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Inflammation as a therapeutic target in heart failure

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Inflammation als therapeutischer Ansatzpunkt bei Herzinsuffizienz

2. Summary

2.1.1 Abstrakt

Inflammation spielt eine wesentliche Rolle bei der Entstehung einer Herzinsuffizienz, deren Prävalenz stetig ansteigt. Trotz der derzeit verfügbaren effektiven pharmazeutischen Behandlung, welche auf die neurohumorale Aktivierung abzielt, ist die Morbidität und Mortalität der Herzinsuffizienz noch immer erheblich, weshalb neue therapeutische Strategien benötigt werden. Aufgrund der Bedeutung der Inflammation für die Pathogenese der Herzinsuffizienz bestand das Ziel dieser gesamten Studie darin, das anti-inflammatorische Potenzial verschiedener Strategien in zwei akuten Modellen zu untersuchen: Lipopolysaccharid (LPS)- und Coxsackievirus B3 (CVB3)- induzierte Inflammation. Das Potenzial des Lipoproteins hoher Dichte (HDL) und seines wichtigsten Apolipoproteins (Apo), Apo A-I, welche für ihre anti-inflammatorischen Eigenschaften bekannt sind, die LPS-induzierte Inflammation zu reduzieren und deren Einfluss auf das angeborene Immunsystem, insbesondere auf den Toll-like Rezeptor (TLR) 4 Signalweg, wurden in der ersten Studie aufgeklärt. Die zweite und dritte Studie dienten der Evaluation des Potenzials mesenchymaler Stromazellen (MSCs) bzw. der erst kürzlich identifizierten und aus dem Herzen gewonnenen adhärenten proliferierenden Zellen (CAPs), die CVB3-induzierte inflammatorische Kardiomyopathie zu reduzieren. Die Wirkungsweise von MSCs und CAPs, speziell deren immunmodulatorische Effekte, und der Einfluss der inflammatorischen Umgebung auf deren Funktionalität wurden analysiert. Apo A-I Gentransfer vor der Anwendung von LPS reduzierte die TLR4 Antwort in der Lunge. Dies äußerte sich in einer verminderten Infiltration von Neutrophilen in die Lunge, Anzahl an Lungenödemen und verminderter Mortalität. Die *in vitro* Zugabe von HDL oder Apo A-I zu Endothelzellen vor dem Hinzufügen von LPS reduzierte die Expression und Antwort von TLR4 unabhängig von der Bildung von HDL-LPS Komplexen. Sowohl MSCs als auch CAPs benötigten Interferon-γ, um die CVB3-induzierte Apoptose der Kardiomyozyten sowie die Aktivität mononukleärer Zellen (aus der Milz) *in vitro* zu reduzieren. Gleiche Ergebnisse, wie die Reduktion der kardialen Apoptose/Verletzungen als auch der kardialen Aktivität mononukleärer Zellen wurden auch *in vivo* erzielt und führten zusätzlich zur Verbesserung der Herzkontraktilität. Abschließend ist zu sagen, dass die anti-inflammatorischen Effekte des HDLs eine Reduktion

der (endothelialen) TLR4 Expression und Antwort beinhaltet. Weiterhin wurde gezeigt, dass MSCs und die den MSCs ähnlichen CAPs die CVB3-induzierte inflammatorische Kardiomyopathie verbessern. Zudem konnte nachgewiesen werden, dass sowohl MSCs als auch CAPs die inflammatorische Umgebung zur Aktivierung ihrer kardioprotektiven/immunmodulatorischen Effekte benötigen. Weitere Studien in (anderen) Modellen der Herzinsuffizienz sind notwendig, um das Potenzial dieser Strategien zur Behandlung dieser Krankheit abschließend beurteilen zu können.

Inflammation as a therapeutic target in heart failure

2.1.2 Abstract

Inflammation plays a major role in the development of heart failure which prevalence is increasing. Despite the current effective pharmaceutical treatment, which targets the neurohumoral activation, the morbidity and mortality of heart failure is still substantial, urging for the search of novel therapeutical strategies. Given the importance of inflammation in the pathogenesis of heart failure, the aim of this cumulative study was to investigate the potential of different strategies to counteract inflammation in two severe models of inflammation: lipopolyssacharide (LPS)- and Coxsackievirus B3 (CVB3)-induced inflammation, in view of finding a novel anti-inflammatory strategy for the treatment of heart failure. The potential of high-density lipoproteins (HDL) and its main apolipoprotein (apo), apo A-I, which are known for their anti-inflammatory properties, to reduce LPS-induced inflammation and their impact on the innate immune system, on Toll-like receptor (TLR) 4 signalling, were elucidated in the first study. The second and third study were directed at evaluating the potential of mesenchymal stromal cells (MSCs) and the recently identified cardiac-derived adherent proliferating cells (CAPs) to reduce CVB3-induced inflammatory cardiomyopathy, respectively. The mode-of-action of MSCs and CAPs, particularly their immunomodulatory effects, and the impact of the inflammatory environment on their functionality were analyzed. Apo A-I gene transfer preceding LPS administration reduced TLR4 signalling in the lung, which was reflected by an attenuation in lung neutrophil infiltration, lung oedema, and mortality. *In vitro*, supplementation of HDL or apo A–I to endothelial cells prior to LPS reduced TLR4 expression and signalling, independently of the formation of LPS-HDL complexes. MSCs as well as CAPs were able to reduce the CVB3-induced cardiomyocyte apoptosis and activity of (splenic) mononuclear cells *in vitro* for which they required interferon-γ. These findings were *in vivo* translated by a reduction in cardiac apoptosis/damage and mononuclear cell activity and by an improvement in cardiac contractility. In conclusion, we demonstrated that the anti-inflammatory effects of HDL comprise the reduction in (endothelial) TLR4 expression and signalling. Furthermore, we showed that MSCs and the MSC-like CAPs improve CVB3-induced inflammatory cardiomyopathy and that MSCs and CAPs require the inflammatory environment to exert their cardioprotective/immunomodulatory effects. Further studies in (other) models of heart

failure are still required to further assess the potential of those strategies for the treatment of heart failure.

2.2 Introduction

Heart failure is one of the most common causes of morbidity and mortality worlwide and its prevalence is increasing. Experimental and clinical studies have consistently demonstrated that inflammation is a major trigger in the development of heart failure[1, 2]. On the other hand promising results of preliminary studies with e.g. anti-tumor necrosis factor-α therapy, have lacked positive clinical outcomes in large randomized studies with patients with heart failure. These results urge for the development of novel anti-inflammatory strategies that may involve a broader spectrum of inflammatory mediators.

Innate immunity is characterized by a natural selection of germ-line encoded receptors, which focus the host response to highly conserved pathogen associated molecular patterns (PAMPs) shared by many micro-organisms[3,4]. Toll-like receptors (TLRs), a group of PAMP recognition receptors, play an important role in innate immune signalling in response to microbial and viral infection. TLR4 is the main protein involved in recognizing lipopolyssacharide (LPS)[5,6,7] present in Gram-negative bacteria and is of importance in the pathogenesis of different cardiovascular disorders[8,9], including inflammatory cardiomyopathy[10,11]. In Coxsackievirus B3 (CVB3)-induced inflammatory cardiomyopathy, the expression of TLR4[10,11] and of its adaptor molecule myeloid differentiation factor (MyD)88[12] are augmented in the heart and consequently promote a burst of pro-inflammatory cytokines. Interestingly, TLR4[8] and MyD88-deficient[12] mice infected with CVB3 develop significantly reduced acute myocarditis and have reduced levels of pro-inflammatory cytokines in the heart compared to CVB3-infected control mice. A recent study demonstrated that in response to CVB3-infection, genes involved in cholesterol metabolisms are upregulated and that the low-density lipoprotein (LDL) transfer and oxidation in macrophages is enhanced, leading to the production of mediators increasing the pro-inflammatory response[13].

High-density lipoproteins (HDL) and its main apolipoprotein (apo), apo A-I, are besides their central role in reverse cholesterol transport also known for their anti-inflammatory properties. However, a direct role of HDL or apo A-I in innate immunity regulation in LPS-induced inflammation has not yet been investigated. There is growing experimental and clinical support[14,15] for the application of cellular transplantation as a strategy to improve myocardial

function. Mesenchymal stromal cells (MSCs) have anti-apoptotic[16], anti-fibrotic[17] and pro-angiogenic features. Especially, their immunomodulatory[18] properties and their low immunogenicity[19], allowing the use of allogeneic MSCs for clinical application[20] make them attractive candidates for the treatment of inflammatory cardiomyopathy, given the importance of the inflammatory component in this disorder. Recently, novel cardiac-derived cells from human cardiac biopsies, cardiac-adherent proliferating cells (CAPs), which share properties with MSCs, have been isolated and identified[21, 22].

2.3. Aim

The aim of this cumulative work was to investigate the potential of different strategies, gene transfer and (stem) cell transfer, to counteract inflammation in two models of severe inflammation. In detail, the aim of the first study was to evaluate the potential of gene transfer with apo A-I to reduce LPS-induced inflammation and to elucidate underlying mechanisms. The purpose of the second and third study was to investigate the potential of MSCs and of the MSC-like CAPs, respectively, to reduce CVB3-induced inflammatory cardiomyopathy. The mode-of-action of MSCs and CAPs, particularly their immunomodulatory effects, and the impact of the inflammatory environment on their functionality were analyzed.

2.4 Methods/experimental design

Eight-week-old male C57BL/6 mice were intravenously (i.v.) injected with $5x10^{10}$ particles of the E1E3E4-deleted adenoviral vector *Ad.hapoA-I*, expressing human apo A-I[23]. As controls, age-matched C57BL/6 mice were injected with the same dose of *Ad.Null*, containing no expression cassette[23]. Fourteen days hereafter, LPS from *E. coli*, serotype 055:B5 (Sigma, Steinheim, Germany), was intraperitoneally (i.p.) injected at a dose of 80 mg/kg. Mice were sacrifice 20 hours (h) after LPS injection. Lung TLR4 expression as well as localisation of TLR4 in lung endothelial cells and neutrophils was determined by immunohistology. In addition, mRNA expression of lung *TLR4*, *MyD88*, *Toll/IL-1R-containing adaptor inducing interferon β (TRIF)* and *ribosomal protein L32* was analysed by real-time PCR and lung myeloperoxidase activity was quantified. *In vitro*, human microvascular endothelial cells-1 (HMEC-1) were incubated in the presence or absence of HDL (50 µg/ml) or apo A-I (35 µg/ml) for 24 h. Next, LPS (100 ng/ml) was supplemented in the absence of HDL or apo A-I for 2 h for TLR4 flow cytometry analysis and *MyD88* mRNA expression analysis or for 4 h

for NF-κB activity analysis.

Human adult MSCs were isolated from iliac crest bone marrow aspirates of normal male donors (n=6) after their written approval and characterized by flow cytometry analysis according to Binger *et al.*[24]. To investigate whether MSCs can be infected with CVB3, MSCs were serum starved or exposed to CVB3 at a multiplication of infection (m.o.i.) of 5 for 1 h. Next, cell morphology, cell viability, and CVB3 RNA copy number were evaluated 4 h, 12 h, 24 h, and 48 h after serum starvation/infection via phase contrast pictures, MTS viability assay, and real-time PCR, respectively. Next, to determine whether MSCs can protect against direct CVB3-induced cardiomyocyte damage, MSCs were co-cultured with uninfected or CVB3-infected HL-1 cardiomyocytes at a ratio of 1 MSC to 10 HL-1. The effect of MSC supplementation on CVB3-induced HL-1 cardiomyocyte apoptosis, oxidative stress, and virion progeny release was determined via annexin V/7AAD flow cytometry and caspase 3/7 activity analysis, DCF flow cytometry, and plaque assay, respectively. To analyse whether the MSC-mediated effects were nitric oxide (NO)-dependent, MSCs were pre-treated with nitro-L-argininmethylesterhydrochloride (L-NAME) for 24 h. To investigate whether MSCs require IFN-γ to exert their protective effects, MSCs were co-cultured with uninfected or CVB3-infected HL-1 cells in the presence of 1 mg/ml of anti-murine IFN-γ antibody. NOx and IFN-γ levels in HL-1 monocultures as well as in co-cultures with MSCs were analysed. Furthermore, the effect of IFN-γ supplementation on NOx production in uninfected or CVB3-infected MSCs was evaluated.

In vivo, 10^6 MSCs or phosphate buffered saline (PBS) was i.v.[25] in 6–8-week-old C57BL/6 mice 1 day after i.p. infection with 5 x 10^5 plaque-forming units (p.f.u.) of CVB3 (Nancy strain). Controls received PBS instead of CVB3. Seven days after CVB3 infection, contractility parameters were analysed as described previously[26].

Left ventricular (LV) damage, apoptosis, and *tumour necrosis factor-α (TNF-α)* mRNA expression were determined via haematoxylin eosin staining, TUNEL staining, and real-time PCR, respectively. Furthermore, the effect of MSC application on cardiac mononuclear cell (MNC) proliferation/activation was evaluated via isolation of cardiac MNCs, followed by carboxyfluorescein succinimidyl ester (CFSE)-staining and flow cytometry. Finally, it was evaluated how MSCs reduce the proliferation of CVB3-induced $CD4^+$ and $CD8^+$ T cell proliferation via (co)-culture of CFSE-labelled splenic MNCs stimulated with inactivated CVB3, with or without MSCs (untreated or pre-treated with L-NAME for 24 h) in the presence or absence of 1 mg/ml of anti-murine IFN-γ antibody, followed by $CD4^+$ and $CD8^+$ T cell staining and flow cytometry.

CAPs were isolated from endomyocardial biopsies taken from the right ventricular side of the interventricular septum[27]. To study the potential infectivity of CAPs, the expression of the Coxsackie- and adenovirus receptor (CAR) and the co-receptor DAF (CD55) on CAPs was evaluated by flow cytometry. Chinese Hamster Ovarian (CHO) cells overexpressing CAR and HL-1 cells, since cardiomyocytes are the target cells of CVB3, were used as positive controls. CHO lacking CAR and fibroblasts, known to express only low CAR, were used as negative controls. Next, CAPs were infected with CVB3 at a m.o.i. of 5 for 1 h. Cell viability was determined 4 h, 12 h, 24 h, and 48 h after CVB3 infection via a MTS assay. To assess whether viral replication took place, the same timeframe experiment was performed and cells were collected to determine CVB3 RNA copy number. The viral replication experiment was performed in parallel with HL-1 cells. LV *interleukin* (*IL)-10* and *IFN-γ* mRNA expression was quantified via real-time PCR.

To determine the effect of CAPs on CVB3-infected HL-1, CAPs were co-cultured with un- or CVB3-infected DiO-labeled HL-1 cells and apoptosis was assessed by Annexin V/7AAD flow cytometry analysis. The effect of CAPs application on the proliferation of cardiac MNCs as well as of $CD4^+$ and $CD8^+$ T cells from the spleen was measured with the CFSE proliferation assay. To investigate whether the anti-apoptotic and immunomodulatory effects of CAPs were NO-dependent, CAPs were pre-treated with the iNOS inhibitor L-NAME. To determine whether CAPs need IFN-γ or mediate their effects in an IL-10-dependent manner, CAPs were co-cultured with HL-1 cells or MNCs, respectively, in the presence of an anti-murine IFN-γ or anti-human IL-10 neutralizing antibody, respectively. To study the effect of IFN-γ stimulation on the production of NOx and IL-10 in un- and CVB3-infected CAPs, NOx and IL-10 were measured with a commercial NOx assay kit and IL-10 ELISA kit, respectively.

To study the effect of CAPs application on the progression of CVB3-induced myocarditis, 10^6 CAPs or PBS was i.v.[25] injected in 6- to 8-weeks-old C57BL/6 mice, one day after i.p. infection with 5×10^5 p.f.u. of CVB3 (Nancy strain) (CVB3-CAPs versus CVB3 mice, respectively). Uninfected controls received PBS instead of CVB3. Seven days after CVB3 infection, hemodynamic parameters were analyzed, followed by harvesting of the LV, which was next snap-frozen for performing molecular biology, viral load analysis, and immuno-histochemistry. For the analysis of MNC proliferation, the heart and spleen were isolated. To evaluate the engraftment of CAPs after i.v. injection, the heart, spleen, lung, kidney, and liver were isolated. To assess the anti-apoptotic effects of CAPs *in vivo*, the presence of cardiac apoptotic cells was detected on 5 µm thick heart sections of control and CVB3-infected mice

receiving PBS or CAPs via TUNEL staining. Furthermore, the caspase 3/7 activity in LV homogenates of control mice and CVB3-infected mice injected with PBS or CAPs was measured with a caspase-Glo 3/7 assay kit. Cardiac damage was assessed via hematoxylin and eosin staining on heart sections. The effect of CAPs application on the percentage of T regulatory cells, excluding the apoptotic T regulatory cells was analysed via flow cytometry. Mouse cardiac troponin-I levels were determined in murine serum with a mouse cardiac troponin-I ELISA kit. Levels of CAPs engraftment in tissues were quantified by real time-PCR with *Alu* specific primers.

2.5. Results

In vivo, HDL cholesterol increased 1.7-fold ($p<0.005$) and lung endothelial TLR4 expression decreased 8.4-fold ($p<0.005$), 2 weeks after apo A-I gene transfer. Following LPS administration in apo A-I gene transfer mice, lung *TLR4* and lung *MyD88* mRNA expression, reflecting TLR4 signalling, were 3.0-fold ($p<0.05$) and 2.1-fold ($p<0.05$) lower, respectively, than in LPS control mice. Concomitantly, LPS-induced lung neutrophil infiltration, lung oedema and mortality were significantly attenuated following apo A-I transfer. *In vitro*, supplementation of HDL or apo A-I to HMEC-1 24 h before LPS administration reduced TLR4 expression by 2.6-fold ($p<0.05$) and 2.5-fold ($p<0.01$) respectively as assessed by flow cytometry analysis. Pre-incubation with HDL or apo A-I decreased the LPS-induced *MyD88* mRNA expression and NF-κB activity by 1.6-fold ($p<0.05$) and 2.0-fold ($p<0.05$), independently of the formation of LPS-HDL complexes.

Phase contrast pictures and MTS viability assay demonstrated that MSCs did not show any significant changes in cell morphology and did not suffer from CVB3 infection 4, 12, 24, and 48 h after CVB3 infection versus serum-starved MSCs. CVB3 RNA copy number decreased in this time frame, suggesting that no CVB3 replication took place. Co-culture of MSCs with CVB3-infected HL-1 cardiomyocytes resulted in a 4.2-fold reduction of CVB3-induced HL-1 apoptosis ($p<0.05$) to levels not significantly different from non-infected cells. Supplementation of MSCs declined the CVB3-induced caspase 3/7 activity by 1.4-fold ($p<0.001$) and reduced the CVB3-induced ROS production in HL-1 cells by 6.3-fold ($p<0.01$), MSCs reduced the viral progeny release 5.1-fold ($p<0.01$) in a NO-dependent manner and diminished the viral progeny release. Moreover, MSCs required priming via IFN-γ to exert their protective effects. Murine IFN-γ treatment in combination with CVB3 infection elevated the NO production in MSCs by 1.2-fold ($p<0.05$), versus non-infected

MSCs. *In vivo*, MSCs application improved the cardiac contractility and relaxation parameters in CVB3-induced myocarditis by a 1.5-fold ($p<0.0005$) and 1.7-fold ($p=0.001$), respectively, which was paralleled with a 3.2-fold ($p<0.05$) and 3.2-fold ($p=0.0001$) reduction in cardiac apoptosis/damage and LV *TNF-α* mRNA expression, respectively. MSCs reduced the cardiac MNC activity in CVB3-infected mice

In vitro, supplementation of MSCs to MNCs isolated from the spleen of control and CVB3-infected mice at a ratio of 1 to 10 reduced the inactivated CVB3-stimulated division index of $CD4^+$ and $CD8^+$ T cells from CVB3-infected mice by 2.5-fold ($p<0.05$) and 2.5-fold ($p<0.05$), respectively. MSCs exerted these anti-proliferative effects in an NO-dependent manner and required IFN-γ priming for their activity.
With respect to CAPs, CAPs only minimally express CAR as well as CD55 compared to CHO-CAR+, CHO-CAR-, HL-1 cells, and cardiac fibroblasts. Moreover, the cell viability of CAPs was not hampered by CVB3 infection. In parallel, CVB3 RNA copy number was only minimally expressed in CVB3-infected CAPs in comparison to CVB3-infected HL-1 cells. CVB3 copy number reduced over time in CAPs, while in CVB3-infected HL-1 cells it dramatically increased over time, indicative for CVB3 replication. CAPs decreased CVB3-induced HL-1 apoptosis by 3.5-fold ($p<0.05$) in an NO- and IL-10-dependent manner and required IFN-γ priming. In line with our *in vitro* findings, application of CAPs in an experimental model of murine acute myocarditis reduced cardiac apoptosis as shown by TUNEL staining and as indicated by a 1.6-fold ($p<0.01$), decrease in caspase 3/7 activity to levels not significantly different from controls. Furthermore, plaque assay demonstrated that CAPs reduced CVB3 viral progeny release by 11–fold ($p<0.05$), an effect, which was blunted in the presence of L-NAME or by blocking either human IL-10 or murine IFN-γ. *In vivo*, CAPs administration reduced cardiac viral load by 5.2-fold ($p<0.05$) as determined by plaque assay on extracts of LVs from CVB3+PBS mice and CVB3+CAPs-injected mice. *In vivo*, CAPs application reduced the CVB3-induced proliferation/activity of cardiac MNCs in murine acute CVB3-induced myocarditis by 2.9-fold ($p<0.05$), which was paralleled with less cardiac damage compared to CVB3+PBS mice. In line with our *in vivo* findings, supplementation of CAPs to MNCs at a ratio of 1 to 10 reduced the PMA/ionomycin-stimulated division index of $CD4^+$ and $CD8^+$ T cells from CVB3-infected mice by 2.7-fold ($p<0.01$) and 2.3-fold ($p<0.001$), respectively. This effect was less pronounced or abrogated when CAPs were pre-treated with L-NAME or co-cultured with MNCs in the presence of an IFN-γ or IL-10 neutralizing antibody. *In vivo*, CAPs increased the percentage of T regulatory

cells and of apoptotic $CD4^+$ and $CD8^+$ T cells in CVB3-infected mice by 9.3-fold ($p<0.05$), 1.4-fold ($p<0.01$) and 1.5-fold ($p<0.01$), respectively. LV *IL-10* and *IFN-γ* mRNA was 1.7-fold ($p<0.005$) and 2.9-fold ($p<0.05$) increased in CVB3-infected CAPs versus CVB3-infected mice receiving PBS. Murine IFN-γ administration raised IL-10 production by 3.8-fold ($p<0.005$) in non-infected CAPs, whereas IL-10 production was 6.7-fold ($p<0.01$) higher in CVB3-infected versus non-infected CAPs. CVB3-infected mice injected with CAPs had significantly improved cardiac contractility and diastolic relaxation compared with CVB3-infected mice receiving only PBS, as indicated by a 1.1-fold ($p<0.05$) increase in LV pressure and a 1.3-fold ($p<0.05$) and 1.3-fold ($p<0.01$) improvement in dP/dt_{max} and dP/dt_{min}, respectively. In parallel, CVB3-infected mice injected with CAPs had 2.0-fold ($p<0.01$) lower serum concentrations of the biomarker cardiac troponin I compared to PBS-injected CVB3-infected mice. CAPs were retrieved in the heart, lung, kidney, liver, and spleen, with the highest entrapment found in the lung. Interestingly, there was a 2.8-fold ($p<0.05$) higher engraftment of CAPs in the heart of CVB3-infected compared to control mice.

2.6. Conclusion

In conclusion, we demonstrated that the anti-inflammatory properties of HDL comprise the downregulation of TLR4 signalling, with TLR4 being important in the pathogenesis of different cardiovascular disorders. Furthermore, we showed that MSCs as well as the MSC-like CAPs improve acute CVB3-induced inflammatory cardiomyopathy involving immunomodulatory effects for which they require IFN-γ. The impact of HDL on TLR4 signalling and the immunomodulatory effects of MSCs/CAPs support the use of HDL raising strategies and MSC/CAPs cell therapy, respectively, for the treatment of heart failure. However, further studies in (other) models of heart failure are still required to further assess the potential of those strategies for the treatment of heart failure.

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3. Anteilserklärung

Kapka Miteva hatte folgenden Anteil an den vorgelegten Publikationen:

Publikation 1:
Van Linthout S, Spillmann F, Graiani G, **Miteva K**, Peng J, Van Craeyveld E, Meloni M, Tölle M, Escher F, Subasigüller A, Döhner W, Quaini F, De Geest B, Schultheiss H-P, Tschöpe C. Down-regulation of endothelial TLR4 signalling after apo A-I gene transfer contributes to improved survival in an experimental model of lipopolysaccharide-induced inflammation. J Mol Med, 2011.
10 Prozent
Beitrag im Einzelnen (bitte kurz ausführen):
- praktische Arbeit:

- Zellkultur
- TLR4 FACS-Analyse

Publikation 2:
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. * equal contribution.
50 Prozent
Beitrag im Einzelnen (bitte kurz ausführen):

- praktische Arbeit:
- Zellkultur
- Apoptose und ROS FACS-Analyse
- Plaque Assay
- Isolierung von Mononukleären Zellen aus der Milz
- Proliferationsanalyse von kardiale Mononukleären Zellen und CD4+ und CD8+ T Zellen aus der Milz
- ELISA

- Etablierung von Methoden:
- Proliferationsanalyse von kardiale Mononukleären Zellen und CD4+ und CD8+ T Zellen aus der Milz
- ...

- Aktive Beteiligung in Literatur-Recherche
- Data Analyse

Publikation 3:
Miteva K, Haag M, Peng J, Savvatis K, Becher PM, Seifert M, Warstat K, Westermann D, Ringe J, Sittinger Mi, Schultheiss H-P, Tschöpe C*, Van Linthout S*. Human cardiac-derived adherent proliferating cells reduce murine acute Coxsackievirus B3-induced myocarditis. PLOS One, 2011. * equal contribution.
85 Prozent

Beitrag im Einzelnen (bitte kurz ausführen):

- praktische Arbeit:
  - Zellkultur
  - Apoptose FACS-Analyse
  - Plaque Assay
  - Isolierung von Mononukleären Zellen aus der Milz und aus peripherem Blut
  - Proliferationsanalyse von kardiale Mononukleären Zellen und CD4+ und CD8+ T Zellen aus der Milz
  - FACS-Analyse regulatorischer T-Zellen
  - ELISA´s
  - NOx Assay
  - Real-time PCRs

- Etablierung von Methoden:
  - Proliferationsanalyse von kardiale Mononukleären Zellen und CD4+ und CD8+ T Zellen aus der Milz
  - FACS-Analyse regulatorischer T-Zellen
  - ...

- Aktive Beteiligung in Literatur-Recherche
- Data Analyse

Promovendin
Kapka Miteva

4. Selbständigkeitserklärung

„Ich, Kapka Miteva, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: „Inflammation as a therapeutic target in heart failure“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

Datum Unterschrift

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