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Autoantibodies against cardiac troponin I in patients presenting with myocarditis

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Summary
Background: Autoantibodies against cardiac troponin I (cTnI) play an important role in the pathogenesis of experimental cardiomyopathy. We developed a new method to measure anti-cardiac troponin I autoantibody (Anti-cTnIAAB) in patients with myocarditis with or without HCV infection.
Methods: Patients with heart failure for up to 2 years, without a distinct cause, were enrolled in the Myocarditis Treatment Trial between 1986 and 1990. Frozen blood samples were available from 1315 to 2233 enrolled patients. Anti-cTnIAAB was determined by a two-step immunoassay.
Results: The mean (±SEM) value of serum Anti-cTnIAAB titer in the 1315 patients was 0.067 ± 0.003 arbitrary unit (AU), significantly higher than in 1115 healthy volunteers (0.053 ± 0.002 AU, $P < 0.01$). The mean Anti-cTnIAAB titer in 88 patients whose endomyocardial biopsies (EMB) satisfied the diagnostic Dallas criteria was 0.086 ± 0.010 AU, versus 0.066 ± 0.004 AU in 1227 patients whose EMB did not satisfy these criteria. The mean Anti-cTnIAAB in both groups was significantly higher than that measured in the healthy volunteers ($P < 0.01$). The mean Anti-cTnIAAB titer in the 88 patients with Dallas criteria-confirmed myocarditis tended to be higher than in the other 1227 patients. Among the 88 patients with Dallas criteria-confirmed myocarditis, the mean Anti-cTnIAAB titer in 5 patients infected with the hepatitis C virus infection (HCV) was significantly higher (0.146 ± 0.047 AU) than in 83 patients without HCV infection (0.082 ± 0.010 AU, $P < 0.05$).

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Conclusions: Elevated autoantibody titers against cTnI were detected in patients with myocarditis, and were higher in HCV-infected patients. The presence of Anti-cTnIAAB might correlate with inflammation and viral infection of the heart.

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Introduction

There is much evidence to support the argument that some forms of human myocarditis and dilated cardiomyopathy (DCM) are the result of a pathogenic autoimmune response. The first line of evidence is the detection of heart-specific antibodies with demonstrated functional effects in a high proportion of patients suffering from these disorders. These antibodies may be present before the onset of DCM, and predict the evolution of disease from the perspective of myocardial function [1]. In addition, the depletion of heart-specific antibodies by extracorporeal immunoadsorption attenuates the disease manifestations in some patients, often for long periods of time [2]. Finally, clinical investigations have shown a benefit conferred by immunosuppression in a subpopulation of patients with DCM, while, in animals, autoimmune myocarditis was induced by viral infection, an autoimmune response that can be duplicated by immunization with cardiac myosin, a well-characterized antigen.

This evidence suggests that some forms of DCM and myocarditis are caused by pathogenic autoimmune responses, representing the final common pathogenetic pathway of various infectious and non-infectious injuries [1]. In a recent study, we showed that mice lacking the T-cell receptor costimulatory molecule PD-1 spontaneously developed autoimmune DCM, along with the production of high autoantibody titers against cardiac troponin I (Anti-cTnI AAB) [3], and that the passive transfer of monoclonal antibodies to cTnI induced myocardial dysfunction [4]. In this study, we developed a new Anti-cTnI AAB detection method, which we applied in a large population of patients with myocarditis and heart failure.

Methods

The design of the trial, methods of random treatment assignments, patient characteristics, and histopathologic techniques used to diagnose myocarditis have been described previously [5,6]. Briefly, consenting patients were eligible to enter the trial if they had (1) suffered from heart failure of undetermined etiology for up to 2 years, (2) a <45% left ventricular ejection fraction by radionuclide left ventriculography, (3) myocarditis proven by endomyocardial biopsy (EMB), and (4) no contraindications to immunosuppressive therapy. EMB was performed by standard techniques according to local institutional practices, and the specimens were fixed in formalin before being processed, sectioned, and stained with hematoxylin and eosin. The initial histopathologic diagnosis of myocarditis was made at each enrolling institution. The EMB samples were reviewed by a panel of seven expert pathologists, and the diagnosis of myocarditis verified on the basis of the Dallas criteria [7]. The Institutional Review Boards of each enrolling medical center reviewed and approved the study protocol, and all study participants granted their consent to the investigative use of all EMB materials and blood specimens.

Among the 2233 patients enrolled in the trial, 111 who satisfied the Dallas criteria were ultimately randomly assigned to a treatment regimen. Frozen blood samples from 1314 enrollees were used for the investigations in this report.

Cloning of human cardiac troponin I and expression of recombinant cardiac troponin I

Human cardiac troponin I (cTnI) cDNA was obtained by reverse transcriptase—polymerase chain reaction (PCR) from total RNA of human heart (Clontech Laboratories Inc., Mountain View, CA).

PCR was carried out using 5′-GACGGATCCATGGCCGATGGTAGCAGCGATGCGG-3′ as the sense primer and 5′-GACGAGCTCTCAGCTCTCAAACTTTTTCTTGCGG-3′ as the antisense primer for cTnI cDNA. The cTnI cDNA encoded amino acids 1—210 and was constructed with a 6xHis tag at the N-terminus. The cTnI cDNA was subcloned into the pQE-30 plasmid (Qiagen K.K., Tokyo, Japan) to yield the pQE-30/cTnI vector. *Escherichia coli* XL-blue (Toyobo, Tokyo, Japan) bearing the pQE-30/cTnI plasmid was cultured at 37 °C in Terrific Broth medium containing ampicillin at a concentration of 50 mg/l. The expression of recombinant cTnI was induced with 1 mM isopropyl thiogalactopyranoside, and the cells were harvested by centrifugation 5 h later. The cells were suspended in phosphate buffer (25 mM sodium phosphate and 0.5 M NaCl, at pH 8.0) and disrupted by sonication. The insoluble fraction was pelleted by centrifugation at 30,000*g* for 30 min at 4 °C, and the pellet was dissolved in phosphate buffer at pH 8.0, which contained 6 M guanidine chloride, followed by sonication. The guanidine-soluble fraction was centrifuged at 30,000*g* for 30 min at 4 °C, and the supernatant was loaded onto a nickel—nitrilotriacetic acid (Ni—NTA) agarose column (Qiagen). The recombinant protein was eluted with Tris buffer (20 mM Tris—HCl, pH 7.5) containing 7 M urea and 0.2 M imidazole, and the eluate was then applied onto a DEAE Sepharose column. The unbound fraction was loaded onto a CM Sepharose column and recombinant cTnI protein was eluted with a linear gradient of 0—0.5 M NaCl.

The partial length of cTnI cDNA (middle-cTnI), corresponding to amino acids 41—110 of cTnI, was obtained by PCR using: 5′-GACGGATCCATCTCCGCCTCGAGAAAATTGCAGC-3′ as the sense primer and 5′-GACGAATTCTCAGTGATGGTGATGGTGATGCTCTTCATCCACCTTGTCCACACGG-3′ as the antisense primer for middle-cTnI cDNA. The middle-cTnI cDNA was constructed with a 6xHis tag at the C-terminus and subcloned into the pGEX-2T plasmid (Qiagen) to yield the pGEX-2T/middle-cTnI vectors. *E. coli* JM-109 (Toyobo) bearing the pGEX-2T/middle-cTnI plasmid was cultured

and expression was induced in TB medium as described earlier. The purity of purified recombinant c-TnI and middle-cTnI, subjected to SDS—PAGE and visualized by Coomassie brilliant blue staining, was ascertained by gel scanning, using the Intelligent Quantifier system (Bio Image Systems Inc., Jackson, MI), as described previously [8].

Identification of autoantibody against cardiac troponin I

The anti-cTnI autoantibody (Anti-cTnIAAB) was identified by a two-step immunoassay. In a first step, recombinant cTnI (0.5 μg/well) was first coated in a microtiter plate (Nunc™ Immuno Plate II) by overnight incubation at 4 °C. The wells were then blocked for 2 h with 200 μl of phosphate buffer solution (PBS) containing 30 g/l of bovine serum albumin (BSA), at room temperature. After washing of the plate with 200 μl of PBS containing 1 g/l of Tween 20, 100 μl serum samples (1:10) diluted with PBS containing 1 g/l of Tween 20 were added and incubated overnight at 4 °C. After the plate had been washed five times, 100 μl of glycine buffer (10 mM glycine—HCl at pH 2.7) was added to each well, and 50 μl from each well was transferred and mixed with 50 μl of PBS at pH 8.0, containing 0.2% BSA and 0.2% Tween 20 in the well of another plate (IgGs solution). In a second step, recombinant middle-cTnI (0.5 μg/well) was coated in a microtiter plate by overnight incubation at 4 °C. The wells were then blocked for 2 h, at room temperature, with 200 μl of PBS containing 30 g/l of BSA. After washing of the plate with 200 μl of PBS containing 1 g/l Tween 20, 100 μl of IgGs solution was added and incubated for 2 h at room temperature. After the plate had been washed five times, 100 μl of horseradish peroxidase-labeled sheep anti-human IgG antibody (1:20,000) was added to each well and incubated for 1 h at room temperature. After the plate was washed, 100 μl of substrate solution (50 mM citrate—phosphate buffer at pH 5.0) containing 0.4 g/l o-phenylenediamine dihydrochloride and 0.15 ml/l H_2O_2 were added to each well. The reaction was stopped after 30 min by the addition of 50 μl of 4 mol/l H_2SO_4. The absorbance was measured at 492 nm with a microplate reader. Serum Anti-cTnIAAB titers are expressed as arbitrary units (AU) [8].

Statistical analysis

Between-groups differences were examined by Student's *t*-test. Other comparisons were made by one-way analysis of variance, with multiple comparisons tested by Fisher's least significant difference. All values are presented as means ± standard error of the mean (SEM). Differences were considered statistically significant when *P* was <0.05.

Results

The mean (±SEM) titer of serum Anti-cTnIAAB (0.067 ± 0.003 AU) measured in the 1315 patients enrolled in the Myocarditis Treatment Trial was significantly higher than that measured in 1115 healthy volunteers (0.053 ± 0.002 AU, $P < 0.01$; Fig. 1). The mean titer of Anti-cTnIAAB in 88 patients whose EMB satisfied the Dallas criteria was

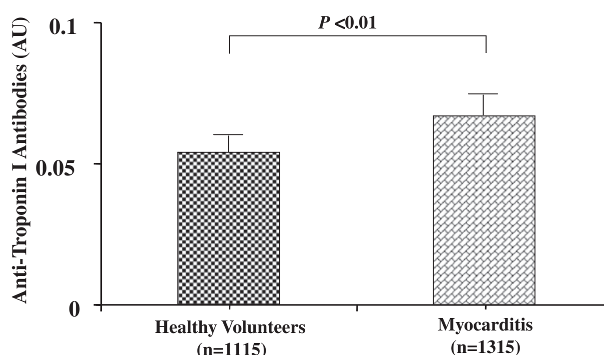

Figure 1 Anti-troponin I antibody in patients with myocarditis versus healthy volunteers.

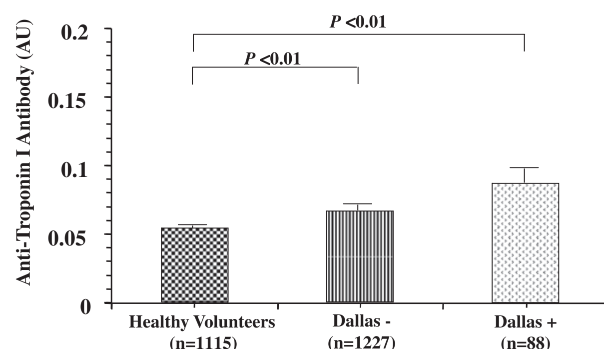

Figure 2 Anti-troponin I antibody titers in healthy volunteers, and in patients whose endomyocardial biopsies satisfied (Dallas +) versus patients whose biopsies did not satisfy (Dallas −) the Dallas diagnostic criteria for myocarditis.

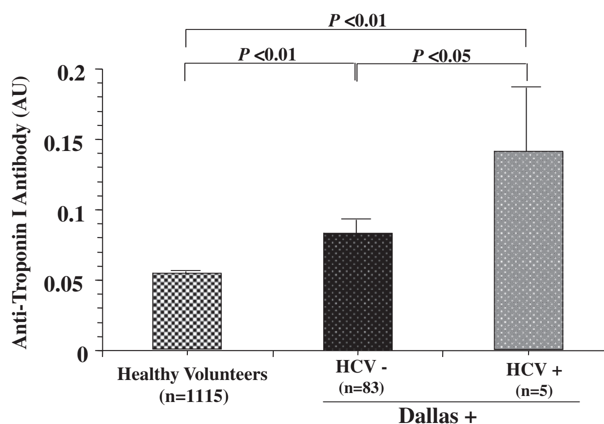

Figure 3 Anti-troponin I antibody titers in healthy volunteers, and in patients whose endomyocardial biopsies satisfied the Dallas diagnostic criteria (Dallas +), with (HCV +) versus without (HCV −) hepatitis C virus infection.

0.086 ± 0.010 AU, versus 0.066 ± 0.004 AU in 1227 patients whose EMB did not satisfy the Dallas criteria. Both values were significantly higher than that measured in healthy volunteers ($P < 0.01$; Fig. 2). The elevated levels of

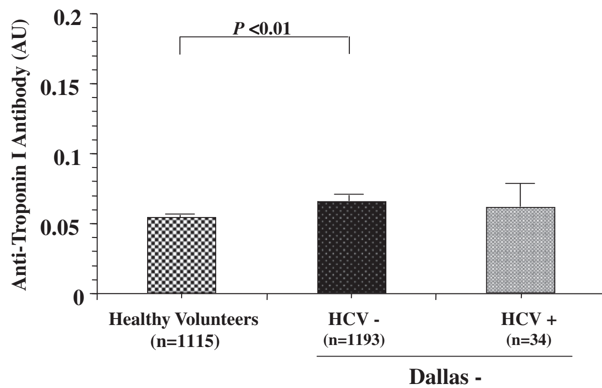

Figure 4 Anti-troponin I antibody titers in healthy volunteers, and in patients whose endomyocardial biopsies did not satisfy the Dallas diagnostic criteria (Dallas −), with (HCV +) versus without (HCV −) hepatitis C virus infection.

anti-troponin I antibody observed in patients whose EMB satisfied the Dallas criteria tended to be higher than those measured in patients whose EMB did not satisfy the Dallas criteria, though the difference did not reach statistical significance (Fig. 2).

Among 88 patients whose EMB satisfied the Dallas criteria, five were infected with the hepatitis C virus (HCV). These five patients had significantly higher Anti-cTnIAAB titers (0.146 ± 0.047) than the 83 patients not infected with the HCV and whose EMB satisfied the Dallas criteria (0.082 ± 0.010, $P < 0.05$; Fig. 3). However, there was no significant difference in Anti-cTnIAAB titers between patients with versus without HCV infection whose EMB did not satisfy the Dallas criteria (Fig. 4).

Discussion

Anti-cardiac antibodies have been observed in patients with myocarditis and DCM [9,10]. Several immunofluorescent patterns have been described, including fibrillary, sarcolemmal, cytoplasmic and others. The presence of anti-fibrillary antibodies suggests that cardiac myofibrils might be a prominent antigen in myocarditis [10]. An antibody to cardiac myosin has often been observed in patients with DCM [11], and alpha and beta myosin heavy chain isoforms both react with that autoantibody [12,13]. Antibodies to the mitochondrial ADP/ATP translocator or β_1-adrenoceptor, often present in patients with DCM, enhance the Ca^{2+} current [14—16]. We have shown that dihydropyridine-insensitive Ca^{2+}-permeable cation channels, but not voltage-gated Ca^{2+} channels, are activated by anti-cardiac antibodies associated with myocarditis in ventricular myocytes. It is likely that the excess Ca^{2+} entry, caused by the activation of this new channel by disease-associated antibodies, is involved in the pathogenesis of myocarditis and DCM [17].

We have recently discovered that mice lacking the T-cell receptor costimulatory molecule PD-1 spontaneously develop autoimmune DCM, along with the production of high titers of antibodies to cTnI. The passive transfer of monoclonal antibodies to cTnI induces myocardial dysfunction in mice [4]. Our experimental observations support the hypothesis of an interaction between cTnI-specific antibodies and cTnI on the surface of cardiomyocytes, enhancing the Ca^{2+} current and, eventually, causing cardiac dysfunction and dilation. However, we cannot exclude the possibility that antibodies to cTnI penetrate the plasma membrane and recognize cTnI in sarcomeres. Our study provided the first direct evidence of an implication of autoimmunity, autoantibodies in particular, in the development of DCM in an animal model.

In the present study, we developed a new method to detect autoantibodies against cTnI, and we measured these autoantibodies in patients with myocarditis. Myocarditis, defined by the Dallas criteria as ''the presence of an inflammatory infiltrate in the myocardium with necrosis and/or degeneration of adjacent myocytes'' remains an etiologic dilemma and a therapeutic challenge [7]. Different microorganisms can cause the same pathologic manifestations, although they mandate different therapies [18]. Several microorganisms have been identified as possible pathogens, including enteroviruses, adenoviruses and HCV [19—23]. We detected higher Anti-cTnIAAB titers in patients with myocarditis whose EMB satisfied the Dallas criteria than in those whose EMB was negative. Furthermore, among those whose EMB satisfied the Dallas criteria, those in whom an anti-HCV antibody was detected had higher titers than those who were not infected with the HCV. Our study showed that Anti-cTnIAAB is often present in patients with active myocarditis, suggesting that their presence correlates with ongoing inflammation.

In our previous studies, we found that HCV infection is often associated with myocarditis [20—23], and that myocardial injury is particularly severe in patients infected with the HCV. In this study, the Anti-cTnIAAB titers were higher in patients with, than patients without, HCV infection. One might hypothesize that infection with the HCV increases the production of Anti-cTnIAAB and further increases the severity of myocardial injury. Therefore, the detection of Anti-cTnIAAB might be helpful in the diagnosis, as well as the evaluation and follow-up, of patients presenting with active myocarditis.

Chronic heart failure patients have been reported to have elevated circulating concentrations of bacterial endotoxin with significant activation of immune cells [24,25], and it is known that bacterial endotoxin stimulates many immunocompetent cells including B lymphocytes [26].

We have recently demonstrated that leukocytes, especially macrophages are the major target of HCV infection [23]. It has been shown that HCV infection can enhance expression of toll-like receptors (TLRs) that are critical to promote innate immune responses [27]. Thus, HCV infection may activate B lymphocytes through up-regulations of TLRs, and increase Anti-cTnIAAB.

In experimental studies, Anti-cTnIAAB was detected from the subacute to chronic stage after infection with coxsackie virus B3 in mice [28]. Immunization with recombinant murine cTnI caused severe inflammation of the myocardium with increased expression of inflammatory chemokines, and the inflammation was followed by cardiomegaly, fibrosis, reduced fractional shortening, and significant mortality. In contrast, mice immunized with murine cardiac troponin T showed little or no inflammation and no death [29]. Furthermore, troponin I-specific T cells induced inflammation and fibrosis in mice [30].

Recently, Anti-cTnIAAB has been shown in normal blood donors [31], and absence of Anti-cTnIAAB was associated with improvement of left ventricular function after acute myocardial infarction [32].

Recent observations suggest that immunoadsorption with a view to removing circulating autoantibodies confers therapeutic benefits [33]. In these clinical experiments, patients with idiopathic DCM underwent extracorporeal adsorption of immunoglobulin by an anti-IgG column. It was further observed that immunosuppression in patients with active myocarditis was most likely to improve cardiac function when circulating cardiac autoantibodies were present [30]. Additional studies might show a benefit conferred by the immunoadsorption of Anti-cTnIAAB, or by immunosuppression to inhibit their production, in patients in whom these antibodies are detected.

Measurement of Anti-cTnIAAB is a simple and specific tool to detect inflammation in the heart, and is suitable for population-based study. Modulation of the production of Anti-cTnIAAB may prevent the development of myocarditis, and could be a cost-effective approach to address this increasing disease entity.

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References

[1] Rose NR. The significance of autoimmunity in myocarditis. In: Schultheiss H-P, Kappa J-F, Grötzbach G, editors. Chronic viral and inflammatory cardiomyopathy. Berlin: Springer-Varlag; 2006. p. 141—5.

[2] Felix SB, Staudt A, Friedrich GB. Improvement of cardiac function after immunoadsorption in patients with dilated cardiomyopathy. Autoimmunity 2001;34:211—5.

[3] Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor deficient mice. Science 2001;291:319—22.

[4] Okazaki T, Tanaka Y, Nishio R, Mitsuiye T, Mizoguchi A, Wang J, et al. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. Nat Med 2003;9:1477—83.

[5] Mason JW, O'Connell JB, Herskowitz A, Rose NR, McManus BM, Billingham ME, et al. A clinical trial of immunosuppressive therapy for myocarditis. The Myocarditis Treatment Trial Investigators. N Engl J Med 1995;333:269—75.

[6] Hahn EA, Hartz VL, Moon TE, O'Connell JB, Herskowitz A, McManus BM, et al. The Myocarditis Treatment Trial: design, methods and patients enrollment. Eur Heart J 1995(Suppl. O): 162—7.

[7] Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio Jr JJ, et al. Myocarditis. A histopathologic definition and classification. Am J Cardiovasc Pathol 1987;1:3—14.

[8] Ishihara M, Kujiraoka T, Iwasaki T, Nagano M, Takano M, Isuji M, et al. A sandwich enzyme-linked immunosorbent assay for human plasma apolipoprotein A—V concentration. J Lipid Res 2005;46:2015—22.

[9] Maisch B, Deeg P, Liebau G, Kochsiek K. Diagnostic relevance of humoral and cytotoxic immune reactions in primary and secondary dilated cardiomyopathy. Am J Cardiol 1983;52:1072—8.

[10] Neumann DA, Burek CL, Baughman KL, Rose NR, Herskowitz A. Circulating heart-reactive antibodies in patients with myocarditis or cardiomyopathy. J Am Coll Cardiol 1990;16:839—46.

[11] Konstadoulakis MM, Kroumbouzou H, Tsiamis E, Trikas A, Toutouzas P. Clinical significance of antibodies against tropomyosin, actin and myosin in patients with dilated cardiomyopathy. J Clin Lab Immunol 1993;40:61—7.

[12] Caforio AL, Grazzini M, Mann JM, Keeling PJ, Bottazzo GF, McKenna WJ, et al. Identification of alpha- and beta-cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy. Circulation 1992;85:173—1742.

[13] Warraich RS, Dunn MJ, Yacoub MH. Subclass specificity of autoantibodies against myosin in patients with idiopathic dilated cardiomyopathy: pro-inflammatory antibodies in DCM patients. Biochem Biophys Res Commun 1999;259:255—61.

[14] Schultheiss HP, Schulze K, Kuhl U, Ulrich G, Klingenberg M. The ADP/ATP carrier as a mitochondrial auto-antigen-facts and perspectives. Ann NY Acad Sci 1986;488:44—64.

[15] Schultheiss HP, Kuhl U, Janda I, Melzner B, Ulrich G, Morad M. Antibody-mediated enhancement of calcium permeability in cardiac myocytes. J Exp Med 1988;168:2105—19.

[16] Staudt A, Mobini R, Fu M, Grosse Y, Stangl V, Stangl K, et al. β(1)-Adrenoceptor antibodies induce positive inotropic response in isolated cardiomyocytes. Eur J Pharmacol 2001;423:115—9.

[17] Tominaga M, Matsumori A, Horie M, Yoshida H, Okada Y. Activation of Ca-permeable cation channels by myocarditis-associated antibody in guinea pig ventricular myocytes. J Clin Invest 1993;91:1231—4.

[18] Feldman AM, McNamara D. Myocarditis. N Engl J Med 2000;343:1388—98.

[19] Towbin JA, Bowles KR, Bowles NE. Etiologies of cardiomyopathy and heart failure. Nat Med 1999;5:266—7.

[20] Matsumori A. Hepatitis C virus infection and cardiomyopathies. Circ Res 2005;96:144—7.

[21] Matsumori A, Shimada T, Chapman NM, Tracy SM, Mason JW. Myocarditis and heart failure associated with hepatitis C virus infection. J Card Fail 2006;12:293—8.

[22] Matsumori A. Global alert and response network for hepatitis C virus derived heart diseases. A call to action. CVD Prev Control 2009;4:109—18.

[23] Matsumori A, Shimada M, Obata T. Leukocytes are the major target of hepatitis C virus infection: Possible mechanism of multiorgan involvement including the heart. CVD Prev Control 2010;5:51—8.

[24] Niebauer J, Volk HD, Kemp M, Dominguez M, Schumann RR, Rauchhaus M, et al. Endotoxin and immune activation in chronic heart failure: a prospective cohort study. Lancet 1999;353:1838—42.

[25] Anker SD, Egerer KR, Volk HD, Kox WJ, Poole-Wilson PA, Coats AJ. Elevated soluble CD14 receptors and altered cytokines in chronic heart failure. Am J Cardiol 1997;79:1426—30.

[26] Roitt IM, Brostoff J, Make D. Immunology. New York: Gower Medical Publishing; 1989, p. 2—9.

[27] Riordan SM, Skinner NA, Kutovic J, Locamini S, Mclver CJ, Williams R, et al. Toll-like receptor expression in chronic hepatitic C: correlation with pro-inflammatory cytokine levels and liver injury. Inflamm Res 2006;55:279—85.

[28] Latva-Hirvela J, Kyto V, Saraste A, Eriksson S, Vuorinen T, Pettersson K, et al. Development of troponin autoantibodies in experimental coxsackievirus B3 myocarditis. Eur J Clin Invest 2009;39:457—62.

[29] Goser S, Andrassy M, Buss SJ, Leuschner F, Volz CH, Otti R, et al. Caediac troponin I but not cardiac troponin T induces

severe autoimmune inflammation in the myocardium. Circulation 2006;114:1693—702.
[30] Kaya Z, Goser S, Buss SJ, Leuschner F, Otti R, Li J, et al. Identification of cardiac troponin I sequence motifs leading to heart failure by induction of myocardial inflammation and fibrosis. Circulation 2008;118:2063—72.
[31] Adamczyk M, Brashear RJ, Mattingly PG. Circulating cardiac troponin-I autoantibodies in human plasma and serum. Ann NY Acad Sci 2009;1173:67—74.
[32] Leuschner F, Li J, Goser S, Reinhardt L, Otti R, Bride P, et al. Absence of auto-antibodies against cardiac troponin I predicts improvement of left ventricular function after acute myocardial infarction. Eur Heart J 2008;29:1049—955.
[33] Frustaci A, Chimenti C, Calabrese F, Pieroni M, Thiene G, Maseri A. Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. Circulation 2003;107: 857—63.