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Efficient Transfer of Genes into Murine Cardiac Grafts by Starburst Polyamidoamine Dendrimers

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ABSTRACT

Starburst dendrimer, a structurally defined, spherical macromolecule composed of repeating polyamidoamino subunits, was investigated to augment plasmid-mediated gene transfer efficiency in a murine cardiac transplantation model. The grafts were directly injected with naked pCH110, a plasmid encoding β-galactosidase (β-Gal), or pCH110-dendrimer complex, and reporter gene expression determined by X-Gal staining. The grafts injected with pCH110-dendrimer demonstrated widespread and extended β-Gal expression in both myocytes and the graft infiltrating cells from 7 to 28 days, compared to the grafts injected with naked pCH110 that expressed β-Gal only in myocytes for less than 14 days. pαMHC-vIL-10, as plasmid encoding viral interleukin-10 (vIL-10) under the control of α-myosin heavy chain promoter, was able to prolong allograft survival from 13.9 ± 0.9 days to 21.4 ± 2.3 days ($p < 0.005$). When dendrimer G5EDA was used with pαMHC-vIL-10, 60-fold less DNA resulted in significant prolongation of graft survival to 38.6 ± 4.7 days ($p < 0.0005$). The dose of DNA, the charge ratio of DNA to dendrimer, and the size generation of the dendrimers were all determined to be critical variables for prolongation of allograft survival in this model system. Thus, the use of the Starburst dendrimer dramatically increased the efficiency of plasmid-mediated gene transfer and expression. Production of immunosuppressive cytokines at higher amounts for longer periods of time in a greater expanse of tissue enhanced the immunosuppressive effect and prolonged graft survival further.

OVERVIEW SUMMARY

Plasmid-mediated gene therapy has been used to deliver immunosuppressive molecules into allografts to prolong graft survival. However, direct injection of naked plasmid DNA is inefficient because transgene expression is low and transient. This study investigated the ability of Starburst dendrimers to augment plasmid-mediated gene transfer efficiency in a murine cardiac transplantation model. The results demonstrate that dendrimers increased the efficiency of transfer and expression of exogenous DNA in cardiac grafts. Improved expression of an immunosuppressive cytokine viral interleukin-10 (vIL-10) by dendrimers significantly prolonged allograft survival. The dose of DNA, the charge ratio of DNA to dendrimer, and the size generation of the dendrimers were all critical for prolongation of allograft survival. Thus, the use of the Starburst dendrimer as a carrier molecule for plasmid-mediated gene transfer improved the efficiency of transfer and expression, providing further therapeutic value for treatment of cardiac allograft rejection.

INTRODUCTION

PREVIOUS STUDIES HAVE SHOWN that a variety of plasmid and viral (retrovirus, herpesvirus, adenovirus) vectors can introduce and express exogenous nucleic acids in isografts and allografts (Gainer *et al.*, 1997; Efrat *et al.*, 1995; Qin *et al.*, 1995; Bengamou *et al.*, 1996; Drazan *et al.*, 1996; Knetchle *et al.*, 1996; Levy and Alexander, 1996) and prolong allograft survival by inhibiting alloantigen-specific immunity (Qin *et al.*, 1996a,b). Despite these successes, indefinite graft survival and tolerance have not yet been achieved. This is likely due to the fact that gene transfer is inefficient and expression is low and transient (Wolff *et al.*, 1990; Jiao *et al.*, 1992; Qin *et al.*, 1995).

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Attempts to improve the transfection efficiency with high-titered viral vectors have met with limited success because of virus specific toxicities and antiviral immune responses (Bramson *et al.*, 1995; Gordon and Anderson, 1994; Yang *et al.*, 1994). It is likely that plasmid DNA, which does not present these difficulties and is easily manipulated and purified, will be the preferred vector system used in clinical transplantation. However, the physical transfer of naked DNA into target cells is currently inefficient (Wolff *et al.*, 1990; Jiao *et al.*, 1992; Qin *et al.*, 1994, 1995). Improved efficacy of plasmid gene transfer will therefore be required to deliver more effective immunosuppression and induce tolerance.

Starburst polyamidoamine (PAMAM) dendrimers are a new class of highly branched spherical polymers that contain large numbers of amino groups on the surface (Tomalia and Durst, 1993; Frechet, 1994), which are positively charged at physiological pH. Dendrimers have been reliably produced in large quantities with several structural characteristics of the molecule (including the overall shape, density, and surface charge) synthesized precisely (Tomalia and Durst, 1993; Frechet, 1994). The defined structure and large number of surface amino groups of PAMAM dendrimers make them good polycations to interact with biologically relevant polyanions, including nucleic acids. PAMAM dendrimers have been used as a substrate for the attachment of antibodies, contrast agents, and radiopharmaceuticals for applications in a number of different areas of biology and medicine both *in vitro* and *in vivo* (Roberts *et al.*, 1990; Barth *et al.*, 1994; Singh *et al.*, 1994), and shown to be nontoxic and able to target biologic agents to specific cells (Roberts *et al.*, 1990; Barth *et al.*, 1994; Singh *et al.*, 1994). Recent studies demonstrated that PAMAM dendrimers can form stable complexes with plasmids and oligonucleotides that dramatically enhance gene transfer and expression in a variety of mammalian cell lines *in vitro* (Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 1996). The stability of DNA/dendrimer complexes and high transfection efficiency with minimal cytotoxicity suggest that this transfection method has the potential for *in vivo* applications. This study investigated the ability of dendrimers to augment plasmid-mediated gene transfer efficiency in a murine cardiac transplantation model.

Interleukin-10 was originally termed cytokine synthesis inhibitory factor (Moore *et al.*, 1990) and is able to regulate a variety of immune responses negatively (Hsu *et al.*, 1990; de Waal Malefyt *et al.*, 1991a,b; Del *et al.*, 1993; Enk *et al.*, 1993; Peguet-Navarro *et al.*, 1994; Qin *et al.*, 1996a), such as inhibition of the synthesis of cytokines by T_H1 cells, particularly interferon-γ (IFN-γ); down-regulation of class II MHC expression on monocytes; and inhibition of the production of monokines, such as interleukin-1 (IL-1), IL-6, IL-8, IL-12, tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), and G-CSF. Viral IL-10, a product encoded by Epstein-Barr virus, is highly homologous to both murine and human IL-10, and shares many immunoinhibitory properties with murine and human IL-10, but does not possess the T cell co-stimulatory activities of authentic cellular IL-10 (Moore *et al.*, 1990; de Waal Malefyt *et al.*, 1991a,b; Hsu *et al.*, 1990). Previous studies have shown that gene transfer of vIL-10 by retroviral vectors prolonged allograft survival without systemic immunosuppression (Qin *et al.*, 1996a). In this study, vIL-10 was used as an immunosuppressant to determine whether enhanced transfer of the cDNA for this cytokine by dendrimer could achieve an improved therapeutic effect.

MATERIALS AND METHODS

Mice

CBA/J (H-2^k) and C57BL/6J (H-2^b) female mice (8–10 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, ME). Timed pregnant C57BL/6 mice were purchased from Harlan-Sprague-Dawley, Inc. (Indianapolis, IN).

Plasmids

pCH110 (Hall *et al.*, 1983), encoding the β-galactosidase (β-Gal) gene under the control of the simian virus 40 (SV40) promoter; and pMC1871 (Shapira *et al.*, 1983), a promoterless plasmid encoding β-Gal, were used. pRαMHCluc (Kitsis *et al.*, 1991), consisting of the firefly luciferase cDNA attached to rat α-cardiac myosin heavy chain (αMHC) 5′-flanking sequences (−613 to +32, relative to the start of transcription); and pHβMHCluc (Yamauchi-Takihara *et al.*, 1989), consisting of the firefly luciferase cDNA attached to human β-myosin heavy chain (βMHC) 5′-flanking sequences (−1,232 to +28, relative to the start of transcription), were provided by Dr. L. Leinwand (Albert Einstein College of Medicine). pcD-SRα-vIL-10 (Takebe *et al.*, 1988), encoding vIL-10 gene under the control of SR alpha promoter, was provided by Dr. H. Tahara (University of Pittsburgh). pαMHC-vIL-10, encoding vIL-10 under the control of αMHC promoter, was constructed by introduction of 0.6 kb of vIL-10 cDNA from pcD-SRα-vIL-10 into the *Xho* I site of pRαMHCluc from which firefly luciferase cDNA was removed by *Bgl* II–*Ppu* MI digestion and blunt end ligation; pβMHCvIL-10, a plasmid encoding vIL-10 under the control of βMHC promoter, was constructed using same strategy but vIL-10 cDNA introduced into pHβMHCluc. All plasmids were purified by two cycles of CsCl gradient centrifugation.

Dendrimer

Starburst PAMAM dendrimers were synthesized and prepared as previously described (Tomalia and Durst, 1993; Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 1996). The fifth and ninth generation of ethylenediamine (EDA) core dendrimer (G5EDA and G9EDA, respectively) (Tomalia and Durst, 1993; Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 1996) were tested in this study. The DNA–dendrimer complexes were prepared by adding 0.31 μg of DNA plus 10.10 μg dendrimer in a total volume of 5 μl in phosphate-buffered saline (PBS), which makes a DNA: dendrimer charge ratio of 1: 50 (Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 1996), and incubated for at least 10 min at room temperature to allow complex formation. Charge ratios in the range of 1:10 to 1:100 were also prepared using constant amounts of DNA and varying the dendrimer concentration.

Antibody

The JES3-19F1.1 rat anti-human IL-10 (Bacchetta *et al.*, 1994) hybridoma was purchased from the ATCC (Rockville, MD), grown in culture, and purified over protein G columns

(Pharmacia-LKB, Piscataway, NJ). This antibody binds both human and viral IL-10.

Cardiac transplantation

The heterotopic, nonvascularized cardiac transplantation model was used. Briefly, donor neonatal C57BL/6 or CBA/J mice were sacrificed, and whole hearts removed and placed subcutaneously in the ear pinnae of CBA/J recipients, as previously described (Qin *et al.*, 1994, 1995, 1996a,b). The DNA–dendrimer complexes were prepared as described above. Twenty micrograms of naked plasmid DNA in 5 μl of PBS/5% sucrose or 5 μl of DNA–dendrimer complex were directly injected into the graft at the time of transplantation. JES3-19F1.1 mAb in 0.5-ml volumes in PBS was injected intravenously at 100 μg every other day for six doses. Survival of cardiac allografts was followed with EKG monitoring (Polygraph 78 Series with pre-amp and filters, Grass Instruments, Quincy, MA) every other day. Cessation of cardiac electrical activity was taken as the determinant of rejection. There were at least 5 mice per group. Statistical comparison was performed with the Student's *t*-test. For histologic studies, ears with transplanted hearts were obtained, frozen, and sectioned at 10 μm for β-Gal determination.

X-Gal staining

The ears with transplanted hearts were quick frozen, embedded in O.C.T. (Miles Scientific, Naperville, IL), sectioned at 10 μm, collected onto gelatin-coated glass slides, fixed at room temperature in 0.25% glutaraldehyde in phosphate buffered saline (PBS) for 30 min, rinsed three times in PBS for a total of 30 min, and incubated at 37°C overnight in X-Gal solution containing 0.5 mg/ml of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 5 m*M* potassium ferrocyanide, 5 m*M* potassium ferricyanide, 2 m*M* $MgCl_2$, 1 m*M* spermidine, 0.02% Nonidet P-40, and 0.01% sodium deoxycholate in PBS. After staining, the sections were fixed with 4% formaldehyde and counterstained with eosin (Qin *et al.*, 1995).

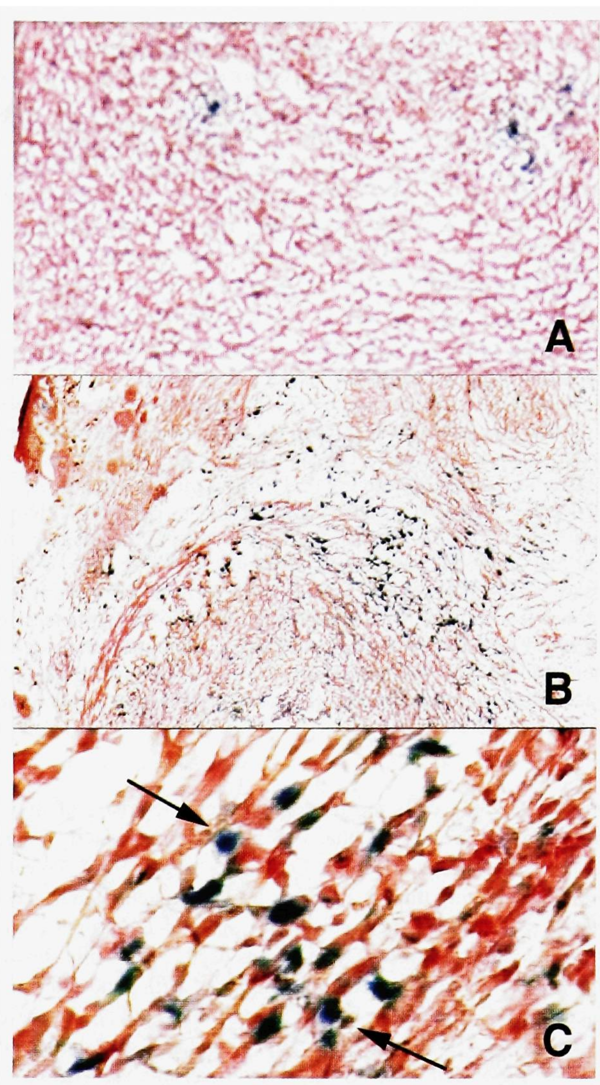

FIG. 1. Dendrimer G5EDA enhances plasmid transfer of β-Gal genes to cardiac isografts *in vivo*. Donor neonatal C57BL/6 mouse hearts were directly injected with naked plasmid (20 μg of DNA) or plasmid–dendrimer complex (0.31 μg DNA) and transplanted into syngeneic recipients. The hearts were harvested 7 or 14 days later and stained with X-Gal for β-Gal activity. A. pCH110 alone, 7 days after transplantation. B and C. pCH110-G5EDA, 14 days after transplantation. Original magnification, 100× (A and B) and 400× (C). In C, arrows point to graft-infiltrating cells whereas other cells all appear to be myocytes. These sections are representative of at least three grafts for each group at each time point.

RESULTS

Dendrimer G5EDA increases the efficiency of plasmid gene transfer in vivo

The charge ratio of DNA to dendrimer is one of the critical parameters in the optimization of *in vitro* transfection (Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 1996). The optimal charge ratio of DNA to dendrimer was selected through *in vitro* cytotoxicity and transfection studies of myocyte cell lines. The highest degree of *in vitro* transfection without toxicity was found at a DNA to dendrimer charge ratio of 1: 50 (not shown). Hence this charge ratio was used for initial *in vivo* studies. Donor hearts from neonatal C57BL/6 mice were transplanted to syngeneic recipients and injected with naked plasmids or plasmid–dendrimer complexes, and reporter gene expression was determined by X-Gal staining. The grafts injected with pCH110–dendrimer complex demonstrated widespread and extended β-Gal expression (Fig. 1B). The high-level expression lasted for at least 14 days after gene transfer, with significant numbers of the cells stained positively by X-Gal (Fig. 1B and Table 1). Reporter gene expression was dramatically decreased by 21 days, and only a few cells were still positive at day 28. Histologic studies demonstrated that both myocytes and the graft infiltrating cells were able to take up dendrimer–plasmid complex and express the transferred gene (Fig. 1C). The sections prepared from the grafts injected with the same amount of naked pCH110 (0.31 μg) displayed no β-Gal activity, whereas injection of a 60-fold higher amount of naked pCH110 (20 μg) resulted in only sparse β-Gal expression at day 7 (Table 1 and Fig. 1A) that was localized to a very few cells staining

Table 1. Dendrimer G5EDA Enhances Reporter Gene Expression in Cardiac Isografts

Treatment	*Days after transplantation and gene transfer*			
	D7	*D14*	*D21*	*D28*
Untreated	−	−		
pMC1871 (20 μg)	−	−		
pCH110 (0.31 μg)	−	−		
pCH110 (20 μg)	+	−		
pMC1871 (0.31 μg)-G5EDA	−	−		
pCH110 (0.31 μg)-G5EDA	+++	+++	++	+

Donor neonatal C57BL/6 mouse hearts were directly injected with naked plasmid DNA (doses in parentheses) or plasmid–dendrimer complex (0.31 μg of plasmid and 10 μg of dendrimer G5EDA, which makes a DNA to dendrimer charge ratio of 1:50) and transplanted into syngeneic recipients. At various time points after transplantation, ears with transplanted hearts were obtained, frozen in O.C.T., sectioned at 10 μm, and stained with X-Gal for β-Gal activity. +++, Widespread cells stained blue; ++, many cells stained; +, few cells stained; −, none of the cells stained. There were at least three grafts for each group at each time point. At least 30 sections per graft were examined.

positively. Only cells morphologically characteristic of myocardial cells were stained blue after naked DNA transfer (Wolff *et al.*, 1990; Jiao *et al.*, 1992; Qin *et al.*, 1994, 1995). To rule out the possibility that endogenous β-Gal was activated as a result of stimulation with dendrimer or plasmid–dendrimer complex, a control plasmid pMC1871 was used. The results shown that pMC1871, both naked and complexed with dendrimer, induced no β-Gal activity (Table 1). Dendrimers did not interfere with normal graft survival because syngeneic grafts injected with 10 μg of dendrimer G5EDA had indefinite survival up to 150 days (not shown). Transplant recipients showed no apparent toxic effects and no mortality associated with the use of dendrimers. Thus, use of dendrimer G5EDA as a carrier molecule dramatically increased the efficiency of transfer and expression of exogenous DNA in cardiac grafts.

Dendrimer-enhanced gene transfer of vIL-10 prolongs cardiac allograft survival

Twenty micrograms of naked pαMHC-vIL-10, a plasmid encoding vIL-10 under the control of αMHC promoter, injected into allografts was able to prolong graft survival significantly from 13.9 ± 0.9 days to 21.4 ± 2.3 days ($p < 0.005$), whereas 0.31 μg of naked DNA had no effect on graft survival. When dendrimer G5EDA was complexed with pαMHC-vIL-10 at a charge ratio of 1:50, 0.31 μg of DNA in a dendrimer complex, or 60-fold less than the optimal dose of naked DNA, resulted in even greater prolongation of graft survival to 38.6 ± 4.7 days ($p < 0.005$ vs untreated group, $p < 0.025$ vs 20 μg of naked pαMHC-vIL-10 treated group) (Table 2). The prolongation of graft survival was dose-dependent, because lower doses of DNA–dendrimer enhanced graft survival less effectively (Table 2).

Because the charge ratio of DNA to dendrimer was a critical determinant in the optimization of *in vitro* transfection (Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 1996), charge ratios in the range of 1:10 to 1:100 were also tested *in vivo*. The results in Table 2 demonstrate that a DNA-to-dendrimer G5EDA charge ratio of 1:50 was superior to either 1:10 or 1:100 in prolonging allograft survival in this model, which may reflect a higher transfection efficiency.

To confirm that prolongation of graft survival was due to vIL-10 production, monoclonal anti-hIL-10 antibody JES3-19F1.1, which cross-reacts with vIL-10 but not murine IL-10, was administered to the graft recipients. This treatment inhibited the effects of pαMHC-vIL-10-G5 administration (11.6 ± 1.0 days, $n = 5$). Specificity was further confirmed using pβMHCvIL-10, as plasmic encoding vIL-10 under the control of the βMHC promoter. In the rodent, the β-myosin heavy-chain promoter is active in embryonic and fetal cardiac ventricles, whereas the α-myosin heavy-chain promoter is active in postnatal and mature ventricles and in atria at all stages of development and maturation (Rindt *et al.*, 1993; Subramaniam *et al.*, 1993). Thus, pαMHC-vIL-10 should be able to direct the transcription of vIL-10 in this model, whereas the pβMHC-vIL-10 should not be capable of directing vIL-10 transcription. As predicted, pβMHCvIL-10, both in naked form and complexed

Table 2. pαMHC-vIL-10-Dendrimer G5EDA Prolongs Cardiac Allograft Survival: Dose and Charge Ratio

pαMHCvIL-10	*Treatment G5EDA*	*Charge ratio*	*Individual survival time (days)*	*MST ± SE (days)*
—	—	—	12,12,14,16,16	14.0 ± 0.9
0.31 μg	—	—	12,12,14,14,16	13.6 ± 0.7
20 μg	—	—	17,17,19,26,28	21.4 ± 2.3*
0.31 μg	10 μg	1:50	21,30,32,45,45	34.6 ± 4.6**
0.1 μg	3.3 μg	1:50	16,16,18,21,23	18.8 ± 1.4
0.03 μg	1 μg	1:50	12,14,18,18,18	16.0 ± 1.3
0.31 μg	2 μg	1:50	18,23,23,25,32	24.2 ± 2.3
0.31 μg	20 μg	1:100	18,18,18,21,21	19.2 ± 0.7

*$p < 0.005$ versus untreated group.
**$p < 0.0005$ versus untreated group, $p < 0.025$ versus 20 μg of naked pαMHC-vIL-10-treated group.
Donor neonatal C57BL/6 mouse hearts were injected directly with naked plasmid DNA or plasmid–dendrimer complex at the indicated doses and transplanted into CBA/J recipients. Survival of cardiac allografts was followed with EKG monitoring every other day.

with G5EDA, had no effect on prolongation of allograft survival (14.0 ± 1.4 days (n = 5) and 13.2 ± 0.7 days (n = 10), respectively). These results demonstrate that enhanced transfer of the cDNA for this cytokine by dendrimer could achieve an improved therapeutic effect.

Dendrimer G5EDA is superior to G9EDA for prolongation of cardiac allograft survival

Because dendrimers can be synthesized with a defined number of rounds of repeating subunits, the optimal number of repeats or generations of dendrimer is different for transfecting different cell lines *in vitro* (Kukowska-Latallo *et al.*, 1996). High generations of dendrimer demonstrated better transfection efficiencies for many cell lines (Kukowska-Latallo *et al.*, 1996), thus we tested whether the ninth generation of dendrimer G9EDA could enhance vIL-10 gene transfer and prolong allograft survival further compare to the fifth generation, G5EDA. The results in Table 3 demonstrate that when complexed with G9EDA, pαMHCvIL-10 significantly prolonged allograft survival in a dose-dependent manner. DNA-to-dendrimer charge ratios from 1:10 to 1:50 were similar and superior to 1:100. Specificity was confirmed by administering anti-vIL-10 antibody JES3-19F1.1, which inhibited the effects of pαMHC-vIL-10-G9EDA (from 24.6 ± 1.1 to 12.4 ± 0.7 days, n = 5). Despite having a slightly higher transfection efficiency in the murine myoblast cell line C2C12 *in vitro* (note shown), G9EDA was not as effective as G5EDA *in vivo* for prolongation of graft survival (27.2 ± 1.7 days vs. 38.6 ± 4.7 days, $p < 0.025$). Because the microenvironment that dendrimer–DNA complexes encounter and/or the cell types that dendrimer–DNA complexes target *in vivo* are very different from those experienced *in vitro*, the data indicate that the optimal generation of dendrimer for an *in vivo* system needs to be empirically determined and is not fully predicted by prior *in vitro* modeling.

DISCUSSION

Previous studies showed that a variety of plasmid and viral (retrovirus, herpesvirus, adenovirus) vectors could successfully transfer reporter genes into murine cardiac grafts (Acsadi *et al.*, 1991; Wang *et al.*, 1992, 1996; Qin *et al.*, 1994, 1995, 1996a,b, 1997; Donahue *et al.*, 1997), and the expression of immunosuppressive cytokines (vIL-10, mTGFβ1) by retroviral (Qin *et al.*, 1995, 1996a), adenoviral (Qin *et al.*, 1997), and plasmid vectors (Qin *et al.*, 1994, 1995, 1996b) prolonged allograft survival without systemic immunosuppression. The problem remains that while allograft survival is prolonged, it is not indefinite and tolerance has not been achieved. This is likely due in part to the fact that gene transfer is inefficient and expression is low and transient (Wolff *et al.*, 1990; Jiao *et al.*, 1992; Qin *et al.*, 1994, 1995). Therefore, gains in allograft survival likely require improvements in gene transfer and expression so that immunosuppressive cytokines can be produced in higher amounts for longer periods of time.

Cationic liposomes have been used to improve plasmid DNA delivery for various application of gene therapy (Nabel *et al.*, 1990; San *et al.*, 1993; Felgner *et al.*, 1995; Schofield and Caskey, 1995; Egilmez *et al.*, 1996). Cationic liposomes bind polyanionic DNA; and by charge attraction and fusion properties, the lipids can adsorb to the cell membrane and deliver the nucleic acid directly into the cytoplasm, bypassing the lysosomal degration pathway. Such liposomes are in widespread use as efficient means of transfecting plasmid DNA into a variety of cell lines grown in tissue culture. Several studies have shown the effectiveness of polycationic liposome delivery of DNA *in vivo* (Nabel *et al.*, 1990; San *et al.*, 1993; Felgner *et al.*, 1995; Schofield and Caskey, 1995; Egilmez *et al.*, 1996). However, most of the commercially available cationic lipids, including LipofectAMINE reagent, Lipofectin, Cellfectin, DC-Cholesterol, and DMRIE-DOPE have a very poor ability to enhance DNA expression above the baseline achieved *in vivo* with naked DNA (Nabel *et al.*, 1990; Felgner *et al.*, 1995; Schofield and Caskey, 1995; Egilmez *et al.*, 1996). Nabel *et al.* reported that fewer than 1% of cells were successfully transduced with a cationic liposome–DNA complex (Nabel *et al.*, 1990). Egilmez *et al.* reported that the overall transfection efficiency of LipofectAMINE reagent, Lipofectin, Cellfectin, DC-Cholesterol, and DMRIE-DOPE did not exceed 0.3% in a tumor model, even under optimal conditions (Egilmez *et al.*, 1996). Felgner *et al.* enhanced CAT expression in mouse lung with DLRIE only 25-fold above the naked DNA level (Felgner *et al.*, 1995). San *et al.* increased cationic liposome dose by 1,000-fold but only enhanced transfection efficiency of DMRIE/DOPE by 2- to 7-fold compared to DC-chol/DOPE (San *et al.*, 1993). In our cardiac model, attempts to use Lipofectin did not enhance plasmid induced prolongation of allograft survival compared to naked DNA alone (not shown). .

Starburst PAMAM dendrimers are a new class of branched

TABLE 3. PαMHC-vIL-10-DENDRIMER G9EDA PROLONGS CARDIAC ALLOGRAFT SURVIVAL

pαMHCvIL-10	*Treatment G9EDA*	*Charge ratio*	*Individual survival time (days)*	*MST ± SE (days)*
0.31 μg	10 μg	1:50	21 × 4,23 × 2,25 ×5,30,35	24.6 ± 1.1*
0.1 μg	3 μg	1:50	16,16,18,21,21	18.4 ± 1.1
0.03 μg	1 μg	1:50	12,14,14,18,18	15.2 ± 1.2
0.31 μg	2 μg	1:10	23,23,30,30,30	27.2 ± 1.7*
0.31 μg	20 μg	1:100	16 × 4,18	16.4 ± 0.4

*$p < 0.005$ versus untreated group.

Donor neonatal C57BL/6 mouse hearts were directly injected with plasmid–dendrimer G9EDA complex at the indicated doses and transplanted into CBA/J recipients. Survival of cardiac allografts was followed with EKG monitoring every other day.

spherical polymers that have a unique surface of primary amino groups (Tomalia and Durst, 1993; Frechet, 1994), which show positive charge densities restricted to the surface of the molecule. These characteristics allow dendrimers to interact simultaneously with both negatively charged phospholipids on cell membranes and DNA. Previous studies demonstrated that DNA–dendrimer complexes were stable for many weeks in solution and capable of mediating high-efficiency transfection to a variety of cell lines, including primary cells and nonadherent cell lines *in vitro* (Kukowska-Latallo *et al.*, 1996). Dendrimers increased and prolonged DNA uptake into cells and the nucleus (Kukowska-Latallo *et al.*, 1996) through energy-dependent endocytosis, which allowed high transfection efficiency compared to naked plasmid DNA alone or lipid-mediated transfection. Dendrimers also increased the stability of DNA inside the cells by protecting DNA from degradation by restriction endonucleases, DNase I, or cellular extracts containing nuclease activity (Bielinska *et al.*, 1996, 1997), which is particularly important for episomal plasmid persistence in transfected cells. Dendrimers are not immunogenic and do not evoke an antibody response when given alone or mixed with adjuvant (Roberts *et al.*, 1996; Baker, personal communication). Our studies demonstrate that Starburst dendrimer can be used to mediate efficient *in vivo* transfer of plasmid DNA into murine cardiac isografts and allografts. Dendrimer G5EDA dramatically increased the efficiency of gene transfer by about 1,000-fold (about 10% of both myocytes and graft infiltrating cells stained by X-Gal compared to less than 0.01% of myocytes only stained after naked plasmid DNA transfer), and extended transgene expression (up to at least 28 days compared to less than 14 days for naked plasmid DNA; Qin *et al.*, 1995). The higher level and longer lasting expression of vIL-10 by dendrimer G5EDA resulted in markedly prolonged graft survival as compared to naked plasmid DNA. Thus, production of immunosuppressive cytokines at higher amounts for longer periods of time in a greater expanse of tissue enhanced the immunosuppressive effect and prolonged graft survival further.

It has been reported that while dendrimers enhanced DNA uptake into cells and the nucleus (Bielinska *et al.*, 1996; Kukowska-Latallo *et al.*, 1996) and increased the stability of DNA inside the cells (Bielinska *et al.*, 1996, 1997), it also inhibited the initiation of transcription *in vitro* from promoters for either T7 RNA polymerase or eukaryotic RNA polymerase II (Bielinska *et al.*, 1997). Both the enhancing and inhibitory effects of the dendrimers are related to the DNA–dendrimer charge ratio and the generation of the dendrimers. For example, G9EDA, which demonstrated better transfection efficiency for many cell lines (Kukowska-Latallo *et al.*, 1996), caused greater inhibition of T7 polymerase transcription activity than G5EDA (Bielinska *et al.*, 1997). Lower charge ratios (*i.e.*, 1:10) demonstrated higher transfection efficiency, but caused greater inhibition of T7 polymerase transcription activity than higher charge ratios (*i.e.*, 1:0.1). In addition, this inhibitory effect varies for different promoters, since the RNA polymerase II-dependent transcription from a CMV promoter was affected to a lesser degree than T7 RNA polymerase-dependent transcription (Bielinska *et al.*, 1997). In this context, the charge ratio of DNA to dendrimer and the generation of the dendrimers were important determinants for prolongation of allograft survival in this model system. The data suggest that several issues, including the optimal generation of dendrimer, the DNA-to-dendrimer charge ratio, and the optimal promoter need to be determined for each model system *in vivo* and can be manipulated to improve gene expression and therapeutic efficacy.

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