Methods: In the coxsackie B3 (CVB3) murine model of viral myocarditis, transcript levels of stabilin-1, a transmembrane glycoprotein, increased 3-fold in heart tissue at 9 days post infection. Immunohistochemical detection of stabilin-1 showed an increase of stabilin-1 expression in mouse heart tissue after CVB3 infection and in endomyocardial biopsies from patients with viral myocarditis. Furthermore, stabilin-1 was expressed on mac-3 positive cells in the mouse model and in human cardiac cells in the human biopsies. Stabilin-1 null (KO) mice showed increased cardiac related mortality when challenged with the CVB3 virus in comparison to wild type (WT) mice. Histological analysis of the heart showed that the stabilin-1 deficient mice had increased cardiomyocyte necrosis and CD3+ lymphocyte infiltration but lower levels of macrophages cells, in comparison to WT animals. In vitro, Stabilin-1 KO monocytes (cd11b+ cells) had a significant decreased adhesion to fibronectin -coated plastic as compared to WT cells.

Conclusions: This study shows that the transmembrane glycoprotein Stabilin-1 is up regulated during myocarditis, both in the animal model and in patients with acute fulminating myocarditis. Stabilin-1 is needed for the recruitment of monocytes to the site of infection and interacts with fibronectin in order for monocytes to adhere. These data demonstrate that Stabilin-1 and monocyte recruitment are necessary in order to eliminate the virus and prevent exaggerated inflammation during viral myocarditis.

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Bone morphogenetic protein (BMP-2) is involved in mononuclear cell function and their interaction with endothelial cells
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Background: Mononuclear cells play a crucial role in vascular remodelling and cardiovascular disease. Monocytes are recruited to sites of collateral growth where they contribute to arteriogenesis. However, prolonged presence or increased accumulation of these cells can compromise tissue integrity and lead to atherosclerosis. Bone morphogenetic proteins (BMPs) are members of the Transforming growth factor (TGF)-β family of cytokines and play important roles in vascular remodelling and function. It was shown that BMP-2 is up-regulated in stabilized atherosclerotic plaques. BMP-2 contributes to calcification and inflammation of the vascular wall. It is unknown whether these effects of BMP-2 are also due to its effects on monocyte function and/or on the monocyte-endothelial cell interactions.

Methods: Human monocytes were isolated from peripheral blood through gradient centrifugation and subsequent negative immunomagnetic isolation. Monocyte mobility was analysed by the modified Boyden chamber migration assay. Protein phosphorylation was assessed by immunoblotting. For endothelial cell adhesion assays, mouse endothelial cells were stimulated with different ligands and mononuclear cell adhesion was analysed. The involvement of different signalling cascades was analysed using specific kinase inhibitors.

Results: Here we show that stimulation of primary monocytes with BMP-2 results in monocyte polarization. BMP-2 stimulated mononuclear cell chemotaxis in a dose-dependent manner, while addition of Noggin, a natural BMP-2/4/7 antagonist, hindered monocyte chemotaxis towards BMP-2. Additionally BMP2 induces mononuclear cell adhesion on fibronectin. BMPs signal into the cells by activating both Smad and non-Smad signalling pathways such as PI3K and p38 MAPK signalling. Our results suggest that both PI3K and p38 pathways are involved in BMP-2 induced monocyte migration. Moreover, BMP-2 stimulated monocyte adhesion to fibronectin by activating inflammatory responses in endothelial cells in a PI3K and p38 dependent manner.

Conclusions: Our results reveal that BMP-2 is a novel stimulator of human monocyte function and suggest that BMP-2 may exert its pro-inflammatory effects and pro-calcification action in arteriosclerosis by activating the endothelium and in addition by recruitment of monocytes.

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Regulation of hypoxia-inducible factors during polarization of human macrophages
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During the last years the concept of macrophage polarization increases the understanding of different behavior of macrophage (MD) subtypes. Whereas classical activated MØs (M1) show a highly inflammatory response, alternative activated MØs (M2) have a more anti-inflammatory phenotype. The gene expression patterns of these two subtypes significantly differ. Furthermore, MØs are often associated to hypoxic areas, like the atherosclerotic plaque. Under these reduced conditions, the hypoxia-inducible factors (Hifs) are of great importance. Until now it is not clear whether the two MD-subtypes show a different hypoxic response. Therefore the aim of the present study was to investigate the expression and regulation of the main hypoxic regulators of the Hif-family in differentially activated MØs.

Therefore MØs were M1 using Interferon-gamma and LPS or to M2 using IL-4. Successful M1 polarization was proven by an increase of IL-1β, -6 and -8 and decrease of SCARβ1. The polarization into M2 was proven by an increase of MRC-1 and SCARβ1. Looking on the expression of the Hif-family members revealed that during M1 polarization the Hif-1α subunit dramatically increases on mRNA and protein level, already under normoxic conditions. Surprisingly, the Hif-2α subunit showed no different expression on mRNA level but is significantly increased on protein level in M1 MDs suggesting a post-transcriptional regulation. In comparison to the pro-inflammatory M1 type, anti-inflammatory M2-MØ showed an alternative expression pattern. Here, no alterations of the Hif-1α subunit were observed compared to unpolarized MØs. In contrast the Hif-2α expression increased on mRNA as well as on protein level. Looking on the expression of Hif target genes revealed that M1 polarization potentiates the hypoxic induction of CCK4, VEGFA and ENO2, whereas M2 polarization did not influence the hypoxic induction of these direct Hif target genes. Interestingly, VEGFA and CXCR4 are already induced under normoxic conditions in M1-MØs, which might be due to the normoxic stabilization of Hif-1α. Recent studies will focus on these different expression patterns and regulations of the Hif-system in the different polarization states of MØs by specific knock-down of the Hif transcription factors and modulate the hypoxic response of the MØs. These important observations open new perspectives for the understanding of the behavior of MØs under different pathological situations.

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NLRP3 inflammasome deletion reduces STAT3 signalling in the heart, without affecting acute ischaemia-reperfusion necrosis or preconditioning in a closed thorax model of cardiac ischaemia-reperfusion
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Background: Acute myocardial infarction is associated with an intense inflammatory response that contributes to ischemic-reperfusion (IR) injury of the heart. Here we examine to what extent the NLRP3 inflammasome, an important component of the innate immune system and therefore inflammation, contributes to the development following 60 min LAD occlusion and 3 h reperfusion. No differences in IS were observed between WT (45±2%) and NLRP3-/- (42±6%). Next we examined in a milder model of ischemia (30 min), whether NLRP3 affected STAT3 signalling in the isolated hearts of NLRP3-/- mice, we examined whether the NLRP3 inflammasome also affected ST3 signalling, cytokine production and IPC in the in vivo condition.

Methods: A closed thorax model was used to induce I (60 min or 30 min) and IPC (3 x 5 min) through LAD occlusion, followed by 3 h reperfusion. Electrocardiograms were used to detect ST-segment elevations. Phosphorylated STAT3, STAT3 expression and cytokines were determined in hearts of non-treated (blanco), IR and IPC+IR animals (n=6-8 per group).

Results: We first examined whether NLRP3 affected acute infarct size (IS) development following 60 min LAD occlusion and 3 h reperfusion. No differences in IS were observed between WT (45±2%) and NLRP3-/- (42±6%). Next we examined in a milder model of ischemia (30 min), whether NLRP3 affected STAT3 signalling, cytokine production and IPC in the in vivo condition. IPC reduced IS in WT from 38±3% to 26±3% and in NLRP3-/- from 39±3% to 27±4%. Conclusion: The NOD-like receptor NLRP3 does not acutely affect cardiac ischemia-reperfusion necrosis or preconditioning in a closed thorax model of cardiac ischaemia-reperfusion, despite its effect on the signalling of the survival protein STAT3. These data are commensurate with reports in the literature that the inflammasome protein complex is mainly present in fibroblasts, which are only activated in later reperfusion.

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Activation of NALP3/inflammasome pathway in circulating monocytes and epicardial adipose tissue of patients with acute coronary syndromes
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Purpose: Interleukin-1 (IL-1) is a potent inflammatory cytokine. Processing of the 32 kDa active IL-1β depends on activation of a protein complex termed “inflammasome”. Several inflammasomes have been described with NALP3 representing the most intensely studied. We sought to evaluate NALP3 (IL-1β) expression by circulating monocytes and epicardial adipose tissue (EAT) of patients with Non-ST ele-