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Letter to the Editor

Adenine nucleotide translocase 1 expression affects enterovirus infection in human and murine hearts ☆

Uwe Kühl [a,1,2], Linda Ebermann [b,1,2], Dirk Lassner [c,2], Karin Klingel [d,2], Inga Klumpe [a,e,2], Julia Winter [a,e,2], Heinz Zeichhardt [f,2], Heinz-Peter Schultheiss [a,2], Andrea Dörner [a,*,2]

[a] Department of Cardiology & Pneumonology, Charité — Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany
[b] Helmhotz Centre for Infectious Research, Braunschweig, Germany
[c] Institute for Cardiac Diagnostics and Therapy (IKDT), Berlin, Germany
[d] Department of Molecular Pathology, University of Tübingen, Tübingen, Germany
[e] Institute of Biochemistry, Free University Berlin, Berlin, Germany
[f] Department of Virology, Charité — Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany

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To the Editor:

Cardiotropic viral infections are important causative factors of dilated cardiomyopathy (DCM) that is frequently a late result of chronic viral myocarditis [1] and the main reason for heart transplantation. Coxsackievirus B3 (CVB3), a member of the enterovirus (EV) group of Picornaviridae, is one of the best characterized infectious agents associated with viral heart disease [2]. EV persistence is associated with a higher risk of death compared with patients capable of inducing spontaneous virus elimination [3].

The mitochondrial function plays a decisive factor in virus elimination of the infected heart by regulating oxidative stress, apoptosis and necrosis of the infected cardiomyocytes [4]. The adenine nucleotide translocase 1 (ANT1), the most abundant mitochondrial protein, facilitates mitochondrial energy transfer and modulates mitochondrion-related cell death [5]. Several viruses target ANT or ANT binding partners to manipulate apoptosis for their own purposes [6]. We previously found a shift towards an elevated ANT1 transcription especially in hearts of EV-infected patients with clinically suspected myocarditis (csMC) [7]. In addition, this shift is linked to increased myocardial ANT protein that has also been detected in patients with DCM [8]. We therefore assumed that the ANT1 expression is significant for EV infection and its prognosis and analyzed this linkage in EV-infected human and murine hearts.

Thirteen patients with csMC, who showed EV persistence or spontaneous EV elimination, were analyzed for their myocardial transcription patterns by microarray analysis (Human Genome U133 Plus 2.0 GeneChip Array; MAS5 method for normalization) (Table 1, set 1). Viral persistence was defined as the presence of EV in the follow-up endomyocardial biopsy (EMB), and spontaneous elimination was assumed if EV genome was absent in the follow-up EMBs within an individual period of 12–18 months. Six EV-negative patients with dilated or inflammatory cardiomyopathy served as control patients. Four additional controls were persons whose examination led to exclusion of cardiac dysfunction, infection, inflammation, or structural cardiac disease. We additionally determined the ANT1/ANT2 + ANT3 mRNA ratio by the PCR technique described in [7] for the biopsies of 17 patients diagnosed as having DCM with an EV infection (Table 1, set 2). The ANT1/ANT2 + ANT3 mRNA ratio has been shown to correlate with the ANT1 protein expression [8] and is identical to that detectable by microarray technique, making the data of sets 1 and 2 comparable. Exclusion criteria for all patients were coronary heart disease, valvular and hypertensive heart disease, the familiar forms of cardiomyopathies, and antiviral treatment. Diagnostic procedures are described in [7]. The study was approved by the ethics committee of the Charité and performed in accordance with the ethics established in the Declaration of Helsinki. All patients gave their written consent.

Compared to controls and EV-negative patients with cardiomyopathy, EV positive patients with EV elimination showed a reduced ANT1 mRNA level, whereas an increased amount of ANT1 mRNA was linked to myocardial EV persistence (Fig. 1A). No correlation was found between ANT1 mRNA levels and hemodynamic parameters or medication. Performing a Pearson correlation analysis of the microarray data, altered ANT1 transcription was found to be strongly co-regulated with components grouped according to their biological function in Table 2.

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* Corresponding author. Tel.: +49 30 8445 4346; fax: +49 30 8445 4648.
E-mail address: andrea.doerner@charite.de (A. Dörner).

[1] Authors contributed equally to this study.

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Table 1
Clinical parameters of enterovirus (EV)-negative and -positive patients.

	Set 1				Set 2
	No EV infection		EV infection		EV infection
	Controls	Cardio-myopathy	EV elimination	EV persistence	DCM
Number of patients	4	6	5	8	17
Age (years)	54.3 ± 9.0	52.7 ± 11.3	49.2 ± 8.9	52.9 ± 16.4	47.7 ± 12.3
Gender (f/m)	1/3	0/6	1/4	1/7	4/13
Hemodynamic data					
LVEF(%)	68.8 ± 11.5	24.5 ± 7.4**	56.4 ± 11.7	39.2 ± 24.1*;##	34.2 ± 10.6**
LVEDD (mm)	47.5 ± 9.0	66.2 ± 3.4**	55.4 ± 1.7	57.6 ± 10.9	70.3 ± 8.9**
Cardiac inflammation	0	3	2	2	0

(f/m), female/male; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter; and DCM, dilated cardiomyopathy.
* $p < 0.05$.
** $p < 0.01$ compared to controls.
\#\# $p < 0.01$ compared to EV elimination.

Interestingly, 34% of these genes were related to virus infection, primarily affecting viral replication. Compared to the uninfected controls, 14 of the 17 EV-positive DCM patients (set 2) showed an increased ANT1 mRNA proportion, additionally confirming the association between increased ANT1 expression and chronic heart disease based on EV infection (Fig. 1B). Thus, ANT1 is part of a gene program that is inversely expressed in patients who performed spontaneous virus elimination or virus persistence linked to chronic heart disease.

Moreover, we verified the link between ANT1 expression and the course of myocardial EV infection in the murine animal models of acute and chronic CVB3-induced viral myocarditis (VM) that are well known to reflect the conditions of human VM. The study was performed according to that described in [4], whereby the study protocol was approved by the institutional animal care committee. CVB3-infected C57BL/6 mice, which developed acute VM, healed and eliminated CVB3, revealed reduced myocardial ANT protein level during the acute phase of MC 8 days post infection (pi) (Fig. 1C). The ANT level almost normalized during recovery at day 28 pi. In contrast, ANT-specific protein increased early in acute VM in CVB3-infected SWR/J mice shown to develop chronic VM and virus persistence (Fig. 1D). ANT remained elevated in these mice during the chronic phase of VM, corresponding to the findings in human biopsies.

To test in depth the significance of elevated ANT1 expression for EV infection, we infected C57BL/6 mice with heart-specifically overexpressed ANT1 with CVB3 [9]. ANT1 overexpression compensated the decrease in ANT1 expression seen in wild-types during CVB3 infection and led to a 6.5 ± 2.2 fold, $p \leq 0.05$ elevation in replicating CVB3 determined by plaque-forming assay. We also found that ANT1 overexpression influenced the expression of genes listed in Table 2 like actin, myosin, and tropomysin that reimbursed their decline during CVB3 infection. Thus, ANT1 overexpression influenced the expression of genes, which were seen to be co-regulated with ANT1 in human biopsies,

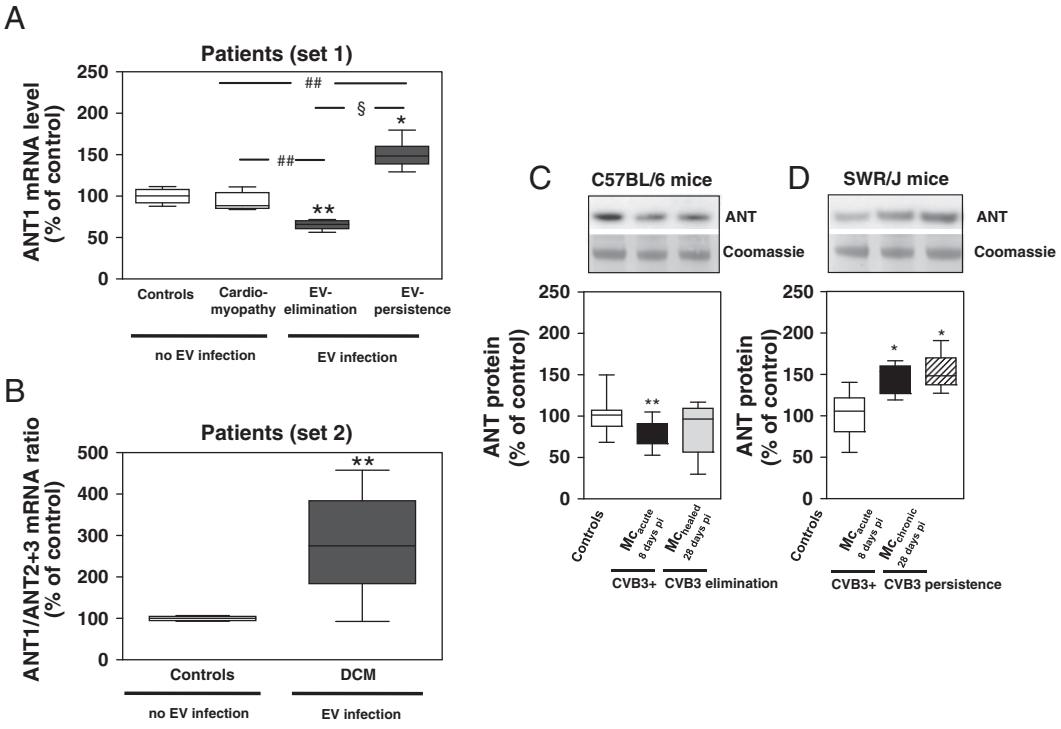

Fig. 1. (A) Myocardial ANT1 mRNA level in EV-infected patients of set 1 detected by microarray analysis and (B) the ANT isoform ratios of EV-positive DCM patients (set 2) determined by PCR [7]. ANT protein was detected by western blot in C57BL/6 (C) and SWR/J mouse hearts (D) (n = 6–8 per group). *$p < 0.05$, **$p < 0.01$ compared to uninfected controls; #$p < 0.01$ vs. patients with cardiomyopathy; and §$p < 0.05$ vs. patients with EV elimination.