P034 / #324, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.03 Macrophages in lipid metabolism and atherosclerosis*

PROLONGED ACTIVATION OF NF-KB SUSTAINS THE PERSISTENT SENSITIZATION INDUCED BY ADVANCED GLYCATED ALBUMIN TO INFLAMMATORY STIMULATION BY LIPOPOLYSACCHARIDE IN MACROPHAGES

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Background and Aims: Advanced glycation end products (AGE) sensitize macrophages to inflammation contributing to intracellular lipid accumulation and atherogenesis. It was investigated how persistent is the effect of AGE-albumin in sensitizing macrophages to the nuclear factor kappa B (NFKB)-mediated inflammatory response elicited by lipopolysaccharide (LPS).
Methods: AGE-albumin was prepared by incubating albumin with 10mM glycoladehyde and control (C) albumin with phosphate buffer saline, for 4 days at 37ºC, in the dark. RAW-264.7 macrophages overloaded with acetylated LDL were incubated for 48h with C or AGE-albumin, rested for different periods of time in a culture medium alone, and challenged by LPS (24h) (n=6). The expressions of RelA, Nfkb1, IL6, and TNF were determined by RT-qPCR, the nuclear content of p50 and p65 by western blot, and the secretion of inflammatory cytokines by ELISA. Statistical comparisons were performed using the Student t test or Mann-Whitney.
Results: AGE-albumin primed macrophages for an LPS challenge, resulting in a 1.5, 9.4, and 5.6-fold increase in TNF, IL-6, and IL-1beta secretion, respectively, compared to C-albumin ($P<0.05$). The increased cytokine secretion persisted for up to 24h, 24h, and 12h, respectively, for TNF, IL-6, and IL-1beta even after the removal of AGE-albumin from the medium. The area under the curve (AUC) was 1.6, 16, and 5.0 times greater, respectively; $P<0.05$). Il6 and RelA expressions were higher in cells treated with AGE after 8h of removing albumins from the culture medium. Nuclear p50 was similar between the groups, but p65 remained increased in cells treated with albumin-AGE by 2.9 times for up to 24h ($P<0.05$).
Conclusions: Elevated RelA expression and augmented nuclear p65 content substantiate the sustained secretion of cytokines in macrophages treated with LPS, following prior sensitization with AGE-albumin. This inflammatory metabolic memory induced by AGE may play a role in promoting atherogenesis.

P035 / #1602, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.03 Macrophages in lipid metabolism and atherosclerosis*

EXPRESSION OF KEY GENES INVOLVED IN KYNURENINE AND NAD+ METABOLISM IS ASSOCIATED WITH MACROPHAGE CONTENT AND POLARIZATION IN CAROTID PLAQUES

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Background and Aims: We previously found that lower tryptophan and higher kynurenine levels are associated with incident cardiovascular disease (CVD). We hypothesize that alterations in kynurenine and NAD+ metabolism are linked to inflammatory processes and macrophage subtypes in atherosclerotic plaques. This study aims to explore the relationship between the expression of key genes involved in the kynurenine pathway and macrophage phenotype, inflammation, and kynurenine derived NAD+ metabolism (salvage pathway) within atherosclerotic plaques, with a focus on elucidating potential therapeutic strategies for CVD.
Methods: We conducted a cross-sectional study using carotid plaques from the Athero-Express (n = 632) and CARIM biobanks. Bulk transcriptomic data were employed to analyze the expression of genes involved in kynurenine and NAD+ metabolism. Within AE macrophages were identified using CD68-staining, in the CARIM biobank M1 & M2 macrophages were differentiated based on iNOS/Cd68 & arginase/Cd68 respectively.
Results: In the Athero-Express biobank, plaques with increased macrophage staining expressed higher levels of IDO2 ($p<0.05$), AFMID ($p<0.05$), and KYNU ($p<0.01$). In the CARIM set, we found a negative correlation between the content of M2 macrophages and IDO1 ($R = -0.49$, $p<0.01$), KYNU ($R = -0.72$, $p<0.001$) and KMO ($R = -0.60$, $p<0.001$). Similarly, several genes involved in the salvage pathway of NAD+ showed also a negative correlation with M2 macrophages such as NADS ($R = -0.49$, $p<0.01$), CD38 ($R = -0.42$, $p<0.05$), CD157 ($R = -0.53$, $p<0.01$) and NAMPT ($R = -0.53$, $p<0.01$). Although not significant, we found positive correlations between M1 macrophage content and the same genes.
Conclusions: Our research reveals a complex interaction between kynurenine metabolism, macrophage polarization, and NAD+ metabolism in atherosclerotic plaques, pointing to new potential treatments for CVD. We found distinct NAD+ metabolic patterns in M1 and M2 macrophages within plaques, suggesting a need for further study to confirm these findings.

P036 / #691, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.03 Macrophages in lipid metabolism and atherosclerosis*

THE ROLE OF MITOCHONDRIAL GENOME MUTATIONS IN MONOCYTES IN THE DEVELOPMENT OF OBESITY AND CHD

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Background and Aims: The immune cells, in particular, monocytes, play an important role in the pathogenesis of obesity and coronary heart disease (CHD). Mitochondrial dysfunction is considered as one of the factors affecting the functional state of monocytes, which leads to inflammation. Previously the role of mtDNA mutations in the development of mitochondrial dysfunction was demonstrated. The aim of this study was to estimate the level of heteroplasmic mtDNA mutations in peripheral blood CD14+ monocytes in patients with CHD and obesity.
Methods: The study included 22 patients aged 50-70 years with BMI>30 kg/m^2 and concomitant CHD and 22 CHD-free participants of control group with normal body weight. CD14+ monocytes were isolated by immunomagnetic method. The isolation of total DNA from CD14+ monocytes was carried out by phenol-chloroform extraction. To measure the level of relative DNA expression, primers specific to the transcripts of the corresponding genes were selected. Mutation analysis was carried out using digital PCR.
Results: It was shown that the level of the SNV A11467G was significantly higher in control group compared with group of study participants with obesity and CHD ($p<0.05$), and the level of the 576insC mtDNA variant was also significantly higher in group of obesity and CHD compared with control ($p<0.001$). The other studied variants of mtDNA heteroplasmy, namely, A1811G and 10958ins, did not differ significantly in the studied groups.
Conclusions: The identified mtDNA heteroplasmic variants, associated with in CHD and obesity, can be used in the development of personalized therapeutic strategies in patients with metabolic disorders accompanied with high cardiovascular risk. Mitochondrial genome editing may be considered as a possible therapeutic approach for CVD in the future. This work was supported by the This work was supported by the Ministry of Science and Higher Education of the Russian Federation—the state task of Petrovsky National Research Centre of Surgery (Project N№ 122030200531-3).

P037 / #1188, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.04 Inflammation, immunity and infection in atherosclerosis*

IMPACT OF IMMUNE SYSTEM HUMANIZATION ON ATHEROSCLEROSIS IN DYSLIPIDEMIC IMMUNOCOMPROMISED MICE

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Background and Aims: Given the key role of immune response during atherosclerosis and the therapeutic interest on biologics targeting human immune cells, the need of experimental models to translate molecular mechanisms and test therapeutic approaches for atherosclerosis is continuously increasing. Here we provide an immune and metabolic characterization of an innovative

immunodeficient mouse humanized with human hematopoietic cells on an atheroprone background.
Methods: LDLR-KO mice were crossed with the immunodeficient C57BL/6J strain Rag2-KO/IL2rg-KO/CD47-KO (TKO, IMSR_JAX:025730) to generate an immunocompromised dyslipidemic mouse (TKO-LDLR KO mice) recipient of human hematopoietic stem cells (hCD34 +).
Results: TKO-LDLR KO were first characterized for their immune and metabolic profile. TKO mice are deficient in mature lymphocytes and NK cells and this profile was conserved in TKO-LDLR KO mice. Under high cholesterol diet, TKO-LDLR KO develop marked dyslipidemia, steatosis and atherosclerosis. This profile confirms the suitability of TKO-LDLR KO mice for atherosclerosis studies. Next we tested the impact of immune system humanization on atherosclerosis. TKO-LDLR KO pups received a low-dose irradiation (200 cGy) and thereafter 2 x 105 hCD34 + were injected in the liver. Engraftment of human leukocytes (hCD45 +) was evaluated after two months by flow cytometry analysis from tail blood. This approach allows to reconstitute between 10-30% of hCD45 + , mainly B and T cells. hCD45 were detected also in the thymus (95%), spleen (20%) and liver (25%). The humanization with hCD34 + cells did not affect cholesterol levels (939,8±94,41 vs 987,4±48,82 mg/dL), but worsen atherosclerosis development by en faceanalysis and lesion area at the aortic valve, compared to non-humanized TKO-LDLR KO mice.
Conclusions: We have generated and characterized the humanized dyslipidemic TKO-LDLR KO mouse. This mouse model presents human B and T cells and represents an important toll to investigate the impact of human adaptive immune cell pharmacological modulation in the context of atherosclerosis.

P038 / #11, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.04 Inflammation, immunity and infection in atherosclerosis*
CCL4 INDUCES VASCULAR ENDOTHELIAL CELL DYSFUNCTION IN AGEING

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Background and Aims: Atherosclerosis can be classified as a disease of ageing, so increasing age is an independent risk factor for the development of atherosclerosis. Given that ageing is a natural process associated with chronic inflammation, we hypothesized that chemokine C-C motif ligands 4 (CCL4), an inflammatory chemokine, might play a vital role in ageing-related vascular endothelial cell dysfunction.
Methods: Human aortic endothelial cells (HAECs) were used in the in vitro study. In the in vivo part, C57BL/6 mice and CCL4 knockout mice were used. Six-month-old mice were defined as the young group and eighteen-month-old mice were defined as the elderly group.
Results: Administration of CCL4 directly induced cell senescence and dysfunction accompanied by enhanced reactive oxygen species (ROS) generation in HAECs. In addition, in vitro CCL4 blockade improved cell function, reduced ROS generation, and reversed senescence, inflammation, and angiogenesis signal pathways in aged HAECs. CCL4 was up-regulated in aged animals in vivo. Compared with aged wild-type mice, aged CCL4 knockout mice showed improved neovascularization, with reduced ageing and inflammatory markers, as well as increased expression of angiogenic proteins in aortic tissues.
Conclusions: These findings indicate that CCL4 may contribute to ageing-related vascular endothelial cell dysfunction via activating oxidative stress and inflammation. CCL4 inhibition may be a potential anti-ageing strategy for vascular protections.

P039 / #283, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.04 Inflammation, immunity and infection in atherosclerosis*
INFLAMMATORY STIMULATION OF MACROPHAGES INHIBITS MITOPHAGY

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Background and Aims: Mitophagy is a degradation mechanism of dysfunctional mitochondria. Cells use mitophagy to control the quality of the mitochondrial population. Recently, mitophagy impairment in inflammatory foci in chronic diseases has been observed. It remains unclear whether the disruption of mitophagy is the cause of chronization of inflammation or the result of an adaptation to an inflammatory state. In our work, we decided to find out the causal relationship between the intensity of mitophagy and the inflammatory response of macrophages.
Methods: Monocytes were isolated by immunomagnetic separation from blood of healthy donors. To assess mitophagy, the cells were stimulated with LPS, then stained with MitoTracker Green and LysoTracker Deep Red. The intensity of mitophagy was quantified by the colocalization area of the dyes. Concentrations of cytokines TNF-α, CCL2, IL-1β, IL-6, IL-8, and IL-10 were evaluated by ELISA.
Results: It turned out that LPS stimulation led to suppression of basal mitophagy. Moreover, we did not observe the activation of mitophagy under the influence of the FCCP connector in LPS-treated macrophages. To study the effect of mitophagy activation on the inflammatory response, we activated mitophagy using FCCP and further stimulated LPS cells, we observed a low level of secretion of the studied cytokines.
Conclusions: It can be assumed that the inflammatory reaction of macrophages is characterized by a decrease in the level of mitophagy. Perhaps prolonged inflammation can lead to the accumulation of dysfunctional mitochondria in the focus of inflammation and contributes to its chronization. Supported by RSF #20-15-00337

P040 / #434, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.04 Inflammation, immunity and infection in atherosclerosis*
$PM_{2.5}$ EXPOSED MACROPHAGES SECRETOME IS ENOUGH TO PROMOTE ENDOTHELIAL DYSFUNCTION

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Background and Aims: Recent experimental evidence suggests that exposure to the air pollutant fine particulate matter ($PM_{2.5}$) exacerbates pre-existent atherosclerotic lesions. In this context, macrophages play an important role in plaque development, releasing oxi-inflammatory molecules that can injure endothelium and lead to its dysfunction, the subclinical phase of atherosclerosis. Here, we aimed to describe the mechanisms involved in $PM_{2.5}$ vascular effects and the possible role of macrophages as mediators of this process.
Methods: CF-1 mice were exposed to $PM_{2.5}$ (30 μg/10 μL) by intranasal instillation once a day, 5 days/wk, for two weeks (CEUA 38492). Aortas were collected for histological analysis, and plasma was used to measure oxidative stress, IL-6, and IL-10 levels, through spectrophotometric techniques and ELISA kits, respectively. Macrophages (RAW264.7 lineage) were exposed to $PM_{2.5}$ [prepared 1 g of filter/125 mL of PBS, ultrasonicated, centrifuged, and diluted 10 times],and inflammation was measured. Culture medium was collected, prepared (10% v/v), and administered to endothelial cells (EOMA lineage). EOMA also received animals' conditioned plasma (1% v/v) for 48 h. Cell viability was verified through MTT assay, nitric oxide through the Griess method, eNOS, p-eNOS, and iNOS by immune cytochemistry, in a flow cytometer. Data were compared through a t-test, P˂0.05.
Results: $PM_{2.5}$ enlarged the aorta vascular wall, increased plasmatic lipoperoxidation, and reduced antioxidant defenses, whilst promoting a disbalance between IL-6 and IL-10 release. We identified that macrophages could be a source of elevated pro-inflammatory cytokines production after direct exposure to $PM_{2.5}$. $PM_{2.5}$ conditioned medium decreased endothelial cell viability, but enhanced p-eNOS and iNOS-dependent NO generation. Plasma exposure also enhanced NO release, with no changes in p-eNOS content.
Conclusions: Altogether, these findings demonstrate a novel link between air pollution $PM_{2.5}$ exposure and inflammatory pathways, highlighting the importance of macrophages as possible exacerbators of cardiovascular diseases.

P041 / #545, Poster Topic: *AS01 PATHOGENESIS OF ATHEROSCLEROSIS / AS01.04 Inflammation, immunity and infection in atherosclerosis*
CHARACTERIZATION OF THE HUMAN MACROPHAGE SPECTRUM: A NOVEL APPROACH TO IDENTIFY NOVEL DRUG TARGETS AND BIOMARKERS IN ATHEROSCLEROSIS

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