Cardiac Troponin I but Not Cardiac Troponin T Induces Severe Autoimmune Inflammation in the Myocardium

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Background—Cardiac troponins in blood are the most preferred markers of myocardial damage. The fact that they are normally not found in the circulation provides a high level of clinical sensitivity and specificity even when cardiac lesions are small. After myocardial injury, the troponins enter the circulation, where they can be used for diagnosis of acute coronary syndromes. Thus, the cardiac troponins are paramount for disease classification and risk stratification. However, little is known about the long-term effects of the released troponins on cardiac function.

Methods and Results—In this study we prepared recombinant murine cardiac troponin I (mc-TnI) and murine cardiac troponin T and used them to immunize mice. We report that A/J mice immunized with mc-TnI developed severe inflammation of the myocardium with increased expression of inflammatory chemokines RANTES (regulated on activation normal T cell expressed and secreted), monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)–1α, MIP-1β, MIP-2, T-cell activation gene 3, and eotaxin and chemokine receptors CCR1, CCR2, and CCR5. The inflammation was followed by cardiomegaly, fibrosis, reduced fractional shortening, and 30% mortality over 270 days. In contrast, mice immunized with murine cardiac troponin T or with the control buffer showed little or no inflammation and no death. Furthermore, we demonstrate that mice preimmunized with mc-TnI before left anterior descending coronary artery ligation showed greater infarct size, more fibrosis, higher inflammation score, and reduced fractional shortening.

Conclusions—Overall, our results show for the first time that provocation of an autoimmune response to mc-TnI induces severe inflammation in the myocardium followed by fibrosis and heart failure with increased mortality in mice. (Circulation. 2006;114:1693-1702.)

Key Words: heart failure ■ inflammation ■ myocarditis ■ troponin

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by progressive depression of myocardial contractile function and ventricular dilation. Despite its rather high frequency and high mortality rate, its pathomechanism is largely unknown. In addition to ischemic, toxic, metabolic, infectious, and genetic causes, autoimmunity has been suspected to be one of the main causes.1,2

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Cardiac troponins in blood are the most preferred markers of myocardial damage. The fact that they are normally not found in the circulation provides a high level of clinical sensitivity and specificity even when cardiac lesions are small. After myocardial injury, the troponins enter the circulation, where they can be used for diagnosis of acute coronary syndromes and for prognosis.3,4 Recently, Nishimura et al5 reported that PD-1 receptor–deficient mice developed autoantibodies to cardiac troponin I (TnI) and severe DCM. They further found that administration of monoclonal antibodies to cardiac TnI induced heart dysfunction and dilation by chronic stimulation of Ca2+ influx in cardiomyocytes.6 It has also been shown that autoantibodies to cardiac TnI are present in patients with acute coronary syndrome and that these antibodies may cause delays in detecting cardiac TnI,7 pointing to an early induction of an autoimmune response to TnI in these patients. However, the possible pathogenic role of an autoimmune response to the troponins is not known. To clarify the effect of this autoimmune response to troponins, we developed an animal model. We constructed a plasmid for production of murine cardiac troponin I (mc-TnI) and murine cardiac troponin T (mc-TnT) and immunized mice with either...
purified mc-TnI, mc-TnT, or control buffer. We studied the effects on production of troponin-specific autoantibodies, cross-reactivity of autoantibodies, troponin-specific cellular response, inflammation, and expression of inflammatory cytokines, chemokines, fibrosis, heart function, ischemia/reperfusion injury, myocardial remodeling, and survival of the animals.

**Methods**

**Mice**

Female BALB/c mice obtained from Charles River (Sulzfeld, Germany) and male and female A/J mice (5 weeks of age) obtained from Harlan Winkelmann GmbH (Borchern, Germany) were maintained in the conventional animal facility at the University of Heidelberg and were used in all experiments. The animal work was approved by the Animal Care and Use Committee of the University of Heidelberg.

**Preparation of Recombinant mc-TnI and mc-TnT**

The cDNAs for mc-TnI and mc-TnT were obtained by reverse transcription–polymerase chain reaction with the use of total RNA isolated from mouse cardiac ventricle (strain C57 Bl/6) as a template. Via primers, Ndel and BamHI restriction sites were introduced at the 5’ and 3’ ends, respectively. The corresponding polymerase chain reaction fragments were digested with Ndel and BamHI and ligated into Ndel/BamHI-digested vector pET11c (Stratagene, Amsterdam, The Netherlands), resulting in pET11c-mc-TnI and pET11c-mc-TnT.

The murine cardiac troponin subunits mc-TnI and mc-TnT were expressed in *Escherichia coli* and purified as previously described.8 In addition to purification via ion exchange chromatography, mc-TnI was applied to a cardiac troponin C affinity column as a second purification step.9 Isolated TnI fractions were dialyzed extensively against 1 mmol/L ammonium hydrogen carbonate, and TnI fractions were dialyzed extensively against 1 mmol/L HCl, then lyophilized and stored at −80°C. The troponin-free fractions of the buffer from the respective second chromatography were dialyzed and used for control immunizations.

**Antigen Preparation and Administration**

Each mouse was injected subcutaneously with 100 μL of emulsion of 120 μg of TnI, troponin T (TnT), or control buffer, or control buffer in supplemented complete Freund’s adjuvant (CFA) containing 5 mg/mL of *Mycobacterium tuberculosis* H37Ra (Sigma, St Louis, Mo) on days 0, 7, 60, and 245.

**Detection of Serum Concentrations of Autoantibody Titers and Cardiac Troponin–Specific Cytokine Production by Splenocytes**

These were done as described previously.10,11 To measure serum anti–cardiac TnI, anti–cardiac TnT, anti–cardiac myosin, or anti–skeletal muscle myosin titers, plates were coated with 100 μL per well of either cardiac TnI, cardiac TnT, cardiac myosin, or skeletal muscle myosin (5 μg/mL) in bicarbonate buffer (pH 9.6) and left overnight. Mouse secondary antibodies, diluted to 1:1000 for IgG (KPL, Gaithersburg, Md), IgG1, IgG2a (Pharmingen, Heidelberg, Germany), and IgG2b (Bethyl, Montgomery, Tex), were used for detection. Serum samples from test mice were diluted to 1:200, 1:400, 1:1600, 1:6400, 1:25 600, and 1:51 200. Normal mouse serum was used as control. Optical densities were determined at 450 nm. Antibody endpoint titers for each individual mouse were calculated as the greatest positive dilution of antibody.

In vitro cytokine production, the splenocytes were cultured at 5 × 10^6 per well in RPMI 1640 complete medium in the presence of 30 μg/mL of either cardiac TnI, cardiac TnT, or medium alone for 48 hours. Supernatant was collected, aliquoted, and frozen at −20°C. Cytokines were measured by Quantikine cytokine ELISA kits (R&D Systems, Wiesbaden-Nordenstadt, Germany), according to the manufacturer’s instructions.

**Histopathologic Evaluation**

For the histopathologic evaluation, mice were euthanized on days 21, 90, and 270, and serial sections were made through the heart. Sections 5 μm thick were cut at various depths in the tissue section and stained with hematoxylin and eosin to determine the level of inflammation and with Masson’s trichrome to detect collagen deposition. Evidence of myocarditis and fibrosis was evaluated in a blind manner by an investigator who used light microscopy, according to a 6-tier scoring system: grade 0, no inflammation; grade 1, cardiac infiltration in up to 5% of the cardiac sections; grade 2, 6% to 10%; grade 3, 11% to 30%; grade 4, 31% to 50%; and grade 5, >50%. The score from the investigator’s reading was taken for statistical analysis with a nonparametric test. Other organs, such as skeleton, kidney, liver, pancreas, or intestine of the immunized mice were taken, and every fifth section was stained with hematoxylin and eosin.

**Echocardiography**

Transtracheal echocardiography was performed as previously described in detail.12 The investigator who conducted the echocardiography was blinded for the treatment status.

**RNA Protection Assay**

The mCR-5 cytokine receptor multiprobe template set (BD Biosciences Pharmingen, Heidelberg, Germany) was used to measure mouse mRNAs encoding CCR1, CCR2, CCR1b, CCR3, CCR4, and CCR5. The mCK-5c multiprobe template set (BD Biosciences Pharmingen, Heidelberg, Germany) was used to measure mouse mRNAs encoding Ltn, RANTES (regulated on activation normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-2, interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), T-cell activation gene 3 (TCA-3), and eotaxin. The measurement was done according to the manufacturer’s guidelines.

**Elution of Autoantibodies From the Affected Hearts**

Heart tissue from A/J mice immunized with either TnI or TnT or control buffer was homogenized, and after centrifugation the pellet was rinsed 3 times with phosphate-buffered saline. Then the pellet was treated with 0.1 mol/L glycine buffer (pH 2.7), followed immediately by neutralization (1 mol/L Tris HCl, pH 9.5). Immunglobulins were purified with protein G-Sepharose beads (Amer sham, GE Healthcare, Freiburg, Germany) according to the manufacturer’s guidelines. Immunoreactivity of the eluates (2 μg/mL) was examined against recombinant mc-TnI and mc-TnT in Western blot.

**Murine Model of Coronary Artery Occlusion**

Female A/J mice (7 to 8 weeks of age) treated with either mc-TnI or control buffer 21 days before instrumentation were anesthetized and subjected to chronic ischemia in the heart. Sham-operated mice were another control group. The coronary artery occlusion procedure was performed as described previously.13 A group of mice was euthanized after 48 hours. The area at risk and infarct size were determined as described previously.14 For the histopathologic evaluation, a second group of mice was euthanized 21 days after left anterior descending coronary artery (LAD) ligation, and serial sections were made through the heart. As described above, hematoxylin and eosin and Masson’s trichrome stainings were done to determine the level of inflammation and fibrosis.

**Statistical Analysis**

Normally distributed data were analyzed by Student *t* test; otherwise, the Mann-Whitney *U* test was used. Disease prevalence was compared by a χ² 2-way analysis. Statistical comparison across 3 groups was calculated by ANOVA followed by appropriately conducted multiple comparisons. Survival data were calculated by the product-limit (Kaplan-Meier) method for 1 or more groups. Probability values of <0.05 were considered significant.
The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Purification of mc-TnI and mc-TnT

The cDNAs for mouse cardiac TnI and TnT were cloned from mouse cardiac ventricle. Mc-TnI and mc-TnT proteins were expressed in *E. coli* and purified (Figure 1). In addition to being purified via ion exchange chromatography, mc-TnI was applied to a cardiac troponin C affinity column. The protein-free fractions of the second purification steps were used for control immunizations. They were treated in the same way as the TnI- and TnT-containing fractions except for lyophilization.

Immunization and Humoral Immune Response

To study the effect of immunization on humoral immune response, we measured mc-TnI- and mc-TnT-specific total IgG, IgG1, IgG2a, and IgG2b autoantibody titers in mc-TnI- and mc-TnT-immunized mice, respectively, at day 21 (Figure 2a to 2d). All troponin-immunized mice had high titers of total IgG, IgG1, IgG2a, and IgG2b autoantibodies against the administered troponin (Figure 2a and 2c), indicating that the immunization was successful. Antibodies associated with both Th1 (IgG1) and Th2 (IgG2a) subclasses were present. To clarify whether these mice also had cross-reacting autoantibodies against the troponin with which they were not injected, we measured mc-TnT autoantibody titers in mc-TnI–immunized mice and mc-TnI autoantibody titers in mc-TnT–immunized mice. No cross-reacting autoantibodies were detectable in the serum of the immunized mice (Figure 2b and 2d). We also tested for the presence of autoantibodies against cardiac myosin. On day 21 we did not find measurable titers of autoantibodies to cardiac myosin in any of the

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**Figure 1.** Two-step purification of mc-TnT and mc-TnI proteins as described in Methods. Samples of different protein preparation steps were separated on a 10% Tricine gel (TnI) and on a 10% to 20% Tricine gel (TnT), respectively. 1, *E. coli* crude extract before induction with Isopropyl β-D-1 thiogalactopyranoside; 2, *E. coli* crude extract 4 hours after induction with Isopropyl β-D-1 thiogalactopyranoside; 3, mc-TnT– and mc-TnI–enriched fraction after cation exchange chromatography at pH 6.0 (TnT) and at pH 8.0 (TnI), respectively; 4, pure mc-TnT and mc-TnI protein after DEAE Sepharose chromatography (TnT) and after cTnC affinity chromatography (TnI), respectively.

**Figure 2.** mc-TnI–, mc-TnT–, and cardiac myosin–specific autoantibody production. Each mouse was immunized subcutaneously with 100 μL of emulsion of 120 μg of mc-TnI, mc-TnT, or control buffer in supplemented CFA containing 5 mg/mL of *M. tuberculosis* H37Ra. a and b, Effects on mc-TnI–specific and mc-TnT–specific autoantibody production on day 21 when mice were immunized with mc-TnI are shown. c and d, Effects on mc-TnT–specific and mc-TnI–specific autoantibody production on day 21 when mice were immunized with mc-TnT are shown. e and f, Effects on cardiac myosin–specific autoantibody production on day 21 and on day 90 when mice were immunized with mc-TnI are shown. Antibody end point titers for each individual mouse were calculated as the greatest positive dilution of antibody above normal mouse serum levels for day 21. *P*<0.05.
groups tested. However, on day 90 antibodies against cardiac myosin associated with both Th1 (IgG1) and Th2 (IgG2a) subclasses were present in mice immunized with mc-TnI but not in mice immunized with mc-TnT or the control buffer (Figure 2e and 2f). Finally, we tested for the presence of autoantibodies to skeletal muscle myosin–specific autoantibodies and found no measurable titers of antibody (data not shown).

**Immunization and Cellular Immune Response**

To determine the effect of immunization on cellular immune response, we measured mc-TnI–specific cytokine production by splenocytes in mc-TnI–immunized mice and mc-TnT–specific cytokine production by splenocytes in mc-TnT–immunized BALB/c and A/J mice at day 21 after immunization (Figure 3). There was a dose-dependent troponin-specific increased production of all tested cytokines, including Th1 (interleukin [IL]-2, interferon [IFN]-γ), Th2 (IL-4, IL-10), and monocyte/macrophage (IL-1, tumor necrosis factor-α) cytokines in both groups of mice. There was no significant difference in antigen-specific cytokine production between TnI- and TnT-immunized mice except for IL-10 and IFN-γ production. The levels of these 2 cytokines were significantly higher in TnI-immunized mice when splenocytes were stimulated with 1 µg/mL antigen. These differences were not significant when splenocytes were stimulated with 30 µg/mL antigen.

**Immunization With mc-TnI Induces Inflammation in the Myocardium**

To study the effect on inflammation, groups of mice were euthanized on day 21, 90, or 270, and serial sections were made through the heart. Every fifth section was stained with hematoxylin and eosin (Figure 4a; Figure 5a to 5e). All 4 BALB/c mice immunized with mc-TnI showed inflammation with a histoscore of ≥1, whereas only 1 of 5 mc-TnT–immunized mice showed any sign of inflammation. In addition, none of the buffer control mice had inflammation. When A/J mice were used, they showed significantly more inflammation compared with BALB/c mice (average histoscore 1.9 ± 0.5 versus 1.1 ± 0.1; P<0.05). Thus, 5 of 9 (on day 21 and 90) and 9 of 16 (on day 270) mc-TnI–immunized A/J mice showed severe inflammation (histoscore of ≥2) (Figure 4a; Figure 5a to 5c); 2 mice (on days 21 and 90) and 3 mice (on day 270) showed mild inflammation (histoscore 1 and 0.5) (Figure 4a; Figure 5d). In contrast, only 3 of 10 (on day 21) and 1 of 10 (day 90) mc-TnT–immunized A/J mice had slight inflammation (histoscore 0.5). Seven of 10 (on day 21), 9 of 10 (on day 90), and 12 of 12 mice (on day 270) had no inflammation (Figure 4a; Figure 5e). In the control group there was only 1 mouse with slight inflammation (histoscore 0.5) on day 90 (Figure 5e). When male mice were used instead of female mice, similar results were obtained, but the inflammation grade was significantly lower in all mc-TnI–immunized male A/J mice compared with female A/J mice (average histoscore 1.0 ± 0.2 versus 1.9 ± 0.5; P<0.05).
Immunization With mc-TnI Causes Myocardial Fibrosis

To study the effect on fibrosis, mice were euthanized on day 21, 90, or 270, and serial sections were made through the heart. Every fifth section was stained with Masson’s trichrome (Figure 4b; Figure 5f to 5i). We observed increased fibrotic myocarditis with deposition of collagen in the myocardium of mice immunized with mc-TnI over time (Figure 4b; Figure 5f to 5i) compared with mice immunized with mc-TnT or control buffer (Figure 4b). Myocardial fibrosis was indicated by bright blue staining for collagen deposition (Figure 5f to 5i) and was associated with myocardial inflammation (Figure 4a).

Echocardiography and Survival

The severe inflammation and fibrosis in the myocardium of the mc-TnI–immunized mice led to enlarged hearts (Figure 6a), significantly increased left ventricular end-systolic and end-diastolic diameter (Figure 6b and 6c), and significantly decreased fractional shortening (Figure 6d). The survival of mc-TnI–immunized mice was reduced over 270 days (P=0.057 versus TnT-immunized mice; Figure 6e).

mRNA Expression of Chemokines and Chemokine Receptors in the Myocardium

To examine the expression of different chemokines and chemokine receptors, we measured the mRNA expression...
levels of the chemokines Lttn, RANTES, MIP-1β, MIP-1α, MIP-2, IP-10, MCP-1, TCA-3, and eotaxin and of the chemokine receptors CCR1, CCR2, CCR1b, CCR3, CCR4, and CCR5 in the myocardium of the immunized mice. We were able to detect mRNA levels for RANTES, MIP-1β, MIP-1α, MIP-2, MCP-1, TCA-3, and eotaxin only in the mc-TnI–immunized mice but not in the mc-TnT– or control buffer–immunized mice (Figure 7a). In addition, we found that the mRNAs for the CCR receptors CCR1, CCR2, and CCR5 were expressed only in the myocardium of mc-TnI–immunized mice (Figure 7b).

**Autoantibodies in Heart Extracts**

Presence and immunoreactivity of heart extracts from TnI-, TnT-, or control buffer–immunized A/J mice was examined against recombinant mc-TnI and mc-TnT in Western blot. Antibodies against mc-TnI could be detected in both serum and heart extracts of mc-TnI–immunized mice, whereas antibodies against mc-TnT could be detected only in serum but not in the heart extracts of mc-TnT–immunized mice (Figure 7c).

**Infarct Size and Inflammation in Mice Preimmunized With TnI**

To study the effects of prior immunization with TnI on regional ischemic injury in vivo, mice preimmunized with either mc-TnI or control buffer (starting 3 weeks before instrumentation) were subjected to ischemia by chronic ligation of the LAD. The infarct size, determined as percentage of area at risk, was significantly greater in the group treated with TnI (74.6 ± 5.75% versus 51.97 ± 8.16%; \( P < 0.001 \)) than in the group treated with control buffer (Figure 8a). In accord with these data, the histological analysis revealed a markedly enhanced ischemia-induced tissue injury due to preimmunization with TnI in comparison with the control group.

Furthermore, mice preimmunized with TnI showed, at 21 days after chronic ligation of the LAD, a higher fibrosis score (3.3 ± 0.2 versus 2.7 ± 0.2; \( P = 0.078 \)), higher inflammation score (average score 2.5 ± 0.2 versus 1.6 ± 0.2; \( P = 0.01 \)), and reduced fractional shortening (25.6 ± 1.7 versus 33.6 ± 2.0; \( P < 0.01 \)) than the control mice (Figure 8b to 8d). Thus, the preimmunized mice showed more inflammatory cells in the infarcted area than the control mice (Figure 8e and 8f).
Furthermore, significantly more mice preimmunized with TnI showed diffuse inflammation in the myocardium, including areas distant from the infarct area (83% versus 43%; \(P<0.029\); Figure 8g).

**Discussion**

Here we show for the first time that inducing an autoimmune response against mc-TnI leads to severe inflammation and fibrosis in mice, resulting in enlarged hearts, increased end-systolic and end-diastolic diameters, reduced fractional shortening, and reduced survival over 270 days compared with mice immunized with mc-TnT or control buffer. Recently, it has been reported that autoantibodies against cardiac TnI induce heart failure by chronic stimulation of \(Ca^{2+}\) influx in cardiomyocytes.\(^5,6\) Now we report that inducing a complete autoimmune response (humoral and cellular) against mc-TnI induces severe inflammation and fibrosis in the myocardium of the mice with persistent, prominent inflammation and fibrosis over 270 days and reduced long-term survival. We also demonstrate that mice preimmunized with TnI before LAD ligation showed greater infarct size, more fibrosis, higher inflammation score, and reduced fractional shortening. Furthermore, significantly more mice showed diffuse inflammation in the myocardium, including areas distal from the infarct area, suggesting generalized autoimmune damage.
The inflammation in mc-TnI–immunized A/J mice was more severe than the inflammation observed in comparable BALB/c mice, and females had more severe disease than male mice, indicating that there is a genetic and sex-based predisposition to mc-TnI–induced inflammation in the myocardium comparable to other models of autoimmune disease.15 Nishimura et al5 did not see much inflammation but only deposition of immune complexes on the cardiomyocytes in the PD-1–deficient mice that developed DCM. The PD-1–deficient mice had a BALB/c background. There are several reasons why this group did not see inflammation in the myocardium and we did. First, they studied PD-1–, immunoregulatory receptor–deficient mice; we used wild-type BALB/c mice for immunization. PD-1–deficient mice develop spontaneous autoimmune diseases5,16; we induced disease by immunization with cardiac troponins. Second, in our experiments BALB/c mice developed inflammation that was found primarily around day 21 after immunization. Okazaki et al6 also used A/J mice in their studies, and they treated them with monoclonal antibodies against Tnl. Thus, they induced only a humoral autoimmune response against Tnl. By immunization with troponins using supplemental CFA, we induced both humoral and cellular immune responses, explaining the severe inflammation observed in Tnl-immunized A/J mice.

Interestingly, when mc-TnT was used for immunization, there was very little or no inflammation in the myocardium of both mouse strains used in our study, even though the immunization induced both strong humoral (antigen-specific autoantibody titers) and cellular (antigen-specific cytokine production) autoimmune responses against mc-TnT. These results support the previously reported finding that cardiac Tnl is not strictly localized in intracellular compartments on normal heart sections.6 In contrast, antibodies against cardiac TnT stain the cytoplasm but not the surface of ventricular cardiomyocytes, indicating that the distributions of the 2 troponins are different.6 Even though we could induce comparable levels of humoral and cellular immune responses by immunization with TnT or Tnl, only mc-TnI–immunized mice developed severe inflammation. We suggest that this is because the Tnl-specific autoantibodies and mc-Tnl–specific T cells could bind the target protein (Tnl) on the surface of cardiomyocytes6 whereas mc-TnT–specific autoantibodies and T cells could not. Thus, we could demonstrate Tnl immunoglobulins in the heart extracts of Tnl-immunized mice, but no significant TnT immunoglobulins could be found in the heart extracts of TnT-immunized mice.

Cardiac myosin is known to be one of the main cardiac proteins reported by us and others to induce experimental autoimmune

Figure 8. a, Each mouse was immunized subcutaneously with either 100 μL of emulsion of 120 μg of Tnl (Tnl+MI) or control buffer (MI) in supplemented CFA containing 5 mg/mL of M tuberculosis H37Ra, and infarct was induced by LAD ligation 21 days after immunization. Sham-operated mice were another control group (control). Effect on infarct size (IA) was determined as percentage of area at risk (AAR) (48 hours after LAD ligation). b, Fractional shortening. Histological examination of the hearts (staining with hematoxylin and eosin [c] or Masson’s trichrome [d to g]) 21 days after LAD ligation is shown.
Therefore, there may be a question of whether heart disease is caused by autoimmunity to cardiac myosin. Our findings clearly show that the initial injury to cardiomyocytes and initiation of myocarditis in our model is caused by cardiac TnI for the following reasons: (1) We used recombinant mouse cardiac troponins, and thus there was no contamination with any other cardiac protein, including cardiac myosin; (2) we administered the emulsion of cardiac troponin and CFA subcutaneously, thereby avoiding direct cardiac injury; (3) the control mice given CFA with buffer did not show any significant signs of inflammation; and (4) on day 21 we had neither significant myosin-specific antibody production nor a significant myosin-specific cellular immune response (evidenced by cytokine production). On the other hand, later the mc-TnI–immunized mice developed high titers of antibodies against cardiac myosin (days 90, 270), indicating that immunization with mc-TnI caused myocardial damage with release of cardiac myosin and induction of an immune response to another cardiomyocyte-specific protein. This broadened autoimmune response may of course aggravate the inflammation and cardiac damage because immunization with cardiac myosin alone is known to induce myocarditis and impaired myocardial function.10,11,15,17

Recently, it has been shown that an adhesive, fibrotic pericarditis is associated with increased DCM and reduced survival in IFN–γ-deficient mice after CVB3 infection.18 The development of fibrosis is an important feature in a number of pathological conditions, including myocarditis, and is a key determinant of the clinical outcome of chronic heart disease.18 Fibrosis involves proliferation of fibroblasts and deposition of extracellular matrix proteins like collagen. Fibroblasts are a major component of cardiac tissue, and therefore it is not surprising that fibrosis is an important contributor to the development of DCM and congestive heart failure.19 In this study we observed increased fibrotic myocarditis in mc-TnI–immunized mice but not in mc-TnT– or control buffer–immunized mice. Myocardial fibrosis was indicated by bright blue staining for collagen deposition and was associated with myocardial inflammation. Thus, mc-TnI–immunized mice developed fibrotic myocarditis, which is associated with enlarged hearts, increased end-systolic and end-diastolic diameters, and reduced fractional shortening and survival over 270 days.

Chemokines such as MCP-1 and MIP-1α and their major receptors CCR2 and CCR5 play important roles in the pathogenesis of many inflammatory diseases.20–22 MCP-1 mRNA expression has been shown in endomyocardial biopsy specimens from patients with DCM and suggested an important role of this chemokine in the regulation of inflammatory cell infiltration into the myocardium.23 Recently, we described an important role for MCP-1 and MIP-1α and their major receptors CCR2 and CCR5 in the initiation of autoimmune myocarditis.17 Not only blocking MCP-1 and MIP-1α with the use of monoclonal antibodies but also immunizing CCR25−/− and CCR55−/− mice reduced the severity of myocarditis. Furthermore, we demonstrated successful gene therapy by blocking MCP-1 activity in vivo using an N-terminal deletion mutant of MCP-1 called 7ND.17 In our experiments, only the mc-TnI–immunized mice showed increased expression of these chemokines, which correlated with increased myocardial inflammation in these mice.

Finally, we studied the effect of prior immunization with mc-TnI on infarct size and postinfarct fibrosis and inflammation in an acute cardiac damage model. Mice preimmunized with mc-TnI showed significantly larger infarct size, more fibrosis, and significantly more inflammation than the control mice, not only in the infarcted area but also in areas distal from the infarcted area. These results indicate that an autoimmune response against TnI aggravates the outcome of acute cardiac damage and may have a significant influence on postinfarct remodeling. This was supported by the findings that tolerance induction by nasal vaccination with troponin reduced ischemia/reperfusion injury.24

The report of the presence of autoantibodies against cardiac TnI in patients with acute coronary syndrome7 points to an early induction of an autoimmune response to cardiac TnI in these patients. The results we obtained from our study suggest that these patients may have a higher risk of heart failure due to inflammation in the myocardium. Thus, the titer of autoantibodies against cardiac TnI may be an additional risk indicator for heart failure. These autoimmune responses may also explain the discrepancy observed in some patients with involvement of 1 or 2 coronary arteries but diffuse hypokinesia of the whole-heart sections. Furthermore, the role of autoimmune cardiomyopathies in heart failure has been supported by many clinical studies demonstrating that by removing immunoglobulins by immunoadsorption, the ejection fraction can be improved and the morbidity can be reduced in patients with DCM.25–27 Our results (induction of an immune response to TnI results in severe inflammation, fibrosis, impaired heart function, and increased mortality) combined with the findings of Okazaki et al16 (TnI antibodies induce dilation by chronic stimulation of Ca2+ channels of cardiomyocytes) demonstrate an important effect for inducing an autoimmune response against TnI, the preferred marker of myocardial damage for classification and risk stratification in acute coronary syndrome,28 in heart failure. These findings may aid in developing new approaches to the early treatment of heart failure in some patients and initiating further (clinical) studies to investigate the role of TnI release during acute cardiac damage in postinfarct remodeling and its role in heart failure.

Acknowledgments

The authors thank Özay Kaya, Theresa Trettter, Jan Torzewski, Jin Li, and Simone Höger for critically reading the manuscript and Frank Autschbach for technical assistance in histopathologic evaluation.

Sources of Funding

This work was supported by the Ernst und Berta Grimmeke Stiftung (Dr Kaya), by the Deutschen Forschungsgemeinschaft, research grants KA 1797/3-1 (Dr Kaya) and SFB 612 (Dr Pfitzer), and in part by National Institutes of Health, research grants HL077611 and HL067290 (Dr Rose).

Disclosures

Hugo A. Katus developed the TnT assay and holds a patent on this assay jointly with Roche Diagnostics. The other authors report no conflicts.

References


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_Circulation._ 2006;114:1693-1702; originally published online October 2, 2006; doi: 10.1161/CIRCULATIONAHA.106.635664

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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