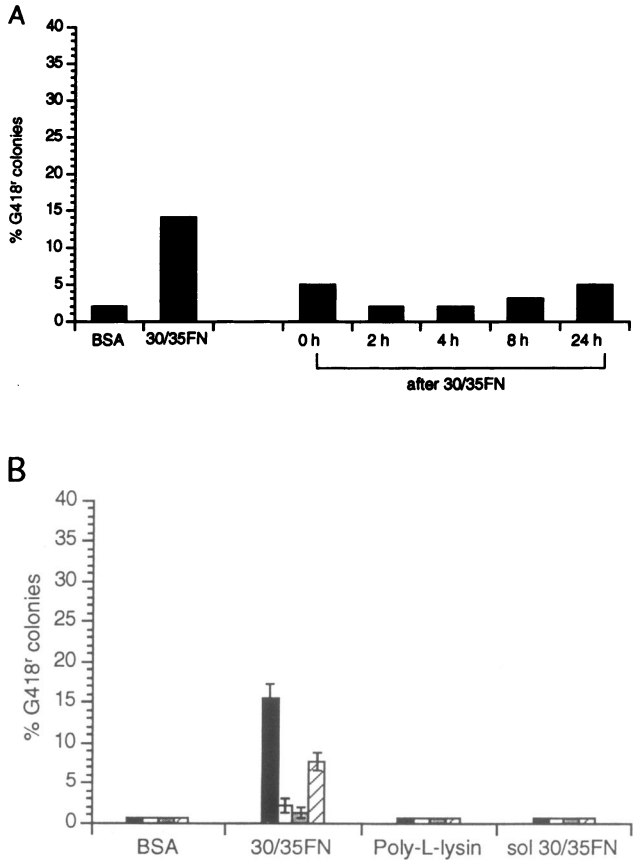

Figure 5. Effect of adhesion on gene transfer efficiency. (*A*) Infection of committed progenitors using the TKNEO vector before (30/35 FN) and after (0–24 h) removal of cells adherent to 30/35 FN. Figure shows percentage of G418^r colonies. Concomitant infection on BSA-coated plates shown as control. (*B*) Infection of committed progenitors using TKNEO vector while plated on 30/35 FN, poly-L-lysine–coated plates, or in the presence of soluble 30/35 FN (*sol 30/35FN*) on BSA-coated plates. BFU-E, *filled bars;* CFU-GM, *open bars;* CFU-Mix, *shaded bars;* and total, *hatched bars.* Figure shows mean±SEM.

control infection (BSA-coated plates). Similar data were observed in two additional independent experiments. In addition, infection was performed in the presence of 5, 10, and 20 μM of soluble 30/35 FN and after nonspecific binding of hematopoietic cells to poly-L-lysine–coated dishes. Neither soluble 30/35 FN (at any concentration) nor nonspecific adherence of hematopoietic cells to poly-L-lysine–coated plates increased the transduction efficiency of committed progenitors (Fig. 5 *B*).

Role of cycling status of hematopoietic cells. Since integration of the retroviral genome requires cell division, we compared the cycling status of committed progenitor cells plated on 30/35 FN with cells plated on BSA-coated plates. After 48 h of prestimulation with IL-6/SCF and incubation for 6 h on protein-coated plates, cycling rates as determined by [^{3}H]-thymidine suicide assays were similar between cells plated on 30/35 FN or BSA (47±5 vs 47±5, respectively, mean±SD of three independent experiments).

Effect of 30/35 FN on viral half-life. To determine whether incubation of virus on 30/35 FN affects the viral half-life, we incubated TKNEO-containing diluted viral supernatant (titer of 5×10^2 G418^r CFU/ml) on 30/35 FN- or BSA-coated dishes and added NIH/3T3 cells after 0, 8, 24, 48, and 72 h. The next day, G418 was added to the medium, and G418^r NIH/3T3 colonies were enumerated after 10 d. While the titer determined on plates containing 30/35 FN were two- to fourfold higher than those determined on plates containing BSA, no differences in recombinant viral titer half-life was noted between the two culture conditions (data not shown).

Discussion

Efficient gene transfer into long-term repopulating hematopoietic stem cells remains problematic in large animal species, preventing the widespread application of gene transfer protocols for curative therapy of hematopoietic diseases. Since direct contact of hematopoietic cells with stromal cells has been demonstrated to facilitate gene transfer into primitive hematopoietic cells including stem cells, we have examined the role of cell adhesion to ECM molecules present in the bone marrow microenvironment on the transduction of hematopoietic cells using retroviral vectors. We have demonstrated that fibronectin, and particularly a chymotryptic fragment of fibronectin containing the alternatively spliced CS-1 adhesion domain of FN, significantly increases gene transfer into committed progenitors (BFU-E, CFU-GM, CFU-Mix) and primitive (LTC-IC) hematopoietic stem cells. We have evaluated the effect of a variety of other ECM molecules on transduction efficiency of committed progenitors, including vitronectin, heparin sulfate, types I, III, IV, and V collagen, thrombospondin, and laminin. Although gene transfer efficiency was higher than infection on BSA for several of these molecules, none improved transduction efficiency to the same level as 30/35 FN or intact FN (Moritz, T., and D. A. Williams, unpublished results).

Our data suggest that specific adhesion of hematopoietic cells to the 30/35 FN matrix fragment is a requirement for improved gene transfer efficiency into this cell population. A variety of ECM molecules, such as type I collagen, laminin, thrombospondin, and fibronectin, has been previously shown to bind to hematopoietic cells, and these interactions are presumed to play a role in regulation of hematopoiesis within the HM. The CS-1 sequence of the 30/35-kD fibronectin fragment used in the experiments reported here has been shown to be a ligand for the very late antigen (VLA)-4 ($\alpha 4/\beta 1$) integrin receptor (35–37). VLA-4 has been demonstrated to be present on primitive hematopoietic cells, and adhesion to the CS-1 sequence of fibronectin via VLA-4 of human CD34$^+$, HLA-DR$^-$ hematopoietic cells (38), murine day 12 CFU-spleen cells, and murine long-term repopulating stem cells has been previously shown (21). However, our laboratory and other investigators have shown little or no adhesion of primitive cells to intact fibronectin, CBD, or 42 FN (38–40). It is intriguing to speculate that adhesion of primitive cells is the basis for the relative selectivity of 30/35 FN in promoting increased gene transfer efficiency into LTC-IC, a cell believed to represent a human hematopoietic stem cell (31). In this regard, the positive effect of intact fibronectin and to a lesser extent 42 FN on gene transfer efficiency into more committed progenitor cells, such as BFU-E, CFU-GM, and CFU-MIX, is consistent with data that adherence of these less primitive cells occurs to other sequences in the FN molecule, such as RGDS and heparin-binding sites (38, 40).

It has become increasingly clear that integrin receptors not only act as specific sites for cellular adhesion but also are involved in the regulation of intracellular processes. In some instances adhesion via integrin receptors has been demonstrated to trigger signal transduction pathways (41, 42). In this way, extracellular matrix molecules may regulate intracellular events. For example, the induction of immediate–early and early response genes has been described after cross-linking of integrin receptors in monocytes, fibroblasts, platelets, and carcinoma cells (43–45). In some experiments, activation of the focal adhesion kinase, pp125^{FAK}, via integrin/ligand interactions has been described (46–48). The data presented here demonstrating increased retroviral infection efficiency on immobilized 30/35 FN but not with soluble 30/35 FN suggest that cell adhesion mediated by the VLA-4 integrin receptor may also be involved in the cellular events controlling viral entry and/or integration.

Alternatively, colocalization of viral particles and hematopoietic cells on 30/35 FN may be involved in the promotion of retroviral-mediated gene transfer by matrix molecules. The latter mechanism predicts that retrovirus may bind directly or indirectly to 30/35 FN. We have observed transduction when hematopoietic cells were incubated on plates coated with 30/35 FN that had been preincubated with viral supernatant and then vigorously washed to remove free virus (Moritz, T., and D. A. Williams, unpublished results). These results suggest that the enhancement of gene transfer efficiency may be because of an increased local concentration of virus. In addition, cointernalization of the occupied VLA-4 receptor and virus bound to the amphotropic cell-surface receptor, similar to receptor-mediated DNA-uptake as described in fibroblasts (49, 50) and hepatic cells (51), remains a possible explanation for the observed results. Furthermore, integrin receptors have been shown to be involved in the internalization by specific target cells of pathogens such as adenovirus, echovirus, and *Staphylococcus aureus* (52–54).

While underlying biochemical and molecular mechanisms remain unclear at present, the data presented here offer an explanation for the observed positive effects of stroma on retroviral infection. The data also may have direct implications for the design of gene therapy protocols. Infection on defined matrix molecules may be a safe and practical way to improve the efficiency of infection of hematopoietic cells via retroviral vec-

tors without the need for cocultivation. Further refinement of this technology may also allow targeting of specific cell populations by using known differences in adhesive properties of different cells.

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