ACQUIRED DISEASES   **RESEARCH ARTICLE**

Decoy against nuclear factor-kappa B attenuates myocardial cell infiltration and arterial neointimal formation in murine cardiac allografts

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Acute rejection and graft arteriopathy in cardiac transplantation limit the long-term survival of recipients; these processes are enhanced by several cytokines and adhesion molecules. Nuclear factor-kappa B (NFκB) is critical in the transcription of multiple genes involved in inflammation and cell proliferation. To test the hypothesis that NFκB decoy can attenuate acute rejection and arteriopathy, we performed single intraluminal delivery of NFκB decoy into murine cardiac allografts using a hemagglutinating virus of Japan (HVJ)-artificial viral envelope (AVE)-liposome method. No decoy or scrambled decoy transfer was performed for control. Hearts were heterotopically transplanted from BALB/c to C3H/He mice (major mismatch group) and from DBA/2 to B10.D2 mice (minor mismatch group). Nontreated or scrambled decoy transfected allografts of the major mismatch group were acutely rejected, while NFκB decoy prolonged their survival. While severe cell infiltration and intimal thickening with enhancement of inflammatory factors were observed in untreated or scrambled decoy-treated allografts of minor mismatch group at day 28, NFκB decoy attenuated these changes. We conclude that NFκB is critically involved in the development of acute as well as chronic rejection of the transplanted hearts. NFκB decoy attenuates both acute rejection and graft arteriopathy by blocking the activation of several genes. Gene Therapy (2000) 7, 1847–1852.

Keywords: *heart transplantation; rejection; arteriosclerosis; adhesion molecule; gene therapy*

Introduction

Cardiac transplantation has been established in humans, however, acute rejection and graft arteriopathy are still problems.[1,2] Acute rejection is enhanced by several cytokines and adhesion molecules; the arteriopathy is characterized by intimal thickening comprised of proliferative smooth muscle cells (SMCs).[3,4]

The use of DNA technology to regulate the transcription of disease-related genes *in vivo* has great therapeutic potential. Antisense cyclin-dependent kinase (cdk) 2 kinase oligodeoxynucleotide (ODN) and double-stranded DNA with high affinity for E2F (E2F decoy)[5,6] inhibit neointimal formation by suppressing multiple gene expression as demonstrated in rat carotid arterial injury models. Recently, we reported antisense cdk2 kinase ODN prevents arteriopathy in murine cardiac allografts; however, the ODN could not attenuate acute rejection.[7]

Nuclear factor-kappa B (NFκB) plays a pivotal role in the coordinated transcription of multiple inflammatory genes.[8–10] However, the role of NFκB in the pathophysiology of cardiac allograft rejection and arteriopathy has not been well investigated. Also, strategies that target this molecule in the attenuation of acute rejection and neointimal formation have not been studied in transplantation. Recently, it has been reported that decoy against the *cis* element of NFκB prevents myocardial infarction by suppressing multiple gene expression.[11] Since NFκB decoy inhibits several inflammatory gene expression, we hypothesized that NFκB decoy could attenuate acute rejection and intimal hyperplasia after transplantation. In this study, we demonstrated that *ex vivo* transfection of NFκB decoy using a hemagglutinating virus of Japan (HVJ)-artificial viral envelope (AVE)-liposome method reduced both acute rejection and graft arteriopathy.

Results

Graft survival

The HVJ-AVE–liposome, phosphorothioate decoy and ODN used in this study were prepared as described previously.[5,6,12] BALB/c (H-2^d) mice hearts were transplanted into C3H/He (H-2^k) mice as the major mismatch group for study of graft survival.[13] DBA/2 (H-2^d) mice hearts were transplanted into B10.D2 (H-2^d) mice as the minor mismatch group for pathological studies.[14] C3H/He mice hearts were transplanted into C3H/He mice as the isografts. Donor hearts were infused with HVJ-AVE–liposome complex and transplanted into

recipients as described previously.[15,16] Isografts and allografts of the minor mismatch group kept beating throughout the observation period. In the major mismatch group, nontreated ($n = 6$; 7.8 ± 0.4 days) or scrambled decoy ($n = 6$; 8.0 ± 0.6 days) transfected allografts were acutely rejected, while NFκB decoy transfection significantly prolonged allograft survival ($n = 6$; 13.7 ± 2.4 days, $P < 0.05$).

Histological findings of the myocardium

Isografts and allografts of the minor mismatch group were harvested at 28 days after transplantation for pathological analysis.[7] Isografts showed no myocardial cell infiltration. In the minor mismatch group, moderate myocardial cell infiltration was observed in nontreated or scrambled decoy transfected allografts at day 28. The cell infiltration scores were not statistically different between the nontreated and scrambled decoy groups. However, NFκB decoy markedly attenuated myocardial cell infiltration, the score was statistically less than those of the other groups. Immunohistochemically,[14–16] native hearts and isografts showed no enhancement of ICAM-1, VCAM-1, MHC class I, MHC class II or NFκB expression. These were enhanced in nontreated or scrambled ODN transfected allografts of minor mismatch group at day 28, mainly observed in myocardial interstitium with mononuclear cell infiltration. However, NFκB decoy transfection markedly attenuated expression of these factors, with scores significantly less than those of the other groups (Table 1, Figures 1 and 2).

Histological findings of the graft arteries

To highlight the internal elastic lamina (IEL) of the graft arteries, serial sections were stained with Elastica van Gieson (EvG). The area of luminal stenosis was calculated according to the formula: luminal occlusion = (IEL area − luminal area)/IEL area. Coronary arteries of native hearts and isografts did not develop intimal thickening. In minor mismatch groups, heavy neointimal thickening was observed in the coronary arteries of untreated or scrambled decoy transfected allografts at day 28. In the NFκB decoy-treated group, arterial intimal thickening was attenuated. The percentage of neointimal thickening statistically differed between NFκB decoy group and other groups. Immunohistochemically, ICAM-1, VCAM-1 and NFκB were expressed strongly and diffusely in the thickened intima of arteries of nontreated or scrambled decoy transfected allografts, while treatment with NFκB decoy suppressed this expression. To detect faint expression in the arteries, we employed *in situ* RT-PCR.[5–7,17–19] The mRNA expression of platelet-derived growth factor (PDGF)-B, cdk2 kinase or cell division cycle (cdc) 2 kinase was

enhanced in the thickened intima of arteries of nontreated or scrambled decoy transfected allografts, while treatment with NFκB decoy suppressed these mRNA expression (Table 2, Figures 1 and 2).

Localization and kinetics of FITC-labeled decoy

To confirm the localization and kinetics of this gene transfer system, FITC-labeled phosphorothioate ODN was transfected into donor hearts.[20,21] Transfer of FITC-labeled double-stranded decoy using the HVJ-AVE–liposome method facilitated widespread distribution of fluorescence in medial vascular cells, localized primarily in cell nuclei; the expression persisted for up to 14 days after transfection, however, it had diminished in the grafts at day 28 (Figure 3).

RT-PCR for HVJ dissemination

We analyzed organs of recipients for systemic dissemination of non-inactivated HVJ to confirm the localization of the *ex vivo* transfected HVJ-AVE–liposome complex using RT-PCR with primers for HVJ F and HN protein.[22,23] RT-PCR studies revealed that HVJ F or NH protein mRNA in the organs of recipients that received HVJ transfected allografts was not amplified; a single band corresponding to the transcription of these proteins was seen in non-inactivated HVJ (Figure 4).

Discussion

The pathological characteristics of acute rejection are myocardial cell infiltration and myocyte damage; chronic rejection includes diffuse intimal thickening comprised of proliferative SMCs.[1] Little is known about the etiology, however, immune reaction to graft endothelial cells via expression of MHC and adhesion molecules is considered the trigger, induced by a complex interaction of multiple cytokines and adhesion molecules. A critical element in the regulation of these genes involves the complex formed by NFκB and IκB.[24] Dissociation of the transcription factor NFκB from this complex is proposed to play a pivotal role in the regulation of rejection by inducing a coordinated transactivation of genes, including MHC, ICAM-1 and VCAM-1, involved in the processes.[8–10] Indeed, administration of monoclonal antibodies against adhesion molecules has been reported to prolong graft survival or induce tolerance.[13]

We report a novel therapeutic strategy to attenuate acute rejection of heart allografts, utilizing *ex vivo* transfer of a 'decoy' *cis* element to bind the critical transcriptional factor NFκB, thereby blocking the coordinated transactivation of the inflammatory genes involved in acute rejection. The specificity of the inhibi-

**Table 1** Degree of cell infiltration and expression scores in the allograft myocardium

Treatment of allograft	No. of grafts	Cell infiltration	Immunohistochemistry				
			ICAM-1	VCAM-1	NFκB	MHC class I	MHC class II
No ODN	8	2.5 ± 0.5	2.8 ± 0.3	2.0 ± 0.5	2.3 ± 0.8	1.8 ± 0.3	1.8 ± 0.3
Scrambled decoy	8	2.3 ± 0.5	2.6 ± 0.2	1.8 ± 0.5	2.2 ± 0.8	1.6 ± 0.2	1.6 ± 0.2
NFκB decoy	8	1.0 ± 0.8*	1.6 ± 0.6*	0.8 ± 0.5*	1.2 ± 0.4*	0.6 ± 0.2*	0.5 ± 0.4*

*$P < 0.05$ *versus* other groups.
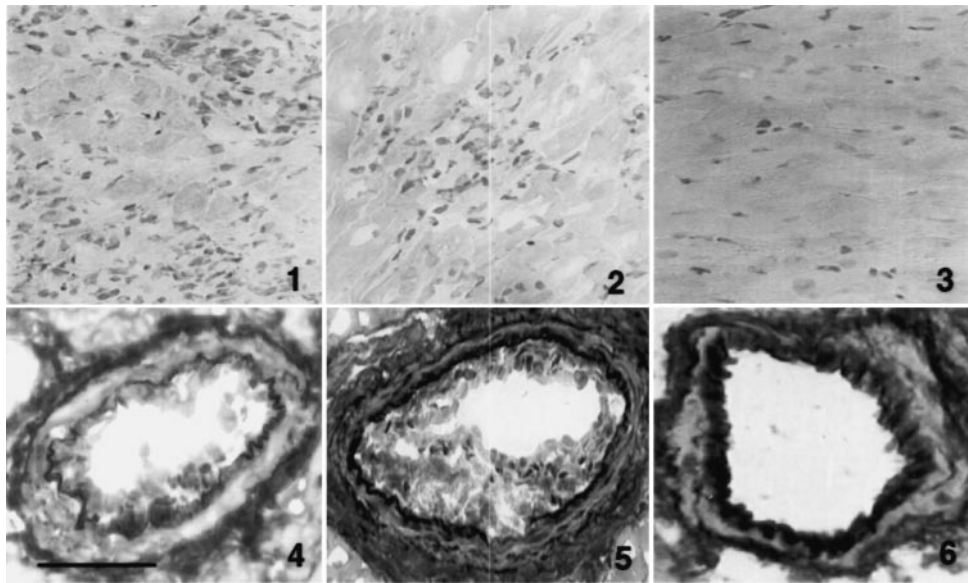
Figure 1 *Representative microscopical findings of allografts of the minor mismatch group at day 28. Upper panels show myocardium stained with HE, and lower panels are coronary arteries stained with EvG to demonstrate intimal thickening. Nontransfected allografts showed massive myocardial cell infiltration (1) and severe progression of intimal thickening (4). Scramble decoy treatment did not attenuate cell infiltration (2) and the neointimal formation (5). NFκB decoy transfection was a effective treatment resulting in not only less myocardial cell infiltration (3) but also suppression of intimal thickening (6). (Scale bar = 50 μm)*
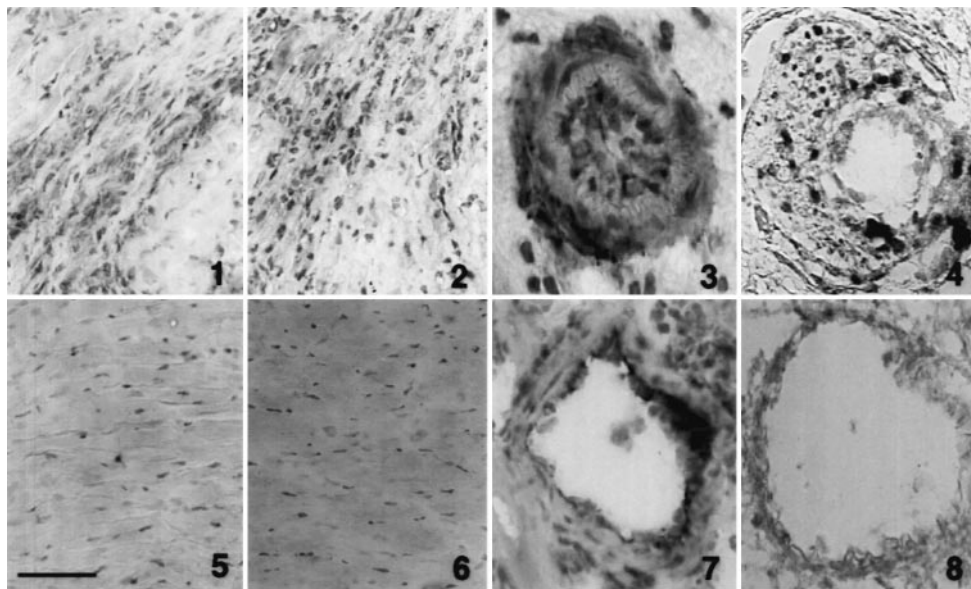
Figure 2 *Representative microscopical findings of allografts of the minor mismatch group at day 28. Upper panels show allografts from nontreated mice; lower panels are from those transfected with NFκB decoy. Panels 1 and 5 show ICAM-1 expression, 2 and 6 show MHC class I expression using immunohistochemistry. ICAM-1 and MHC class I were strongly and diffusely expressed in the myocardial interstitium of allografts from untreated recipients, while expression was weak in allografts transfected with NFκB decoy. Panels 3 and 7 show VCAM-1 expression, 4 and 8 show PDGF-B mRNA expression in graft coronary arteries. They were strongly expressed in the nontreated thickened intima, while expression was weak in the allografts with NFκB decoy. (Scale bar = 50 μm).*

tory effect of NFκB decoy on acute rejection is supported by several points of evidence. First, this study has documented that NFκB decoy selectively and directly inhibits the expression of targeted multiple factors, including MHC and adhesion molecules,[11] which are crucial for antigen presentation in transplanted organs. Second, administration of NFκB decoy markedly inhibits myocardial cell infiltration, which may be regulated by the inflammatory network which includes cytokines and chemokines.[7] In the latest studies, NFκB decoy attenuates IL-8 which is a polymorphonuclear neutrophil chemotaxin,[25] and *in vivo* administration of NFκB decoy reduces monocyte infiltration and VCAM-1 expression in a rat renal transplant model.[26] Therefore, NFκB is one of the key regulators promoting acute rejection; the decoy strategy against NFκB may provide

Table 2 Degree of intimal thickening and expression scores in the allograft arteries

Treatment of allograft	No. of grafts	No. of arteries	Intimal thickening	Immunohistochemistry			In situ RT-PCR		
				ICAM-1	VCAM-1	NFκB	PDGF-B	cdk2 kinase	cdc2 kinase
No ODN	8	24	60.0 ± 22.8	2.3 ± 0.8	1.8 ± 0.4	2.2 ± 1.1	2.3 ± 0.8	2.3 ± 0.8	2.3 ± 0.8
Scrambled decoy	8	24	50.6 ± 31.8	2.2 ± 0.8	1.8 ± 0.4	2.0 ± 1.0	2.2 ± 0.8	2.0 ± 0.9	2.0 ± 0.8
NFκB decoy	8	24	20.8 ± 17.5*	0.7 ± 0.8*	1.2 ± 0.4*	0.3 ± 0.8*	1.2 ± 0.4*	1.2 ± 0.4*	1.2 ± 0.4*

*$P < 0.05$ *versus* other groups.
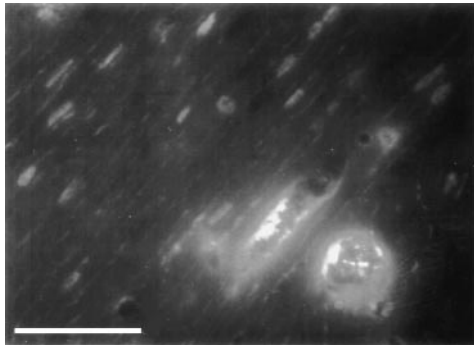
Figure 3 Representative fluorescence microscopical result of ex vivo transfection of FITC-labeled decoy; transfer of FITC decoy using the HVJ-AVE–liposome method resulted in widespread distribution of fluorescence in vascular and myocardial cells of murine allografts at day 14. (Scale bar = 50 μm).
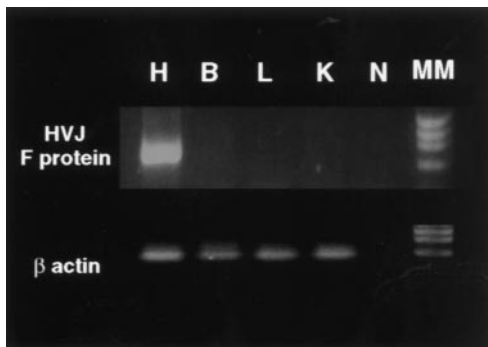
Figure 4 Representative results of RT-PCR showing HVJ F protein mRNA. A single band corresponding to the HVJ F protein mRNA was seen in non-inactivated HVJ, there were no bands in the organs of murine recipients. β Actin was used as an internal control. H, inactivated HVJ with murine liver; B, brain; L, liver; K, kidney; N, amplification without RNA; MM, molecular marker.

a new and specific therapeutic modality in the treatment of acute rejection.

It is noteworthy that NFκB decoy not only attenuates myocardial cell infiltration but also inhibits arterial neointimal formation in cardiac allografts. Several therapeutic trials have been undertaken to attenuate this arteriopathy but without wide-spread use.[27,28] In this study, we demonstrated that NFκB decoy inhibits intimal hyperplasia effectively; the prevention of neointimal formation was associated with suppressed expression of adhesion molecules which are directly affected by NFκB decoy; it can be deduced that NFκB must play an important role in the SMC proliferation. Suppressed cell cycle regulatory genes, growth factors and NFκB expression were also observed, and may be controlled indirectly by NFκB decoy throughout the cytokine network.

To confirm the infection resistance of UV-irradiated HVJ in organs after *ex vivo* transfection, we performed RT-PCR analysis of recipient organs for detection of HVJ mRNA. It is reported that UV-irradiation of 198 mJ/cm² HVJ greatly reduces infectivity while maintaining the fusion activity.[29] However, it remains to be elucidated whether liposomes fused with UV-irradiated HVJ cause dissemination of intact HVJ in the recipients. This study revealed that HVJ mRNA was not amplified in the organs of recipients, while bands corresponding to these proteins were seen in non-inactivated HVJ. This contrasts with other methodology, eg *in vivo* injection of genes using adenovirus, in which viral dissemination is revealed using RT-PCR.[30] These results further characterize the use of HVJ-AVE–liposome *ex vivo* transfer into transplant organs and suggest that clinical trials using this approach may be feasible.

Previous approaches that utilized gene transfer as a therapeutic agent *in vivo* were limited by short half-life, nonspecific toxicity at high doses and the inefficiency of cellular uptake.[31] The HVJ-AVE–liposome method increases the efficiency of cellular uptake of ODN without significant side-effects.[12,32] In this study, a single intraluminal *ex vivo* application of HVJ yields sustained ODN stability within allografts and attenuates myocardial infiltration and arterial neointimal formation. Intraluminal *ex vivo* delivery of ODN during the interval between donation and implantation is clinically feasible; thus it is a particularly attractive approach in cases of acute rejection and graft arteriopathy after cardiac transplantation.

In this report, we demonstrate that *ex vivo* transfection of NFκB decoy using HVJ-AVE–liposome attenuates both acute rejection and arterial neointimal formation by inhibition of multiple genes without complications. Further studies should be conducted in other models to explore the clinical utility of this technology for prevention of acute rejection and graft arteriopathy.

Materials and methods

DNA sequences and preparation of HVJ-AVE–liposomes

Egg yolk phosphatidylcholine, dioleoylphosphatidylethanolamine, egg yolk sphingomyelin, bovine brain phosphatidylserine and cholesterol were mixed at a molar ratio of 13.3:13.3:13.3:10:50 and liposomes were prepared

by shaking and sonication.[5,12] The NFκB decoy sequences were 5′-CCT TGA AGG GAT TTC CCT CC-3′ and 3′-GGA ACT TCC CTA AAG GGA GG-5′; the scrambled decoy sequences were 5′-TTG CCG TAC CTG ACT TAG CC-3′ and 3′-AAC GGC ATG GAC TGA ATC GG-5′.[6] Dried lipid was hydrated in 200 μl balanced salt solution (BSS) containing NFκB or scrambled decoy (15 μM). Purified HVJ (Z strain) was inactivated by ultraviolet irradiation (198 mJ/cm^2) for 3 min just before use. The liposome suspension (1 ml, containing 15 μM of lipids) was mixed with HVJ (30 000 U) in a total volume of 4 ml BSS. The mixture was incubated at 4°C for 5 min and then at 37°C for 30 min while gently shaking. Free HVJ was removed from the HVJ-AVE–liposomes using sucrose density gradient centrifugation.[12]

Murine cardiac transplantation and ex vivo gene transfer

The major mismatch combination male BALB/c (H-2^d) and C3H/He (H-2^k) mice (4–6 weeks, 20–25 g) was used as the major mismatch group for study of graft survival.[13] Minor mismatch combination male DBA/2 (H-2^d) and B10.D2 (H-2^d) mice (4–6 weeks, 20–25 g) was used as the minor mismatch group for pathological studies; the amount of intimal thickening development in this combination at 1 month has been seen in previous studies to resemble that in extended human chronic rejection.[14] C3H/He (H-2^k) mice hearts were transplanted into C3H/He (H-2^k) mice as the isografts. They were obtained from Japan Charles River Laboratories (Tokyo, Japan) and were anesthetized by intraperitoneal injection of 3.6% hydrochloride (0.2 ml/20 g mouse). Ischemic time averaged 60 min, and the overall success rate was greater than 90%. Donor hearts were infused via the aorta with HVJ-AVE–liposome complex and incubated for 10 min on ice. After transfection, donor hearts were immediately and heterotopically transplanted into recipients as described previously.[15,16] Graft survival was assessed by graft palpation. This investigation conforms with the Guide for the Care and Use of Laboratory Animals at Shinshu University.

Ex vivo transfection of FITC-labeled ODN

FITC-labeled phosphorothioate ODN was provided by Greiner Japan (Tokyo, Japan). FITC was anchored to the 3′ and 5′ ends of the double-stranded ODN using fluorescein-ODN phosphoramidite. Surgery and transfection of the FITC ODN (3 μM) was performed as described above. The grafts were transplanted immediately, harvested at days 14 and 28 after transfection and stored in Tissue-Tek optimum cutting temperature (OCT) compound (Sakura Finetechnical, Tokyo, Japan). Sections were cut (6 μm) and stained with erichrome black T solution and examined using fluorescence microscopy.[20,21]

Histological examination and morphometry

Isografts and allografts of the minor mismatch group were harvested at 28 days after transplantation for analysis of coronary arterial neointimal formation and myocardial cell infiltration. Grafts were sectioned transversely at the maximal circumference of the ventricle. As previously described, serial sections (6 μm) from tissue embedded in OCT were stained with hematoxylin and eosin (HE) and Elastica van Gieson (EvG) to highlight the internal elastic lamina (IEL). Myocardial cell infiltration was scored as: 0, no cell infiltration; 1, faint and limited cell

infiltration; 2, moderate cell infiltration; or 3, severe and diffuse cell infiltration.[7] The graft arteries were photographed, videodigitized and processed using an image analysis system (NIH Image). The area encompassed by the lumen and IEL was traced carefully, and the area of luminal stenosis in each cross section was calculated according to the formula: luminal occlusion = (IEL area – luminal area)/IEL area. Scores of two independent reviewers were averaged.

Immunohistochemistry

Serial sections (6 μm) were cut and dipped in cold acetone for 10 min. The sections were rehydrated in PBS and incubated with 5% normal goat serum to block nonspecific reactions. Sections were incubated with primary antibodies against murine intercellular adhesion molecule (ICAM)-1 (YN1/1.7), vascular cell adhesion molecule (VCAM)-1 (MK/2) (provided by Prof Ko Okumura, Juntendo University, Japan), MHC class I and class II (Funakoshi, Tokyo, Japan) and NFκB (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 12 h at 4°C.[14–16] Antibody–biotin conjugate was detected with Vectastain ABC Kit (Vector, Burlingame, CA, USA) used according to the manufacturer's instructions. Enzyme activity was detected with diaminobenzidine (0.5 mg/ml) with 0.05% NiCl in 50 mM Tris buffer, pH 7.5. Intensity of expression was scored as follows: 0, no visible staining; 1, few cells with faint staining; 2, moderate staining; or 3, intense diffuse staining.[16] The scores of two independent reviewers were averaged.

In situ RT-PCR

Serial sections (6 μm) were cut and dipped in cold acetone for 30 min, then in 4% buffered paraformaldehyde for 20 min. The sequences of PCR primers used in this study have been described previously.[5–7,17] RT solution contained PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$), 25 mM dNTP mix, 1 mM DTT, 0.05 pM antisense primer, 75 U/100 μl M-MLV reverse transcriptase, 40 U/100 μl RNase inhibitor (Perkin Elmer, Norwalk, CT, USA) and dH$_2$O. The RT solution was applied to each slide and incubated at 42°C for 15 min. The PCR solution contained PCR buffer, 1 mM DTT, 3 pM each of sense and antisense primers, 5 U of *Taq* DNA polymerase and 5 μl of 10× DIG DNA labeling mix (Boehringer Mannheim, Indianapolis, IN, USA). PCR amplification protocol was as follows: 94°C, 1 min; 55°C, 2 min; 72°C 1 min; 30 cycles. Digoxigenin-labeled cDNA segments were detected with enzyme-linked immunoassay using anti-digoxigenin-AP, Fab fragments; an enzyme-catalyzed color reaction with 5-bromo-4-chloro-3 indolyl phosphate (x-phosphate) and nitroblue tetrazolium salt (Boehringer Mannheim) produced an insoluble blue.[18,19] Scoring was as follows: 0, no visible staining; 1, few cells with faint staining; 2, moderate staining; and 3, intense diffuse staining.[7] Positive controls (no DNase digestion) and negative controls (no RT reaction) were also examined using serial sections.[16] The scores of two independent reviewers were averaged.

RT-PCR for detecting HVJ

The sequences of PCR primers were as follows: sense 5′ GTG ATT GGT ACT ATC GCA CTT ′3 antisense 5′ CTG GCT GTC AGG TAT CAG TTG 3′ for detecting HVJ F protein,[22] sense 5′ ACT TCT CCT AGT GGT AGC ACT

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3′; antisense 5′ CCG ATT GTC GTT GAT GTC ATA 3′ for detecting HVJ HN protein.[23] RNA was extracted from cardiac allografts, native hearts, brains, aortas, lungs, livers, kidneys, spleens, testes and skeletal muscles were also removed for studies at day 28 from HVJ-AVE–liposome complex transfected allograft recipients. RNA derived from organs was amplified using RT-PCR (35 cycles) and compared with positive (using RNA from non-inactivated HVJ and murine liver) and negative controls (primers without RNA). Amplification products were subjected to electrophoresis through 1.5% agarose gels stained with ethidium bromide.

Statistical analysis

All data are expressed as mean ± s.d. Scores were compared among the groups using a Scheffe's ANOVA. Differences with values of $P < 0.05$ were considered significant.

Acknowledgements

References

1 Hosenpud JD *et al*. The registry of the international society for heart and lung transplantation: sixteenth official report--1999. *J Heart Lung Transplant* 1999; **17**: 611–626.

2 Johnson DE *et al*. Transplant coronary artery disease: histopathologic correlations with angiographic morphology. *J Am Coll Cardiol* 1991; **17**: 449–457.

3 Suzuki J *et al*. Nonmuscle and smooth muscle myosin heavy chain expression in rejected cardiac allograft. A study in rat and monkey models. *Circulation* 1996; **96**: 1118–1124.

4 Hosenpud JD. Immune mechanism of cardiac allograft vasculopathy: an update. *Transplant Immunol* 1993; **1**: 237–249.

5 Morishita R *et al*. Intimal hyperplasia after vascular injury is inhibited by antisense cdk2 kinase oligonucleotides. *J Clin Invest* 1994; **93**: 1458–1464.

6 Morishita R *et al*. A gene strategy using a transcription factor decoy of the E2F binding site inhibits smooth muscle proliferation *in vivo*. *Proc Natl Acad Sci USA* 1995; **92**: 5855–5859.

7 Suzuki J *et al*. Prevention of graft coronary arteriosclerosis by antisense cdk2 kinase oligonucleotide. *Nature Med* 1997; **3**: 900–903.

8 May MJ, Ghosh S. Signal transduction through NF-kappa B. *Immunol Today* 1998; **19**: 80–88.

9 Sha WC. Regulation of immune responses by NF-kappa B/Rel transcription factor. *J Exp Med* 1998; **187**: 143–146.

10 Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *New Engl J Med* 1997; **336**: 1066–1071.

11 Morishita R *et al*. *In vivo* transfection of cis element 'decoy' against nuclear factor-κB binding site prevents myocardial infarction. *Nature Med* 1997; **3**: 894–899.

12 Saeki Y *et al*. Development and characterization of cationic liposomes conjugated with HVJ (Sendai virus); reciprocal effect of cationic lipid for *in vitro* and *in vivo* gene transfer. *Hum Gene Ther* 1997; **8**: 2133–2141.

13 Isobe M, Yagita H, Okumura K, Ihara A. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. *Science* 1992; **255**: 1125–1127.

14 Furukawa Y, Matsumori A, Hirozane T, Sasayama S. Angiotensin II receptor antagonist TCV-116 reduces graft coronary artery disease and preserves graft status in a murine model. A comparative study with captopril. *Circulation* 1996; **93**: 333–339.

15 Isobe M *et al*. Immunosuppression to cardiac allografts and soluble antigens by anti-vascular cellular adhesion molecule-1 and anti-very late antigen-4 monoclonal antibodies. *J Immunol* 1994; **153**: 5810–5818.

16 Suzuki J *et al*. Inhibition of accelerated coronary atherosclerosis with short-term blockade of ICAM-1 and LFA-1 in a heterotopic murine model of cardiac transplantation. *J Heart Lung Transplant* 1997; **16**: 1141–1148.

17 Akai Y *et al*. Intraglomerular expressions of IL-1 alpha and platelet-derived growth factor (PDGF-B) mRNA in experimental immune complex-mediated glomerulonephritis. *Clin Exp Immunol* 1994; **95**: 29–34.

18 Patel GV *et al*. Detection of epidermal growth factor receptor mRNA in tissue sections from biopsy specimens using *in situ* polymerase chain reaction. *Am J Pathol* 1994; **144**: 7–14.

19 Nuovo GJ, Gallery F, MacConnell P, Braun A. *In situ* detection of polymerase chain reaction-amplified HIV-1 nucleic acid and tumor necrosis factor-alpha RNA in the central nervous system. *Am J Pathol* 1994; **144**: 659–666.

20 Sawa Y *et al*. Efficiency of *in vivo* gene transfection into transplanted rat heart by coronary infusion of HVJ liposome. *Circulation* 1995; **92** (Suppl. II): 479–482.

21 Morishita R *et al*. Pharmacokinetics of antisense oligodeoxyribonucleotides (cyclin B1 and CDC2 kinase) in the vessel wall *in vivo*: enhanced therapeutic utility for restenosis by HVJ-liposome delivery. *Gene* 1994; **149**: 13–19.

22 Miura N. Okada Y. Use of the deoxyinosine-containing probe to isolate and sequence cDNA encoding the fusion (F) glycoprotein of Sendai virus (HVJ). *Gene* 1985; **38**: 271–274.

23 Miura N *et al*. Molecular cloning of a full-length cDNA encoding the hemagglutinin-neuraminidase glycoprotein of Sendai virus. *FEBS Lett* 1985; **188**: 2789–2793.

24 Lenardo MJ, Fan CM, Maniatis T, Baltimore D. The involvement of NF-kappa B in beta-interferon gene regulation reveals its role as widely inducible mediator of signal transduction. *Cell* 1989; **57**: 287–294.

25 Cooper JA Jr *et al*. Attenuation of interleukin-8 production by inhibiting nuclear factor-kappaB translocation using decoy oligonucleotides. *Biochem Pharmacol* 2000; **59**: 605–613.

26 Vos IH *et al*. NFkappaB decoy oligodeoxynucleotides reduce monocyte infiltration in renal allografts. *FASEB J* 2000; **14**: 815–822.

27 Uretsky BF *et al*. Development of coronary artery disease in cardiac transplanted patients receiving immunosuppressive therapy with cyclosporine and predonisolone. *Circulation* 1987; **76**: 827–834.

28 Pollak R, Fabrega AJ. Diltazem in the prevention of coronary artery disease in heart-transplant recipients. *New Engl J Med* 1993; **328**: 1851–1852.

29 Okada Y, Tadokoro J. Analysis of giant polynuclear cell formation caused by HVJ virus from Ehrlich ascites tumor cells. *Exp Cell Res* 1962; **26**: 106–118.

30 Hwang HC *et al*. Gene therapy using adenovirus carrying the herpes simplex-thymidine kinase gene to treat *in vivo* models of human malignant mesothelioma and lung cancer. *Am J Resp Cell Mol Biol* 1995; **13**: 7–16.

31 Morishita R *et al*. Novel strategy of gene therapy in cardiovascular disease with HVJ-liposome method. In: Koide H, Ichikawa I (eds). *Progression of Chronic Renal Diseases. Contrib Nephrol.* Karger: Basel, 1996; **118**: 254–264.

32 Okada Y *et al*. Modification of cell membranes with viral envelopes during fusion of cells with HVJ (Sendai virus). *Exp Cell Res* 1975; **93**: 368–378.