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The immune protein MD-2 is associated with early death in dilated cardiomyopathy and increases M1 macrophage polarization and recruitment in vitro

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Objective: Dilated cardiomyopathy (DCM) is characterized by systolic dysfunction and dilatation of ventricles. Myocardial inflammation and leukocyte activation and recruitment play a major role in the development and progression of disease. Myeloid differentiation factor-2 (MD-2) is the TLR (toll like receptor)-4 co-receptor and has been shown to be an important risk predictor for mortality of DCM patients. It is expressed in various cell types and mediates TLR-4 dependent inflammation/activation processes.

Purpose: We examined the impact of MD-2 on mortality of DCM patients and on polarization and recruitment of monocytes in vitro.

Methods: In 77 DCM patients, divided by median time point of death after first hospital admission into early and late death and alive group, MD-2 was quantified by means of ELISA. In THP-1 monocytes, cytokine secretion was quantified by ELISA after 72h treatment with MD-2 (5μg/mL). Bone marrow derived macrophages (BMDM) were generated from MD-2 KO and WT mice. NFkB phosphorylation (10min) and changes in gene expression (4h) of different adhesion molecules was quantified after treatment with 1 or 10ng/mL LPS. In human umbilical vein endothelial cells (HUVEC), protein kinase B (PKB) phosphorylation was quantified after 15min of treatment with 10ng/mL LPS or 5μg/mL MD-2. CCL2 gene expression in lysed cells (4h) and CCL2 secretion in supernatants (48h) were quantified to. Adhesion of monocytes on treated HUVEC was determined by FACS (Fig.1a). Initial HUVEC treatment with MD-2 (5μg/mL) or LPS (10 or 100ng/mL) took place for 48h.

Results: We found significant increased MD-2 in early (591.3ng/mL; N=18) vs late death (p=0.015) (369.2ng/mL; N=17) and alive (p≤0.0001) (303.2ng/mL; N=42) patients. Treatment of THP-1 cells (N=5) with MD-2 lead to a significantly increased secretion of inflammatory cytokines IL-8 (p=0.012), IP-10 (p=0.029), and MCP-1 (p=0.032) but not of anti-inflammatory cytokines IL-4, IL-10 and IL-13. Treatment of BMDM obtained from MD-2 KO and WT mice with 10ng/mL LPS lead to a increased phosphorylation of NFkB (N=4; p=0.022) and increased gene expression (N=6) of adhesion molecules VLA-4 (p=0.006) and ICAM-1 (p=0.049) in WT mice but not in KO mice. In HUVEC, LPS (p=0.008) and MD-2 induced a comparable increased phosphorylation of PKB (p=0.008) as well as an increase of CCL2 gene expression (p=0.029) and protein amount (p=0.039). Furthermore, treatment of HUVEC with both MD-2 (p=0.015) and LPS (p=0.0001) lead to a significant increase in monocyte adhesion (Fig.1).

Conclusion: The impact of MD-2 on cardiac inflammation and macrophage recruitment has not been described yet. In this study, we showed that, in DCM, elevated levels of sMD-2 are associated with early death. Furthermore, we could demonstrate that MD-2 enhances the process of HUVEC based monocyte recruitment. Finally, we could show that MD-2 induces inflammatory monocyte activity and triggers polarization of macrophages towards an inflammatory phenotype.
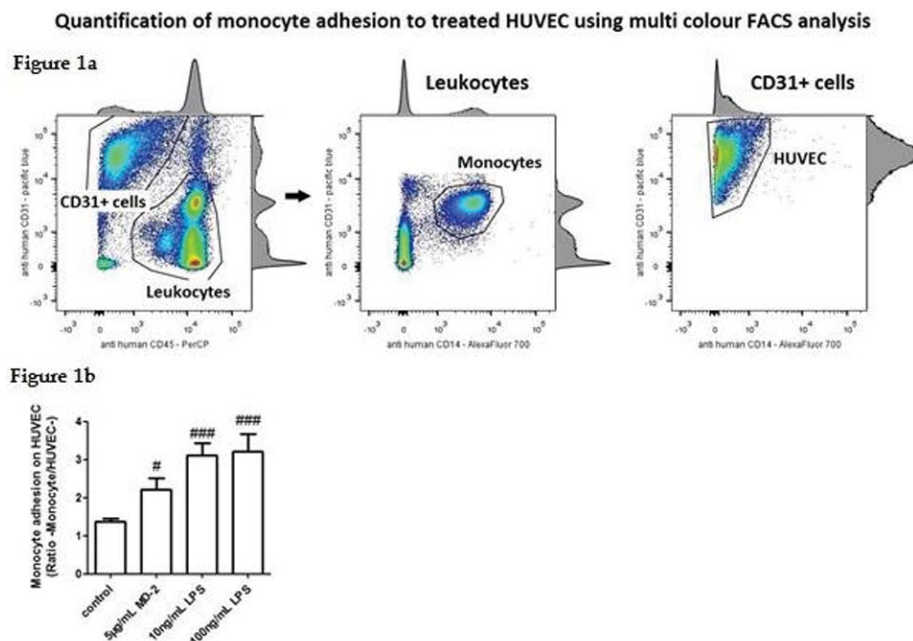

Figure 1: Figure 1a: illustrates the gating process by which monocytes and HUVEC are separated and quantified. Therefore, three different antibodies against CD45 (pan leucocyte marker), CD14 (monocyte marker) and CD31 (endothelial cell marker) were used.
Figure 1b: illustrates the monocytes/HUVEC ratio quantified by multicolour FACS analysis. Data are given as mean ±SEM, the significance threshold was set at 0.05 (# p<0.05; ### p<0.001). # indicates the significance in comparison to an untreated control.