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The involvement and interplay of HMGB1 with soluble MD-2 in dilated cardiomyopathy and its impact in immune cell recruitment

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Objective: Dilated cardiomyopathy (DCM) is characterized by systolic dysfunction and simultaneous dilatation of the left or both ventricles. Besides other causes, the innate immune system plays a major role in the development and progression of the disease. To uncover links between molecular mechanisms and disease progression our group has focused on the toll like receptor 4 / myeloid differentiation factor-2 (MD-2) system.

Purpose: We already reported that soluble MD-2 (sMD-2) is a risk factor for survival in patients with DCM. High mobility group box protein 1 (HMGB1) is a potent intrinsic interaction partner of MD-2. In the current study, we quantified HMGB-1 in plasma from patients with DCM at baseline, upon first hospital admission. Furthermore, we studied the impact of different HMGB-1 isoforms on monocyte adhesion in vitro.

Methods: We included 77 DCM patients divided by median time point of death after first hospital admission into "early death", "late death" and "alive" group. MD-2 was quantified by means of ELISA. MD-2 and HMGB1 was quantified by means of ELISA. Statistical analysis was performed using a linear regression model.

Human umbilical vein endothelial cells (HUVEC; n=6) were treated for 48h with two isoforms of HMGB1 (disulfide (ds) and fully reduced (fr)) alone and in combination with MD-2. Subsequently, those activated HUVEC were incubated with fresh isolated peripheral blood mononuclear cells (PBMCs) for 20 min. Finally, monocyte adhesion was quantified using multicolour FACS.

Results: At baseline, we found significantly increased sMD-2 level in the "early death" group (591.3±75.5 ng/ml) compared to the "later death" group (369.2±46.5 ng/ml; p=0.015) and the "alive" group (303.2±18.1 ng/ml; p<0.001). Likewise, we could demonstrate significantly increased levels of HMGB1 in the "early death" group (0.93±0.14 ng/ml) compared to the "later death" (0.57±0.17 ng/ml; p=0.04) and the "alive" group (0.49±0.06 ng/ml; p<0.001). In all patients who died during the observation period, sMD-2 and HMGB1 plasma levels showed a positive correlation.

In vitro, we could demonstrate a significantly increased monocyte adhesion on HUVECs in the dsHMGB1 and the frHMGB1 group compared to controls (p=0.001; p=0.004). In contrast, the dsHMGB1 MD-2 group showed a significantly decreased monocyte adhesion on HUVECs compared to dsHMGB1 treatment alone (p=0.049). In the frHMGB1 MD-2 group, however, the reduction of the monocyte adhesion was less pronounced and did not reach significance (Fig. 1).

Conclusion: Our findings give a first hint that the interplay between HMGB1 and MD-2 is particularly involved in the development and progression of DCM. Furthermore, the data suggest that soluble MD-2 is capable of reducing the pro-inflammatory effects of dsHMGB1 but not of frHMGB1

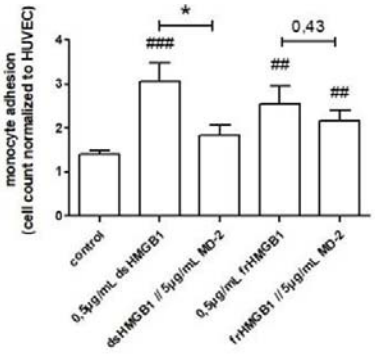

Figure 1: Monocyte adhesion on HUVECs (n=7).

Significant monocyte adhesion could be demonstrated for the dsHMGB1 and the frHMGB1 group. The dsHMGB1 MD-2 group showed significantly decreased adhesion compared to dsHMGB1 treatment. In contrast, adhesion of the frHMGB1 MD-2 group was still significantly elevated compared to the untreated group. Data are given as mean ±SEM. The significance threshold was set at 0.05 (* p<0.05; ## p<0.01; ###<0.001). # indicates the significance in comparison to an untreated control. * indicates the significance in between groups.