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Proteoglycans Secreted by Packaging Cell Lines Inhibit Retrovirus Infection

JOSEPH M. LE DOUX,[1] JEFFREY R. MORGAN,[2] RICHARD G. SNOW,[2] AND MARTIN L. YARMUSH[1,2]*

Department of Chemical and Biochemical Engineering, Rutgers University, Piscataway, New Jersey 08854,[1] *and Surgical Services, Massachusetts General Hospital, and Shriners Burns Institute, Boston, Massachusetts 02114*[2]

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Using a model recombinant retrovirus encoding the *Escherichia coli lacZ* gene, we have found that medium conditioned with NIH 3T3 cells and packaging cell lines derived from NIH 3T3 cells inhibits infection. Most of the inhibitory activity was greater than 100 kDa and was sensitive to chondroitinase ABC digestion, which is consistent with the inhibitor being a chondroitin sulfate proteoglycan. Proteoglycans secreted by NIH 3T3 cells and purified by anion-exchange chromatography inhibited amphotropic retrovirus infection. Pretreatment of amphotropic retrovirus stocks with chondroitinase ABC boosted the level of transduction efficiency by more than twofold. The implications of these findings with respect to retrovirus-cell interactions and the production of high-titer retroviral stocks are discussed.

Recombinant retroviruses are a common and effective means for conducting experimental and clinical gene transfer. Recombinant retroviruses are widely used primarily because they infect a variety of cell types and stably integrate the transgene into the genome of the host cell and the construction of cell lines to produce stocks of replication-defective recombinant virus is relatively straightforward (5, 37). Retrovirus-mediated gene transfer, although far more efficient than many traditional gene transfer techniques, such as calcium phosphate precipitation and electroporation, is either too inefficient or too inconsistent for many potential applications (21, 27). Improvements in the efficiency of retrovirus-mediated gene transfer are clearly needed and will most likely result from an improved understanding of retrovirus-cell interactions.

Retrovirus infection requires the completion of a series of steps which begins with diffusion and binding of the virus particle to a cell surface (41). This overall binding process is slow and inefficient, and some recent studies suggest that improving the rate and efficiency of retrovirus binding can significantly increase the transduction efficiency of retrovirus-mediated gene transfer (4, 7, 19, 25, 44). As with most viruses, the attachment of murine leukemia retroviruses is mediated by specific interactions between the envelope proteins (gp70) which protrude from the surface of a retrovirus particle and the receptors for the virus on the surface of the host cell. The domains of gp70 which determine the receptor specificity (host range) of various retroviruses have been identified (8, 11, 26, 29). In addition, the cellular receptors for many retroviruses have been cloned and their normal cellular functions have been determined (2, 9, 18, 22, 28, 40, 43). For example, the receptor for ecotropic virus (MCAT) is a cationic amino acid transporter whereas the receptors for amphotropic viruses (Ram-1 and Glvr-2) are sodium-dependent phosphate symports (15, 16, 23). Both the ecotropic and the amphotropic receptors have multiple extracellular domains, and some of the domains which are critical for virus binding and infection have been identified (1, 31, 45).

* Corresponding author. Mailing address: Bigelow 1401, Surgical Services, Massachusetts General Hospital, Boston, MA 02114. Phone: (617) 726-3474. Fax: (617) 374-5665.

Retrovirus binding is strongly influenced by the specific binding reactions between the envelope proteins and cellular receptors but is also significantly affected by many other factors, including temperature, pH, medium composition, and the surface charge densities of the virus particles and cells (3, 13, 32, 38). For example, exogenous factors, such as cationic polymers, are routinely added to a culture medium (Polybrene, protamine, or DEAE-dextran) prior to or during retroviral infection to enhance virus binding and transduction efficiency (20, 38). Conversely, many anionic polymers, including sulfated polysaccharides, such as heparin and dextran sulfate, inhibit retrovirus attachment and infection (6, 35).

The dose-response curves of amphotropic and ecotropic *lacZ* viruses are not linear. In a previous study, we used a model recombinant amphotropic retrovirus (α-SGC-*lacZ* produced by the ψ-CRIP packaging cell line) encoding the *Escherichia coli lacZ* gene and quantitative assays to measure infection to show that the efficiency of retroviral infection was linearly proportional to virus concentration at low virus concentrations but that it plateaued at a maximum level at high virus concentrations (25). Our results suggested that endogenous factors that limited retroviral infection and gave rise to the plateau in the dose-response curve were present in the culture media of retrovirus stocks. We conducted similar studies using a recombinant ecotropic retrovirus (Cre BAG 2 *lacZ* virus; American Type Culture Collection, CRL 1858) encoding the *E. coli lacZ* gene. Dilutions of *lacZ* virus stock, composed of volumes of ecotropic *lacZ* virus and fresh medium totaling 100 μl and adjusted to 8 μg of Polybrene per ml, were added to wells in which 5,000 NIH 3T3 cells had been plated 24 h previously. Two days later infectivity was determined by measuring β-galactosidase activity in extracts of the cells. As shown in Fig. 1, the overall shape of the dose-response curve of the ecotropic virus was similar to that of the amphotropic retrovirus. At low concentrations (6 to 25 μl per well) a linear dependence was observed, whereas at intermediate concentrations (above 25 μl) infectivity remained virtually constant, and at the highest doses of virus (above 75 μl) infectivity declined slightly. A similar trend was observed if, instead of overall β-galactosidase activity, the percentage of *lacZ*$^+$ cells after infection was measured (25). We found that the shapes of the dose-response curves were somewhat variable (data not shown) and de-

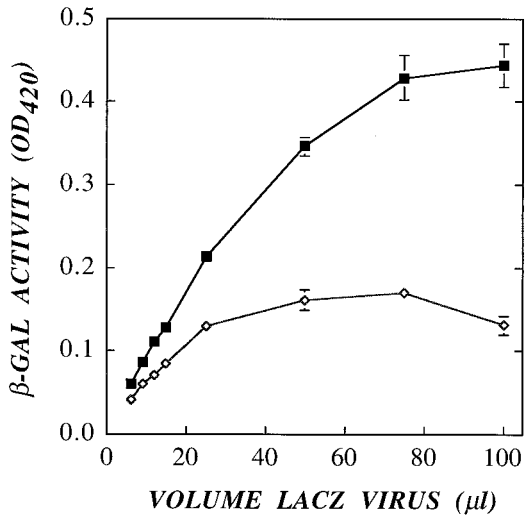

FIG. 1. Dose-response curves for amphotropic (filled squares) and ecotropic (open diamonds) *lacZ* recombinant retroviruses. NIH 3T3 cells were plated in a 96-well dish (5,000 cells per well) and the next day infected with 100 μl of medium containing various amounts of *lacZ* virus with 8 μg of Polybrene per ml. Two days later the cells were assayed for β-galactosidase (β-GAL) activity. The infected 3T3 cells were washed with 100 μl of PBS–1 mM $MgCl_2$, lysed with 50 μl of lysis buffer for 30 min at 37°C, and incubated with an additional 50 μl of lysis buffer containing 6 mM *o*-nitrophenyl-β-D-galactopyranoside (ONPG) at 37°C. After 15 min, the reactions were stopped by the addition of 20 μl of 1 M Na_2CO_3. The optical density at 420 nm (OD_{420}) was read, and the optical density at 650 nm was subtracted, with uninfected 3T3 cells being used as a plate blank. Each point shows the mean and standard deviation of three replicate experiments.

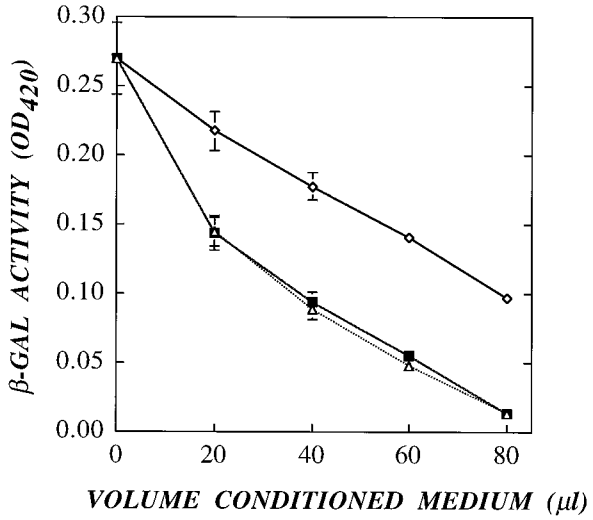

FIG. 2. Effect of conditioned medium on retroviral infection. NIH 3T3 cells were plated in a 96-well dish (5,000 cells per well), and the next day the medium was replaced with medium containing various volumes of medium conditioned with NIH 3T3 (open triangles), ecotropic packaging (open diamonds) or amphotropic packaging (filled squares) cells, 20 μl of amphotropic *lacZ* virus, and 8 μg of Polybrene per ml and brought to a final volume of 100 μl with fresh medium. The wells were assayed for β-galactosidase (β-GAL) activity 2 days after infection. Each point shows the mean and standard deviation of three replicate experiments. OD_{420}, optical density at 420 nm.

pended on the culture conditions used to produce the virus stocks, e.g., the density of the packaging cell line as well as the number of times the packaging cell line had been passaged in culture.

Fibroblast-conditioned medium inhibits infection. Each of the retrovirus solutions tested in the dose-response experiments contained various amounts of viral stocks adjusted to 100 μl with fresh medium. Since stocks of retrovirus were composed of both virus particles and medium conditioned by the fibroblast packaging cell line, the highest doses of virus also contained the highest doses of conditioned medium. To determine the effects of conditioned medium, virus particles were pelleted from amphotropic virus stocks (generated by incubating confluent 10-cm-diameter dishes of amphotropic virus producer cells with 10 ml of fresh medium for 24 h at 37°C) by centrifugation at 40,000 rpm in a Beckman SW55Ti rotor for 1.5 h at 4°C. Virus-free conditioned medium was decanted from the virus pellet, and various amounts were mixed with 20 μl of amphotropic *lacZ* virus, adjusted to 100 μl with fresh medium, and used to infect NIH 3T3 cells. Infectivity was inhibited in a dose-dependent manner by increasing concentrations of virus-free medium conditioned by the amphotropic packaging cell line (Fig. 2). Likewise, infectivity of the amphotropic virus was inhibited by virus-free medium conditioned by ecotropic producer cells and by medium conditioned with normal NIH 3T3 cells. These results demonstrated that infection is inhibited by conditioned medium and suggests that the plateau seen in the dose-response experiments is not due to saturation of virus receptors but is primarily due to the effects of increasing concentrations of conditioned medium.

Inhibitory activity is between 100 and 1,000 kDa in size. In order to obtain an enriched fraction containing the inhibitory activity, we generated serum-free conditioned medium by incubating 30 ml of serum-free Dulbecco's modified Eagle's medium in a confluent T175 flask of NIH 3T3 cells for 4 h at 37°C. The serum-free conditioned medium was concentrated fourfold by ultrafiltration with a range of molecular size cutoff membranes (30, 100, 300, and 1,000 kDa), and both the high-molecular-size fraction and the low-molecular-size fraction were assayed for inhibitory activities. Fractionated conditioned medium (80 μl, adjusted to 10% bovine calf serum) was mixed with 20 μl of amphotropic *lacZ* virus and used to infect NIH 3T3 cells. Fresh medium and unfractionated medium mixed with 20 μl (each) *lacZ* virus were used as controls. After 4 h the infection was halted by replacing the virus-laden medium with fresh medium. Two days later the β-galactosidase activities of the lysates of the infected cells were measured and the inhibitory activities were calculated. Inhibitory activity was defined as the β-galactosidase activity of cell lysates in the control serum-free fresh medium wells minus the β-galactosidase activity of cell lysates in the control serum-free conditioned medium wells divided by the β-galactosidase activity of cell lysates in the serum-free fresh medium wells. When the 30- and 100-kDa molecular size cutoff membranes were used to fractionate conditioned media, the inhibitory activity in the high-molecular-size fraction was about twofold higher than that of the unfractionated conditioned medium. Conversely, the inhibitory activity in the low-molecular-weight fraction was about twofold lower than the inhibitory activity of unfractionated conditioned medium (Table 1). A similar trend was seen with the 300-kDa membrane. The 1,000-kDa membrane failed to partition the inhibitory activity. These results suggest that a significant portion of the inhibitory activity was larger than 100 kDa but smaller than 1,000 kDa.

Inhibitory activity is sensitive to chondroitinase ABC. To further characterize the inhibitory activity, we tested the cells' sensitivity to various agents. The inhibitory activity was abolished after incubation for 5 min at 100°C, was increased slightly by heating to 65°C for 15 min, and was not affected by heating

TABLE 1. Effects of various treatments on the inhibitory activity of conditioned medium

Treatment	Inhibitory activity (% of control)[a]
Control (no treatment)	100
Fractionation by ultrafiltration	
High-molecular-size fractions (kDa)	
>30	173 ± 11
>100	173 ± 31
>300	129 ± 8
>1,000	104 ± 11
Low-molecular-size fractions (kDa)	
<30	58 ± 13
<100	45 ± 15
<300	45 ± 11
<1,000	78 ± 9
Heat	
37°C, 60 min	109 ± 6
65°C, 15 min	143 ± 8
100°C, 5 min	−1.0 ± 0.1
Acid (pH 2.0, 24 h)	163 ± 26
Protease digestion (37°C, 60 min) with trypsin (0.1 mg/ml)	37 ± 4
Nucleases (37°C, 60 min)	
DNase (200 U/ml)	93 ± 15
RNase (10 μg/ml)	102 ± 23
Glycosaminoglycan lyases	
Chondroitinase ABC (0.1 U/ml, 37°C, 3 h)	49 ± 6
Heparinase I (3 U/ml, 31°C, 3 h)	101 ± 15
Polybrene (μg/ml)	
8	100 ± 12
16	113 ± 15
24	86 ± 9
32	93 ± 8

[a] The data are the inhibitory activities of treated conditioned media as percentages of the inhibitory activities of sham-treated conditioned media.

to 37°C for 1 h (Table 1). The inhibitory activity was not altered by nuclease digestion (1 h, 37°C, with 200 U of DNase I per ml or 10 μg of RNase per ml) but was enhanced by acid treatment (pH 2.0, 15 h, 4°C) and partially destroyed by protease digestion (1 h, 37°C, 0.1 mg of diphenylcarbamyl chloride [DPCC]-treated bovine pancreas trypsin per ml). Most notably, the inhibitory activity level was significantly reduced by treatment with chondroitinase ABC (3 h, 37°C, 0.1 U of chondroitinase ABC [Seikagaku, Kogyo, Japan] per ml, 0.01% bovine serum albumin [BSA]) whereas it was unaffected by treatment with heparinase (3 h, 31°C, 3 U of heparinase I [Sigma, St. Louis, Mo.] per ml, 0.01% BSA, 2 mM calcium chloride). These results suggested that the inhibitor might be a proteoglycan, because both chondroitinase ABC and trypsin significantly reduced its level of inhibitory activity. It is not known why heat and acid treatment enhanced inhibitory activity, but these results suggest that the inhibitor was denatured, or partially denatured, by these treatments and that the inhibitor does not need to be in its native conformation to block infection.

We confirmed that the inhibitory activity of stocks of amphotropic retrovirus was also sensitive to chondroitinase ABC. Virus-free conditioned medium was treated with 0.1 U of chondroitinase ABC or enzyme buffer (phosphate-buffered saline [PBS]) for 1 h at 37°C and then incubated for 15 min at 65°C to inactivate the chondroitinase ABC. Various amounts of the enzyme-treated or untreated conditioned medium were mixed with 20 μl of amphotropic *lacZ* virus, adjusted to 100 μl with fresh medium, and used to infect NIH 3T3 cells. Chondroitinase ABC treatment abolished virtually all of the inhibitory activities at the lowest doses of conditioned medium tested and about 50% of the inhibitory activities at the highest doses of conditioned medium tested (data not shown). These results demonstrated that a major portion of the inhibitory activities of medium conditioned by the packaging cell lines was sensitive to chondroitinase ABC.

Polybrene does not block inhibitory activity. Since inhibitory activity was sensitive to chondroitinase ABC, it appeared likely that the inhibitor contained highly negatively charged chondroitin sulfate glycosaminoglycan chains. Polybrene, a cationic polymer that is routinely added during retroviral infections to enhance infectivity, is presumed to act by reducing electrostatic repulsion between the negatively charged cells and virus particles (38). In an attempt to overcome the negative charges of the chondroitin sulfates and thereby neutralize the inhibitory activity of conditioned medium, we increased Polybrene concentrations up to fourfold above normal concentrations. Inhibitory activity was not reduced by increasing Polybrene concentration, however, which suggests that the inhibitor did not block infection solely by increasing the electrostatic repulsion between the cells and virus particles.

Proteoglycans inhibit amphotropic retrovirus infection. Because our initial results suggested that the main inhibitory activity was chondroitin sulfate proteoglycans, we directly determined if proteoglycans secreted by NIH 3T3 cells inhibited infection. Low-serum (2% bovine calf serum) medium, previously incubated overnight at 4°C with Q-Sepharose (1 ml of Q-Sepharose for every 25 ml of medium) to remove any serum proteoglycans, was conditioned with confluent NIH 3T3 cells for 20 h (40 ml of medium per T175 flask). The conditioned medium was filtered (pore size, 0.45 μm), brought to 1 M urea–2 mM EDTA–0.3 M NaCl–50 mM Tris (pH 7.5), and then chromatographed by gravity flow through a Q-Sepharose column (3 ml). The column was washed with 30 ml of equilibration buffer (1 M urea, 2 mM EDTA, 0.3 M NaCl, 50 mM Tris [pH 7.5]) to remove any unbound protein. Bound proteoglycans were removed with elution buffer (4 M guanidine chloride, 0.1 M sodium sulfate, 2.5 mM EDTA, 0.1 M Tris [pH 7]), extensively dialyzed (in a membrane with a molecular size cutoff of 3,500 Da) against 2,000 volumes of 5 mM Tris (pH 7) at 4°C, and concentrated by ultrafiltration. The concentration of glycosaminoglycans in the proteoglycan solution was estimated by a dimethylene blue assay that had been modified for use in a 96-well-plate format and calibrated with shark cartilage chondroitin sulfate C (10).

To determine if the purified proteoglycans inhibited infection and if their inhibitory activities were sensitive to chondroitinase ABC, the proteoglycan solution was mixed with fresh medium, treated with chondroitinase ABC (0.1 U/ml, 1 h, 37°C) or enzyme buffer (PBS), and then incubated at 65°C for 15 min to inactivate the enzyme. Various volumes of the treated medium were mixed with 20 μl of amphotropic *lacZ* virus, adjusted to 100 μl with heat-treated (65°C, 15 min) fresh medium, and then used to infect NIH 3T3 cells. As shown in Fig. 3, the proteoglycan solution strongly inhibited infection in a dose-dependent manner. Infection was blocked by more than 50% at a concentration of proteoglycan equivalent to 0.2 μg of chondroitin sulfate C per ml. The concentration of chondroitin sulfate C in medium conditioned with NIH 3T3 cells is typically in this range (data not shown). Moreover, inhibition by the

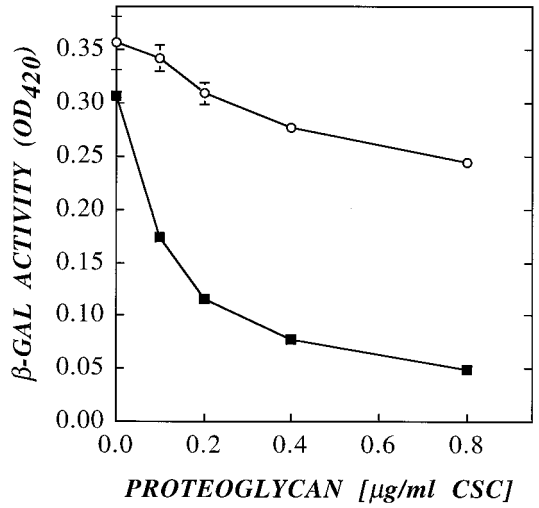

FIG. 3. Inhibition of retroviral infection by proteoglycans purified by anion-exchange chromatography. Fresh medium was brought to a known proteoglycan concentration (measured as the concentration of proteoglycan equivalent to a known concentration of shark cartilage chondroitin sulfate C [CSC; Sigma] with the dimethylene blue [DMB] assay) with proteoglycan solution purified by anion-exchange chromatography, digested with chondroitinase ABC (0.1 U/ml, 1 h, 37°C [open circles]) or enzyme buffer (PBS [filled squares]), and then incubated for 15 min at 65°C to inactivate the enzyme. Various volumes of the enzyme-treated medium were mixed with 20 μl of *lacZ* virus, brought to 100 μl with heat-treated fresh medium (65°C for 15 min) and 8 μg of Polybrene per ml, and then used to infect NIH 3T3 cells in the β-galactosidase (β-GAL) assay. Each point shows the mean and standard deviation of three replicate experiments. OD_{420}, optical density at 420 nm.

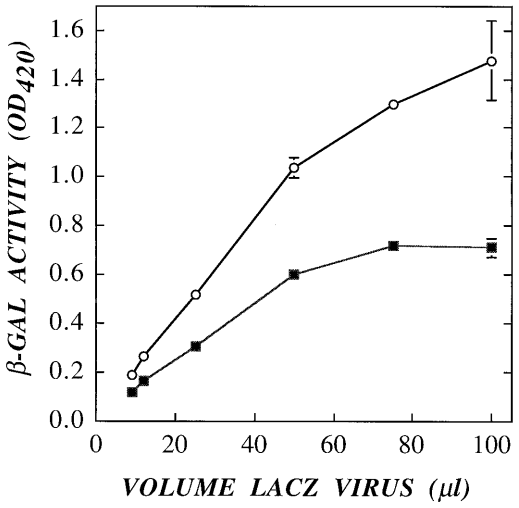

FIG. 4. Dose responses of chondroitinase ABC-treated and sham-treated amphotropic retrovirus stocks. Amphotropic *lacZ* retrovirus stocks were digested with chondroitinase ABC (0.1 U/ml, 37°C [open circles]) or sham digested with enzyme buffer (PBS [filled squares]) for 1 h. Various volumes of the treated virus stocks were brought to 100 μl with fresh medium and then used to infect NIH 3T3 cells in the β-galactosidase (β-GAL) assay. Each point shows the mean and standard deviation of three replicate experiments. OD_{420}, optical density at 420 nm.

proteoglycan solution was significantly reduced but not abolished by chondroitinase ABC treatment. Proteoglycans also inhibited retroviral infection of primary human fibroblasts, and the inhibition was substantially reduced by chondroitinase ABC treatment (data not shown). This suggests that proteoglycan inhibition is not cell-type specific. Chondroitinase ABC treatment of fresh medium increased the level of infection slightly over that of sham-treated fresh medium even when no proteoglycan solution was added, which suggests that fresh medium contained an inhibitory substance, sensitive to chondroitinase ABC, that was most likely contributed by the bovine calf serum.

Treatment of viral stocks with chondroitinase ABC boosts infectivity. To determine if chondroitinase ABC treatment would boost infectivity at high virus concentrations, virus stocks were incubated at 37°C for 1 h with either 0.1 U of chondroitinase ABC per ml or enzyme buffer (PBS). After enzyme digestion, various volumes of *lacZ* virus (enzyme treated or control) were diluted to 100 μl with fresh medium and used to infect NIH 3T3 cells. As shown in Fig. 4, the level of infectivity of the amphotropic retrovirus was boosted by more than twofold at the highest doses of virus and the dose-response curve was somewhat more linear than that of the untreated retrovirus.

Our study demonstrates that the flattening of the dose-response curve is largely due to inhibitors of retroviral infection present in the conditioned medium of retroviral stocks and is not due solely to retrovirus particles, proteins, or other functions specific to the packaging cell line. We identified chondroitin sulfate proteoglycans, secreted by the packaging cell lines, as one of the major inhibitors present in retrovirus stocks. Our results also suggest that the plateau of the dose-response curve is not due to saturation of the cells with retrovirus. Treatment of virus stocks with chondroitinase ABC more than doubled the efficiency of infection, demonstrating that the plateau region did not represent an absolute maximum level of infection of the cells.

Proteoglycans are complex, heterogeneous macromolecules that are found inside the cell, on the cell surface, in the extracellular matrix, and in the surrounding medium (17). They are composed of a protein core and one or more covalently bound glycosaminoglycan (GAG) chains which are large, negatively charged, linear polymers of repeating disaccharides. The three most prevalent types of sulfated GAGs are heparan sulfate, chondroitin sulfate, and chondroitin sulfate's epimerized homolog dermatan sulfate. The number, compositions, and molecular weights of GAGs can vary widely with cell type, culture conditions, and age of the cell line (12, 33, 36, 39). Fibroblasts and other connective tissues often produce several types of proteoglycans, including decorin, whose core protein (36 kDa) is covalently bound to a single GAG (chondroitin sulfate or dermatan sulfate), and versican, whose core protein (≈260 kDa) is covalently bound to up to 25 GAGs (chondroitin sulfate) (42).

The presence of chondroitin sulfate proteoglycans in viral stocks may complicate the concentration or purification of retrovirus particles. Several methods have been developed to concentrate retrovirus stocks, including tangential-flow filtration, hollow-fiber filtration, and ultrafiltration (19, 30, 34). All of these methods concentrate virus against porous membranes that restrict the passage of large molecules and virus particles but permit the free passage of low-molecular-weight components of the culture medium. The linear dimensions of a large chondroitin sulfate proteoglycan can approach those of a retrovirus particle; thus, concentration by any of these methods could inadvertently concentrate chondroitin sulfate proteoglycans as well as virus particles. In one study, the titers and transduction efficiencies of unconcentrated and concentrated stocks were compared (14). Although concentration increased virus titer 10-fold, the transduction efficiency was increased by only 20 to 30%. The investigators speculated that damaged

virus particles or other inhibitors might be present in the concentrated stocks and concluded that concentration was not a viable method to increase the transduction efficiency level of retroviral stocks. Our results suggest that chondroitin sulfate proteoglycans, if coconcentrated with virus particles, would inhibit infection of the concentrated stock.

Methods which eliminate proteoglycans may be one means to improve the effectiveness of retrovirus stocks. In principle, proteoglycans could be removed by any number of methods, including gel filtration and anion-exchange chromatography, but use of these or similar methods may not be straightforward since retrovirus particles are sensitive to shear stress and osmotic pressure gradients (24, 30). Our results show that the inhibitory activity of proteoglycans can be eliminated by GAG lyases, but this approach may not be useful for large-scale procedures. Alternatively, new packaging cell lines based on cells which secrete fewer proteoglycans could be constructed.

Other inhibitors may also be present in the conditioned medium. Infection in the plateau region of the dose-response curve was enhanced by treatment with chondroitinase ABC, but at high concentrations transduction efficiency was still not linearly proportional to virus concentration. Likewise, chondroitinase ABC digestion of virus-free medium conditioned by producer cell lines did not remove all inhibitory activity, especially at high concentrations of conditioned medium. Some of these inhibitors are most likely highly negatively charged molecules, because conditioned medium purified by anion-exchange chromatography contained inhibitory activity which was not sensitive to chondroitinase ABC. One additional possibility is that the core protein of the proteoglycan also inhibits infection but to a lesser extent than the intact proteoglycan molecule.

Chondroitin sulfate proteoglycans could inhibit retroviral infection by several mechanisms. The negatively charged GAGs of the proteoglycans might block the effects of Polybrene, a positively charged polymer known to enhance infection more than 10-fold when added to the culture medium during infection (38). Indeed, dextran sulfate and heparin, both negatively charged polymers, block the enhancement of infection by positively charged polymers, such as Polybrene and protamine, possibly by counteracting the charge effects of these molecules (38). Our results suggest that proteoglycan-mediated inhibition may be more complex. Inhibition by negatively charged sulfated polysaccharides, such as heparin, can be overcome by high concentrations of Polybrene (38), but in our studies, the inhibitory activity of conditioned medium could not be overcome by increasing the Polybrene concentration.

Another possibility is that chondroitin sulfate proteoglycans bind to specific domains of the virus envelope proteins or to the cellular receptor (or to a site adjacent to the cellular receptor) and block virus attachment and infection. Proteoglycans and GAGs, by virtue of their strong negative charge, often bind to clusters of positively charged amino acids, and there are several such clusters present in the primary sequences of the amphotropic and ecotropic envelope proteins. It is unlikely, however, that cell surface chondroitin sulfate proteoglycans act as receptors for amphotropic murine leukemic retroviruses or are essential for infection, because transduction efficiency was increased, rather than abolished, by infection in the presence of chondroitinase ABC. Finally, it is possible that chondroitin sulfate proteoglycans bind to the cellular retrovirus receptor or to an adjacent site. Binding to the receptor or an adjacent site would effectively reduce the number of virus binding sites available on the cell surface and therefore reduce levels of virus binding and infection.

In this study, we have demonstrated that chondroitin sulfate proteoglycans are present in retroviral stocks and inhibit infectivity. These findings have implications for the conduct of gene therapy protocols, for the production of high-titer retroviral stocks for the purposes of gene therapy, and for the understanding of retrovirus-cell interactions. Future studies should focus on developing methods to eliminate proteoglycans from retroviral stocks, since concentration of proteoglycan-free viral stocks should result in significant increases in transduction efficiency. The usefulness of proteoglycans as antiviral agents should also be assessed. Elucidation of the mechanism of proteoglycan inhibition of retroviral infection should also be pursued, since a thorough understanding of the mechanism would undoubtedly improve our understanding of retrovirus-cell interactions.

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