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Immune Tolerance to Cardiac Myosin Induced by Anti-CD4 Monoclonal Antibody in Autoimmune Myocarditis Rats

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Autoimmune myocarditis is a T-cell-mediated autoimmune disease. CD4-positive T cells are believed to be the most important for the initiation and mediation of the disease. This study was aimed at evaluating whether anti-CD4 monoclonal antibody could induce immune tolerance to porcine cardiac myosin and whether the immune tolerance could protect rats with autoimmune myocarditis from myocardial injury. Lewis rats were immunized with porcine cardiac myosin to induce experimental autoimmune myocarditis. Immune tolerance was induced by injections of anti-CD4 monoclonal antibody on days -2, -1, 0, and 1. Results showed that cardiac function of antibody-treated rats was significantly increased compared with untreated rats 18 days postimmunization examined by transthoracic echocardiography. Typical cardiac histopathological changes were observed obviously in untreated group but not in antibody-treated group. Lymphocytes obtained from antibody-treated group had no proliferative response to porcine cardiac myosin examined by lymphocyte proliferation assay. Serological examination showed that rats immunized with cardiac myosin could produce high levels of anti-cardiac myosin antibody. The administration of anti-CD4 monoclonal antibody significantly prevented the increase of them. Serum levels of Th1 cytokines were significantly down-regulated by antibody administration, while the production of Th2 cytokines were up-regulated or unaffected evaluated by enzyme-linked immunosorbent assay. It concluded that immune tolerance to porcine cardiac myosin could be induced by anti-CD4 monoclonal antibody *in vivo*, and cardiac dysfunction and myocardial injury could be prevented by induction of immune tolerance.

KEY WORDS: anti-CD4 monoclonal antibody; myosin; immune tolerance; myocarditis.

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INTRODUCTION

Myocarditis is characterized by myocyte necrosis and degeneration with mononuclear cell infiltration and can lead to sudden death (1, 2). Patients with myocarditis may develop dilated cardiomyopathy, a major cause of morbidity and mortality among young adults (3). The pathogenesis of myocarditis remains to be defined. Increasing studies have shown that autoimmune reactions to heart antigens, particularly cardiac myosin, after viral infection contribute to the disease process (4, 5). Injection of porcine whole or rod cardiac myosin into susceptible strains of rats and mice has been shown to cause experimental autoimmune myocarditis (EAM) (6, 7), which resembles myocarditis in humans and can lead to dilated cardiomyopathy.

Because the pathogenesis of myocarditis remains unclear, treatment is not direct at the diseases themselves but instead of managing the symptoms and complications, such as congestive heart failure, cardiogenic shock, conduction abnormalities, and arrhythmia. Recently, because evidences suggest that autoimmune response plays important role in the development of myocarditis, immunomodulatory therapies are being taken more and more attention for the treatment of myocarditis. However, their effects are still controversial (8, 9).

CD4 molecule is a trans-membrane glycoprotein expressed on the surface of a subset of mature T helper lymphocytes that recognize antigenic peptides in the context of major histocompatibility complex (MHC) class II molecule. Binding CD4 to a nonpolymorphic region of MHC class II molecule is thought to both stabilize the T-cell receptor (TCR)–peptide–MHC class II complex and increase intracellular signal transduction of T cells. Specific immune tolerance can be induced in animals, given antigen under the treatment of anti-CD4 monoclonal antibody (McAb), a kind of immunomodulatory reagent. In this study, the effect of a nondepleting McAb on EAM was studied. We investigated whether

immune tolerance to porcine cardiac myosin could be induced by injection of McAb in rats, and whether induction of immune tolerance could protect rats with myosin-induced myocarditis from myocardial injury and cardiac dysfunction.

METHODS

Animals and Reagents

Inbred 6-week-old male Lewis rats weighting 170 ± 5 g were purchased from Beijing Vitalriver Laboratory Animal Inc. (Beijing, China). They were housed in Laboratory Animal Center with controlled temperature (22–26°C), humidity (50–60%), and lighting (12 h cycle), and given free access to standard feed and sterile water. All experiments involving rats were performed in accordance with the Guidelines for Animal Experiments of Medical College of Shandong University and were approved by the Institutional Authority for Laboratory Animal Care.

W3/25 (mouse IgG1, anti-rat CD4) was bought from Serotec Company (Oxford). This kind of monoclonal antibody can recognize the CD4 molecular on rat T cells and down-regulate $CD4^+$ cell function when administered *in vivo*.

Preparation of Cardiac Myosin

Porcine cardiac myosin was prepared from porcine heart mainly according to the methods established by Murakami *et al*. (10). Briefly, porcine heart tissues were minced with scissors and homogenized in 0.05 mol/L potassium phosphate buffer (pH = 6.8). Then the homogenized tissues were centrifuged and the pellet obtained was homogenized in 3 volumes of solutions containing 0.3 mol/L KCl, 0.1 mol/L KH_2PO_4, 0.05 mol/L K_2HPO_4, 0.01 mol/L EDTA, and 0.002 mol/L β-mercaptoethanol. Then the mixture was stirred for 2 h and centrifuged for 30 min at 12,000*g*. The supernatant was filtered and then precipitated with 9 volumes of cold water containing 0.001 mol/L EDTA. The pellet was dissolved in 0.05 mol/L Tris-HCl buffer (pH = 7.5) and centrifuged at 125,000 *g* for 2 h. The supernatant was precipitated with 14 volumes of cold water containing 0.001 mol/L EDTA. The pellet was suspended in 0.05 mol/L pyrophosphate buffer (pH = 7.5). Then undissolved materials were centrifuged out. Saturated ammonium sulfate solution was added and the fraction between 36 and 45% saturation was collected and dissolved in 0.15 mol/L potassium phosphate buffer (pH = 7.5). This was designed as crude myosin. Further purification was through chromatography on a column of DEAE Sephadex A-50 resin.

Induction of EAM and Immune Tolerance

All Lewis rats ($n = 24$) were divided into three groups at random: control group ($n = 8$), McAb-treated group ($n = 8$), and saline-treated group ($n = 8$). Porcine cardiac myosin was dissolved in 0.01 mol/L potassium phosphate buffer (pH = 7.4), containing 0.6 mol/L potassium chloride, at a concentration of 10.0 g/L. The protein was then mixed with an equal volume of Freund's complete adjuvant containing 10.0 g/L of heat-killed mycobacterium tuberculosis. A total volume of 0.2 mL of the emulsion was injected subcutaneously in each rear footpad of rats in McAb-treated group and saline-treated group on days 0 and 7, respectively. Rats in control group were given equal volume of potassium phosphate buffer in Freund's complete adjuvant in the same manner.

Immune tolerance was induced by injections of 4 mg/kg of McAb on days -2 (intravenous), -1, 0, 1 (intraperitoneal) in the McAb-treated group. McAb was replaced by physiological saline with the same volume in saline-treated group as a control.

Rat serum samples were obtained 18 days postimmunization after echocardiography examination and stored at –80°C. Myocardial samples were fixed in formalin as soon as they were removed from rats and were processed histopathological examination.

Echocardiography Examination

Eighteen days after the first immunization, transthoracic echocardiography was performed on animals using Agilent sonos 5500 echocardiograph with 11–13 L transducer. Intraperitoneal administration of pentobarbital sodium (20–35 mg/kg) was used for anesthesia. The M-mode echocardiogram was obtained along the short axis view of the left ventricular at the chordae tendineae level. Left ventricular end-diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), left ventricular diastolic interventricular septum thickness (IVS), and left ventricular posterior wall thickness (LVPW) were measured. Left ventricular fractional shortening (LVFS) was calculated as described previously (11).

Histopathology

The animals were sacrificed on day 18 after the first immunization. Hearts were removed from the rats, weighted, and sliced transversely and fixed in 10% formalin and then embedded in paraffin. Five micrometers transverse sections from the base of the hearts, at the midventricular level and near the apex were cut from the paraffin-embedded samples and stained with hematoxylin and eosin or Masson′s trichrome according to standard

procedures. All sections examined were blinded for the presence of myocarditis by light microscopy.

Macroscopic findings of the heart were classified into five grades as described by Matsui *et al*. (12) and Okura *et al*. (13): 0, no inflammation; 1, presence of a small discolored focus; 2, presence of multiple discolored foci; 3, diffuse discolored areas not exceeding a total of one-third of the cardiac surface; 4, diffuse discolored areas totaling more than one-third of the cardiac surface. Microscopic findings were expressed in terms of myocardial infiltration and fibrosis scores, and the severity of myocardial infiltration and the extent of fibrosis were graded as follows (14): 0 (no involvement), 1 (<25% involvement), 2 (25–50% involvement), 3 (50–75% involvement), 4 (>75% involvement).

Lymphocyte Proliferation Assay

Spleens were collected from rats in control group, saline-treated group, and McAb-treated group on sacrifice. Cell suspensions were prepared by passage through a 200-gauge stainless steel mesh. Lymphocytes were recovered by Ficoll-Hypaque. A total of 2×10^6 cells per mL were incubated in triplicates for 72 h in 0.2 mL of RPMI 1640 (Gibco, USA) containing 10% fetal bovine serum in 96-well, flat-bottomed, microtiter plates in the presence or absence of 10 μg/mL porcine cardiac myosin or 5 μg/mL Con A (Sigma, USA). Proliferation was measured by the incorporation of 0.5 μCi [^{3}H] thymidine into DNA during the final 12 h of incubation. The proliferation responses of lymphocytes to myosin or Con A were presented as counting per minute (cpm).

Anti-Cardiac Myosin Autoantibody Assay

Porcine cardiac myosin (10 μg/mL) in phosphate buffered saline (pH = 7.2) was coated in flat-bottom 96-well ELISA plates by overnight incubation at 4°C. After being washed with 0.05% PBS-Tween, and blocked with 2% BSA in PBS, sera were added in different dilutions (1:50, 1:100, 1:200, in PBS) and incubated for 1 h at room temperature. Alkaline-phosphatase-conjugated goat anti-rat IgG (Sigma, USA) was added and incubated for 1 h at room temperature, followed by four washings with PBS-Tween. After being extensively washed, 1 mg/mL *para*-nitrophenylphosphate (Sigma, USA) in 0.05 mol/L carbonate buffer containing 0.001 mol/L $MgCl_2$ (pH = 9.8) was added as a substrate. The reaction was stopped after 30 min by the addition of 1 mol/L NaOH. The optical density (OD) at 405 nm wavelength was read and each sample was analyzed in duplicate.

Cytokine Assay

Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum concentrations of interferon (IFN)-γ, interleukin (IL)-2, IL-6, and IL-10. The serum levels of these cytokines were determined with ELISA kits (Jingmei Biotech. Co., China) according to the manufacture′s instructions.

Statistical Analysis

All data were expressed as means ± standard deviation. Data from heart weight/body weight ratio, echocardiographic parameters, incorporation thymidine, serum titer of anti-cardiac myosin antibody, and cytokines were compared employing one-way ANOVA test followed by a Newman–Keuls *t* test. Macroscopic score and microscopic score were compared between groups employing nonparametric statistics. All data were analyzed using SPSS10.0 software (SPSS Institute, Inc.). Significance were considered at $P < 0.05$.

RESULTS

Changes of Body and Heart Weights

Changes of the body weight are shown in Fig. 1. In control group, the average body weight of the rats increased from 174 ± 4 g at the beginning to 258 ± 12 g at the end of the experiment, a gain of 84 g. In saline-treated group, body weight of the rats decreased from 171 ± 3 g at the beginning to 153 ± 9 g 18 days after immunization, a loss of 18 g. Weight gain was not significantly changed in McAb-treated group at 74 g (169–243) compared with control group. With regard to heart weight, there was a significant difference between saline-treated group and the other two groups. The mean heart weight was heaviest in saline-treated group and lighter in control group and McAb-treated group. When normalized to body weight at the time of sacrifice, there was a marked increase in saline-treated group compared with control group and McAb-treated group, and there was not significant difference between control group and McAb-treated group (Table I).

Prevention of Heart Failure and Ventricular Remodeling by McAb

Changes of heart structure and cardiac function of EAM rats and the effects of McAb on them were examined using echocardiography 18 days after the first immunization (Table II, Fig. 2). All rats in saline-treated group showed massive pericardial effusion. Both IVS and LVPW were

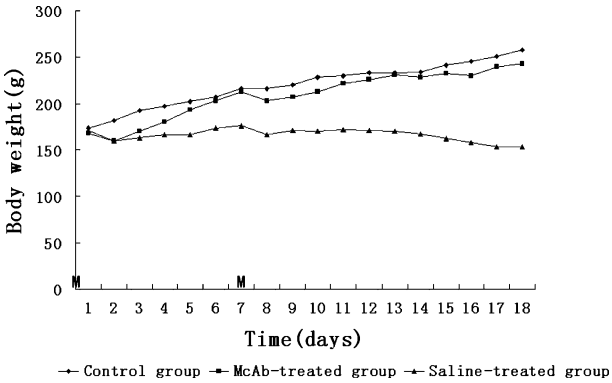

Fig. 1. Time course of the effect of anti-CD4 monoclonal antibody on body weight of rats. M: Induction of experimental autoimmune myocarditis.

significantly increased, while LVDd and LVFS were significantly decreased in saline-treated group, relative to the values in control group. A slight increase in LVSd was observed in saline-treated group in comparison to the control group. A significant reduction in IVS and LVPW and a significant increase in LVFS and LVDd were found in McAb-treated group, along with a slight decrease in LVSd. The changes of HR were insignificant. Echocardiography analysis demonstrated that heart structure and cardiac function were greatly impaired in EAM rats, and the treatment with McAb significantly prevented the left ventricular dysfunction and ventricular remodeling.

Inhibitory Effects of McAb on Inflammation and Fibrosis

No rat died prior to day 18. On day 18 after the first immunization, all rats in saline-treated group developed typical severe autoimmune myocarditis. Macroscopically, the hearts were markedly enlarged and the surfaces of the hearts were discolored. There was massive pericardial effusion. However, gross analysis of the hearts of rats in McAb-treated group revealed a significant decrease in the typical signs of myocarditis: hypertrophy, pericardial effusion, and pallor. Microscopically, in saline-treated group, large numbers of inflammatory cells including lymphocytes, macrophages, and multinucleated giant cells, infiltrated all layers of ventricular wall, especially epicardium. Degeneration and necrosis were also evident. Masson′s trichrome staining showed the existence of massive fibrosis. In sharp contrast, very little infiltration of inflammatory cells and fibrosis in myocardium was observed in McAb-treated rats (Figs. 3 and 4). Pathological scores of rats in McAb-treated group were significantly lower than those of rats in saline-treated group (Table III).

Table I. Changes of Heart and Body Weights in Autoimmune Myocarditis Rats ($n = 8$)

Group	BW (g)	HW (g)	HW (mg)/BW (g)
Control group	258 ± 12^a	0.7946 ± 0.0720	3.08 ± 0.17^a
McAb-treated group	243 ± 15	0.7630 ± 0.0675	3.14 ± 0.15
Saline-treated group	153 ± 9	0.9503 ± 0.0809	6.19 ± 0.18

Note. Values are expressed as mean ± SD. BW: body weight; HW: heart weight; HW/BW: heart weight/body weight ratio.
$^aP > 0.05$ vs. McAb-treated group and $P < 0.01$ vs. saline-treated group.

Suppression of Lymphocyte Proliferation by McAb

Effects of McAb treatment on lymphocyte proliferation were examined as shown in Fig. 5. Lymphocytes obtained from rats in saline-treated group had remarkable proliferative response to porcine cardiac myosin. In McAb-treated group, however, lymphocytes showed no significant proliferation to this antigen. When stimulated with the nonspecific mitrogen Con A, Lymphocytes obtained from control group, saline-treated group and McAb-treated group displayed similar thymidine incorporation.

Inhibitory Effect of McAb on Serum Anti-Cardiac Myosin Autoantibody

Effect of McAb treatment on serum anti-cardiac myosin autoantibody was examined, as shown in Fig. 6. Rats in saline-treated group produced large numbers of anti-cardiac myosin autoantibody and the titer of the autoantibody was much higher in saline-treated group than in control group and McAb-treated group. Rats in McAb-treated group produced very little anti-cardiac myosin autoantibody and there was no significant difference between McAb-treated group and control group. The results showed that McAb administration markedly impeded the production of anti-cardiac myosin antibody.

Changes of Th1 and Th2 Balance

Th1 and Th2 balance of the three groups was examined. The serum levels of Th1 cytokines IFN-γ and IL-2 were significantly higher in saline-treated group than in control group and treatment with McAb significantly reduced them (Fig. 7A and B). Moreover, treatment with McAb significantly up-regulated the serum level of Th2 cytokine IL-10 of EAM rats and did not significantly

Table II. Changes of Echocardiographic Parameters in Autoimmune Myocarditis Rats ($n = 8$)

Group	HR (beats/min)	IVS (mm)	LVPW (mm)	LVDd (mm)	LVSd (mm)	LVFS (%)
Control group	391 ± 27	1.11 ± 0.05	1.12 ± 0.05	6.07 ± 0.26	2.90 ± 0.17	52.29 ± 1.93
McAb-treated group	406 ± 29^a	1.14 ± 0.06^b	1.19 ± 0.09^b	5.99 ± 0.22^b	3.00 ± 0.11	49.87 ± 0.97^b
Saline-treated group	433 ± 28	1.92 ± 0.11	2.08 ± 0.14	4.99 ± 0.26	3.13 ± 0.23	37.10 ± 3.63

Note. Values are expressed as mean ± SD. HR: heart rate; IVS: left ventricular diastolic interventricular septum thickness; LVPW: left ventricular posterior wall thickness; LVDd: left ventricular end-diastolic dimension; LVDs: left ventricular end-systolic dimension; LVFS: left ventricular fractional shortening.
[a] $P > 0.05$ vs. control group and saline-treated group.
[b] $P > 0.05$ vs. control group and $P < 0.01$ vs. saline-treated group.

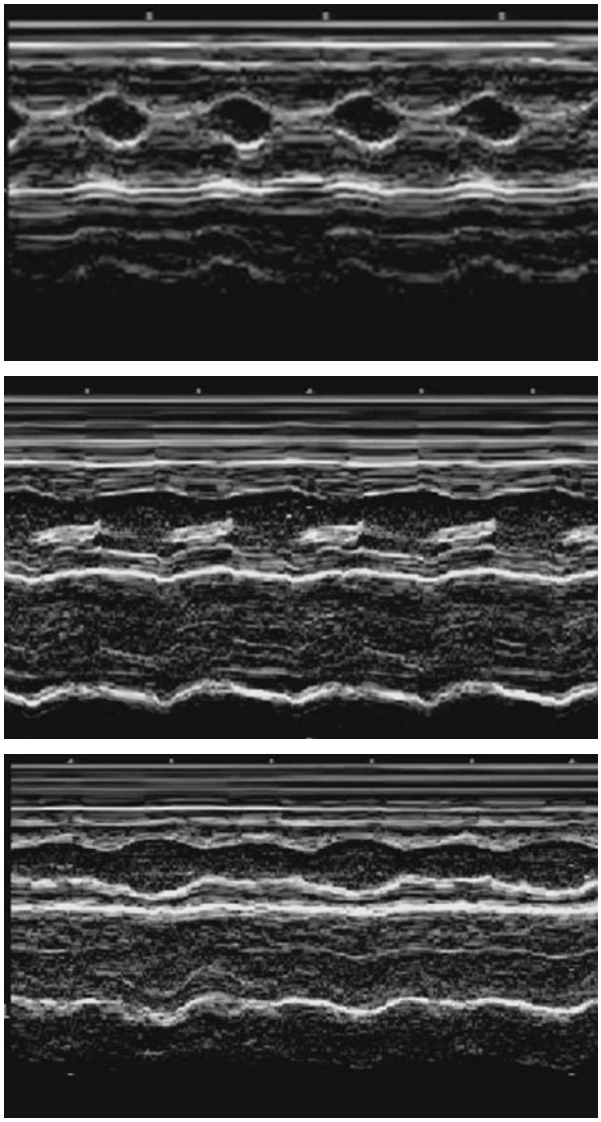

Fig. 2. M-mode echocardiograms of rats in (A) control group; (B) saline-treated group; (C) McAb-treated group.

affect the serum level of IL-6 (Fig. 7C and D). These results suggested that treatment with McAb changed the helper T cells balance from Th1 to Th2 in EAM rats.

DISCUSSION

In this study, immunization of Lewis rats with porcine cardiac myosin elicited typical autoimmune myocarditis 18 days after the first immunization. The results were the same as those reported by Kodama *et al*. (15). Its characteristics including marked cellular infiltration, extensive myocardial necrosis, fibrosis, cardiac dysfunction and cardiac remodeling, high titers of anti-cardiac myosin autoantibody in sera were similar to those in human severe virus myocarditis. It provided an important animal model for the study of human severe virus myocarditis. At the same time, successfully establishment of this kind of animal model revealed that autoimmune response played a crucial role in the development of myocarditis.

Because autoimmune injury play a critical role in the development of autoimmune myocarditis, immunosuppressive therapies have received increased attention. A principal disadvantage of current therapeutic management of myocarditis in humans is the induction of general immune suppression by nonselective agents such as steroids and cytotoxic drugs. They have many side effects and, moreover, they may accelerate the replication of viruses and diffuse the inflammation. A new strategy for the treatment of autoimmune disease is to administer monoclonal antibodies to block the immunological responses or inflammatory cascades (16, 17). The advantage of monoclonal antibody over conventional therapy is their potential for great specificity, which allows them to act selectively on a specific lymphocyte subset *in vivo*, rather than general immunosupression (18). As far as autoimmune myocarditis is concerned, it is a T-cell-mediated

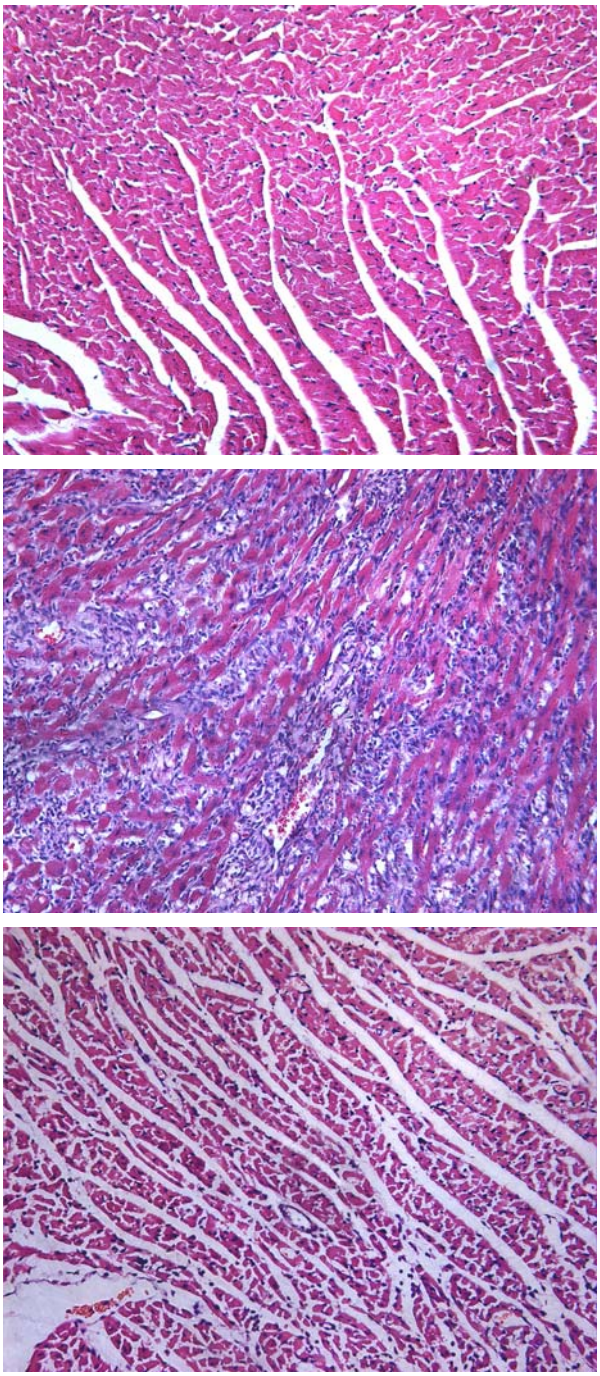

Fig. 3. Hematoxylin-eosin staining of the hearts, 200×: (A) control group; (B) saline-treated group; (C) McAb-treated group.

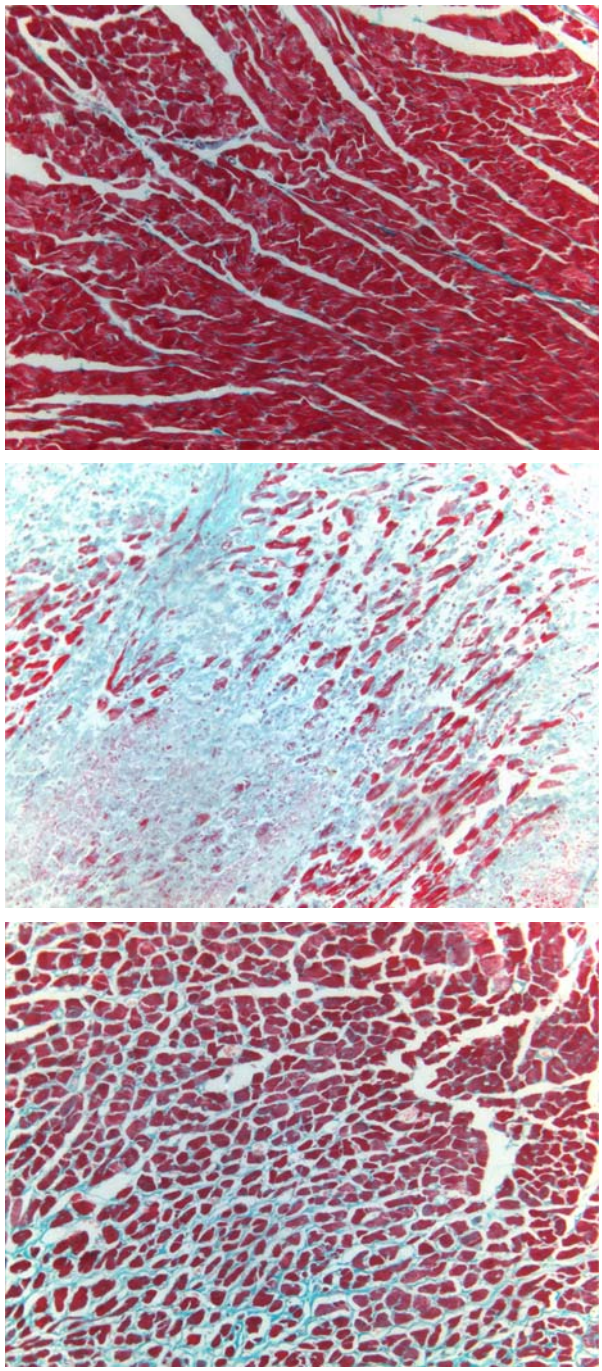

Fig. 4. Masson's trichrome staining of the hearts, 200×: (A) control group; (B) saline-treated group; (C) McAb-treated group.

autoimmune disease (19, 20); CD4-positive T cells, so-called helper T cells, are believed to be the most important for the initiation and mediation of the disease (21). They are responsible for the production of most of the cytokines that are necessary to stimulate an immune response. In keeping with the primary role of CD4-positive T cells in autoimmune myocarditis, we chose monoclonal antibody which was bind to CD4 molecular and disturbed the activation of CD4-positive T cells as a reagent. The aim is to

Table III. Changes of Cardiac Histopathology in Autoimmune Myocarditis Rats ($n = 8$)

Group	Macroscopic scores	Microscopic scores	
		Infiltration	Fibrosis
Control group	0	0	0
McAb-treated group	0.4 ± 0.5^a	0.6 ± 0.5^a	0.8 ± 0.6^a
Saline-treated group	3.5 ± 0.5	3.8 ± 0.2	3.9 ± 0.4

Note. Values are expressed as median ± SEM.
$^aP < 0.01$ vs. saline-treated group.

provide a new approach for the treatment of myocarditis and dilated cardiomyopathy.

McAb is the most potent and extensively studied reagent in monoclonal antibody therapy of autoimmune disease. It has been reported effective in impeding allograft rejection after transplantation and in other autoimmune disease, such as rheumatoid arthritis (22). But the effects of McAb have not been well studied in autoimmune myocarditis. In the present study, McAb was used as a drug for the treatment of EAM rats. We demonstrated that short-time administration of McAb prevented the development of EAM. In McAb-treated group, after four administrations of McAb, HW/BW ratio was not significantly elevated, and cardiac function was not markedly decreased, and very little anti-cardiac myosin autoantibody was found in sera 18 days after the first immunization, and there were not obvious myocardial pathological changes in the hearts. These results suggested that cardiac dysfunction and myocardial injury mediated by immune response to cardiac myosin were prevented. It means that short-time treatment with McAb is effective for acute autoimmune myocarditis.

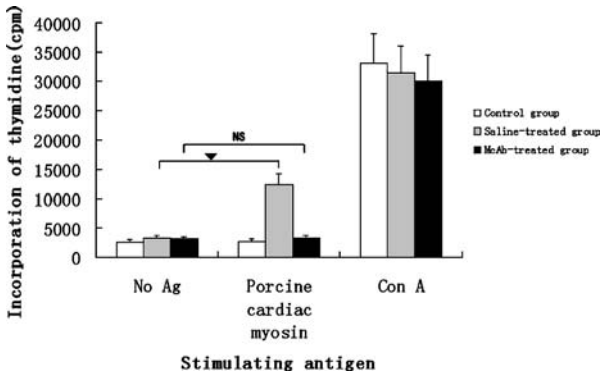

Fig. 5. Effects of anti-CD4 monoclonal antibody on lymphocyte proliferation in autoimmune myocarditis rats. $^*P < 0.05$; NS: not significant.

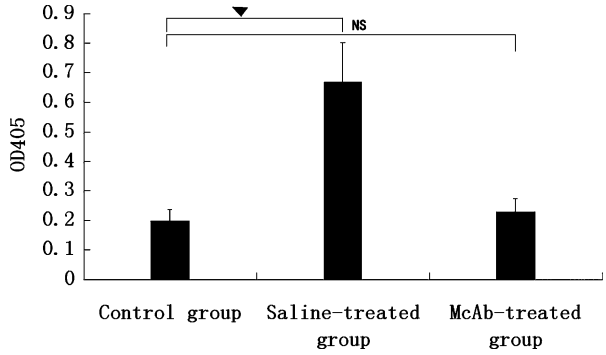

Fig. 6. Effects of anti-CD4 monoclonal antibody on serum level of anti-cardiac myosin antibody in autoimmune myocarditis rats. $^*P < 0.05$; NS: not significant.

In this study, the changes of the special cellular immunity and humoral immunity of EAM rats after McAb administration were evaluated. Results showed that proliferative response to cardiac myosin was markedly diminished in lymphocytes of EAM rats after administration of McAb. In addition, proliferative responses to Con A stimulation were still preserved in those cells. The results revealed that antigen-specific cellular immunity was inhibited by McAb administration. Humoral immunity was evaluated by detecting the serum level of anti-cardiac myosin autoantibody. Results showed that McAb administration significantly impeded the production of anti-cardiac myosin antibody. These data demonstrated that four consecutive injection of McAb, before the immunization of cardiac myosin, immune tolerance to cardiac myosin was successfully induced and maintained, which suggested that tolerant condition could be maintained by repeated contact with the same antigen, but without the need for further administration of McAb. Thus, side effects induced by long-time treatment with immunosuppressive drugs could be avoided.

CD4 target therapy can be categorized into depleting and nondepleting types *in vivo*, depending on multiple factors, such as IgG isotope and specific CD4 epitope characteristics (23). Depleting monoclonal antibodies were initially applied to kill target cells as a form of global transient immunosuppression. More recently, however nondepleting monoclonal antibodies are taken more attention. In the present study, immune tolerance to cardiac myosin is successfully induced by nondepleting monoclonal antibody, which means that $CD4^+$ T-cell depletion is not a key to monoclonal antibodies functions. At the same time, nondepleting monoclonal antibodies have shown to be at least as effective as depleting monoclonal antibodies in transplantation or autoimmune disease models (24). Direct compassion of the efficiency of depleting McAb with nondepleting isotope to induce transplant tolerance

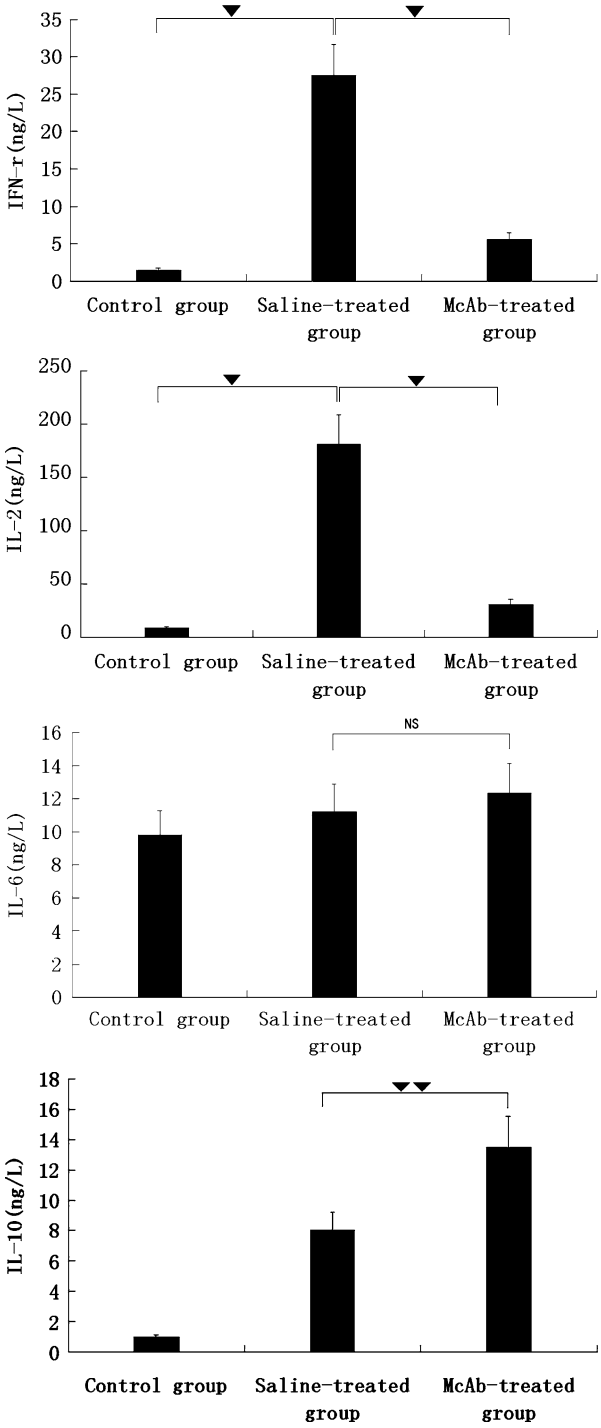

Fig. 7. Effects of anti-CD4 monoclonal antibody on Th1 and Th2 cytokines in autoimmune myocarditis rats. $^{*}P < 0.01$;$^{**}P < 0.05$; NS: not significant.

has been performed (25, 26). Results showed that depleting isotope generated a more profound, but transient immunosuppression *in vivo*, with a rapid effect on rejection rather than on tolerance induction. Results also showed that nondepleting isotopes were significantly superior in extending graft survival.

The mechanism of immune tolerance induced by McAb is still not well understood. Plain *et al.* observed that W3/25 did not markedly deplete the $CD4^+$ T cell population in peripheral blood lymphocytes, and the maximum depletion was two-fifths of the circulating $CD4^+$ T cell, even given at a high dose of 7 mg/kg (27). In the present study, four consecutive injections in rats with a regular dose of 4 mg/kg of McAb successfully induced immune tolerance to cardiac myosin. Those results suggest that there are some other mechanisms involved in the induction of immune tolerance except the depletion of $CD4^+$ T cells. The research about the mechanism of immune tolerance focuses on two aspects. The first aspect is the role of immune regulation in tolerance induction, especially the regulation of Th1 and Th2 (27, 28). The present study showed that treatment of rats with McAb selectively inhibited the production of Th1 cytokines, but the production of Th2 cytokines was unaffected or even upregulated. These results demonstrated that treatment with McAb changed the Th1/Th2 balance from Th1 to Th2. The results contrasted with pervious reports by Plain *et al.* (27). Their results showed that induction of tolerance with nondepleting McAb was associated with down-regulation of Th2 cytokines. Given that, additional research will be required to elucidate the mechanisms involved in the role of Th1/Th2 balance in immune tolerance induction. The second aspect is the signal transduction pathways of T cell activation (29). *In vitro* studies evidenced that McAb, when applied to T cells, not only reduced the signal intensities through TCR/CD3, but also disrupt the downstream signaling events of TCR (30).

In conclusion, this study demonstrates that anti-CD4 monoclonal antibody can successfully prevent the initiation of myocarditis in Lewis rats. It accomplishes this through the induction of immune tolerance to cardiac myosin. This study suggests that anti-CD4 monoclonal antibody may have great potential as a therapy for human myocarditis.

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