P2397 | BENCH
Direct infusion of bone marrow-derived progenitor cells delays atherosclerotic plaque progression and decreases inflammatory mediators in a murine model of atherosclerosis

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Purpose: The effects of direct infusion or indirect mobilization of progenitor cells on atherosclerotic plaque progression are not clear. We sought to investigate the effects of lin-/sca+ cells, endothelial progenitor cells (EPCs) and granulocyte colony-stimulating factor (G-CSF) administration on atherosclerotic plaque progression.
Methods: Apolipoprotein E-deficient (apoE-/-) mice were splenectomized and treated with high-cholesterol diet for 6 weeks in order to induce atherosclerotic plaque development. Bone marrow derived Lin-/sca-1+ cells were isolated and further cultured to early growth endothelial progenitor cells (EPCs). Mice were divided in four groups (n=10/group) and received two intravenous injections of 5x105 cells (lin-/sca-1+ or EPCs), or granulocyte colony-stimulating factor (G-CSF 100mcg/kg/day) for 7 days or normal saline. The same interventions were administered to animals, which had undergone unilateral hind-limb ischemia. Effects on inflammatory parameters, lesion severity, and atherosclerotic plaque area size were assessed.
Results: The administration of both G-CSF and progenitor cells significantly decreased the levels of IL-6, 6 weeks after the initiation of treatment. The effect of treatment on pro-inflammatory molecules 7 days post-treatment was not significant. Atherosclerotic lesion area was reduced by G-CSF (atherosclerotic plaque area percentage 22.94%±3.68, p=0.001), by lin-/sca-1+ (23.27%±5.98, P=0.002) and cultured EPCs (23.16±4.86%, p=0.002) compared to control (32.75%±7.05). The percentage of severe lesions was higher in the control group compared to treatment groups [(80% in controls vs 50% in the G-CSF group ($p< 0.05$), 46% in the lin-/sca-1+ group ($p< 0.05$) and 56% in the EPC group ($p<0.05$)]. In the atherosclerotic mice that underwent limb ischemia, the atherosclerotic plaque area degree was not significantly different between the treatment groups [(G-CSF (26.86%±4.84, P=0.105), lin-/sca-1+ (29.15%±5.42, P=0.188), cultured EPCs-treated mice (28.07±5.89%, P=0.202)] and the control (31%±7.39).
Conclusions: Direct infusion of progenitor cells and indirect mobilization of hematopoietic progenitor cells decreased plaque progression and levels of inflammatory molecules in a murine model of atherosclerosis. Treatment with G-CSF, lin-/sca-1+, or EPCs may exert beneficial effects on vascular inflammation and atherosclerotic plaque progression. However, the effects are diminished in an ischemic setting.

P2398 | BENCH
M1 macrophages are an early feature of shear stress modulated vulnerable atherosclerotic plaques

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Purpose: Atherosclerosis occurs in vascular sites subjected to complex flow, causing oscillatory shear stress (OSS) or low shear stress (LSS). Macrophages can be skewed towards a pro-inflammatory phenotype (called M1) or a regulatory one (broadly named M2) and may determine atherosclerotic plaque outcome. The aim of this study is to establish the polarisation of macrophages in different plaque phenotypes and shear stress conditions.
Methods: A perivascular shear stress modifying cast was tied around a carotid artery for 6 or 9 weeks, mimicking LSS and OSS in ApoE-/- mice fed a high fat diet (HFD) from 17 weeks of age. We examined the expression of the pan-macrophage marker CD68 and polarisation markers iNOS (M1), CD206 (M2) and HO-1 (Mox subset) in the aortic root at 12, 20, 28, 35 and 53 weeks of age, and cast-treated carotid artery by Immunohistochemistry and Confocal Immunolocalisation
Results: In the aortic root, most CD68+ cells stained positively for HO-1, particularly at 28 weeks (36.3±1.2% of lesion area; $p<0.001$) while iNOS and CD206 were expressed by few macrophages (max 5.6±2.3% and 4.2±1.1% at 20 weeks respectively). In stark contrast, most macrophages in early LSS-induced carotid artery lesions express iNOS (23.6±5.1% vs. 5.4±2.7%; p=0.006) while at 9 weeks, HO-1 expression becomes more abundant.
Conclusions: The majority of macrophages in aortic root lesions express HO-1 (Mox phenotype). While in the carotid artery, M1 macrophages are significantly more prevalent in the low shear stress-modulated plaque. These differences suggest low shear stress promotes polarisation towards the M1 macrophage phenotype.

P2399 | BENCH
Circulatory inflammation molecules and extracellular matrix proteoglycans: local and systemic modulated markers in an atherogenesis model

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Purpose: Inflammatory cells and mediators play a key role in the pathogenesis of atherosclerosis (ATS) and systemic inflammation is known to enhance atherogenesis. In this process extracellular matrix proteoglycans (ECM-PGs) may contribute to arterial lipoprotein retention. Overexpression of PGs, as well as biglycan, leads to the trapping of lipoproteins in arterial wall and it is recognized as one initiating factor in ATS.
In this study, soluble ICAM-1 and its monocyte (MN) receptor were measured as inflammation markers and related to end-diet average coronary lesion grade (morphometry) as well as to expression of ECM-PGs released from coronary segments in a swine model of high cholesterol diet-induced coronary ATS.
Methods: Farm pigs were fed either standard (CTRL) or high cholesterol diet for 2 (HF) and 4 months (HHF). Elisa assay for ICAM-1 and its MN receptor CD18a positivity % (PP) and Relative Fluorescence Intensity (RFI) following flow cytometry were measured on pre- and end-diet blood samples. All three main coronary arteries were analysed by digital microscopy for intimal thickness (IT) and lesional area (H&E and Masson's trichrome stains) (LA). In addition, segments of right coronary arteries were incubated for 24 hours in serum-free medium to collect secreted proteins and their expression analysed by high performance liquid chromatography connected with mass spectrometry.
Results: Although average values of ICAM-1 and its CD18a MN receptor (PP and RFI) are not significantly different in HF and HHF cases as compared to CTRL due to strong inter-animal variation, both are positively and significantly correlated to IT and LA within HF and HHF group cases at end-diet. In addition, variation in inter-animal baseline ICAM-1 values is significantly correlated to extent of coronary ATS in HHF group. ECM proteoglycans (biglycan and dermatan), are also overexpressed in coronary samples of HHF cases as compared to CTRL.
Conclusions: These results indicate that, in diet-induced coronary ATS swine model, systemic inflammation marker ICAM-1 and its MN receptor CD18a correlate with coronary ATS burden as assessed by hystomorphometry as well as with coronary-released ECM-proteoglycans. Despite the irreplaceable utility of plasma inflammatory markers for risk prediction in ATS, ECM markers and tissue factors, due to the possibility of their detection in blood, may be exploited as more specific biomarkers of atherosclerosis onset and progression than conventional inflammatory molecules as ICAM-1.

P2400 | BENCH
Crucial role of hyaluronan derived from macrophages in neointimal formation after vascular injury

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Background: Hyaluronan (HA) is a primary component of the extracellular matrix of cells, and it is involved in the pathogenesis of atherosclerosis. The purpose of this study was to investigate the role of HA in neointimal formation after vascular injury and determine its cell-specific role in macrophages by using a cre-lox conditional transgenic (cTg) strategy.
Methods and results: HA was found to be expressed in neointimal lesions in humans with atherosclerosis and after wire-mediated vascular injury in wild-type (C57BL/6) mice. Oral treatment with 4-methylumbelliferone, a specific inhibitor of HA production, significantly attenuated neointimal formation after vascular injury (I/M ratio: 1.84±0.07 vs. 0.63±0.03, $p<0.0001$). In vitro experiments revealed that low-molecular-weight HA (LMW-HA) induced murine macrophages activation, including migration, and production of inflammatory cytokines. Because HAS2 is thought to be a key enzyme responsible for HA production, we generated cTg mice that overexpress the murine HAS2 gene specifically in macrophages (cHAS2/CreLys mice) and showed that HA overexpression markedly enhanced neointimal formation after cuff-mediated vascular injury (I/M ratio: 1.44±0.08 (cHAS2/CreLys) vs. 0.54±0.07 (cHAS2) and 0.50±0.04 (CreLys), $p<0.0001$). Further, HA-overexpressing macrophages isolated from cHAS2/CreLys mice showed augmented migration, and production of inflammatory cytokines.
Conclusion: Macrophages-derived HA promotes neointimal formation after vascular injury, and HA may be a potential therapeutic target for cardiovascular disease.