marrow and subsequent movement are critically regulated by the CXCR4 receptor, here we sought to investigate whether they promptly respond to metabolic adaptations induced by a short-term HFD feeding as a function of the CXCR4 signaling.
Methods: We studied the metabolic and the immunophenotypic profile of mice harboring a conditional deletion of CXCR4 (CXCR4fl/flMrp8Cre+) versus wild-type counterpart (CXCR4fl/flMrp8Cre-), when fed a HFD (60% Kcal from fat) for a short timeframe (seven days). The prandial profile of surrogate indicators of systemic metabolic adaptations to HFD feeding were measured, the expression of membrane immune markers was studied by flow-cytometry. To verify whether phenotypic changes on neutrophils indicate the movement towards peripheral sites, we initially explored the plasma quantity of proteins involved on neutrophils behavior by untargeted plasma proteomics.
Results: Short-term HFD feeding resulted into an expected dysmetabolic impact and increasing number of circulating neutrophils in CXCR4fl/flMrp8Cre-; by contrast, CXCR4fl/flMrp8Cre+ mice displayed persistent neutrophilia, which was partially increased by HFD feeding. Moreover, the reduced expression of CXCR4 and CD62L along with an increased expression of CD11b on the membrane that we observed in CXCR4fl/flMrp8Cre- neutrophils was also recapitulated in CXCR4fl/flMrp8Cre+ neutrophils. This suggests a phenotypic remodeling toward enhanced migratory and activated features, independent from the neutrophilia when the CXCR4 signaling is altered, but, rather, partially recapitulated by the quantity of proteins involved in inflammation and chemotaxis. Metabolically, this derailment of neutrophil behavior impacted systemic metabolism, as CXCR4fl/flMrp8Cre+ displayed increased triglyceridemia upon standard and HFD feeding.
Conclusions: Short-term HFD feeding results into metabolic adaptations, which could affect neutrophil behavior. Whether this is of interest for the development of immunoinflammatory disturbances in cardio-metabolic diseases warrants more evidence.

SaaG138 / #760
SAAG SESSION: IMMUNE CELL DYNAMICS IN CVD: MONOCYTES, T CELLS, NEUTROPHILS AND MACROPHAGES
06-05-2025 1:30 PM - 2:30 PM

Hemin-driven epigenetics of atherosclerotic risk gene SMARCA4 switches human blood-derived macrophages from leukocyte clearance to erythrocyte clearance

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Background and Aims: Putative genetic risk loci for atherosclerotic vascular disease include *SMARCA4*, a chromatin-remodeller important for gene activation. Its role in atherosclerosis has been uncertain. Intraplaque hemorrhage is a late event in atherosclerosis, countered by Mhem macrophages. which have hemin-mediated induction of Heme Oxygenase 1 (*HMOX1*) via Activating Transcription Factor 1 (ATF1). *Atf1* deficiency *in vivo* impairs hematoma clearance, promoting inflammation and oxidative stress. Like its homologue CREB1, ATF1 is normally cyclic-AMP activated. Cyclic-AMP is more linked to leukocyte clearance. We postulated that SMARCA4 regulates ATF1-mediated activation promoting erythrocyte clearance over leukocyte clearance.
Methods: Human blood-derived macrophages (hMDM) were cultured, stimulated with hemin or PGI2. Gene expression was measured by RT-qPCR, ChIP, 3C, phagocytic index (fluorescence microscopy). SMARCA4 was tested with si-SMARCA4. In vivo was studied with a recently developed clearance models for killed leukocytes and erythrocytes. Human carotid plaques were immunostained for SMARCA4 and marker molecules..
Results: *SMARCA4* was genetically independent of the adjacent *LDLR* locus ($p<0.05$). In hMDM hemin triggered histone acetylation (H3K9Ac) and SMARCA4 recruitment in advance of p-ATF1 recruitment at the *HMOX1* enhancer. si-RNA-mediated *SMARCA4*-knockdown suppressed p-ATF1 binding to *HMOX1* but increased binding to cyclic-AMP responsive genes *FOS* and *NR4A2*, with corresponding changes in mRNA levels. This functionally correlated with *SMARCA4*-knockdown switching hemin to mimic prostacyclin (PGI_2), for induced genes and phagocytic disposal of leukocytes rather than erythrocytes. In carotid endarterectomies, SMARCA4 was not detected in media but highly expressed in macrophages in healing mural thrombus. Leukocytes and erythocytes mutually impeded clearance in vivo, where si-SMARCA4 delayed hematoma clearance but accelerated clearance of mixed leukocytes / erythrocytes.
Conclusions: *SMARCA4* is an independent atherosclerosis risk gene. In hMDM it allows hemin to promote erythrocyte clearance over leukocyte clearance, has this role in vivo and is induced in plaque mural thrombi. This may have important clinical implications for late atherosclerosis.

SaaG139 / #871
SAAG SESSION: IMMUNE CELL DYNAMICS IN CVD: MONOCYTES, T CELLS, NEUTROPHILS AND MACROPHAGES
06-05-2025 1:30 PM - 2:30 PM

Metabolic adaptations during feeding associate with phenotypic changes of neutrophils in circulation

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Background and Aims: Feeding promotes the development of immune-inflammatory pathways. While this is well described in chronic pathological conditions, it is less clear if every time we eat inflammatory pathways, unique to a specific immune cell type, are activated. Here, we sought to find surrogate indicators of a cell-specific activation that emerge in the circulation during feeding.
Methods: In C57Bl/6j mice we characterized both the metabolic profile (inverse calorimetry, glycemia, insulinemia and triglyceridemia) and the blood immunophenotype (to detect cell-specific markers of leukocytes via flow-cytometry), either in prandial state (feeding a chow diet) or during two hours of refeeding ad libitum after fasting. We also investigated the enrichment of plasma proteins (by untargeted proteomics) that could represent surrogate indicators of immune cell-specific activation (by pathway clustering analysis) during refeeding.
Results: Chow feeding, which maintained higher prandial glycaemia, insulinemia and triglyceridemia compared to fasting, did not induce significant change in the immunophenotype, except for a tendency towards a higher abundance of CD19+ B cells. However, during re-feeding program, where glucose and triglycerides levels increased by two-to-three-fold comparated to fasting unmasked a prominent increase in the blood count of Ly6G+ neutrophils, which increased one-fold compared to fasting. Of note, the counts of other leukocytes (CD3+T, CD19+ B cells and Ly6c+ monocytes) did not change. Plasma proteomics revealed enrichment of immune pathways that connects with neutrophils activation and extra-vasation.
Conclusions: Our data suggest an innate, but acute, regulation of neutrophils in response to re-feeding. Further studies are needed to elucidate the underlying mechanisms and to assess the effect of more caloric diets.

SaaG140 / #1137
SAAG SESSION: NEUROLOGICAL DISORDERS AND CARDIOVASCULAR INTERPLAY: EPIGENETIC AND METABOLISM
06-05-2025 1:30 PM - 2:30 PM

Impact of Western diet and PCSK9 on the expression of amyotrophic lateral sclerosis-predisposing genes

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Background and Aims: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive degeneration of motor neurons. Mutations in three genes, C9orf72, SOD1, TARDBP, account for the disease in about 70% of patients with familial ALS. Since cholesterol may play a role in the onset/progression of ALS, the impact of PCSK9, as well as that of different dietary lipid contents, on the expression of genes relevant in ALS and cholesterol metabolism was evaluated in brain and liver.
Methods: Six-week-old C57BL/6J WT and PCSK9-KO female mice were fed for 16 weeks either standard chow diet (CD), or Western-type diet (WD). After sacrifice, gene expression in liver and brain was evaluated via qPCR.
Results: When comparing dietary treatments, in the liver of both genotypes, WD significantly increased the expression of Sod1, Tardbp and C9orf72, as well as that of Dhcr24, Cyp27a1, Cyp46a1 and Soat1 vs. CD. In the brain, WD did not

alter the expression of any of the genes considered. When comparing genotypes, the hepatic expression of Sod1, Tardbp and C9orf72 was comparable in PCSK9-KO and WT when fed the same diet. On WD, increased hepatic expression of Dhcr24, Dhcr7, Msmo1, Hmgcr and Ldlr was observed in PCSK9-KO vs. WT. Conversely, in the brain, PCSK9-KO mice showed increased expression of C9orf72, Tardbp, Ldlr and Hmgcr, vs. WT when fed the same diet.
Conclusions: WD administration increases the expression of ALS-predisposing genes only in the liver but not in the brain in a genotype-independent manner. The lack of PCSK9 consistently results in increased expression of C9orf72 and Tardbp in the brain. Additional studies may shed light on the role of PCSK9 in the onset of ALS.

SaaG141 / #314
SAAG SESSION: NEUROLOGICAL DISORDERS AND CARDIOVASCULAR INTERPLAY: EPIGENETIC AND METABOLISM
06-05-2025 1:30 PM - 2:30 PM

Cerebrospinal fluid cholesterol esterification is hampered in patients with amyotrophic lateral sclerosis

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Background and Aims: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by motor neuron degeneration. Metabolic abnormalities, in both the bloodstream and central nervous system, have been observed in ALS. Similarly to plasma, cholesterol in the cerebrospinal fluid (CSF) is transported by "HDL-like particles", which closely resemble plasma HDL in density and composition. Lecithin:cholesterol acyltransferase (LCAT), a pivotal enzyme in HDL metabolism, catalyzes cholesterol esterification in both fluids, facilitating HDL maturation. Recent findings from our laboratory revealed impaired cholesterol esterification in the CSF of Alzheimer's disease patients. This study aimed to explore whether similar alterations occur in ALS.
Methods: The study included 20 ALS patients (9 females and 11 males, mean age of onset 52.9±10.1 years) and 20 matched controls. Lipid and lipoprotein profiles and cholesterol esterification were evaluated in both plasma and CSF.
Results: Plasma lipids were similar between patients and controls; however, ALS patients exhibited a significant reduction in the proportion of discoidal preβ-HDL compared to controls (8.5±4.9% vs. 13.6±4.1%, $p<0.0001$). In the CSF, unesterified cholesterol (UC) levels were significantly higher in ALS patients than controls (0.22±0.07 mg/dL vs. 0.15±0.04 mg/dL, $p<0.01$), leading to an increased unesterified/total cholesterol (UC/TC) ratio in ALS patients (0.52±0.12 vs. 0.40±0.12, respectively). Importantly, stratification based on the presence of the *SOD1* variant showed that the UC/TC ratio was not influenced by genetic background (0.23±0.06 vs. 0.21±0.07 for carriers vs. non-carriers). While plasma cholesterol esterification was not altered in ALS patients, the CSF-cholesterol esterification rate was significantly reduced (0.16±0.10 nmol/mL/h vs. 2.41±1.98 nmol/mL/h, $p<0.01$).
Conclusions: In conclusion, these findings highlight a specific impairment in cholesterol esterification within the CSF of ALS patients, aligning with similar alterations observed in other neurodegenerative diseases. This dysfunction may represent a common pathogenic mechanism in neurodegeneration.

SaaG142 / #257
SAAG SESSION: NEUROLOGICAL DISORDERS AND CARDIOVASCULAR INTERPLAY: EPIGENETIC AND METABOLISM
06-05-2025 1:30 PM - 2:30 PM

Cerebral abnormalities and functional changes in familial hypercholesterolemia

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Background and Aims: Familial hypercholesterolemia (FH) is an autosomal disease characterised by increased level of serum low-density lipoprotein cholesterol and risk of cardiovascular disease. Cerebral changes of cerebral small vessel disease are associated with an increased risk for myocardial infarction, stroke and cognition. In this study we aimed to assess the cerebral abnormalities and functional changes in FH.
Methods: Eleven patients with homozygous FH (HoFH), thirteen patients with heterozygous FH (HeFH) and fourteen controls were enrolled in this study. All participants underwent cognitive function evaluations on the day of the MRI scan. Infarcts, white matter hyperintensity, cerebral microbleeds, perivascular space counts and volume were quantitatively evaluated.
Results: The demographic and imaging characteristics of the participants are shown in Table 1. The prevalence of white matter hyperintensity, microbleeding, perivascular space, and atrophy were significantly higher in FH patients (Figure 1-3). Compared with controls, cognitive function declined in the FH. What's more, the cerebral abnormalities and functional decline were most obvious in HoFH.

Characteristics	HoFH (n=11)	HeFH (n=13)	Control(n=14)	p value
Age(years)	31.73±7.60	30.64 + 6.95	36.36 + 4.85	0.061
Sex(male/female)	7/4	8/5	6/8	0.508
Infarction,subiects(%)	0(0%)	0(0%)	0(0%)	NA
Deep white matter hyperintensity	1(1-2)	1(0-1)	0(0-1)	0.002
Periventricular hyperintensity	1(0-2)	1(0-1)	0(0-1)	0.028
Cerebral microbleeds counts	1(0-33)	0(0-4)	0(0)	0.007
Perivascular space counts	130.23±43.47	95.13±20.47	60.48±14.56	<0.001
Perivascular space volume	1000.20±241.56	876.43±153.94	763.43±260.24	<0.001
Atrophy, subjects	1(0%)	0(0%)	0(0%)	0.293
Montreal Cognitive Assessment	24.45±2.62	26.46±1.61	28.00±0.68	0.002
Alzheimer disease assessment scale-cog	12.09±4.32	7.00±2.08	5.14±1.29	<0.001

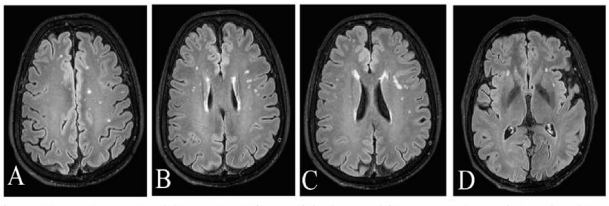

Figure 1. A 47 years old HoFH patient with deep white matter hyperintensity (2-point), periventricular hyperintensity(2-point) and obvious brain atrophy.