Macrophages: New Frontier in Cardiovascular Medicine

Session held on 10 July 2016
doi:10.1093/cvr/cww144

464

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

L. Xu; S. Deng; J. Yang; X. Yang; J. Ge
1Zhongshan Hospital, Fudan University, Cardiology Department, Shanghai, China People’s Republic of
2Shanghai Institute of Cardiovascular Diseases, Shanghai, China People’s Republic of

Background: Atherosclerosis (AS) is a chronic inflammatory disease of large and medium size vessels. Signal transducer and activator of transcription 4 (STAT4) has been reported to regulated the proliferation and differentiation of myeloid cells. However, the role of STAT4 in atherosclerotic progression 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 cholesterol, 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 atheroma area, neointima area and percentage of plaques formation in DKO mice compared to control group. Although both groups displayed a similar increase of total myeloid cells and 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 (60µg/ml). Enhanced differentiation of CD11b+Ly6C+ cells into M1 macrophage and increase formation of foam cells were detected in DKO group. IFN-γ in the supernatant increased while IL-10 decreased in DKO group, indicating enhanced polarization of M1 macrophage 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). Mechanistically, STAT4 deficiency significantly promoted the formation of foam cells by inhibiting of phosphatidylinositol-3 kinase (PI3K)/akt and STAT4 signal transduction. Activating of PI3K/AKT pathway leads to increased expression of acyl coenzyme A: cholesterol acyltransferase-1 (ACAT-1), an enzyme that esterifies cholesterol and promotes its storage in macrophages.

Conclusions: Our studies identified STAT4 as a regulator of the proliferation and differentiation of myeloid cells and atherogenesis. PI3K/AKT/ACAT-1 signaling was the molecular mechanisms of STAT4 functioning in the process. These findings points towards the development of STAT4 as a novel 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 atherosclerosis for future study.

465

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

C. Ongenhouw; W. Bakker; K. Loderd; M. Goumans
Leiden University Medical Center, molecular cell biology, Leiden, Netherlands

Background: Hereditary Hemorrhagic Telangiectasia type 1 (HHT-1) is a genetic dominant vascular disorder caused by haploinsufficiency of the TGFβ3 co-receptor Endoglin. Although the pathology of HHT-1 suggests that mainly vascular endothelial cells are involved, we have previously shown dys-regulation of the expression of acyl coenzyme A: cholesterol acyltransferase-1 (ACAT-1), an enzyme that esterifies cholesterol and promotes its storage in macrophages.

Methods: We isolated CD11b+ myeloid cells and atherogenesis. PI3K/AKT/ACAT-1 signaling was the molecular mechanisms of STAT4 deficiency in myeloid cells. However, the role of STAT4 in atherosclerotic progression is not well defined.

Results: Genetic deletion of STAT4 significantly exacerbated AS as evidenced by markedly increased atheroma area, neointima area and percentage of plaques formation in DKO mice compared to control group. Although both groups displayed a similar increase of total myeloid cells and 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 (60µg/ml). Enhanced differentiation of CD11b+Ly6C+ cells into M1 macrophage and increase formation of foam cells were detected in DKO group. IFN-γ in the supernatant increased while IL-10 decreased in DKO group, indicating enhanced polarization of M1 macrophage 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). Mechanistically, STAT4 deficiency significantly promoted the formation of foam cells by inhibiting of phosphatidylinositol-3 kinase (PI3K)/akt and STAT4 signal transduction. Activating of PI3K/AKT pathway leads to increased expression of acyl coenzyme A: cholesterol acyltransferase-1 (ACAT-1), an enzyme that esterifies cholesterol and promotes its storage in macrophages.

Conclusions: Our studies identified STAT4 as a regulator of the proliferation and differentiation of myeloid cells and atherogenesis. PI3K/AKT/ACAT-1 signaling was the molecular mechanisms of STAT4 functioning in the process. These findings points towards the development of STAT4 as a novel 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 atherosclerosis for future study.

466

Myeloid cell regulation by CD200 signalling in atherosclerosis

C. Kasimendi; J. Cole; M. Goddard; P. Green; I. Park; D. Danso-Abeam; C. Monaco
University of Oxford, Kennedy Institute of Rheumatology, Oxford, United Kingdom

Background: Atherosclerosis, the major risk factor for cardiovascular disease and the leading cause of death worldwide, is a multifactorial chronic inflammatory disease. CD200 has been examined in a murine model of carotid injury. ApoE-/-mice underwent surgery for placement of a perivascular collar and were treated with 10mg/kg of a CD200-Fc fusion protein. Three weeks post injury, carotid arteries were removed and neointima formation was assessed.

Methods and Results: To understand the role of CD200 signalling, both the effect of CD200 deletion 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 accelerates advanced atherosclerotic lesion formation in the aortic roots, as shown by the morphometric 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 myelial cell population in spleen, blood and aorta. Secondly, the role of CD200R ligation has been examined in a murine model of carotid injury. ApoE-/-mice underwent surgery for placement 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 fusion protein affects macrophage accumulation and polarization.

Conclusions: Our data indicate that CD200 is a 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.

Purpose: Our aim is to increase homing of the HHT-1 MNC and improve cardiac recovery and function 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 Diprotin A (2.5mg/kg/day). The infarcted hearts were assessed at day 4 and at day 14 post MI.

Results: DPP4 inhibition restored the number of MNC present in the infarcted hearts and significantly reduced infarct size (eng+/-: 46.60 ± 9.35% vs. eng+/- treated 77.02 ± 3.49%, P=0.03), 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 infract 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 compared to WT mice which continued to day 14 post-MI, together with an overall increase in macrophage phenotype. 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 macrophages to wildtype levels.