Protection Against Autoimmune Myocarditis by Gene Transfer of Interleukin-10 by Electroporation

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Background—Although immunosuppressive therapy for myocarditis has attracted a great deal of attention, its effectiveness is controversial. Interleukin (IL)-10 has a variety of immunomodulatory properties. Among the nonviral techniques for gene transfer in vivo, the direct injection of plasmid DNA into muscle is simple, inexpensive, and safe.

Methods and Results—We examined the applicability of murine IL-10 (mIL-10) gene transfer to the treatment of rats with experimental autoimmune myocarditis. Nine-week-old Lewis rats were inoculated with pig myosin (day 0). A plasmid vector expressing mIL-10 cDNA (800 μg per rat) was transferred into the tibialis anterior muscles by electroporation 3 times (5 days before immunization and at days 4 and 13); control rats received empty plasmid. Electroporation increased the serum mIL-10 levels to >250 pg/mL. The 21-day survival rate in rats treated with mIL-10 cDNA was higher (15 of 15; 100%) than that of the control group (9 of 15; 60%). Furthermore, mIL-10 treatment significantly attenuated myocardial lesions and improved hemodynamic parameters.

Conclusions—These findings showed that gene transfer into muscle by electroporation in vivo is an effective means of delivery of IL-10 for the treatment of autoimmune myocarditis. (Circulation. 2001;104:1098-1100.)

Key Words: interleukins ■ myocarditis ■ gene therapy ■ immune system ■ cardiomyopathy

Human myocarditis can be classified into lymphocytic myocarditis and giant cell myocarditis according to histopathological findings. Giant cell myocarditis is a fatal disease, and survivors are more likely to develop dilated cardiomyopathy than patients with lymphocytic myocarditis.1,2 The efficiency of immunosuppressive therapy for this disease is controversial.1,2

In a rat model in which myocarditis was induced by purified cardiac myosin, T cells were reported to play an important role in inducing myocarditis.3–5 Immune dysfunction associated with autoimmune disease may be related to an imbalance between T helper type 1 and 2 cells.6 The T helper type 2–associated cytokine interleukin (IL)-10 has a variety of immunomodulatory properties, including the inhibition of T helper type 1 cells and the production of proinflammatory cytokines.7,8 Recent reports have suggested that the immunosuppressive effects associated with IL-10 are effective in suppressing the rejection of transplanted organs and immune complex diseases, and clinical trials of IL-10 have been performed in patients with these disorders.9,10

Electroporation has been widely used to introduce DNA into various types of cells in vitro. Gene transfer by electroporation in vivo has been effective for introducing DNA into mouse skin, chick embryos, rat liver, and murine melanoma and muscle.11–13 We previously showed that gene transfer into muscles by electroporation in vivo can be used to deliver cytokines systemically.13,14 In the present study, we applied this method for the delivery of IL-10 in a rat model of autoimmune myocarditis.

Methods

Animals

Nine-week-old male Lewis rats were injected with the antigen-adjuvant emulsion in their foot pads according to the procedure described previously.3–5 The morbidity of experimental autoimmune myocarditis was 100% in rats immunized by this method.3–5 Throughout the studies, all animals were treated in accordance with the guidelines for animal experiments of our institute.5

Construction of Mouse IL-10 Expression Vector

Mouse IL-10 (mIL-10) cDNA cloned by polymerase chain reaction was inserted into the unique Xho I site between the cytomegalovirus immediate early enhancer-chicken β-actin hybrid promoter and rabbit β-globin poly A site of the pCAGGS expression plasmid.13,14 The resulting plasmid, pCAGGS-IL-10, was grown in Escherichia coli DH 5α and prepared using plasmid purification columns (EndFree plasmid giga kit; Qagen). The plasmid DNA was dissolved in a buffer

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(10 mMol/L Tris-HCl and 1 mMol/L EDTA; pH 8.0). The purified plasmid DNA was stored at −20°C and diluted to 4 µg/µL with phosphate-buffered saline (pH 7.4) immediately before use.

Intramuscular DNA Injection and Electroporation

Rats were anesthetized with diethyl ether. Aliquots of 50 µL of plasmid DNA (pCAGGS-IL-10 or control pCAGGS) at 4 µg/µL in phosphate-buffered saline were injected 4 times (total amount of DNA was 800 µg per rat) into the bilateral tibialis anterior muscles using a disposable insulin syringe with a 27-gauge needle. A pair of electrode needles with a gap of 5 mm were inserted into the muscle to a depth of 5 mm to encompass the DNA injection sites, and electrical pulses were delivered 4 times at 100 V using an electrical pulse generator (Electro Porator CUY21; TR Tech).

Treatment Protocols

Protocol 1: Time Course of Changes in Serum mIL-10 Levels After Administration of mIL-10 Plasmid to Normal Rats

Single Administration of mIL-10 Plasmid

The mIL-10 expression plasmid pCAGGS-IL-10 was transferred once at a dose of 800 µg per rat into the tibialis anterior muscles of normal Lewis rats (n=5) by electroporation in vivo, and the serum mIL-10 levels were monitored. The time course of changes in serum mIL-10 levels was examined on days 0, 2, 4, 6, 8, 10, and 14 after transfer of pCAGGS-IL-10 using an ELISA kit (Endogen).

Administration of mIL-10 Plasmid 3 Times

The mIL-10 plasmid was administered 3 times to normal rats (n=5). After the first injection of mIL-10 plasmid, second and third injections were added on days 10 and 20. Serum mIL-10 levels were examined on days 6, 16, and 26.

Protocol 2: Preventive Effects of mIL-10 Plasmid Administration in Myocarditis

Lewis rats were inoculated with pig myosin (day 0). The results of protocol 1 indicated that the serum levels of mIL-10 were enhanced to >50 pg/mL until day 10 after mIL-10 plasmid administration. pCAGGS-IL-10 (at a dose of 800 µg per rat) was administered 3 times (5 days before immunization and on days 7 and 14) to rats (group IL-10; n=15), and controls received empty pCAGGS (group V; n=15). Lewis rats without any treatment were used as age-matched normal controls (group N; n=10).

Myocardial histopathology and hemodynamic parameters were examined on day 21 as described previously. Briefly, rats were anesthetized with 2% halothane in O2 during surgical procedures, and then the halothane concentration was reduced to 0.5% to minimize hemodynamic effects. Mean blood pressure, central venous pressure, peak left ventricular pressure, left ventricular end-diastolic pressure, and dP/dt were recorded as described previously. The heart weight was measured, and the ratio of heart weight to body weight (g/kg) was calculated. After embedding in paraffin, several transverse sections were cut from the mid-ventricle slice and stained with hematoxylin-eosin and Azan-Mallory.

Statistical Analysis

Data are presented as mean±SEM. Statistical analysis between the groups was performed by one-way ANOVA followed by Tukey’s method. Differences were considered significant at P<0.05.

Results

Protocol 1: Time Course of Changes in Serum mIL-10 Levels After Administration of mIL-10 Plasmid to Normal Rats

Single Administration of mIL-10 Plasmid

The levels of serum mIL-10 on days 2 (126±14 pg/mL), 4 (308±16 pg/mL), 6 (320±23 pg/mL), 8 (192±34 pg/mL), 10 (82±9 pg/mL), and 14 (35±8 pg/mL) were significantly higher (all P<0.01) than on day 0 (not detected). The level of mIL-10 peaked on day 6. These mIL-10 levels were >50 pg/mL until day 10 and were greater than the peak value of serum rat IL-10 during the natural course of progression of myocarditis in this model (42±4 pg/mL on day 27).

Administration of mIL-10 Plasmid 3 Times

The levels of serum mIL-10 on days 6, 16, and 26 were 332±46, 286±48, and 218±42 pg/mL, respectively.

Protocol 2: Preventive Effects of mIL-10 Plasmid Administration in Myocarditis

Clinical Course in Rats with Myocarditis

Rats in the myosin-immunized group became ill and immobile on day 14. The 21-day survival rates in groups IL-10 and N were higher (15 of 15 and 10 of 10; 100%) than that in group V (9 of 15; 60%). Six rats in group V died between days 19 and 21, and all hearts from these rats showed extensive myocarditis and massive pericardial effusion.

Body Weight, Heart Weight, and Serum Levels of mIL-10

Body weights did not differ between rats in groups IL-10 and V. Heart weight and the heart weight to body weight ratio were significantly greater in group V (1.62±0.05 g and 6.50±0.23 g/kg, respectively) than those in groups IL-10 (1.17±0.02 g and 4.80±0.10 g/kg; both P<0.01) and N (1.02±0.02 g and 2.45±0.06 g/kg; both P<0.01).

Although serum levels of mIL-10 in both groups V and N were below the level of detection, that in group IL-10 was 125±16 pg/mL on day 21.

Hemodynamic Parameters

Central venous pressure and left ventricular end-diastolic pressure were significantly higher and mean blood pressure,

![Graphs showing hemodynamic parameters](image-url)
left ventricular pressure, and +dP/dt were lower in group V than in group N (all \( P<0.01; \) Figure 1).

Central venous pressure was significantly lower and the mean blood pressure, left ventricular pressure, and −dP/dt were significantly higher in group IL-10 than group V. The left ventricular end-diastolic pressure and +dP/dt did not differ between groups IL-10 and V.

**Quantitative Analysis of Myocardial Inflammation**

Although massive pericardial effusion was observed in most of the surviving rats in group V on day 21, there was little effusion in group IL-10.

Figure 2 shows representative photographs of thin sections stained with hematoxylin-eosin and Azan-Mallory. The normal heart did not show inflammation, but hearts in group V rats showed massive inflammation (stained light blue, which indicates inflammation). The area of myocarditis in group IL-10 was smaller than that in group V (Figure 2).

**Discussion**

In the present study using a rat model of autoimmune myocarditis, we examined the effects of IL-10 on the prevention of myocarditis. We found that IL-10 reduced heart weight and myocardial inflammation and increased positive dP/dt.

Human dilated cardiomyopathy is thought to have a variety of causes. Cardiac myosin-induced autoimmune myocarditis, which is not exclusively related to viral infection, develops clinicopathologically to resemble dilated cardiomyopathy in the chronic phase. Thus, the present results provided some insight into the effectiveness of IL-10 treatment against not only myocarditis but also dilated cardiomyopathy after myocarditis.

Gene transfer by electroporation, which uses plasmid DNA as a vector, has several advantages over transfer using viral vectors. A large quantity of highly purified plasmid DNA can be obtained easily and inexpensively. Gene transfer can be repeated without apparent immunological responses to the DNA vector. There is less likelihood of recombination events with the cellular genome, eliminating the risk of insertional mutagenesis that is associated with the use of viral vectors.

The present study indicated that delivery of IL-10 expression plasmid DNA by electroporation provided marked cardioprotection in a rat model of autoimmune myocarditis and may help clarify the mechanisms of the protective effect of IL-10 when used for the treatment of myocarditis in humans.

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