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# HUMAN GENE THERAPY

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# High-Efficiency Gene Transfer Mediated by Adenovirus Coupled to DNA-Polylysine Complexes

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## ABSTRACT

Employment of recombinant viruses as gene transfer vectors is limited by constraints on the size and functional design of the genetic material to be transferred as well as potential safety hazards deriving from obligatory co-transfer of viral genetic elements. As an alternative strategy that capitalizes on the efficient cellular entry mechanisms of viruses, we have derived adenovirus-polylysine-DNA complexes whereby foreign DNA is transferred bound to the exterior of the virion. This linkage was accomplished utilizing an antibody bridge in which a monoclonal antibody was rendered competent to carry DNA by the attachment of a polylysine residue. Attachment of the antibody-polylysine to the virus was by virtue of the antibody's specificity for the virion. The resulting vector system mediates high-efficiency gene transfer to target cells *in vitro*. In addition, this vector design allows greatly enhanced flexibility in terms of the size and design of heterologous sequences that can be transferred. Since this strategy selectively exploits viral entry functions, which are independent of viral gene expression, the potential exists to derive vectors that avoid the hazards deriving from transfer of parent virus genome.

## OVERVIEW SUMMARY

Gene transfer is being applied to more and more clinical problems. Not unexpectedly, ingenious new ways are being found to improve gene transfer efficiency. Curiel *et al.* have developed a clever system where they attach the DNA of interest to the *outside* of a virion, rather than putting it inside.

## INTRODUCTION

RECOMBINANT VIRAL GENE TRANSFER VECTORS accomplish gene transfer by capitalizing on the efficient entry mechanisms of their parent viruses. This strategy has been employed in the design of recombinant retroviral and adenoviral vectors to achieve high-efficiency gene transfer *in vitro* and *in vivo* (Berkner, 1988; Eglitis and Anderson, 1988). Despite their efficacy, these agents possess limitations related to the size and

construction of the heterologous DNA to be transferred. In addition, these agents pose safety hazards deriving from the co-transfer of viable genetic elements of the parent virus.

To circumvent these limitations, alternative strategies have been developed to accomplish gene delivery by exploiting cellular mechanisms of macromolecular transport. For example, the highly efficient receptor-mediated endocytosis pathway has been exploited to achieve gene transfer (Wu and Wu, 1987; Wagner *et al.*, 1990). This approach utilizes bifunctional molecular conjugates that possess a domain to bind DNA and a separate domain with specificity for a cell-surface receptor (Wu and Wu, 1987; Wagner *et al.*, 1990; Cotten *et al.*, 1991). When the ligand domain is recognized by the appropriate cell-surface receptor, the conjugate is internalized by the receptor-mediated endocytosis pathway, co-transporting the bound DNA. Gene transfer efficiencies equal to or greater than other DNA-mediated techniques have been accomplished by this route (Zenke *et al.*, 1990; Cotten *et al.*, 1991).

Although this vector system can achieve high levels of DNA delivery to cells possessing the appropriate cell-surface recep-

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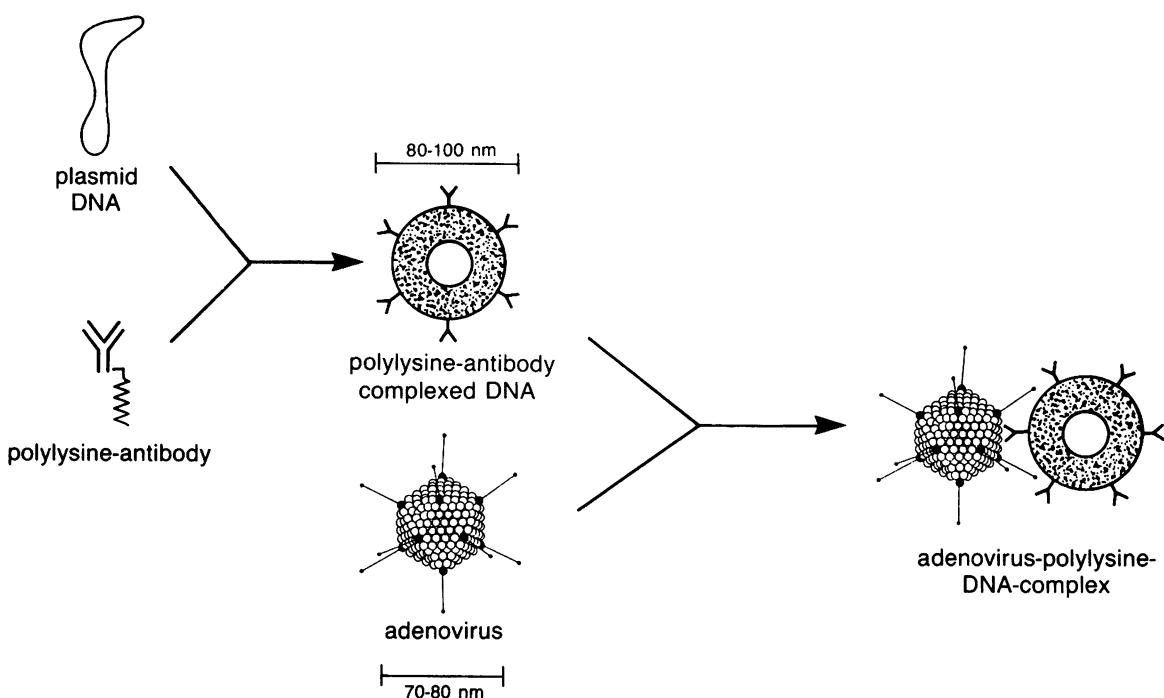
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tor, corresponding gene expression is often idiosyncratic (Cotten *et al.*, 1990, 1991). It has been suggested that the basis for this observation is that DNA delivered by the receptor-mediated endocytosis pathway is targeted for lysosomal degradation (Cotten *et al.*, 1990, 1991; Zenke *et al.*, 1990). Thus, the fact that the endosome-internalized DNA lacks a specific mechanism to escape from the intracellular vesicle system represents an intrinsic limitation of conjugate-mediated gene delivery. Recently, we have observed that gene transfer by transferrin-polylysine conjugates is greatly augmented by infection with adenovirus (Curiel *et al.*, 1991). This effect was achieved by capitalizing on the fact that adenoviruses enter cells *via* the receptor-mediated endocytosis pathway and possess a specific mechanism to escape the vesicle system of the cell by mediating disruption of the endosomes, thus allowing entry of the transferrin-polylysine DNA complexes into the cytoplasm (Pastan *et al.*, 1986). On the basis of these findings, we have constructed a conjugate system that possesses the capacity to mediate endosome disruption by incorporating the adenovirus into its functional design. The adenovirus-polylysine-DNA complexes mediate highly efficient gene transfer *in vitro* and, by virtue of their design, allow enhanced flexibility in terms of genetic material to be transferred compared to conventional recombinant viral gene transfer vectors.

## MATERIALS AND METHODS

### *Preparation of components of adenovirus-polylysine complexes*

To accomplish linkage between adenovirus and a polycationic DNA-binding moiety without functional disruption of the capsid proteins, an antibody bridge was employed whereby a polylysine moiety was conjugated to a monoclonal antibody with specificity for the adenoviral capsid (Fig. 1). Since the adenoviral fiber and penton proteins are important in mediating viral attachment and entry while the major capsid protein, hexon, appears less important in these processes (Seth *et al.*, 1984), antibody attachment was *via* the hexon protein. This specific attachment was achieved utilizing a chimeric adenovirus (P202-Ad5) that has a foreign epitope in the surface region of its hexon protein in conjunction with a monoclonal antibody specific for the heterologous epitope (MP301). Control experiments demonstrated that attachment of the monoclonal antibody MP301 was nonneutralizing for adenovirus P202-Ad5. To prepare the antibody-polylysine monoclonal antibody MP301 (20.6 nmoles) in 1 ml of 200 mM HEPES pH 7.9 was treated with a 5 mM ethanolic solution of *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) (100 nmoles). After 3 hr at 22°C, purifica-



**FIG. 1.** Schematic of approach to derive adenovirus-polylysine-DNA complexes containing heterologous DNA attached to exterior of adenovirus capsid. To accomplish linkage of an adenovirus and a polycationic DNA-binding domain, the chimeric adenovirus P202-Ad5 containing a heterologous epitope in the exterior domain of its hexon protein was employed in conjunction with the monoclonal antibody MP301 specific for this epitope. The monoclonal antibody was rendered competent to carry foreign DNA sequences by attaching a polylysine moiety. Interaction of the polylysine-antibody complexed DNA with adenovirus P202-Ad5 occurs *via* the specificity of the conjugated antibody.

tion was performed by a Sephadex G25 gel filtration to give 19 nmoles of MP301 modified with 62 nmoles of dithiopyridine linker. The modified antibody was reacted with 3-mercaptopropionate-modified poly(L-lysine) (22 nmoles, with an average chain length of 300 lysine residues, FITC-labeled, and modified with 56 nmoles of mercaptopropionate-linker) in 100 mM HEPES buffer pH 7.9 under argon atmosphere. Conjugates were isolated by cation-exchange chromatography on a Mono S HR 5/5 column (Pharmacia) using a gradient from 20% A to 100% B (A, 50 mM HEPES pH 7.9; B, A + 3 M NaCl). The main product fraction, eluting between 1.65 M and 2 M salt, was pooled and dialyzed against HBS (20 mM HEPES pH 7.3, 150 mM NaCl) to yield MP301 (9.1 nmoles) conjugated to 9.8 nmoles of polylysine. To derive adenovirus P202-Ad5, site-directed mutagenesis was performed on the adenovirus 5 hexon gene to replace amino acids 188–194 with 15 amino acids from the *Mycoplasma pneumoniae* P1 protein (Inamine *et al.*, 1988). This insertion is in loop 11 of the hexon protein, which is located on the outer surface of the virion (Roberts *et al.*, 1986). These 15 amino acids contain the epitope for the monoclonal antibody MP301. The details of this work will be described elsewhere.

#### *Preparation of adenovirus-polylysine-DNA complexes*

Complexation of the DNA to the polylysine monoclonal antibody was accomplished by diluting 6 µg of DNA to a 350 µl total volume in HBS and combining with 9.5 µg MP301-polylysine conjugate in 150 µl total volume of the same buffer. The DNA plasmid pRSVL containing the *Photinus pyralis* luciferase gene under the control of the Rous sarcoma virus long terminal repeat enhancer/promoter was used as a reporter gene (De Wet *et al.*, 1987). The reaction mixture was incubated 30 min at room temperature. This amount of polylysine-antibody was calculated to be the amount necessary to achieve electro-neutrality of the input DNA. The polylysine-antibody-complexed DNA was diluted in HBS to achieve a final concentration of  $5 \times 10^{10}$  DNA molecules per milliliter. Adenovirus P202-Ad5 was diluted in ice-cold Dulbecco modified Eagle medium (DMEM) supplemented with 2% fetal calf serum (FCS) to achieve a concentration of  $5 \times 10^{10}$  viral particles per milliliter. Equal volumes of the polylysine-antibody-DNA and virus were combined and incubated for 30 min at room temperature. The HeLa target cells for the gene transfer experiments were maintained in DMEM supplemented with 5% FCS, penicillin at 100 international units (IU)/ml, and streptomycin 100 µg/ml in 60-mm tissue culture plates. For comparison to HeLa cells, the cell lines HBE1, KB, and MRC-5 were evaluated. HBE1, a respiratory epithelial cell line, was grown in F12-7X medium as described (Willumsen *et al.*, 1989). KB and MRC-5 were grown in Eagle minimal essential medium, 10% heat-inactivated (h.i.) FCS, penicillin at 100 IU/ml, streptomycin at 100 µg/ml, 10 mM nonessential amino acids, and 2 mM glutamine. Prior to application of the reaction mixture, the plates were cooled by incubation at 4°C for 30 min. After this incubation, the media was removed from the tissue culture dishes and 1 ml of complex applied. The treated cells were incubated at 4°C for 2 hr. This step allowed binding of formed complexes without cellular internalization. After this binding step, plates were washed three times with ice-cold 2% FCS/DMEM to

remove unbound reaction components in the fluid phase. The plates were then gradually warmed up after the addition of 2 ml ice-cold 2% FCS/DMEM and returned to the incubator (37°C, 5% CO<sub>2</sub>) for 16 hr. To evaluate reporter gene expression, cell lysates were prepared, standardized for total protein content, and analyzed for luciferase activity as previously described (Wagner *et al.*, 1990). Aliquots of cell lysate containing 50 µg of total cellular protein were analyzed. The luminometer had been calibrated such that 1 pg of luciferase produced 50,000 light units. Reporter plasmid DNA pRSVL was combined with adenovirus P202-Ad5 (DNA + P202-Ad5) and pRSVL DNA complexed to the polylysine monoclonal antibody was employed in the absence of the specific virus (DNA + MP301pLys) for comparison to the complete combination of the complex components (DNA + MP301pLys + P202-Ad5). The identical complex reaction was evaluated utilizing specific adenovirus that had been heat inactivated (45°C, 30 min) prior to complex formation (DNA + MP301pLys + P202-Ad5[h.i.]). Competition experiments were performed by incubating the specific adenovirus in the presence of the polylysine monoclonal antibody MP301pLys plus a 10-fold molar excess of nonlysinated monoclonal antibody MP301 (DNA + MP301pLys + MP301 + P202-Ad5) or polylysine monoclonal antibody MP301pLys and a 10-fold molar excess of nonlysinated irrelevant, monoclonal antibody, anti-rat IgG (DNA + MP301pLys + anti-rat IgG + P202-Ad5). DNA was also complexed with nonconjugated polylysine (4 µg) in amounts equimolar to polylysine-antibody prior to incubation with the specific virus (DNA + pLys + P202-Ad5). Complex formation reactions containing adenovirus WT300, which lacks the heterologous epitope recognized by MP301, were exactly as for the specific virus P202-Ad5 (DNA + MP301pLys + WT300). To determine the optimum ratio of complex components, various molar ratios of complex components were evaluated for the capacity to mediate gene transfer to HeLa cells. Complex formation reactions were as before except that  $2.5 \times 10^{10}$  polylysine-antibody-complexed DNA molecules were used for each reaction in combination with variable amounts of the specific adenovirus P202-Ad5. Application of complexes to cells and analysis of reporter gene expression was exactly as before. To determine gene transfer efficiency mediated by adenovirus-polylysine-DNA complexes, limiting dilutions of the complex were evaluated for the capacity to mediate detectable levels of reporter gene expression in HeLa cells. After formation, logarithmic dilutions of the complex were performed in 2% FCS/DMEM. One-milliliter aliquots of the various dilutions of complex were applied to 60-mm tissue culture dishes containing  $5 \times 10^5$  HeLa cells. After 1 hr incubation (37°C, 5% CO<sub>2</sub>), 3 ml of 5% FCS/DMEM was added and the plates were incubated for an additional 16 hr as before. Determination of reporter gene expression was as before. Experiments were performed in triplicate and data depicted represents the mean ± SEM.

#### *Preparation of chimeric complexes containing adenovirus and human transferrin*

To prepare ternary complexes containing a combination of adenovirus and human transferrin domains, the epitope-tagged adenovirus P202-Ad5 ( $2.5 \times 10^{10}$  particles) was diluted in 750 µl of 2% FCS/DMEM and combined with polylysine mono-

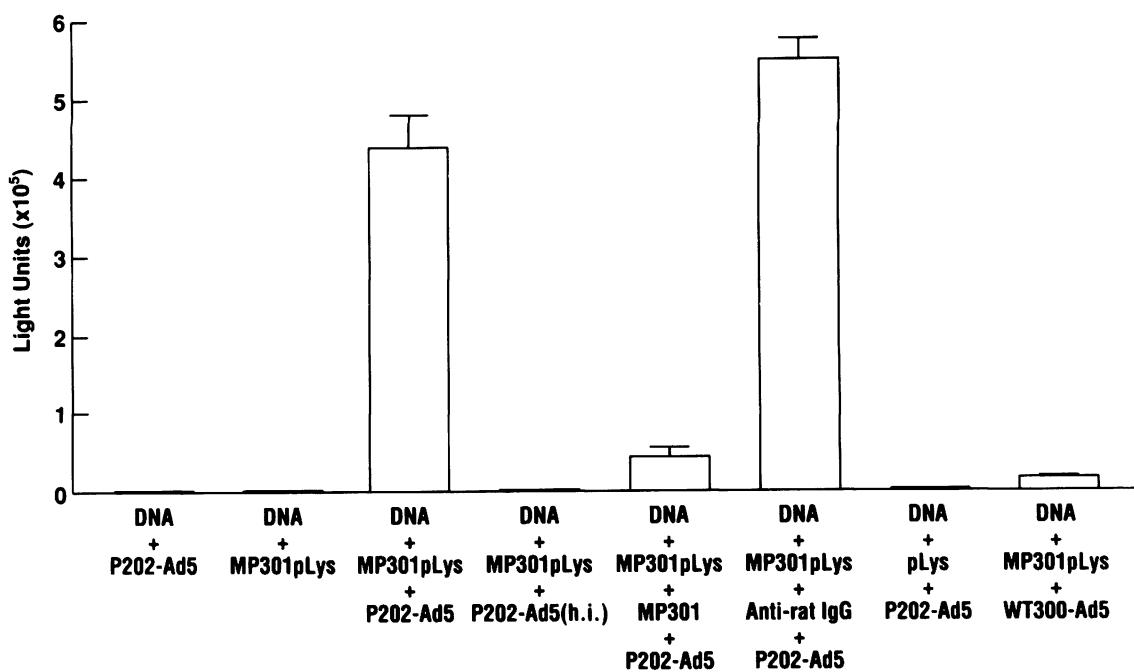
clonal antibody MP301pLys (2 µg) diluted in 250 µl of HBS. Incubation was performed for 30 min at room temperature. Plasmid DNA pRSVL (6 µg) diluted in 250 µl of HBS was then added to the mixture and incubated for an additional 30 min at room temperature. The resulting adenovirus–polylysine–DNA complexes were predicted to possess incompletely condensed DNA based upon total polylysine content. To complete DNA condensation and contribute a human transferrin moiety to the complexes, human transferrin polylysine conjugates (Wagner *et al.*, 1990) (9 µg) diluted in 250 µl of HBS were added to the adenovirus–polylysine–DNA complexes. A final incubation of 30 min at room temperature was performed. The resulting chimeric complexes were incubated with tissue culture cells to achieve specific binding of the formed complexes (4°C, 2 hr). The plates were then washed three times with ice-cold 2% FCS/DMEM and returned to the incubator (37°C, 5% CO<sub>2</sub>) for 16 hr after the addition of 2 ml of 2% FCS/DMEM. Evaluation of reporter gene expression was as before.

## RESULTS

### *Gene transfer mediated by adenovirus–polylysine–DNA complexes*

To evaluate the capacity of the adenovirus–polylysine–DNA complexes to mediate gene transfer, a plasmid encoding the

firefly luciferase gene was used as a reporter (De Wet *et al.*, 1987). The epithelial cell line HeLa was used as a target for the complexes because these cells possess a defined population of cell-surface receptors for adenoviruses (Philipson *et al.*, 1968). When employed in combination, the components of this conjugate system mediated high levels of expression of the luciferase reporter gene (Fig. 2). Control experiments demonstrated that the adenovirus did not significantly augment target cell delivery of uncomplexed plasmid DNA. Reporter DNA complexed by the polylysine monoclonal antibody was also not appreciably transferred to the target HeLa cells. In marked contrast, when the monoclonal antibody-bound DNA was allowed to interact with the epitope-tagged adenovirus, the resulting complex mediated high-level gene expression. This effect was abolished by heat treatment of the virions prior to complex formation. Since heat treatment selectively abrogates adenoviral entry functions without perturbing viral structural integrity (Defer *et al.*, 1990), it is apparent that the specific internalization functions of the adenovirus comprise a significant component of the gene transfer capacity of the complexes. Competition for the heterologous epitope on the surface of the chimeric adenovirus by nonlysinated specific monoclonal antibody also attenuated the net gene expression accomplished by the complex. This effect was not seen with an irrelevant monoclonal antibody. Thus, the specific interaction between the monoclonal antibody-bound DNA and the corresponding adenoviral surface epitope is important in permitting functional gene delivery by the complex. Consistent



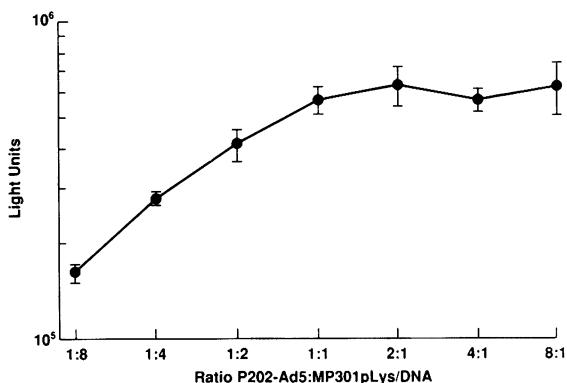
**FIG. 2.** Gene transfer mediated by adenovirus–polylysine–DNA complexes. Various combinations of specific and nonspecific complex components were evaluated for the capacity to mediate reporter gene transfer to HeLa cells. Reporter plasmid pRSVL (6 µg) [DNA] was complexed with either antibody–polylysine (9.5 µg) [MP301pLys] or equimolar amounts of unconjugated polylysine (4 µg) [pLys]. The complexed DNA was diluted prior to combination with adenovirus. Epitope-tagged virus [P202-Ad5] or irrelevant adenovirus [WT300] ( $2.5 \times 10^{10}$  particles) was added to the DNA complexes ( $2.5 \times 10^{10}$  DNA molecules) in the absence or presence of nonlysinated monoclonal antibody [MP301] or equimolar amounts of irrelevant monoclonal antibody [anti-rat IgG]. For some experiments adenovirus P202 was heat inactivated [P202-Ad5(h.i.)] prior to combination with antibody–polylysine–DNA complexes. After incubation, cell lysates were evaluated for luciferase reporter gene expression.

with this concept, an adenovirus lacking the epitope recognized by the polylysine antibody was not capable of mediating the high levels of gene expression achieved by the virus that possessed this epitope. Further, polylysine-complexed DNA was not appreciably transferred to target cells by the adenovirus, indicating that the gene transfer capacity of the complexes was not on the basis of DNA condensation but contingent upon the antibody-mediated attachment of the reporter gene to the virion.

Interaction of plasmid DNA with polylysine conjugates results in significant structural alterations of the DNA molecule, characterized most prominently by marked condensation into a 80- to 100-nm diameter toroid structure (Wagner *et al.*, 1991). The diameter of the adenovirus is also on the order of 70–80 nm (Philipson, 1983). When evaluated experimentally, it was shown that adenovirus input in molar excess of polylysine-antibody-complexed DNA yielded a plateau of reporter gene expression at a ratio of unity (Fig. 3). Thus, the optimized conjugate consists of a single adenoviral cognate domain in association with a single polylysine-antibody DNA-binding domain.

We next examined the efficiency of gene transfer of the adenovirus-polylysine conjugates at this optimized ratio. Logarithmic dilutions of the complex administered to target cells yielded a corresponding logarithmic decrease in reporter gene expression (Fig. 4). Significantly,  $5 \times 10^6$  DNA molecules delivered by this vector system to  $5 \times 10^5$  HeLa cells produced detectable levels of reporter gene expression. Thus, administration of as few as 10 DNA molecules per cell in the form of adenovirus-polylysine-DNA complexes accomplished detectable foreign gene expression. This is in marked contrast to the amount of input DNA required by DNA-mediated gene transfer vectors, where on the order of 500,000 DNA molecules per cell are required (Felgner *et al.*, 1987, 1989; Maurer, 1989).

In the configuration of the adenovirus-polylysine-DNA complexes, the adenovirus moiety functions both in the capacity of an endosome disruption agent and as the ligand domain of the complex. Thus, the gene transfer efficiency of the complexes for a given target cell should reflect the relative number of adenoviral cell surface receptors. Both HeLa and KB, cell

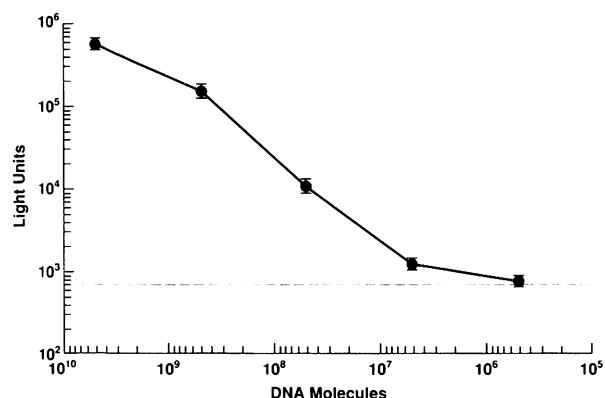


**FIG. 3.** Determination of optimum ratio of complex components to mediate gene transfer. A fixed amount of antibody-polylysine-DNA complex was combined with various molar ratios of epitope-tagged adenovirus and evaluated for the capacity to mediate gene transfer to HeLa cells.

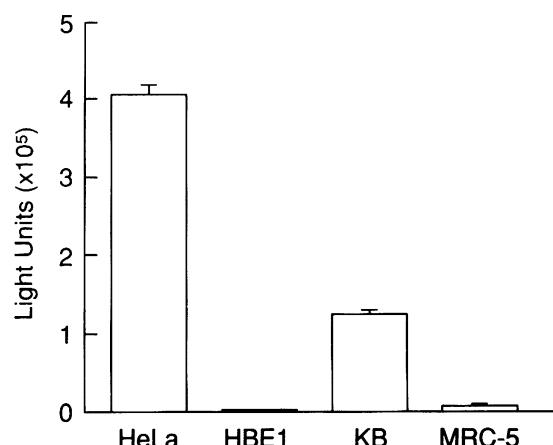
lines known to possess high levels of adenoviral receptors (Philipson *et al.*, 1968), demonstrated a corresponding high degree of susceptibility to gene transfer *via* adenoviral-polylysine-DNA complexes (Fig. 5). In contrast, the relatively low number of adenoviral receptors characterizing MRC-5 (Precious and Russell, 1985) and HBE1 (data not shown) is reflected in a lower level of gene transfer to these cell lines mediated by the complexes.

#### Gene transfer mediated by chimeric complexes containing human transferrin and adenovirus domains

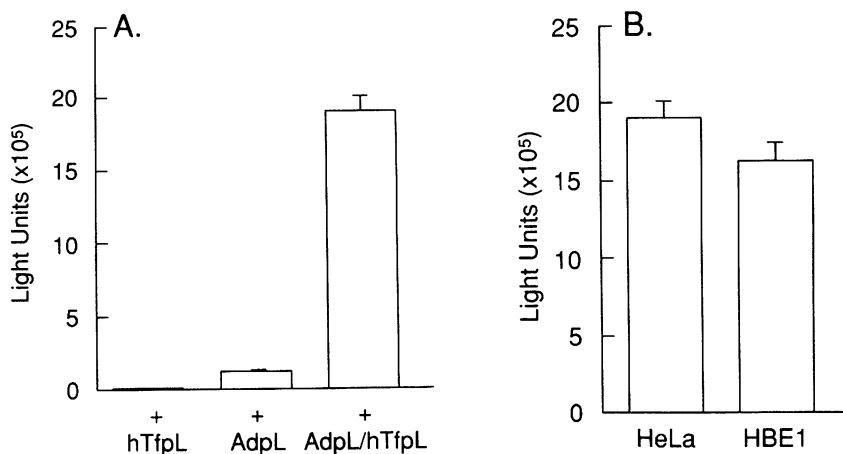
Specific internalization of molecular conjugate gene transfer vectors is dictated by the tropism of the conjugate ligand do-



**FIG. 4.** Determination of gene transfer efficiency mediated by adenovirus-polylysine-DNA complexes. Limiting dilutions of the complex were evaluated for the capacity to mediate detectable levels of luciferase gene expression in HeLa cells. Dotted line indicates background levels of luciferase gene expression in untreated HeLa cells.



**FIG. 5.** Gene transfer to various cell lines mediated by adenovirus-polylysine-DNA complexes. Epitope-tagged adenovirus P202-Ad5 ( $2.5 \times 10^{10}$  particles) was added to complexes formed between antibody-polylysine MP301pLys and reporter plasmid DNA pRSV-L. The resulting adenovirus-polylysine-DNA complexes were incubated with the cell lines HeLa, HBE1, KB, and MRC-5 as for Fig. 2. Reporter gene expression in the cell lysates was evaluated as before.



**FIG. 6.** Gene transfer mediated by chimeric complexes containing adenovirus and human transferrin. A. Relative levels of gene expression mediated by human transferrin–polylysine–DNA complexes, adenovirus–polylysine–DNA complexes, and ternary complexes containing a combination of adenovirus and human transferrin domains. Transferrin–polylysine–DNA complexes were formed by combination of pRSV-L DNA (6 µg) with human transferrin–polylysine conjugate (12 µg) as described (Wagner *et al.*, 1990) [hTfpL]. Adenovirus–polylysine–DNA complexes [AdpL] were prepared as in Fig. 2. Chimeric complexes were formed by sequential addition of polylysine monoclonal antibody MP301pLys (2 µg), plasmid DNA pRSV-L (6 µg), and human transferrin–polylysine (9 µg) to the epitope-tagged adenovirus P202-Ad5 ( $2.5 \times 10^{10}$  particles) [AdpL/hTfpL]. Delivery to HeLa cells and evaluation of reporter gene expression was as before. B. Relative susceptibility to gene transfer by adenovirus–human transferrin ternary complexes. Chimeric complexes containing a combination of adenovirus and human transferrin domains were formed as above. Delivery to the cell lines HeLa and HBE1 and analysis of reporter gene expression was as for Fig. 2.

main for receptors on the cell surface. Subsequent to internalization, gene transfer efficiency is limited by the conjugate's lack of a specific mechanism to escape entrapment within the cell vesicle system. Adenovirus–polylysine–DNA complexes exploit adenoviral-mediated endosome disruption to enhance gene transfer efficiency. In this specific configuration, however, the adenoviral domain also functions as the ligand moiety of the conjugate, limiting the efficacy of this vector to target cells containing surface receptors for adenovirus. As an alternative means to exploit the endosome disruption capacity of the adenovirus, ternary complexes were constructed that contained an alternate cell-surface ligand moiety in conjunction with an adenovirus moiety. In this arrangement, it was hypothesized that the adenovirus moiety would mediate endosome disruption after internalization *via* the adenoviral pathway or the pathway of the alternate ligand. To evaluate this concept, ternary complexes were constructed that contained a human transferrin ligand domain in conjunction with an adenoviral domain. The gene transfer efficiency of these chimeric complexes was compared to human transferrin–polylysine–DNA complexes (Wagner *et al.*, 1990) and adenovirus–polylysine–DNA complexes in HeLa cells, a cell line containing cell-surface receptors for both adenovirus and transferrin (Huebers and Finch, 1987). The ternary complexes accomplished significantly greater levels of reporter gene expression than the conjugates that possessed exclusively transferrin or adenovirus ligand domains (Fig. 6A). The magnitude of this augmentation was clearly not on the basis of an additive effect of human transferrin–polylysine–DNA complexes plus adenovirus–polylysine–DNA complexes. Since the ternary complexes may be internalized *via* the adenoviral or transferrin pathways, this apparent cooperativity suggests that

the adenovirus domain facilitates entry *via* either pathway, likely on the basis of adenovirus-mediated endosomolysis.

To demonstrate the selective employment of the endosome disruption capacity of the adenoviral domain of the ternary complex, the chimeric complexes were delivered to cell lines that exhibited variable susceptibility to adenovirus–polylysine–DNA complexes (Fig. 6B). The respiratory epithelial cell line HBE1 shows very low levels of gene transfer mediated by adenovirus–polylysine–DNA complexes, compared to HeLa cells (Fig. 5), reflecting the relatively low cell surface population of adenoviral receptors characterizing this line. In marked contrast, employment of the adenovirus–transferrin ternary complexes resulted in levels of gene expression comparable to those seen in HeLa cells. The susceptibility of this cell line to gene transfer *via* the ternary complexes is consistent with the concept that the adenoviral domain is internalized *via* the transferrin pathway, where it augments gene transfer by mediating endosome disruption. Thus, it appears feasible to exploit selectively the endosomolysis property of adenovirus in the design of molecular conjugate vectors that thereby possess the capacity to escape the cell vesicle system.

## DISCUSSION

The design of molecular conjugate gene transfer vectors capitalizes on the receptor-mediated endocytosis pathway to accomplish DNA delivery (Cotten *et al.*, 1991). Despite the efficient cellular internalization, however, DNA delivered by this route is largely trapped within the cell vesicle system, due to the fact that conjugate vectors lack a specific mechanism to facili-

tate DNA escape from the endosome (Cotten *et al.*, 1990). This concept led us to explore the utility of employing the adenovirus as an endosome disruption agent in conjunction with molecular conjugate-mediated gene delivery. In this regard, it was found that adenoviral-mediated endosomolysis dramatically augmented the gene transfer efficiency of transferrin-polylysine conjugates (Curiel *et al.*, 1991). Because molecular conjugate gene transfer vectors are limited by their lack of a specific mechanism to accomplish escape from the cell vesicle system, and because the adenovirus possesses such a mechanism, it was logical to incorporate the adenovirus into the functional design of the conjugate. Thus, the adenovirus-polylysine-DNA conjugates circumvent the principal limitation inherent in described molecular conjugate systems in possessing a specific mechanism to accomplish escape from the cell vesicle system.

The coupling of DNA to the exterior of the virion represents a radical conceptual departure from recombinant viral vectors utilized heretofore. Important advantages derive from this design whereby the foreign DNA to be transferred is not integrated into the genome of the parent virus, as is the standard design of recombinant viral vectors (Berkner, 1988; Egliitis and Anderson, 1988). These advantages include a much greater flexibility in the functional design of the foreign gene sequences expressed, as transcription is not dependent on promoters in the parent virus genome. In addition, this strategy allows a greatly increased size of foreign DNA that can be transferred, because the packaging constraints of the virus do not limit the amount of DNA that can be carried on the exterior. Over and above these practical and immediate advantages, important potential safety features derive from the design of the vector. Conventional recombinant viral vectors mediate obligatory co-delivery of genome elements of the parent virus, from which potential safety hazards derive (Anderson, 1984; Ledley, 1989). Because the adenovirus-polylysine complexes selectively exploit viral entry features, the viral genome is not an essential feature. This design allows the possibility of modifying the present system with a functionally and/or structurally inactivated viral genome to minimize the safety hazards deriving from the transfer of viable genes from the parent virus.

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