PAR2 expression differentially regulates immune response upon treatment with Poly(I:C) and LPS
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Introduction: The protease activated receptor 2 (PAR2) is a G-protein coupled receptor, that is activated by serine proteases such as trypsin, trypstatin and the complex composed of tissue factor (TF)/factor VII (FVII) and factor X (FX). PAR2 is also involved in modulating immune response after infections with pathogens. The activation of PAR2 is associated with the induction of a pro-inflammatory response by induction of gene expression of cytokines, such as IL8. Here we studied role of PAR2 for the innate immune response in the heart.

Methods: Biopsies of patients suffering from cardiomyopathy and mouse hearts of wt and PAR2ko mice were examined for IFNβ, TNFα and IL6 mRNA expression with real time PCR. Fibroblasts were isolated from wildtype (wt) and PAR2 knockout (ko) mouse hearts and stimulated with poly(I:C) and LPS for different time points. IFNβ, IL6 and TNFα mRNA expression was determined with real time PCR. For IFNβ pathway analysis western blot and co-immunoprecipitation was performed.

Results: The stimulation with poly(I:C) led to an increased expression of antiviral IFNβ and CCL5 in PAR2ko fibroblasts compared to wt fibroblasts (n=3-5, p<0.05 wt vs PAR2ko). Furthermore, the transcription factors NFκB and Stat1 were stronger activated in PAR2ko cells. Poly(I:C) activates TLR3 and co-immunoprecipitations revealed that the negative regulation of IFNβ expression by PAR2 was dependent on a physical interaction between PAR2 and TLR3. When stimulating TLR4 with LPS the pro-inflammatory cytokine IL6 was stronger up-regulated in PAR2ko fibroblasts than in wt fibroblasts (n=3, p<0.05). Contrarily, the gene expression of TNFα was lower in PAR2ko fibroblasts than in wt fibroblasts (n=3-5, p<0.05). Thus, the stimulation of TLR3 and TLR4 led to opposite immune responses dependent on the presence of PAR2 in the fibroblasts. In human hearts a low PAR2 expression was associated with a low expression of the pro-inflammatory cytokines TNFα and IL6 and vice versa (n=100, p<0.05). Further, a low PAR2 expression was associated with a high IFNβ expression in the heart (n=100, p<0.05). These data point to PAR2 to regulate innate immune responses in the heart.

Conclusion: The lack of PAR2 leads to an enhanced antiviral immune response after stimulation of TLR3 with poly(I:C). In contrast, PAR2 deficiency evokes a pro-inflammatory response after stimulation of TLR4 with LPS. Thus, dependent on the pathogen PAR2 functions as a pro- or an anti-inflammatory mediator of the immune system.