3.1 About project Z4 (E)

3.1.1 Title: Phenotypic, Molecular Pathological, and Immunological Characterization of Murine Inflammatory Cardiomyopathy Models

3.1.2 Principal investigator

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3.2 Project history

Cardiotropic viruses have been implicated as major pathogenetic agents in acute and chronic forms of myocarditis. The most reliable data for a direct role of viral infection of the myocardium leading to myocarditis and dilated cardiomyopathy as long term sequela were derived from enterovirus infections and especially from coxsackievirus B3 (CVB3) myocarditis.
Regarding the aetiopathogenetic mechanisms in viral heart disease, a considerable portion of our knowledge originates from murine models of CVB3-myocarditis. Dependent on the genetic background, susceptible animals such as ABY/SnJ, A/J, ASW/J, SWR/J, Balb/c develop a chronic myocarditis on the basis of virus persistence which may last for several months. However, clearance of the virus which is observed in C57BL/6 mice two weeks post infection (pi), results in the downregulation of the cardiac inflammation by the mediation of anti-inflammatory cytokines such as transforming growth factor beta (TGFβ) and IL-10. Factors which were found to influence the course of myocarditis comprise those of the innate immunity such as TLR expression on NK cells, DCs and macrophages. In addition, components of the adaptive immune response including CD4+ and CD8+ T lymphocytes were found to contribute to the successful elimination of CVB3 from the heart. On the other hand we have shown that ongoing cardiac inflammation in susceptible mice is associated with a considerable cytokine-mediated upregulation of fibrosis-relevant molecules including connective tissue growth factor (CTGF) and osteopontin. These findings explain well the interrelationships between inflammation and the development of fibrosis, cardiac remodelling and heart failure, thus reflecting our observations in patients with inflammatory heart disease.

3.2.1 Report

In the second period of the SFB-TR 19 (8/2008-12/2013), the **Z4** (Klingel) core project was created to support the different groups working on inflammatory cardiomyopathy with animal models on viral myocarditis.

Aim of many projects of the SFB-TR 19 was to further evaluate mechanisms, including different signaling pathways mainly involved in the different regulation of the immune system, which are capable to explain the various outcome of enteroviral myocarditis. In addition, in order to explore novel therapeutic options preventing the consecutive remodelling processes, numerous investigations were performed in immunocompetent or knockout mice which are either susceptible or resistant to chronic CVB3 myocarditis. The task of the core project **Z4** was to provide different model systems of immunocompetent, transgenic, gene-deleted, drug-treated mice being infected with well-defined CVB3 variants. It is absolutely necessary to keep uniformity of the viral and animal parameters in the experiments in order to compare and exchange data within the SFB-TR 19. Some immunocompetent mouse strains such as ABY/SnJ mice were not available in other breeding facilities or companies and thus, many hundreds of these mice had to be bred, infected and kept for many weeks in the animal facility of the Department of Molecular Pathology. At various time points after infection, different organs were investigated by light microscopic techniques. The patterns of inflammatory lesions and fibrosis were evaluated, quantified and interpreted by an experienced scientist. Immunohistology was applied to differentiate and quantify the subsets of inflammatory infiltrates, and molecular techniques

such as radioactive *in situ* hybridization, RT-PCR and plaque assays were used to quantify and localize specific patterns of virus infection especially in the heart. Various radioactively labeled probes were generized capable to localize different mRNAs in the organs and to correlate them with histopathological findings. Data of microarrays regarding the differential expression of mRNA in models systems of acute and chronic myocarditis were allocated for all SFB-TR 19 projects. In addition, in the Z4 project investigations were performed to evaluate whether the different cardiac expression of miRNA in susceptible (e.g.TLR3-deficient, ABY/SnJ) mice and resistant C57BL/6 mice may contribute to chronic myocarditis and the development of fibrosis.
Besides extensive studies on animals, the **Z4** project was also involved in histological and immunohistological as well as virological investigations on human heart tissue for various projects.

3.2.2 Project-related publications of the investigator

Starting from 08/2008 the **Z4** project took active part in **72 publications** including one paper in N Engl J Medicine and three publications in Nature Med/ Immunol/ Comm which are listed here to demonstrate the successful interaction of the **Z4** project with the other SFB-TR19 groups:

1. Ursu ON, Sauter M, Ettischer N, Kandolf R, Klingel K. Heme Oxygenase-1 Mediates Oxidative Stress and Apoptosis in Coxsackievirus B3-Induced Myocarditis. **Cell Physiol Biochem**. 2014; 33(1):52-66.
2. Tank J, Lindner D, Wang X, Stroux A, Gilke L, Gast M, Zietsch C, Skurk C, Scheibenbogen C, Klingel K, Lassner D, Kühl U, Schultheiss HP, Westermann D, Poller W. Single-target RNA interference for the blockade of multiple interacting proinflammatory and profibrotic pathways in cardiac fibroblasts. **J Mol Cell Cardiol**. 2014; 66:141-56.
3. Kühl U, Ebermann L, Lassner D, Klingel K, Klumpe I, Winter J, Zeichhardt H, Schultheiss HP, Dörner A. Adenine nucleotide translocase 1 expression affects enterovirus infection in human and murine hearts. **Int J Cardiol**. 2014 Jan 21 [Epub ahead of print].
4. Schumm J, Greulich S, Wagner A, Grün S, Ong P, Bentz K, Klingel K, Kandolf R, Bruder O, Schneider S, Sechtem U, Mahrholdt H. Cardiovascular magnetic resonance risk stratification in patients with clinically suspected myocarditis**. J Cardiovasc Magn Reson**. 2014; 16(1):14-26.
5. Brunner S, Theiss HD, Leiss M, Grabmaier U, Grabmeier J, Huber B, Vallaster M, Clevert DA, Sauter M, Kandolf R, Rimmbach C, David R, Klingel K, Franz WM. Enhanced stem cell migration mediated by VCAM-1/VLA-4 interaction improves cardiac function in virus-induced dilated cardiomyopathy. **Basic Res Cardiol**. 2013;108(6):388-402. **Klingel and Franz share last-authorship.**
6. Steinke K, Sachse F, Ettischer N, Strutz-Seebohm N, Henrion U, Rohrbeck M, Klosowski R, Wolters D, Brunner S, Franz WM, Pott L, Munoz C, Kandolf R, Schulze-Bahr E, Lang F, Klingel K, Seebohm G. Coxsackievirus B3 modulates cardiac ion channels. **FASEB J**. 2013; 27(10):4108-21. **Klingel and Seebohm share last-authorship.**
7. Lurz P, Eitel I, Klieme B, Luecke C, de Waha S, Desch S, Fuernau G, Klingel K, Kandolf R, Grothoff M, Schuler G, Gutberlet M, Thiele H. The potential additional diagnostic value of assessing for pericardial effusion on cardiac magnetic resonance imaging in patients with suspected myocarditis. **Eur Heart J Cardiovasc Imaging**. 2013 Dec 29. [Epub ahead of print]
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9. Jacoby C, Borg N, Heusch P, Sauter M, Bönner F, Kandolf R, Klingel K, Schrader J, Flögel U. Visualization of immune cell infiltration in experimental viral myocarditis by (19)F MRI in vivo. **MAGMA**. 2013 Jul 4. [Epub ahead of print]
10. Nishtala K, Phong TQ, Steil L, Sauter M, Salazar MG, Kandolf R, Felix SB, Völker U, Klingel K, Hammer E. Proteomic analyses of age related changes in A.BY/SnJ mouse hearts. **Proteome Sci.** 2013; 11(1):29. **Klingel and Hammer share last-authorship.**
11. Seizer P, Klingel K, Stickel J, Dorn C, Horger M, Kandolf R, Bigalke B, May AE, Gawaz M, Schreieck J. Left ventricular site-directed biopsy guided by left ventricular voltage mapping: a proof of principle. **Int J Cardiol**. 2013; 168(3):3113-4.
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3.1.2 Project staff in the ending funding period

Funding of the project within the Collaborative Research Centre started July 2008. Funding of the project ended December 2013.

	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	Karin Klingel, Prof. Dr. med., Adjunct Professor	Molecular pathology	Dept. of Molecular Pathology, UKT*)	6		UKT
	2	Martina Sauter, Dr. med. vet.(DVM)	Molecular pathology	Dept. of Molecular Pathology, UKT	5		UKT
Non-research staff							
Requested							
Research staff	1	Nicole Ettischer, Dr. rer. nat., Postdoc	Molecular pathology	Dept. of Molecular Pathology, UKT	39	Postdoc	
Non-research staff	2	Patrizia Dietze, Medical, MTA		Dept. of Molecular Pathology, UKT	38,5	Medical technical assistent	
	3	Schwandt, Anna, MTA		Dept. of Molecular Pathology, UKT	38,5	Medical technical assistent	
	4	Bundschuh, Sandra, MTA		Dept. of Molecular Pathology, UKT	38,5	Medical technical assistent	

*) University Hospital Tübingen

Job description of staff (supported through available funds):

1. Prof. Dr. med. Karin Klingel. The project leader is adjunct Professor in the Department of Molecular Pathology at the University Hospital Tübingen. Besides her tasks in the diagnosis of heart diseases by histology, immunohistology, electron microscopy, and molecular pathology on more than 2000 endomyocardial biopsies per year she is adjunct lecturer of the Department. Since 25 years she is working in the field of viral myocarditis and has a longstanding experience in experimental model systems of CVB-induced myocarditis. This resulted in more than 230 publications with an impact factor of more than 1000. Her expertises in numerous virological, molecular pathological, immunological and electron microscopic techniques were available for the SFB-TR19. In the Z4 core project she planned and coordinated the activities and surveyed the results acquired for the different projects.

3. Dr. med. vet. Martina Sauter. Dr. Sauter has experience in the murine model of virus myocarditis for more than 16 years. She was responsible for handling, breeding, infection and survey of CVB3-infected immunocompetent and immunodeficient mice.

Job description of staff (requested):

1 Dr. rer. nat. Nicole Ettischer. The task of the Z4 project was to support the SFB-TR19 projects with virological, histological, immunohistological, immunological and molecular pathological investigations in murine models of DCMi. Being a virologist Dr. Ettischer was responsible for generating and titrating CVB3 virions and to perform all experiments which are related to virology. Her main tasks included the performance and analyses of the molecular biological experiments (qRT-PCR, cloning of probes for radioactive *in situ* hybridization, sequencing, miRNA experiments, etc.) and virological investigations (plaque assays, qRT-PCR for the quantification of viral RNA) performed in the Z4 core project. She was responsible for the validation of the established methods and the results in the projects.

2-4 Patrizia Dietze, Anna Schwandt, Sandra Bundschuh (MTA). The Medical technical assistents processed the tissue (paraffin embedding) and performed 5um thick tissue sections of the organs from CVB3-infected mice. This required thousands of slides for the different projects. In addition, they performed all histological stainings of the organs (HE, Masson Trichrom, Picrosirius red etc.) and the majority of the immunohistochemical stainings. They isolated mRNA from the tissues and cells (in vitro experiments) and performed quantitative RT-PCR for the projects. Without the assistents it would not have been possible to run the core project Z4.