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464 STAT4 deficiency exacerbates atherosclerosis by promoting mobilization of myeloid cells, polarization of M1 macrophages and formation of foam cells

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Background: Atherosclerosis (AS) is a chronic inflammatory disease of large and medium size ves-
sels. Signal transducer and activator of transcription 4 (STAT4) has been reported to regulate the prolifera-
tion and differentiation of myeloid cells. However, the role of STAT4 in atherosclerotic pro-
gression is not well defined.

Methods: We constructed APOE/STAT4 double knock out (DKO) mice via hybridization of ApoE-/-
and STAT4-/- mice. 10 ApoE-/- mice (control) and 10 DKO mice were challenged with high-fat diet for 12 weeks. The extent of AS was determined by oil-red staining and HE staining. Plasma chole-
terol, triglyceride and cytokines were assessed by ELISA. Changes in subsets of immune cells were evaluated by flow cytometry. Microarray analysis was applied to detect gene expressions while Western blot was used to assess protein levels.

Results: Genetic deletion of STAT4 significantly exacerbated AS as evidenced by markedly increased oil-red-positive lipid-rich lesion in DKO mice accompanied by reduction of collagen fiber and increase of necrotic core lesion in plaques. Higher level of TC, TG and LDL-C in the serum and more abdom-
inal fat were detected in DKO mice. Increased percentage of CD11b+Gr-1+ myeloid cells and Ly6-
Chygh M1 macrophage in peripheral blood, bone marrow and spleen of DKO mice suggested that STAT4 signal may play a critical role in regulating the proliferation and mobilization of myeloid cells and polarization of macrophages. To further explore the impact of STAT4 deficiency in myeloid cells, we isolated CD11b+ myeloid cells from bone marrows of ApoE-/- and DKO mice and incubated them with GM-CSF (60ng/ml) plus ox-LDL (60ug/ml). Enhanced differentiation of CD11b+Ly6GChygh macrophage and increase formation of foam cells were detected in DKO group. IFN-γ in the superna-
tant increased while IL-10 decreased in DKO group, indicating enhanced polarization of M1 macro-
phage from CD11b+ cells of DKO mice. Meanwhile, microarray data demonstrated that STAT4 KO increased expression levels of M1-related genes such as inducible nitric oxide synthase (iNos). Mech-
anistically, STAT4 deficiency significantly promoted the formation of foam cells by inhibiting of phosphatidylinositol-3 kinase serine-threonine kinases (PI3K/AKT) signal and consequently up-regulating of the expression of pro-inflammatory cytokine, tumor necrosis factor-α. STAT4 deficiency significantly decreased the expression of CD200 and up-regulated the expression of CD200R in macrophages.

Conclusions: Our studies identified that STAT4 is a regulator of the proliferation and differentiation of myeloid cells and atherogenesis. PI3K/AKT/STAT4 knockout signaling was the molecular mechanisms of STAT4 functioning in the process. This findings points towards the development of STAT4 as a no-
vel pharmacotherapeutic target for the treatment of atherosclerotic diseases and APOE/STAT4 DKO mice with hyperlipidemia and hyper-inflammation as a novel mouse model more susceptible to ath-
erosclerosis for future study.

465 Effects of DPP4 inhibition on cardiac regeneration and macrophage balance in a mouse model of HHT-1

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Background: Hereditary Hemorrhagic Telangiectasia type 1 (HHT-1) is a genetic dominant vascular

Vorban caused by haploinsufficiency of the TGFB3 co-receptor Endoglin. Although the pathology of HHT-1 suggests that mainly vascular endothelial cells are involved, we have previously shown dys-
functional homing of mononuclear cells (MNC) towards the infected myocardium (M). This is most likely due to enhanced expression of dipetidyl peptidase-4 (DPP4). DPP4 inactivates SDF-1α, thereby inhibiting recruitment of CXCR4 expressing cells.

Purpose: Our aim is to increase homing of the HHT-1 MNC and improve cardiac recovery and func-
tion in HHT-1 mice following MI, by inhibition of DPP4 activity.

Methods: MI was induced in wildtype (WT) and endothelin heterozygous (eng+/−) as a model of HHT-
1 mice by ligation of the left anterior descending (LAD) coronary artery, followed by 5 days of daily treatment with the DPP4 inhibitor Daprotin A (2.5mg/kg/day). The infarcted hearts were as-
essed at day 4 and at day 14 post MI.

Results: DPP4 inhibition restored the number of MNC present in the infarcted hearts and significant-
antly reduced infarct size (eng+/- vs. eng−/- treated 71.07 ± 3.44% vs. 37.40 ± 17.4%, P=0.003), as measured by myocardial collagen formation. Analysis of cardiac function using ultrasound demonstrated that treatment of WT mice improved ejection fraction, however showed a slightly deteriorating effect in the eng+/- animals. Investigating the infarct borderzone, the number of capillaries increased (eng+/- 61.63 ± 1.43 vs. eng−/- treated 74.30 ± 17.4%, P=0.001) while the number of arteries decreased (eng+/- 11.88 ± 0.63 vs. eng−/- treated 6.38 ± 0.97, P<0.003), suggesting that angiogenesis is upregulated, though the maturation of the new vessels is still impaired. Furthermore at day 4 post-
MI, during the peak of inflammatory cell influx, eng+/- mice show a significant decrease (WT 29.88 ± 1.52 vs. eng−/- 12.34 ± 1.64, P<0.0001) of regenerative M2 macrophages in the heart com-
pared to WT mice which continued to day 14 post-MI, together with an overall increase in macro-
phage presence. DPP4 inhibition corrected the M2 levels at day 4 and even at 14 days post MI the increased M2 levels had persisted.

Conclusions: The findings show we can restore impaired MNC homing in eng+/- mice by systemic DPP4 inhibition, though we see no effect on cardiac ejection fraction, cardiac repair is improved as demonstrated by a decreased fibrotic response, resulting in a decreased infarct size. Furthermore our results suggest eng+/- mice have a defect in macrophage differentiation and function. We observe that inhibition of DPP4 results in an increase in angiogenesis and rescues the amount of M2 macro-
phages to wildtype levels.

466 Myeloid cell regulation by CD200 signalling in atherosclerosis

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Background: Atherosclerosis, the major risk factor for cardiovascular disease and the leading cause of death worldwide, is a multifactorial chronic inflammatory disease. CD200 is reported to be an en-
dogenous myeloid suppressor. Deletion of CD200 in vivo increases myeloid cell numbers and acti-
vation resulting in enhanced susceptibility to autoimmune diseases and infection. However, the importance of CD200 in atherosclerosis development is still unknown.

Methods and Results: To understand the role of CD200 signalling, both the effect of CD200 de-
letion and provision were assessed in a murine model of atherosclerosis. Firstly, CD200-deficient (CD200-/-) mice were crossed with apolipoprotein E deficient (ApoE-/-) mice. CD200 deficiency ac-
celerates advanced atherosclerotic lesion formation in the aortic roots, as shown by the morphomet-
ic measurement of aortic root atherosclerotic lesion development. Moreover, the leukocyte content of various tissues was assessed by flow cytometry. APOE-/-CD200-/- mice exhibit significant increase in specific myeloid cell populations in spleen, blood and aorta. Secondly, the role of CD200R+/- mice underwent surgery for place-
ment of a perivascular collar and were treated with 10μg/kg of a CD200-Fc fusion protein. Three weeks post injury, carotid arteries were removed and neointima formation was assessed. CD200-Fc fusion protein treatment attenuated neointima development. Interestingly, CD200-Fc fu-
sion protein affects macrophage accumulation and polarization.

Conclusions: Our data indicate that CD200 is an important modulator of myeloid cell function and phenotype in atherosclerosis and suggest that targeting the CD200-CD200R pathway holds promise as a potential therapeutic strategy in atherosclerosis.