Editorial on the manuscript entitled “Impact of Troponin I-Autoantibodies in Chronic Dilated and Ischemic Cardiomyopathy” by Andreas O. Doesch and co-workers

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Despite advances in medical treatment, the prognosis of heart failure due to left ventricular systolic dysfunction is poor. In many cases in which disease progression cannot be inhibited by pharmacotherapy, heart transplantation remains the only therapeutic option. The most common causes of heart failure due to myocardial diseases are coronary heart disease, valve diseases, and cardiomyopathies [4]. Dilated cardiomyopathy (DCM) is the third most common cause of heart failure and the most frequent reason for heart transplantation [18]. This myocardial disease is characterized by ventricular chamber enlargement and progressive systolic dysfunction. In approximately 20–35% of DCM cases, a genetic background has been reported [18]. In addition, both experimental and clinical data indicate that viral infection and inflammatory processes may be involved in the pathogenesis of DCM [3, 6, 11, 27]. Abnormalities of the cellular immune system are a common feature of myocarditis and DCM. For patients with DCM, immunohistological methods have been introduced for diagnosis of myocardial inflammation. The term “inflammatory cardiomyopathy” has therefore been coined to describe the etiology of acquired cardiomyopathy [18]. Infiltrations with lymphocytes and mononuclear cells, as well as increased expression of cell adhesion molecules, are frequent phenomena in DCM [14, 21, 26]. These findings support the hypothesis that the immune process is still active.

Patients with myocarditis and DCM demonstrate abnormalities of the humoral immune system as well. Furthermore,

various autoantibodies have been detected in DCM patients: these include antibodies against mitochondrial proteins [23], cardiac myosin [2], cardiac β_1 -adrenergic receptors [25], muscarinic receptors [8], and the sarcolemmal Na-K-ATPase [1]. The functional role of cardiac autoantibodies in DCM is under debate. Antibodies may reflect an inflammatory response to myocyte necrosis, thereby representing an epiphenomenon or, alternatively, may play an active role in the disease process. In patients with chronic myocarditis and cardiomyopathy, the prevalence of antibodies against cardiac myosin is associated with the deterioration of cardiac function [15]. Experimental data indicate that certain cardiac autoantibodies may play a functional role in DCM: antibodies against the ADP/ATP carrier interact with the calcium channel and possess cardiotoxic properties [23]. In the plasma of patients with DCM, negative inotropic antibodies are detectable that decrease the calcium transients of isolated cardiomyocytes [7]. A recent study by Li et al. [17] demonstrated that anti-cardiac myosin antibodies induced by immunization of rats with cardiac myosin target the β -adrenergic receptor on the heart cell surface. Passive transfer of purified antibodies from cardiac myosin-immunized rats results in IgG deposition and apoptosis in the heart, leading to a cardiomyopathic heart disease phenotype in recipients.

Immunization of rodents against peptides derived from cardiovascular G-protein receptors induces morphological changes of myocardial tissue resembling DCM [9, 19]. A recent study by Jahns et al. [12] investigated the pathogenic significance of autoantibodies that target cardiac β_1 -adrenoceptors in DCM: rats immunized against the second extracellular loop of cardiac β_1 -receptors developed progressive left-ventricular dilatation and dysfunction. Sera transferred from these immunized animals to unsensitized

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rats induced a similar cardiomyopathic phenotype, thus demonstrating the pathogenic potential of a particular antibody for development of DCM. Further confirmation of the principle that autoantibodies contribute to induction of the disease process and to progression to DCM has been provided in a study by Nishimura et al [20]. The authors of this study showed that mice deficient in the programmed cell death-1 (PD-1) immunoinhibitory coreceptor develop autoimmune DCM with production of high-titer circulating IgG autoantibodies reactive to a 33-kDa protein expressed specifically on the sarcolemma of cardiomyocytes. Okazaki et al. [22] were recently able to identify this antigen as cardiac troponin I (cTNI). Interestingly, transfer of cTNI-specific T cells induces inflammation and fibrosis in wild-type mice [13]. Furthermore, cTNI—but not cardiac troponin T—induces severe inflammation in the myocardium followed by fibrosis and heart failure, with increased mortality in mice [10]. The question arises whether release of cTNI into the circulation after damage or cell death of cardiomyocytes due to various aetiologies will likewise induce the production of auto-antibodies against cardiac troponin I. Interestingly, a recent study investigated the clinical relevance of detected antibodies to cTNI (cTNI-ab) in patients after acute myocardial infarction [16]. Absence of cTNI-ab predicts improvement of left ventricular function after acute myocardial infarction, whereas no significant increase in LVEF was seen in cTNI-ab-positive patients.

These above experimental and clinical data indicate that antibodies against cardiac epitopes may play a causal role in the development of myocardial diseases, rather than being epiphenomena. In this matter, investigations of the prognostic value of circulating TNI-autoantibodies in plasma of patients with chronic heart failure are of particular scientific interest. In this issue of the *Journal*, Doesch et al. [5] present data on the impact of autoantibodies to troponin I (TNI) in chronic dilated and ischemic cardiomyopathy.

The authors tested sera of 249 patients with dilated cardiomyopathy (DCM) and 141 patients with ischemic cardiomyopathy (ICM) for the presence of antibodies to TNI (TNI-ab) by a customized enzyme-linked immunosorbent assay. The primary aim of the study was to ascertain whether there was an association of detection of TNI-ab with the combined end-point of death ($n = 118$, 30.3% of total) or heart transplantation. Subgroup analysis showed a favorable outcome in TNI-ab-positive patients with heart failure if the patients suffered from DCM, whereas TNI-ab status in patients with ICM was not associated with survival. Multivariate analysis in DCM patients showed that a negative TNI-ab serostatus was still an independent risk associated with higher all-cause mortality reaching borderline significance ($p = 0.0492$). From

the data of the present study, the authors deduce that in DCM patients the presence of TNI-ab is associated with improved survival, whereas detection of TNI-ab has no association with prognosis in ischemic cardiomyopathy. The authors conclude that TNI-ab may be protective in DCM.

The study by Doesch et al. [5] clearly contrasts with most experimental and clinical data published until now. As the authors of the present study rightly mention, all experimental data suggest detrimental effects of TNI-ab. Antibodies to cTNI induce heart dysfunction and ventricular dilatation [20]. Subsequent animal studies found that immunization with cTNI, but not troponin T, leads to myocardial inflammation and a DCM-like phenotype [22]. Clinical studies point in the same direction: patients with acute myocardial infarction and cTNI-ab detection had significantly worse LVEF when compared to cTNI-ab-negative patients [16]. Based on these findings, speculation may arise that exposure of cardiac proteins such as myosin or cTNI to the circulation and, subsequently, to the immune system initiates production of cardiac antibodies that induce myocardial damage.

Why does the present study fail to support most of the published data? Is it justified to conclude from the present data that TNI antibodies are unexpectedly protective in DCM? To begin, one potential strength of the study by Doesch et al. [5] is the high number of patients included into this monocentric analysis. However, tempting as it may be, conclusions should not be heedlessly drawn from the present study, e.g., concerning a protective role of antibodies. Additional analysis of cardiovascular mortality may have been appropriate to ascertain potential (cardio)protective effects of cTNI-ab in DCM. The small number of autoantibody-positive patients with ICM and DCM (42 patients with DCM and 31 patients with ICM) may inhibit analysis of the outcome of these subgroups. Association of negative auto-antibody status with all-cause mortality was of borderline significance in multivariate analysis ($p = 0.049$). Moreover, clinical data at baseline differed between the two patient groups. The ICM group was not aged-matched to the DCM group, which impedes the comparison of the two groups. One criterion for inclusion of ICM patients was left-ventricular ejection fraction less than 55%. Some patients of this cohort may therefore have had almost normal LV function although LV function at baseline was not different from patients with DCM. Furthermore, no healthy control group was chosen for comparison of antibody status in patients.

There are some other points that should be considered concerning interpretation of the data. The ELISA method may entail several shortcomings: in estimating an antibody titer, one usually assumes homogeneous binding of the respective antibody to the solid phase, despite reports in

the literature that this may be not true [24]. Determining the optimum cut-off point and the manner of accounting for positive and negative control values are problematic. Moreover, the immunologic response is polyclonal, and at least two epitopes of cTnI can induce myocardial damage in murine models [13]. The exact binding site of the troponin antibodies measured in the present study remains unclear.

Finally, this study does not contain mechanistic data that support the conclusion that TNI-ab are protective in DCM. Hence, as the authors rightly mention, a potential protective role of TNI-ab is still based on speculation. Further studies that provide mechanistic data are required to support the hypothesis that TNI-ab are protective in DCM. Nevertheless, this provocative study challenges the hypothesis that a particular antibody plays a causal pathophysiological role in cardiac dysfunction in DCM. These data require confirmation by both clinical and experimental findings.

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