Research Focus 1:

miRNAs and myocarditis

Patients have different susceptibility for the development of a myocarditis. This fact can be displayed by various mice strains, which mimic the human disease and respond to the induction of an experimental autoimmune myocarditis (EAM) with diverse levels of sensibility. Our preliminary work revealed variation in the expression profils of some miRs in different mice strains, which could explain the various outcomes. Thus, some of these potential miRs and their targets are now under deeper investigation with regard to their role in the development and pathogenesis of a myocarditis. One interesting target is the protein called PNUTS, which is part of the protein phosphatase 1. Subsequently, the role of PNUTS in this disease will be explored. Therefore, changes in cardial function will be monitored via echocardiography, different immune histological analysis will be performed, the antibody titer will be measured by ELISA and gene regulation will be examined via qPCR.

Research Focus 2:

Role of immunocheckpoints PD-1, PD-L1 and CTLA-4 in the pathogenesis of autoimmune myocarditis and dilated cardiomyopathy.

The inhibition of immunocheckpoints such as PD-1, PD-L1 and CTLA-4 plays an important role in cancer therapy. Monoclonal antibodies are used to block the immunocheckpoints in order to trigger the immune response. However, this often leads to autoimmune reactions, which affect the heart muscle and can lead to myocarditis and dilated cardiomyopathy (DCM). Therefore, this project aims to elucidate the underlying mechanisms of these side effects in order to identify solutions and potential therapeutic approaches. Therefore, the effects of the blockade will be investigated using a myocarditis mouse model. This includes, the effect of PD-1 and CTLA-4 gene knockout on the development and progression of myocarditis, as well as the investigation of chemokine regulation (interferons, interleukins, etc.) in comparison to wild-type mice. This is done by means of histology, immunohistology and echocardiography, investigation of cytokine production by FAC's analysis, determination of antibody titers by ELISA, and gene regulation by qPCR.

Research Focus 3:

Regulatory B cells in the pathogenesis and progression of autoimmune myocarditis.

B cells play an important role in the development and progression of autoimmune diseases. They costimulate T-cells and produce high-affinity pathogenic autoantibodies. In addition, they secrete pro-inflammatory cytokines such as TNF-alpha, IL-6 and IL-1ß, but can also halt the progression of the autoimmune disease by producing anti-inflammatory cytokines such as IL-10 and IL-35. In previous work it was shown that the transfer of T-cells from mice with cardiac troponin-induced myocarditis to non-immunized mice was milder when autologous B-cells were added to the T-cells. This supports the hypothesis of the regulatory function of B cells in the course of the disease. Thus, optimally stimulated B cells could be of therapeutic importance in autoimmune myocarditis (EAM). Therefore, in this study the influence of differently stimulated B-cells on the transmission of experimental autoimmune myocarditis (EAM) by adoptive T-cell transfer will be investigated in detail. Another goal of this work is to elucidate the mode of action of regulatory B cells (Breg) in EAM by ELISA or flow cytological methods, as well as EAM in B-cell deficient mice.

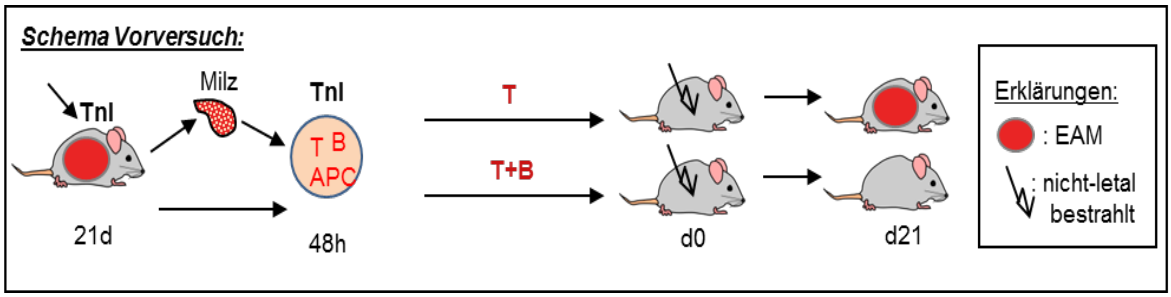

Figure 1: The preliminary experiment shows the effects of co-transfer of T and B cells from TnI-immunized mice spleens to non-lethal irradiated mice.

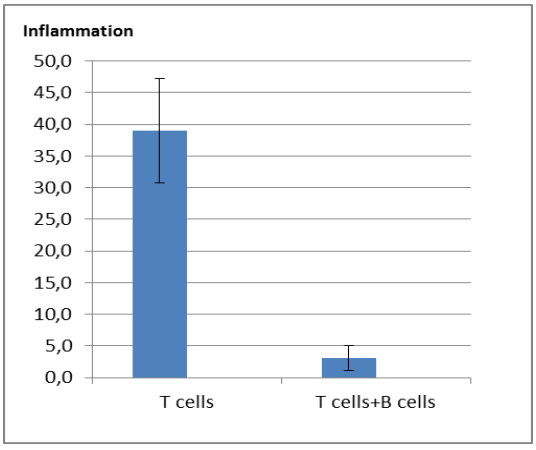

Figure 2: The co-transfer of T- and B-cells with TnI-immunized mice spleens into non-lethal irradiated mice resulted in a significantly reduced inflammation rate compared to mice that received only T-cell transfer.

Research Focus 4:

The role of lactate dehydrogenase B in experimental autoimmune myocarditis (EAM).
Previous studies showed that the lactate dehydrogenase subunit B (LDHB) plays a role in autophagy, apoptosis and ROS production and is thus involved in inflammatory reactions. In previous work a cardioprotective effect of a microRNA specific LNA (MB_1114) could be detected. By RACE PCR the lactate dehydrogenase B subunit was identified as a potential target. Further investigations revealed that MB_1114 induces overexpression of cardiac mLDHB and protects mice from developing experimental autoimmune myocarditis (EAM).
Therefore, this work will investigate the extent to which there is a direct link between the amount of cardiac mLDHB and the ability to develop EAM in different mouse lines. To this end, overexpression of mLDHB in the heart of A/J mice will be induced by AAV9 and the animals will be immunized with TnI. Furthermore, we will investigate whether a comprehensive mLDHB knock-out in C57BL/6 mice leads to the loss of their immunity against EAM and if reactivation by AAV9-mediated LDHB overexpression restores this immunity. This should lead to a better understanding of the immunological processes in EAM and the development of potential targets and therapeutic approaches.

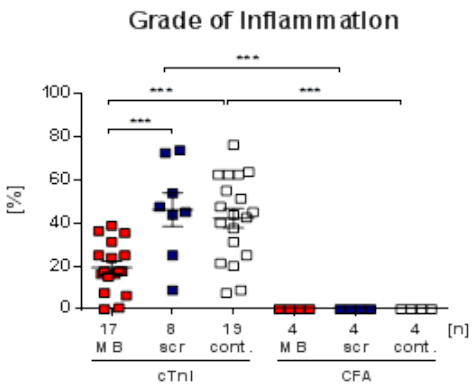

Figure 3: Degree of inflammation of the heart after immunization with cardiac troponin I (cTnI) or CFA (control) in combination with MB_1114 LNA (MB), inactivated MB_1114 (scr) and a TE control (cont.)

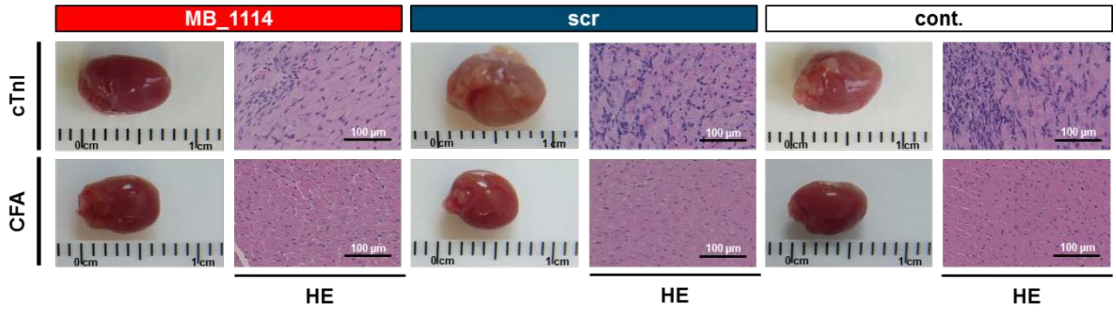

Figure 4: Visualization of the heart muscle and the results of the histological HE staining after immunization with cardiac troponin I (cTnI) or CFA (control) in combination with MB_1114 LNA (MB), inactivated MB_1114 (scr) and a TE control (cont.)