3.1 About project Z3 (E)

3.1.1 Title: Characterization of cardiovascular phenotype and experimental and therapeutic approaches in animal models

3.1.2 Principal investigator

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3.2 Project history

Summary

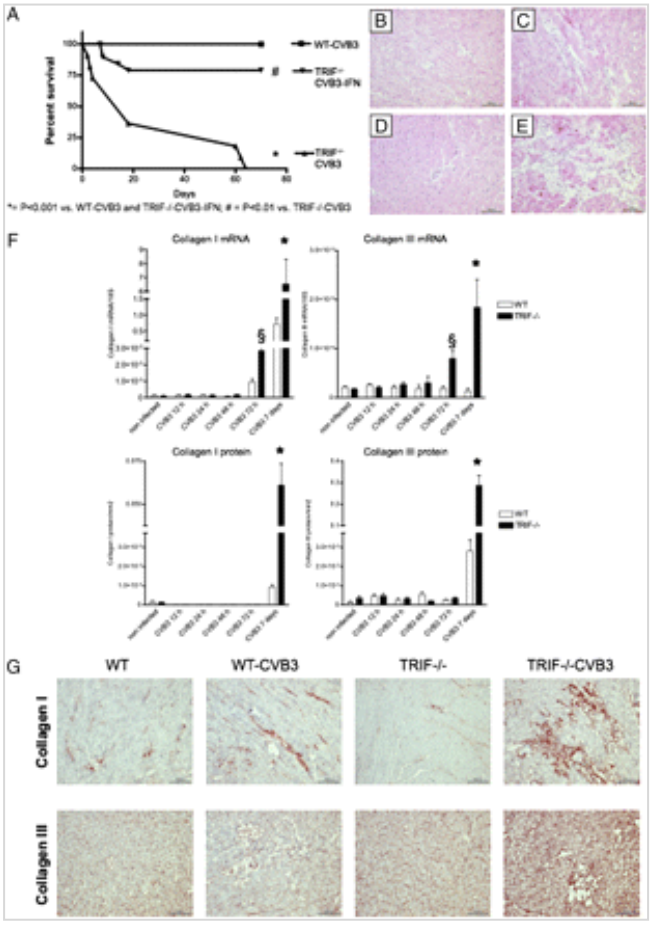
During FP2 the core project Z3 focused on the detailed hemodynamic characterization of cardiac inflammatory animal models, especially the coxsackievirus B3-induced myocarditis model in various knockout mice. The aim of this service project is to get a better and sufficient understanding of the hemodynamic consequences concerning the cardiac inflammatory immune response and their regulation in *in vivo* settings. The scientific focus of the core project Z3 is the establishment of methods for the hemdynamic characterization of cardiac phenotypes, which are offered for all research groups of the SFB-TR19. The hemodynamic characterization of cardiac phenotypes should be measured by this central service project in order to identify valid physiological mechanisms between the different animal settings and models and in order to guarantee a high scientific standard of the hemodynamic characterization of various cardiac phenotypes. This aspect is very significant for the transmissibility of experimental settings when compared to the concrete human situation of the congruent cardiovascular disease (e.g. viral- or autoimmune induced myocarditis). In addition, invasive pressure volume loops recorded by conductance catheter technique measured in real-time the working diagram of the heart. Moreover, different non-invasive and invasive application strategies for therapeutic substances are available are this core project during intervention in animal studies. In detail, oral, subcutaneous, intraperitoneal and intramyocardial application strategies (e.g. adeno-vectors) are well established in various animal models. The characterization of the initial and follow-up cardiac function is well established and used during clinical course of animal settings. As a service project of the SFB-TR19 we contributed to 8 projects and were involved in more than 23 publications. In the FP2 the explicit role of the innate immunity was detailed investigated by using different Toll-like receptor knockout mice. In detail, we were able to show that the cardiac immune response in highly associated with the cardiac inflammation and the regulation of the extracellular matrix. Moreover, the exact physiological role of various matrix metalloproteinases (MMPs) and their regulators were investigated in special knockout mouse models, too.

3.2.1 Report

In the period of the SFB-T1R19, the Z3 core project have been maintained to support different research groups working in the field of inflammatory cardiomyopathy with animal models of viral-induced myocarditis. The Z3 core project was involved in breeding, viral infection, hemodynamic measurements of the left ventricular function and tissue preparation of different mice and knockout mice strains. Moreover, the Z3 core project performed numerous immunological and molecular analyses of animal samples of different studies on mice.

One research topic of this core project was to investigate to the role of Toll-like receptor and their function as inductors of the innate immunty in viral-induced cardiomyopathy. Furthermore, viral

infection of the heart is a major cause of unexpected and sudden death in patients under 40 y of age. The clinical course of viral myocarditis varies from limited cardiac disease to fulminant cardiac injury and severe heart failure leading to increased morbidity and mortality in those patients. The endemic single-stranded coxsackievirus group B serotype 3 (CVB3) is among other things cytopathic for cardiac cells in both humans and mice and can cause severe myocarditis. Intense inflammatory response, including proinflammatory cytokine activation and immune cell infiltration, is often observed under those conditions and are thus suggested to be involved in cardiac damage such as left ventricular (LV) dysfunction and remodeling. Additionally, we and others have shown previously in experimental and clinical settings that the cytokine IFN-β can lead to an elimination of viral genomes and to an improvement of LV function in patients and animals with enteroviral or adenoviral persistence and LV dysfunction. The production of IFN-β, especially during its early activation, is substantially under control of the intracellular TRIF-dependent pathway. TRIF is a part of the innate immune system that acts as an intracellular adaptor molecule of Toll-like receptor (TLR)3 and TLR4. In brief, TLR3 and TLR4 are members of 13 functional mammalian TLRs that have been discovered so far. They are localized subcellulary either on the cell surface or in intracellular vesicular compartments. Although TLRs are expressed at low levels in a large number of cells, including nonimmune cells, the highest expression is generally on immune cells such as macrophages or lymphocytes, indicating a critical role of TLRs in function of those cells, including cytokine production. In the case of TLR4, this receptor has been demonstrated to modulate virus load and cardiac function in viral myocarditis. However, the physiological role of TRIF in cardiac diseases, including viral myocarditis, still remains unclear. In the present study, we investigated whether TRIF affects the development of CVB3-induced myocarditis in a mouse model.



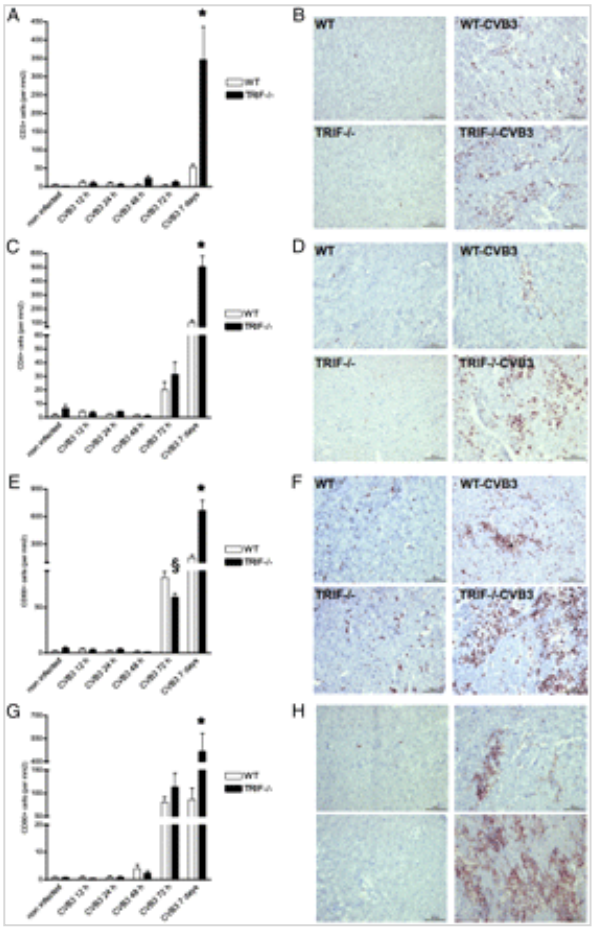


Figure 1: Mortality study, matrix remodelling in TRIF deficient and wildtype mice seven days after viral infection.
Figure 2: Cardiac immune cell infiltration in TRIF deficient and wildtype mice seven days after viral infection.

In summary, this study showed that TRIF is essential in virus control of CVB3 in the heart. Loss of TRIF led to exacerbation of viral replication, heart failure, and mortality in viral myocarditis. Our data suggest that early impaired IFN-β response may contrubute to the consequences of TRIF deficiency seen in CVB3-induced murine myocarditis. To our knowledge, this study provided the first evidence for

TRIF as an essential cardioprotective TLR adaptor in viral myocarditis and anticipates a novel role for the TLR system concerning this disease.

An additional research topic of this core project was to investigated the role of cell therapy strategies in inflammatory cardiomyopathy. Experimental and clinical studies have consistently supported the application of cellular transplantation as a strategy to improve myocardial function. Whereas experimental studies as well as clinical trials have been performed with stem cells for the treatment of myocardial infarction or chronic myocardial ischemia, only few experimental cell-based studies are directed at treating nonischemic cardiomyopathies. We recently isolated and identified novel cardiac-derived cells from human cardiac biopsies: cardiac-derived adherent proliferating cells (CAPs) characterized as $CD105^+$, $CD73^+$, $CD166^+$, $CD44^+$, $CD90^-$, $CD14^-$, $CD34^-$ and $CD45^-$. CAPs have similarities with mesenchymal stromal cells (MSCs), which are known for their anti-apoptotic and immunomodulatory features and have been shown to reduce CVB3-induced and autoimmune myocarditis. MSCs suppress T cell responses induce apoptosis of activated T cells and increase T regulatory cells. As in the case of MSCs, CAPs are low immunogenic, whereas in contrast to MSCs, CAPs do not have a multilineage differentiation potential.

This study explored whether CAPs share these anti-apoptotic and immunomodulatory features with MSCs and whether they are potential agents for the treatment of acute CVB3-induced inflammatory cardiomyopathy. To address potential safety concerns, we first investigated whether CAPs express the Coxsackie- and adenovirus receptor (CAR) and the co-receptor CD55, which are both necessary for effective CVB3 infectivity. Furthermore, we analyzed whether and how CAPs can reduce CVB3-induced HL-1 cardiomyocyte apoptosis, viral progeny release, and T cell activation *in vitro* and whether our findings can be extrapolated into a murine experimental model of acute CVB3-induced myocarditis.

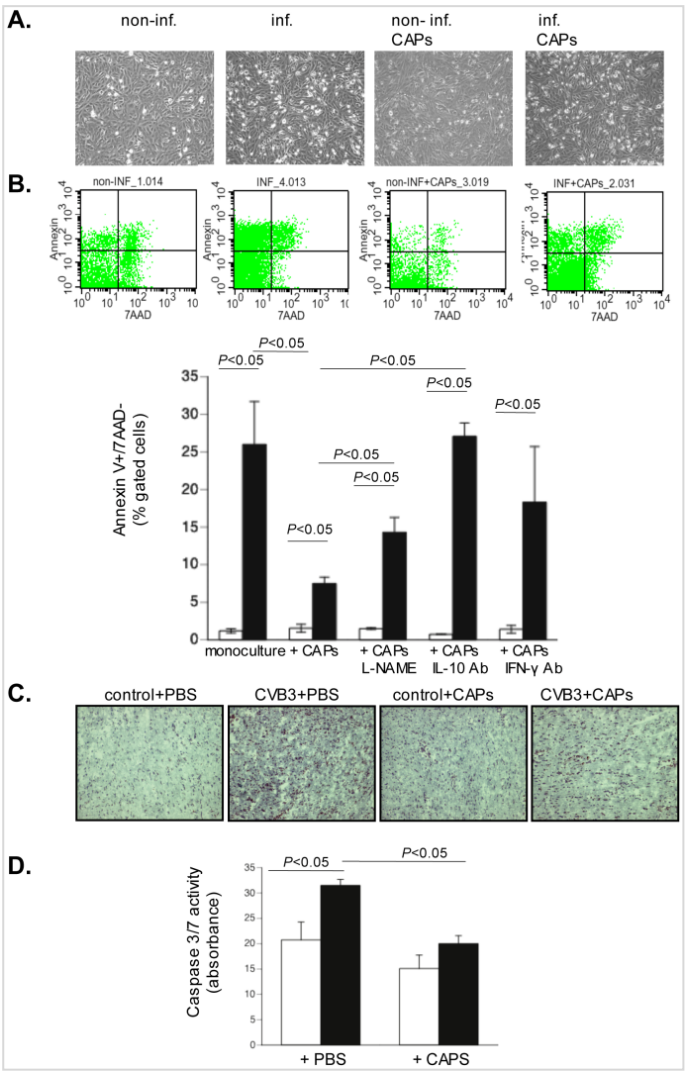


Figure 1: Cardiac adherent proliferating cells reduce Coxsackievirus B3-induced HL-1 apoptosis in a nitric oxide- and interleukin-10-dependent manner and require interferon-γ priming.

3.2.2 Project-related publications of the investigator

1. Becher PM, Lindner D, Miteva K, Savvatis K, Zietsch C, Schmack B, Van Linthout S, Westermann D, Schultheiss HP, **Tschope C**. Role of heart rate reduction in the prevention of experimental heart failure: comparison between If-channel blockade and ß-receptor blockade. **Hypertension**, 2012 ahead of print. Original article

2. Miteva K, Haag M, Peng J, Savvatis K, Becher PM, Seifert M, Warstat K, Westermann D, Ringe J, Sittinger M, Schultheiss HP, **Tschope C***, Van Linthout S*. Human cardiac-derived adherent proliferating cells reduce murine acute coxsackievirus b3-induced myocarditis. * contributed equally. **PLoS One**. *2011;6(12):e28513. Epub 2011 Dec 9*. Original article.

3. Van Linthout S, Savvatis K, Miteva K, Peng J, Ringe J, Warstat K, Schmidt-Lucke C, Sittinger M, Schultheiss HP, **Tschöpe C.** Mesenchymal stem cells improve murine acute coxsackievirus B3-induced myocarditis. **Eur Heart J***; 2011 32:2168-78.* Original article.

4. Westermann, D, Savvatis, K, Lindner, D, Zietsch, C, Becher, PM, Hammer, E, Heimesaat, MM, Bereswill, S, Volker, U, Escher, F, Riad, A, Plendl, J, Klingel, K, Poller, W, Schultheiss, HP, and **Tschope, C,** Reduced Degradation of the Chemokine MCP-3 by Matrix Metalloproteinase-2 Exacerbates Myocardial Inflammation in Experimental Viral Cardiomyopathy. **Circulation,** 2011 124: 2082-2093. Original article.

5. Riad, A, Westermann, D, Zietsch, C, Savvatis, K, Becher, PM, Bereswill, S, Heimesaat, MM, Lettau, O, Lassner, D, Dorner, A, Poller, W, Busch, M, Felix, SB, Schultheiss, HP, and **Tschope, C,** TRIF is a critical survival factor in viral cardiomyopathy. **J Immunol**, 2011 186: 2561-2570. Original article.

6. Riad, A, Westermann, D, Escher, F, Becher, PM, Savvatis, K, Lettau, O, Heimesaat, MM, Bereswill, S, Volk, HD, Schultheiss, HP, and **Tschope, C,** Myeloid differentiation factor-88 contributes to TLR9-mediated modulation of acute coxsackievirus B3-induced myocarditis in vivo. **Am J Physiol Heart Circ Physiol**, 2010 298: H2024-2031. Original article.

7. Knöll R, Kostin S, Klede S, Savvatis K, Klinge L, Stehle I, Gunkel S, Kötter S, Babicz K, Sohns M, Miocic S, Didié M, Knöll G, Zimmermann WH, Thelen P, Bickeböller H, Maier L, Schaper W, Schaper J, Kraft T, **Tschöpe C**, Linke WA, Chien KR. A common MLP (Muscle LIM Protein) variant is associated with cardiomyopathy**.** ***Circ Res****.; 2010, 106:695-704.* Original article.

8. Riad, A, Bien, S, Westermann, D, Becher, PM, Loya, K, Landmesser, U, Kroemer, HK, Schultheiss, HP, and **Tschope, C,** Pretreatment with statin attenuates the cardiotoxicity of Doxorubicin in mice. **Cancer Res**, 2009 69: 695-699. Original article.

9. Westermann, D, Riad, A, Richter, U, Jager, S, Savvatis, K, Schuchardt, M, Bergmann, N, Tolle, M, Nagorsen, D, Gotthardt, M, Schultheiss, HP, and **Tschope, C,** Enhancement of the endothelial NO synthase attenuates experimental diastolic heart failure. **Basic Res Cardiol**, 2009 104: 499-509. Original article.

10. Westermann D, Riad A, Richter U, Jäger S, Savvatis K, Schuchardt M, Bergmann N, Tölle M, Nagorsen D, Gotthardt M, Schultheiss HP, **Tschöpe C**. Enhancement of the endothelial NO synthase attenuates experimental diastolic heart failure. **Basic Res Cardiol**.; 2009, *104:499-509.* Original article.

11. Westermann, D, Kasner, M, Steendijk, P, Spillmann, F, Riad, A, Weitmann, K, Hoffmann, W, Poller, W, Pauschinger, M, Schultheiss, HP, and **Tschope, C,** Role of left ventricular stiffness in heart failure with normal ejection fraction. **Circulation**, 2008 117: 2051-2060. Original article.

12. Westermann, D, Riad, A, Lettau, O, Roks, A, Savvatis, K, Becher, PM, Escher, F, Jan Danser, AH, Schultheiss, HP, and **Tschope, C,** Renin inhibition improves cardiac function and remodeling after myocardial infarction independent of blood pressure. **Hypertension**, 2008 52: 1068-1075. Original article.

3.3 Funding

Funding of the project within the Collaborative Research Centre started July 2004. Funding of the project ended December 2013.

3.3.1 Project staff in the ending funding period

	No.	Name, academic degree, position	Field of research	Department of university or non-university institution	Commitment in hours/week	Category	Funded through:
Available							
Research staff	1	Tschöpe, Carsten, Prof. Dr. med., Project leader	Animal models and heart failure	Dept. of Cardiology and Pneumology, Charité Berlin	42		Charité
Non-research staff							
Requested							
Research staff	2	Becher, Moritz, Dr. med., Postdoc	Animal models and heart failure	Dept. of Cardiology and Pneumology, Charité Berlin	40	Postdoc	
	3	Pappritz, Kathleen, Doctoral student	Animal models and heart failure	Dept. of Cardiology and Pneumology, Charité Berlin	39	Doctoral student	
Non-research staff	3	Orrin, Nadine, Technical assistent		Dept. of Cardiology and Pneumology, Charité Berlin	39	Technical assistent	

Job description of staff (supported through available funds):

1 Prof. Dr. Tschöpe, Carsten, Coordination of the experimental study designs, interpretation and publication of results, communication with the other *CRC* working groups.

Job description of staff (requested):

1 Dr. Becher, Moritz, Postdoc, Coordination of animal studies, immunohistological stainings, animal surgeries

2 Pappritz, Kathleen, Doctoral student, Hemodynamic measurements of animal studies, immunohistological stainings, coordination of animal studies

3 Orrin, Nadine, technical assistant, Hemodynamic measurements of the animal studies, immunohistological stainings