cis Element Decoy Against Nuclear Factor-κB
Attenuates Development of Experimental Autoimmune
Myocarditis in Rats

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Abstract—Nuclear factor-κB (NFκB) plays a significant role in the coordinated transactivation of cytokine, inducible NO synthase (iNOS), and adhesion molecule genes. Although inflammation is an essential pathological feature of myocarditis, the role of NFκB in this process remains obscure. We examined the role of NFκB in the progression of rat experimental autoimmune myocarditis (EAM) and tested the hypothesis that NFκB blockade with a decoy against the cis element of NFκB can prevent the progression of EAM. Lewis rats were immunized with purified porcine cardiac myosin to establish EAM on day 0. NFκB decoy was infused into the rat coronary artery on day 0 (group NF0), 7 (group NF7), or 14 (group NF14) and harvested on day 21. Scrambled decoy was infused on day 0 (group SD0), 7 (group SD7), or 14 (group SD14) and served for control groups. The ratios of myocarditis-affected areas to the ventricular cross-sectional area of all treatment groups were significantly lower than those of the control groups (group NF0, 33±18% versus SD0, 53±14%; group NF7, 19±15% versus SD7, 50±16%; and group NF14, 34±10% versus SD14, 52±14%). Immunohistochemical and immunoblot analyses showed expression of ICAM-1, iNOS, IL-2, and TNFα in myocardium of scrambled decoy groups, and this expression was effectively suppressed by NFκB decoy treatment. Thus, we found that NFκB is a key regulator in the progression of EAM and that in vivo transfection of NFκB decoy reduces the severity of EAM. (Circ Res. 2001;89:000-000.)

Key Words: myocarditis ■ gene therapy ■ nuclear factor-κB

Myocardial damage induced by acute myocarditis is a major cause of dilated cardiomyopathy.1 The etiology of myocarditis is unclear. Autoimmunity, however, is known to play a role in its development.2–5 Although this condition is sometimes fatal, methods for treating myocarditis in humans have not yet been developed. Experimental autoimmune myocarditis (EAM) in rats is an autoimmune inflammatory cardiac disorder that is an animal model for human giant cell myocarditis.4–6 The therapeutic efficaciousness of various immunosuppressive agents have been reported in the treatment of human giant cell myocarditis7 and EAM in rats.8–12 The mechanisms underlying the effectiveness of such drugs are unknown but are believed to be associated with suppression of the T cell–mediated immune response.6 In addition, acute inflammatory processes are involved in this response. Several cytokines, nitric oxide (NO), and adhesion molecules such as intercellular adhesion molecule (ICAM)-1 have been recognized as important factors in the pathogenesis and pathophysiology of EAM.5,13 In addition, NO produced by inducible NO synthase (iNOS) in response to cytokines plays a role in the pathological process, because it is cytotoxic when present at high concentrations.11

Nuclear factor-κB (NFκB), a transcription factor, is involved in the coordinated transcription of genes encoding cytokines, iNOS, and adhesion molecules.14,15 Therefore, blockade of NFκB activation may inhibit expression of essential cytokines, iNOS, and adhesion molecules.16,17 Many stimuli activate NFκB, including cytokines, activators of protein kinase C, viruses, and oxidants.15 Some inflammatory cytokines, including tumor necrotic factor α (TNFα) and interleukin 1β, released from macrophages in the process of development of EAM13 may activate NFκB.

A decoy against the cis element of NFκB blocks binding of NFκB to the promoters of target genes and reduces the extent of myocardial infarction by suppressing expression of the target genes.17 In the present study, we used transfection of
NFκB decoy into EAM rat myocardium by the hemagglutinating virus of Japan (HVJ)–artificial viral envelope (AVE)–liposome method to examine whether NFκB plays a significant role in the inflammatory process of EAM and whether blocking NFκB binding can attenuate the progression of EAM.

Materials and Methods

Animals

Adult male Lewis rats (10 weeks old; body weight 250 to 300 g) were obtained from Japan Charles River Laboratories (Tokyo, Japan). They were housed under conventional conditions, fed water and a standard diet, and were maintained in compliance with animal welfare regulations of the Institute of Experimental Animal, Shinshu University School of Medicine.

Antigen and Immunization

Purified porcine cardiac myosin (Sigma, St Louis, Mo) was dissolved in 0.01 mol/L phosphate buffered saline (PBS) at a concentration of 10 mg/mL and emulsified with an equal volume of complete Freund’s adjuvant (Difco) supplemented with Mycobacterium tuberculosis H37RA (Difco, Detroit, Mich) at a concentration of 10 mg/mL. On day 0, rats were injected subcutaneously in footpads with 0.2 mL of emulsion, yielding an immunizing dose of 1.0 mg/body of the cardiac myosin for each rat.

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 13.3:13.3:10:50. Purified HVJ (Z strain) was inactivated by ultraviolet irradiation (110 erg/mm²/second) for 3 minutes just before the addition of the labeled probe. As a control, EAM samples were incubated with an excess (10X×, 100X×, 1000X×) of the unlabeled NFκB decoy ODN or with scrambled sequence ODN. One sample of EAM was preincubated with 3 μg of anti-NFκB p65 antibody (Santa Cruz Biotechnology) before incubation with binding mixture. Gels were analyzed by autoradiography.

In Vivo Gene Transfer

The method of in vivo gene transfer has been previously described.17 Fluorescein isothiocyanate (FITC)–labeled phosphorothioate ODN was provided by Greiner Japan. FITC was anchored to the 3′ and 5′ ends of the double-stranded ODN using fluorescein-ODN phosphoramidite. Rats were anesthetized with 3.6% chloral hydrate IP at a dose of 1 mL/100 g, and the right common carotid artery was surgically exposed. For infusion of HVJ-liposome complex with ODNs (2 mL), polyethylene cannula (PE50) was introduced into the common carotid artery, passed as far as the aortic valve via the ascending aorta, and positioned at the aortic sinus of Valsalva. To confirm the transfection, we introduced FITC-labeled ODNs against NFκB and scrambled decoy ODNs (3 μmol/L) into the normal and EAM rat myocardium. HVJ-liposome complex containing NFκB decoy ODNs (15 μmol/L) were administered on day 0 (group NF0, n=8), day 7 (group NF7, n=8), or day 14 (group NF14, n=8) after emulsion injection. Scrambled decoy ODNs (15 μmol/L) were transfected on day 0 (group SD0, n=6), day 7 (group SD7, n=6), or day 14 (group SD14, n=6) and served for controls. Twelve rats received no transfection (group UT). After infusion, the infusion cannula was removed, and the wound was closed. Four rats received no immunization and were euthanized seven days after sham-operation for a negative control (group N).

Histologic Examination and Morphometric Analysis

FITC-labeled ODN-transfected rat hearts were harvested 1 hour after transfection, and the sections were stored in Tissue-Tek optimum cutting temperature (OCT) compound (Sakura Finetechnical). Sections were cut (6-μm thick), stained with erichrome black T solution, and examined with fluorescence microscopy.20,21 All rat hearts transfected with NFκB decoy and scrambled decoy were harvested on day 21. In group UT, hearts were harvested on days 10 (n=3), 14 (n=3), and 21 (n=8). Hearts were removed immediately after death and cut into 2-mm thick slices. We obtained 5 transverse sections per heart. Aper midventricular, and basal level slices were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin (HE). The remaining sections were snap-frozen in Tissue-Tek OCT compound. The extent to which the myocardial and nonmyocardial regions were affected by myocarditis (consisting of inflammatory cell infiltration and myocardial necrosis) was determined with HE and a computer assisted analyzer (NIH Image, version 1.56). The area ratio (percentage affected/entire area) was calculated as previously described.12,13 Values for the three ventricular regions were averaged for each heart, and the mean percent of affected area in each treatment group and the control group was calculated in the hearts that were harvested on day 21. Data were obtained blindly by two independent observers and were averaged.

Immunohistochemistry

Serial sections (6 μm) were cut and dipped in cold acetone for 10 minutes. To reduce nonspecific reactivity, sections were preincubated with 0.3% hydrogen peroxide and normal rabbit serum.

Gel Shift Assay

Nuclear extracts were prepared from the myocardium of EAM and sham-operated rats. Rat hearts were homogenized with a Potter-Elvehjem homogenizer in 4 volumes of ice-cold homogenization buffer (10 μmol/L HEPES [pH 7.9], 0.5 mmol/L phenylmethylsulfonyl fluoride [PMSF], 1.5 mmol/L MgCl₂, 10 mmol/L KCl, and 0.5 mmol/L dithiothreitol [DTT]). After centrifugation at 15 000g for 15 minutes at 4°C, each pellet was dissolved in 1 volume of ice-cold homogenization buffer containing 0.5 mol/L PMSF, 20 mmol/L HEPES (pH 7.9), 0.5 mmol/L DTT, 0.2 mmol/L EDTA, 0.1 mol/L KCl, 20% glycerol) within dialysis tubing (GIBCO BRL). After centrifugation at 15 000g for 20 minutes at 4°C, the supernatant was stored at −70°C. An NFκB primer was labeled at the 3′ end by a 3′-end-labeling kit (Clontech).32P-labeled ODNs were then purified by application to a nick column (Pharmacia). Binding mixtures (10 μL) including 32P-labeled primer (0.5 to 1 ng, 10 000 to 15 000 cpm) and 1 mg polydeoxyinosinic-deoxyctydic acid (Sigma) were incubated with 10 mg nuclear extract for 30 minutes at room temperature and then loaded onto a 5% polyacrylamide gel. The gels were subjected to electrophoresis, dried, and incubated with parallel samples 10 minutes before the addition of the labeled probe. As a control, EAM samples were incubated with an excess (10X×, 100X×, 1000X×) of the unlabeled NFκB decoy ODN or with scrambled sequence ODN. One sample of EAM was preincubated with 3 μg of anti-NFκB p65 antibody (Santa Cruz Biotechnology) before incubation with binding mixture. Gels were analyzed by autoradiography.

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Sections were incubated with primary antibody 1A29, a mouse monoclonal antibody to rat ICAM-1, for 12 hours at 4°C. A rabbit polyclonal antibody to iNOS (BIOMOL Research Laboratories, Plymouth, Penn), a goat polyclonal antibody to tumor necrotic factor α (TNFα, Genzyme/TECHNE, Cambridge, Mass), and a goat monoclonal antibody to Interleukin-2 (IL-2, Genzyme/TECHNE) were also used as primary antibodies. Antibody–biotin conjugate was detected with a Vectastain ABC Kit (Vector) according to the manufacturer’s instructions. Enzyme activity was detected with diaminobenzidine (0.5 mg/mL) plus 0.05% NiCl in 50 mmol/L Tris buffer, pH 7.5. Native rat hearts were used as controls (n = 3).

Western Blot Analysis

Left ventricular myocardial samples from EAM rats were lysed and homogenized in two ways. For IL-2 and TNFα immunoprecipitation, cardiac tissue was homogenized in 9 volumes of 1.15% KCl solution at 4°C. The homogenate was centrifuged at 1000g for 10 minutes at 4°C to extract nuclei. Nuclei were resuspended in 1.2 volumes of an extraction solution consisting of 10 mmol/L HEPES (pH 7.9), 0.4 mol/L NaCl, 1.5 mmol/L MgCl2, 0.1 mmol/L EGTA, 0.5 mmol/L DTT, 5% glycerol, and the following protease inhibitors: 5 mmol/L leupeptin, 1.5 mmol/L aprotinin, and 2 mmol/L PMSF. For ICAM-1 and iNOS immunoprecipitation, another homogenization buffer was applied that contained 10 mol/mL HEPES buffer, pH 7.4, 320 mmol/mL sucrose, 1 mmol/mL EDTA, 1 mmol/mL DTT, 10 μg/mL leupeptin, and 2 μg/mL aprotinin at 0 to 4°C, and tissues were lysed with a polytron homogenizer. Homogenates were centrifuged at 1000g for 5 minutes at 4°C, and the resulting supernatants were used as a postnuclear fraction. Tissue extracts containing 30 μg of total protein were separated by sodium dodecyl sulfate (SDS)–polyacrylamide gels (6% for ICAM-1 and iNOS, 12.5% for IL-2 and TNFα) and transferred to nitrocellulose membranes (Bio-Rad Laboratories). After being blocking for 1 hour (20% milk, 0.1% Tween 20), membranes were incubated with primary antibodies overnight at room temperature. The membranes were washed in PBS and incubated for 2 hours with secondary antibodies (sheep anti-mouse IgG, donkey anti-rabbit IgG [Amersham Corp Buckinghamshire, England] or rabbit anti-goat IgG [Kirkegaard & Perry Laboratories, Gaithersburg, MD], respectively) and conjugated to horseradish peroxidase. The membranes were washed, developed with ECL reagent (Amersham), and exposed to Kodak X-AR film (Eastman Kodak).

Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Levels of ICAM-1, iNOS, and TNFa were detected by RT-PCR.24 Total RNA was extracted from the PBS-perfused, snap-frozen rat cardiac muscles using RNAzol (Tel-Test), cDNA was synthesized using a SuperScript first-strand synthesis system for RT-PCR (Life Technologies). Glycerinaldehyde-3-phosphate dehydrogenase (GAPDH) expression was used as an unregulated control and amplified using primers: GAPDHF, 5′-TGGAGGGAGCTACTGA-3′ and GAPDHR, 5′-TCCCCACCTTTGGTGTA-3′. iNOS, ICAM-1, and TNFa cDNA were amplified by specific primer pairs: iNOSF, 5′-CAGGTGCACACAGGCTACT-3′ and iNOSR, 5′-CTTCACCTCATACTCAATCC-3′; ICAMF, 5′-CTATCGGGATGGTGAAGTCT-3′ and ICAMR, 5′-CITCTGGCCGTATATAGGTGA-3′; TNFaF, 5′-ACTACGTGCTCCCTCACCCA-3′ and TNFaR 5′-GTACTTTGGCCAGGTACC-3′. RNA Amplification Kit SYBR Green I was used in all PCR reactions that were performed in a real-time LightCycler system (Roche Molecular Biochemicals). The level of specific mRNA was quantified and expressed as the cycle number at which the LightCycler system detected the upstroke of the exponential phase of PCR product accumulation and normalized by the level of GAPDH expression in each individual sample.

Statistical Analysis

Values are expressed mean ± SD. Results were compared among the groups using a Scheffe’s ANOVA. A value of P < 0.05 was considered statistically significant.

Results

Gel Shift Assay

The binding affinity of NFκB was increased markedly in EAM rat myocardium compared with that in myocardium from sham-operated hearts. Moreover, competition for increased binding of NFκB was observed by an excess amount of NFκB decoy ODN but not of scrambled decoy ODN (Figure 1).

In Vivo Transfection of FITC-Labeled ODNs Into Rat Hearts

Transfection of FITC-labeled ODNs via infusion into the rat aortic Valsalva sinus resulted in diffusely distributed fluorescence in transfected hearts (Figures 2A and 2B). Similar results were observed in EAM rat hearts (Figure 2C). Fluorescence was localized mainly in the nuclei of cardiac myocytes and microvascular endothelial cells. These results are consistent with those of our previous study.25

Figure 1. Gel shift assay of NFκB binding site. The binding affinity of NFκB is markedly greater in experimental autoimmune myocarditis (EAM) rat myocardium than in sham-operated heart. Anti-NFκB caused a supershift (arrow). Complete competition for the increased binding of NFκB by excess NFκB decoy ODN (ND) but not by scrambled decoy ODN (SD) was observed.
Expression of Cytokines and Adhesion Molecules

Immunohistochemical analysis of rat hearts in group UT and group SD revealed enhanced expression of IL-2, TNFα, ICAM-1, and iNOS compared with that in normal rat hearts. On day 10, ICAM-1 and IL-2 were detected in EAM myocardium before the appearance of mononuclear cell infiltration. ICAM-1 expression was enhanced in vascular endothelial cells (Figure 3A), and IL-2 was detected in interstitial cells (Figure 3C). On day 10, expression of ICAM-1 (Figure 3B) and IL-2 (Figure 3D) in group NF0 rats was lower than that in group SD0 rats.

In control EAM rats, ICAM-1 immunoreactivity was present in cardiac myocytes and interstitial cells on days 14 and 21 (Figure 4A), and iNOS and TNFα were not detected until day 14. TNFα labeling was observed in interstitial cells (Figure 4E), and iNOS was detected in infiltrating large mononuclear cells in the area of myocardial necrosis (Figure 4G). Expression of these factors was reduced in parallel with the attenuation of myocardial damage in group NF7 rats (Figures 4B, 4D, 4F, and 4H).

Immunoblot analysis of group NF7 and group SD7 on day 21 is shown in Figure 5. In these groups, NFκB decoy effectively suppressed ICAM-1, IL-2, TNFα, and iNOS in EAM hearts. Bands were barely detectable in samples from the treatment groups and the sham-operated rats, whereas bands were easily detected in samples from group SD7.

Real-time quantitative RT-PCR analysis showed that NFκB decoy suppressed expression of ICAM-1, iNOS, and TNFα mRNAs in group NF7 (Figure 6).

Clinical Effects on EAM in Rats

Rats in group UT and group SD became ill, had ruffled fur, and were immobile after the second week. Rats in the treatment group, however, remained active and appeared healthy until the experiment was terminated. No EAM rats in the study died spontaneously.

The ratios of heart weight to body weight in all NFκB decoy treatment groups were significantly lower than those in the scrambled ODN groups (group NF0 versus SD0, 0.64±0.11% versus 0.87±0.06%; group NF7 versus SD7, 0.43±0.09% versus 0.79±0.08%; and group NF14 versus SD14, 0.52±0.12% versus 0.81±0.11%). The ratio of groups NF7 and NF14 were significantly lower than that of group UT (0.83±0.16%). The ratio of group NF0 was also lower than that of UT, but the difference was not statistically significant (Figure 7A). The ratio of myocarditis-affected areas were significantly lower in all NFκB decoy-treated groups than those in the scrambled ODN group (group NF0 versus SD0, 33±18% versus 53±14%; group NF7 versus SD7, 19±15% versus 50±16%; and group NF14 versus SD14, 34±10% versus 52±14%). The ratios of groups NF0 and NF7 were significantly lower than that of group UT (49±21%). The ratio of group NF14 was also lower than that of UT, but the difference was not statistically significant (Figure 7B).

Severe myocardial lesions were visible in control group hearts (Figure 8A), whereas myocarditis was suppressed in the NFκB decoy–treated groups (Figure 8B). Histopathological findings revealed extensive cell infiltration and necrosis in the control groups (Figure 8C). Inflammatory cell infiltration consisted of mononuclear cells and polymorphonuclear neutrophils (group SDB, Figure 8E). Multinucleated giant cells were also observed frequently in the lesions (Figure 8F). In the hearts of NFκB decoy–treated rats, there was a significant reduction in the extent of inflammation, but infiltration of mononuclear and polymorphonuclear cells into the hearts was still present (Figure 8D).

Discussion

NFκB Is Involved in EAM

The EAM rat model resembles human giant cell myocarditis in its pathological features. Although the exact mechanism...
underlying myocyte injury in EAM is not fully understood, the disease process in EAM is thought to be initiated by autoreactive T cells and macrophages. NFκB is an ubiquitous, rapid-response transcription factor that regulates the expression of genes encoding cytokines, chemokines, growth factors, iNOS, and cell adhesion molecules. Activation of NFκB also regulates a number of genes necessary for normal T cell response. Therefore, NFκB may play a pivotal role in the regulation of myocardial damage in EAM. In the present study, we investigated the role of NFκB in the progression of EAM and found that the binding affinity of NFκB, as assessed by gel shift assay, was markedly increased in the EAM rat myocardium.

**NFκB Decoy Attenuates EAM**

Decoy cis elements block binding of various nuclear factors to the promoters of target genes, resulting in inhibition of gene expression. In the present study, gel shift assay with EAM rat heart samples showed complete competition for the increased NFκB binding by excess NFκB decoy ODNs but not by scrambled decoy ODNs. In vitro experiments documented that NFκB decoy ODN inhibits expression of cytokine and adhesion molecule genes. In the present study, we showed that in vivo transfection of NFκB decoy reduced the severity of EAM in rats, including those treated after the initiation of inflammation.

Blocking NFκB is thought to inhibit activation of T cells and expression of genes encoding many inflammatory factors regulated directly and indirectly by T cells in EAM. Many inflammatory factors, such as IL-2, IL-12, IL-1β, TNFα, and iNOS, are expressed in the inflammatory phase of EAM on approximately day 19 after immunization. Our immunohistochemical and immunoblot analyses showed that IL-2 and ICAM-1 were expressed in EAM myocardium before cell infiltration, whereas iNOS and TNFα were not expressed until the onset of myocarditis, and these inflammatory factors were suppressed in the decoy-treated groups.

The pathogenic mechanism of EAM in this animal model involves 3 sequential processes. The first step is activation of hibernating autoreactive T cells by a fragment of cardiac myosin. The second step is recruitment of activated T cells to the target organ. The third step appears to involve an effector-target interaction. Cell infiltration of the myocardium of EAM Lewis rats occurs approximately 14 days after the first immunization. Therefore, the fact that the intracoronary-transfected decoy ODN remain in the myocardium for 7 to 14 days suggests that the NFκB decoy attenuated the early response in group A in the present study. In addition, the decoy inhibited the late phase response in group C, with alleviation of myocardial damage that occurred after the inflammatory process began. However, the inhibitory effect

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**Figure 3.** Immunohistochemical findings in hearts from the control rats (group SD0) at day 10 (left column) and that in those from group NF0 at day 10 (right column). Panels A and B show ICAM-1 expression, and panels C and D show IL-2 expression. In group SD0, ICAM-1 is enhanced in vascular endothelial cells (A) and IL-2 is enhanced in interstitial cells (C). ICAM-1 and IL-2 expression on day 7 is lower in group NF0 than in group SD0 (B and D). Group SD0, the scrambled decoy were transfected on day 0. Group NF0, the NFκB decoy were transfected on day 0. Bar indicates 20 μm; all photographs are the same magnification.
on EAM was most remarkable in group B. The NFκB decoy was effective from well before EAM onset to after onset in this group. The possibility that the NFκB decoy ODN attenuates not only the effector phase of the inflammatory process but also the afferent response cannot be excluded, because IL-2 and IL-12 play major roles in the initial process of EAM through activation of T cell–mediated immune responses.28 NFκB may be involved in this process by activation of costimulator molecules such as ICAM-1.

**NFκB Decoy Treatment Is a Potent Strategy Against Acute Myocarditis**

Although the effectiveness of immunosuppressive agents and iNOS inhibitors on rat EAM has been reported,8-12 FK506 was the only agent reported to suppress inflammation of established EAM effectively.8 In the present study, we showed that NFκB decoy transfected after the onset of EAM effectively suppresses the inflammatory process. This finding has important clinical implications. If patients with giant cell myocarditis are treated during the active phase, inflammation

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**Figure 4.** Immunohistochemical findings in hearts from control rats (group SD7) at day 21 (left column) and in those from group NF7 at day 21 (right column). Panels A and B show ICAM-1 expression, C and D show IL-2 expression, E and F show TNFα expression, and G and H show iNOS expression. ICAM-1 immunoreactivity is also visible in cardiac myocytes and interstitial cells (A). IL-2, TNFα, and iNOS are expressed in infiltrating mononuclear cells and in interstitial cells (C, E, and G). In group NF7, expression of these factors is reduced in parallel with the attenuation of myocardial damage (B, D, F, and H). Group SD7, the scrambled decoy were transfected on day 7. Group NF7, the NFκB decoy were transfected on day 7. Bar indicates 20 μm; all photographs are the same magnification.
and subsequent myocardial necrosis might be satisfactorily suppressed.

Recently, a strategy for blocking NFκB activation was reported, and in vivo NFκB decoy transfection with HVJ-AVE-liposomes was used successfully to treat several experimental diseases, including angiopathy after subarachnoid hemorrhage, adenocarcinoma, and collagen-induced arthritis. In vivo gene transfection into the myocardium has been used to treat ischemia-reperfusion myocardial damage in animal models. We also found previously that this technology effectively suppresses acute rejection of murine cardiac allografts.

The HVJ-AVE-liposome method increases the efficiency of cellular uptake of ODN and is much less immunogenic and cytotoxic than other viral-vector systems. The efficiency of gene transfer in the technique we used in the present study is unclear. ODN injected into the aortic root enters the systemic circulation and is distributed to other organs. Decoy ODN may work systemically to suppress local inflammation, and this possibility must be evaluated in the future. Possible adverse effects of NFκB decoy treatment remain undetermined. Further studies are necessary to confirm the safety and to investigate the possible side effects associated with this treatment.

**Conclusions**

We demonstrated that in vivo transfection of NFκB decoy with HVJ-AVE-liposome attenuates the development of EAM. Transfection starting after the onset of cell infiltration was effective in reducing the extent of inflammation. Further
studies are needed to explore the clinical usefulness of this novel strategy for treatment of acute myocarditis.

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References


Figure 7. A, the ratios of heart weight to body weight in all NFκB decoy treatment groups were significantly lower than those in scrambled ODN group. The ratios of groups NF7 and NF14 were significantly lower than that of group UT. B, The ratios of myocarditis-affected areas were significantly lower in all NFκB decoy treatment groups than those in scrambled ODN group. The ratios of groups NF0 and NF7 were significantly lower than that of group UT. Groups SD0, SD7, and SD14, the scrambled decoy were transfected on day 0, 7, and 14, respectively. Groups NF0, NF7, and NF14, the NFκB decoy were transfected on day 0, 7, and 14, respectively. *P<0.05, **P<0.01.

Figure 8. Representative cross sections of hearts from control EAM rats (group SD7) (A) and the NFκB decoy-treated rats (group NF7) (B). The ratios of myocarditis-affected areas in panels A and B are 59% and 19%, respectively. Bar indicates 2.5 mm. C through F shows representative histopathologic findings of hearts from group SD7 and group NF7. C, Severe myocardial lesions are visible (group SD7). D, There was reduction in the extent of inflammation (group NF7). Bar indicates 250 μm. E, Lesions in the hearts of control rats are composed of inflammatory mononuclear cells and polymorphonuclear neutrophils (group SD7). Bar indicates 80 μm. F, Multinucleated giant cells (arrow) are visible in the lesions (group SD7). Bar indicates 20 μm.


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