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 macrophages in the mouse model and in macrophages 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 KO mice had increased cardiomocyte necrosis and CD3+ lymphocytic infiltration but lower levels of macrophages cells, in comparison to WT animals. In vitro, Stabilin-1 KO monocytes (c11+ cells) had a significant decreased adherence to fibronectin coated plastic as compared to WT cells. Conclusions: This study shows that the transmembranal glycoprotein Stabilin-1 is up regulated during myocarditis, both in the animal model and in patients with acute fulminant 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.

2789 | BENCH
Bone morphogenetic protein (BMP-2) is involved in mononuclear cell function and their interaction with endothelial cells
E. Pardi, L. Merle, J. Shi, J. Waltherbecker. University Hospital Münster, Muenster, Ger Germany
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)-beta family of cytokines and play important roles in vascular remodelling and function. It was shown that BMP-2 is up-regulated in aortic and esophageal 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 interaction.
Methods: Human monocytes were isolated from peripheral blood through gradient centrifugation and subsequent negative immunological magnet isolation. Monocyte motility 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 monocyte 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 pathway. Our results suggest that both PI3K and p38 pathways are involved in BMP-2-induced monocyte migration. Moreover, BMP-2 stimulated monocyte adhesion to endothelial cells by inducing 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 pro-inflammatory effects and pro-calciﬁcation action in arteriosclerosis by activating the endothelium and in addition by recruitment of monocytes.

2790 | BENCH
Regulation of hypoxia-inducible factors during polarization of human macrophages
D.M. Poitz, F. Hantsche, A. Augstein, S. Jellinghaus, M. Christoph, K. Ibrahim, R.H. Strasser. Dresden University of Technology, Heart Center University Hospital, Dresden, Germany
During the last years the concept of macrophage polarization increases the understanding of different behavior of macrophage (MD) subtypes. Whereas classical activated MDs (M1) show a highly inflammatory response, alternative activated MDs (M2) has a more anti-inflammatory phenotype. The gene expression pattern of these two subtypes significantly differs. Furthermore, MDs are often associated to hypoxic areas, like the atherosclerotic plaque. Under these reduced conditions, the hypoxia-inducible factors (Hif) are of great importance. Until now it is not clear whether the two MD subtypes differ a 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 MDs. Therefore MDs were polarized to M1 using Interferon-gamma and LPS or to M2 using IL-4. Successful M1 polarization was proven by an increase of IL-1β, IL-8 and IL-12/23 and decrease of SCARB1. The polarization into M2 was proven by an increase of MRC-1 and SCARB1. 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 compared to un-polarized MDs. In contrast to the pro-inflammatory M1 type, anti-inflammatory M2-MD showed an alternative expression pattern. Here, no alterations of the Hif-1α subunit were observed on mRNA level but protein level showed a significant reduction. 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 a highly inflammatory response and a hypoxic induction of CXCR4, 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-MDs, which might be due to the hypoxic stabilization of Hif-1α. Recent studies focus on these different expression patterns and regulations of the Hif-system in the different polarization states of MDs by specific knock-down of the Hif-subunits. In conclusion, the present study shows that polarization of human MDs dramatically modulates the expression pattern of the Hif transcription factors and modulates the hypoxic response of the MDs. These important observations open new perspectives for the understanding of the behavior of MDs under different pathophysiological situations.

2791 | BENCH
NLRP3 inflammasome depletion reduces STAT3 signalling in the heart, without affecting acute ischemia-reperfusion necrosis or preconditioning in a closed thorax model of cardiac ischemia-reperfusion
W.M.C. Jong, M.W. Hollmann, C.J. Zuurbier. Academic Medical Center, University of Amsterdam, Department of Anaesthesiology, Amsterdam, Netherlands
Background: Acute myocardial infarction is associated with an intense inflammatory response that contributes to ischemia-reperfusion 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 inflammatory response after ischemia-reperfusion (IR). NLRP3 inflammasome and IR response of the heart in vivo. In addition, as triggers for the activation of NLRP3 inflammasome and ischaemic preconditioning (IPC) overlap and we previously reported decreased IL-6/STAT3 signalling in the isolated hearts of NLRP3-/-/ mice, we examined whether the NLRP3 inflammasome also affected STAT3 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 I 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 generation and ischemic preconditioning. Both STAT3 expression and its activation-Signalling were decreased in NLRP3-/- hearts, for all three groups (blanco, IR, and IPC-IR). Although cardiac IL-1β and IL-6 were increased in IR and IPC-IR hearts as compared to non-IR hearts, NLRP3 deletion did not result in a significant effect on cytokine generation. However, NLRP3 deletion decreased (p=0.06) plasma IL-6 in IPC-IR animals (45.8±7.8 to 29.1±2.6 pg/ml for WT and NLRP3-/- animals, respectively). Despite the attenuated STAT3 signalling, NLRP3 deletion was without effect on ischemic preconditioning 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 ischemic preconditioning in a closed thorax model of ischemia-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.

2792 | BENCH
Activation of NALP3/inflammasome pathway in circulating monocytes and epicardial adipose tissue of patients with acute coronary syndromes
S. Ucci, D. Pedicono, D. Fiego, C. Zara, A. Severino, F. Trotta, M. Prevello, G. Massaro, F. Crea, G. Liuzzo. Catholic University of the Sacred Heart, Institute of Cardiology, Rome, Italy
Purpose: Inteleukin-1β (IL-1β) is a potent inflammatory cytokine. Processing of 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 and IL-1β expression by circulating monocytes and epicardial adipose tissue (EAT) in patients with Non-ST ele
Ectopic calcification is often associated with soft tissue inflammation and is seen in a multitude of clinical settings such as chronic renal failure and primary hyperparathyroidism. To further evaluate the specific pathophysiological role of T cells in nephrocalcinosis and dystrophic cardiac calcification, we used DBA/2 mice that have a natural splic variant in the ABCC6 gene and are prone to develop ectopic calcifications under high-phosphate diet.

Methods: Female DBA/2 mice were depleted of T cells (n=10) or regulatory T cells (Tregs) (n=15) using either an anti-CD3 antibody, a ROSERkv2, a KO anti CD3. Female DBA/2 mice were depleted of T cells (n=10) or regulatory T cells (Tregs) (n=15) using either an anti-CD3 antibody, a ROSERkv2, a KO anti CD3.

Conclusions: Circulating monocytes of ACS had a constitutive higher and a sustained expression of NALP3 and proIL-1β. In ACS, NALP3 and proIL-1β expressions were still elevated after 24h of LPS-challenge, while in SA and C they returned to baseline (Fig. 1A). Moreover, ACS had higher NALP3 expression in EAT (Fig. 1B).

Fig 1. Circulating Monocytes

Conclusions: Circulating monocytes of ACS had a constitutive higher and a sustained expression of NALP3 and proIL-1β. In ACS, NALP3 and proIL-1β expressions were still elevated after 24h of LPS-challenge, while in SA and C they returned to baseline (Fig. 1A). Moreover, ACS had higher NALP3 expression in EAT (Fig. 1B).

2794 | BENCH
Apolipoproteins in High-Density Lipoproteins (HDL) and their modifications determine the Sphingosine 1-Phosphate (SIP) content of HDL: effects on the HDL-mediated, SIP-dependent signaling

K. Satller1, M. Graefer1, G. Heuschen1, B. Lekava1, 1University of Essen Medical School, Institute of Pathophysiology, Essen, Germany; 2Charité - Molecular Cancer Research Centre, Berlin, Germany

Purpose: The bioactive sphingolipid sphingosine 1-phosphate (SIP) binds in plasma mainly to high-density lipoproteins (HDL) via apolipoprotein M (apoM) and mediates many of the atheroprotective and cardioprotective properties of HDL. Consequently, we demonstrated that the SIP-content of plasma and HDL is reduced in patients with coronary artery disease (CAD). In the current study, we tested the hypothesis that the reduction of SIP in CAD-HDL is due to modifications of apolipoproteins in HDL. In addition, we tested whether the extent of cellular responses upon stimulation with HDL was related to the SIP content of HDL.

Methods: HDL were isolated by density gradient ultracentrifugation from plasma of patients with CAD (n=68) and of controls (n=68). The contents of apoM, apoAI and SIP were detected by Western blotting, ELISA and mass spectrometry, respectively. The uptake capacity of HDL for SIP was measured in native and oxidized HDL as well as in the subfractions HDL2 and HDL3. Induction of the phospholysis of SIP/44 Erks upon stimulation with HDL was determined in SIP-receptor 1-overexpressing CHO-cells. The same assays were performed in HDL isolated from plasma of apoM-knock out, apoAI knock-out, and apoAI-transgenic mice.

Results: While the SIP content was lower in CAD-HDL, the contents of apoM and apoAI were similar in CAD-HDL and control HDL. Oxidation of HDL reduced their baseline SIP content by 44% and their uptake capacity for SIP by 68%. HDL had less SIP than HDL2 not only at baseline (122 pmol/mg of protein [102-171] vs. 225 pmol/mg of protein [216-448], P=0.04) but also after loading HDL with SIP (1510 pmol/mg of protein [930-1990] vs. 573 pmol/mg of protein [503-1252], P=0.004). HDL isolated from apoAI-deficient and apoAI-transgenic mice had a significantly reduced content of SIP (6.3% and 20.2%, respectively). In addition, intracellular phosphorylation of SIP/44 Erks after stimulation with apoAI-deficient HDL was greatly diminished. An enormous capacity of HDL to uptake SIP was found (20 fold of baseline). Loading apoAI-deficient HDL with SIP normalized the defective activation of SIP/44 Erks. HDL mediated cell signaling was completely blocked by a SIP neutralizing antibody.

Conclusion: Changes in the apolipoprotein composition of HDL, posttranslational modifications of apolipoproteins, or the disarranged ratio of HDL2/HDL3 in patients with CAD might be causes for the reduction of SIP in plasma and in HDL in CAD. As the content of SIP in HDL seems to determine the extent of HDL-mediated effects, quality and function of HDL might be improved by targeting their SIP content.

2795 | BENCH
Idiopathic dilated cardiomyopathy shows increased distribution of low-density lipoprotein receptor-related protein 1 in membrane lipid rafts

S. Roura Ferrer1, R. Perez Quevedo2, C. Galvez Montorn1, L. Nasarre1, A. Marti1, L. Astil1, A. Bayes-Genis1, V. Lorente Cortes1, ICREC research group, Institut d’Investigació en Ciències de la Salut Germans Trias i Pujol, Badalona, 1Cardiovascular Research Center, CSIC-ICCC, IIB-Sant Pau, Hospital de la Santa Creu i Sant Pau, Barcelona, 4Cardiology Service, Hospital de la Santa Creu i Sant Pau, Barcelona, 5Cardiology Service, Hospital universitari Germans Trias i Pujol, Badalona, Spain

Purpose: Compelling evidence recognises cholesterol-enriched membrane domains (lipid rafts) and their associated proteins as cardiac modulators and novel targets for human therapy. Idiopathic dilated cardiomyopathy (IDCM) is linked to α-2 macroglobulin (α-2M) and extracellular signal-regulated kinase (ERK)-mediated signalling which are, in turn, related to low-density lipoprotein receptor-related protein 1 (LRP1). The aim of this study was to compare the activation levels and distribution of LRP1 and ERK1/2 in the lipid rafts derived from IDCM and control myocardium.

Methods: Left ventricle samples from IDCM hearts collected at transplant (n=10) were compared with those from control hearts from non-cardiac deceased donors (n=5). Intersitial collagen was assessed by Picrosirius Red staining. Gene and protein expression analysis were carried out using quantitative RT-PCR, Western blotting and indirect immunofluorescence. Lipid raft isolation was performed by non-ionic surfactant solubilisation and sucrose gradient ultracentrifugation. Distribution of LRP1 and ERK1/2 into the lipid rafts derived from IDCM and control hearts were compared using quantitative RT-PCR, Western blotting and indirect immunofluorescence. Lipid raft isolation was performed by non-ionic surfactant solubilisation and sucrose gradient ultracentrifugation. Distribution of LRP1 and ERK1/2 into the lipid rafts derived from IDCM and control hearts were compared using quantitative RT-PCR, Western blotting and indirect immunofluorescence.

Results: Total levels of α-2M, p-ERK1/2 and p-LRP1 expression in IDCM myocardium, which shows altered total collagen deposition (P<0.005), were higher than those in control myocardium (P=0.028, P=0.044 and P=0.032, respectively). Although no differences in total levels of LRP1 were detected, increased amounts of the receptor were found in the lipid rafts from IDCM in comparison with those derived from controls (P=0.036). Moreover, increased distribution of p-ERK1/2 into lipid rafts was observed in IDCM myocardium (P<0.001).