of patients with CAVS (48.5%) and high Lp(a) compared to controls (13%, p=0.006).
Conclusions: Genetically elevated Lp(a) level is causally associated with CAVS, independently of CAD. First-degree relatives of patients with CAVS and Lp(a) may be at high CAVS risk.

Workshop track 3
Workshop: Vascular ageing

EAS19-0431.
GENETIC RISK LOCI FOR AAA ARE ASSOCIATED WITH INFLAMMATORY BIOMARKERS WITHIN THE ANEURYSM-EXPRESS BIOBANK STUDY

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Background and Aims: Abdominal aortic aneurysms (AAA) have a multifactorial pathology with both genetic and environmental risk factors. Genome-wide association studies have discovered nine genetic risk loci for AAA. Our aim was to investigate to what extent these genetic variants contribute to the aneurysm pathology.

Methods: The nine risk variants for AAA were selected for analysis. Individual variants and a weighted genetic risk score (GRS) were tested for association with eight inflammatory biomarkers (IL-6,IL-8,MMP-8,MMP-9,MCP-1,IFN-gamma,TNF-alpha, and -beta) in AAA tissue from the Aneurysm-Express Biobank Study cohort. In total 220 tissue samples were included. We corrected models for age, sex, ancestral background, smoking status and maximum diameter of the aneurysm sac, and data was normalized.
Results: For one variant (rs10757274, A>G on 9p21), a nominal association with IFN-gamma was found (β = 0.216 pg/mL ± se 0.108, per A-allele, p=0.048, effect allele frequency = 0.485). Another variant (rs10985349, C>T on 9q33.2) was nominally associated with MCP-1 (β = -0.296 ± se 0.133, per T-allele, p=0.028, effect allel frequency = 0.232), and the variant(rs6511720, G>T) on 19p13.2 with IL-8 (β = -0.424 ± se 0.189, per T-allele, p=0.026, effect allele frequency = 0.096). The GRS based on the nine risk variants showed no significant correlation with the studied biomarkers.
Conclusions: Within our AAA cohort, three risk loci (rs10757274,rs10985349,rs6511720) were nominally associated with IFN-gamma, MCP-1 and IL-8 respectively. Given the limited sample size, these results require validation within future biobank collaborations to assess the potential causal role of these genetic risk variants in the inflammatory pathology of AAA.

Workshop track 1
Workshop: Lipoproteins and Immunity

EAS19-0579.
B CELL UPTAKE OF MODIFIED LDL RESULTS IN MODULATION OF B CELL ACTIVATION AND FUNCTIONS

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Background and Aims: Uptake of mLDL by macrophages are key events in atherosclerosis. Our data shows that B cells can also uptake mLDL. The focus of this study is to determine the role of mLDL uptake in B cell biology.

Methods: To test a T-independent immune response, BΔDhcr24 cells and BWT cells were adoptively transferred in B cell deficient mice that were immunized with DNP-Ficoll. To measure intracellular calcium changes, B cells were preincubated with Fluo3, activated with IgM F(ab')2, and calcium changes were measured via FACS.
Results: Upon mLDL uptake, B cells dampen activating signaling molecule such as SYK, ERK, and BTK, but upregulate inhibitory molecules such as phospho-Lyn (Tyr507). mLDL+ B cells mobilize less intracellular calcium upon BCR cross-linking. B cells are also less efficient in response to DNP-Ficoll immunization upon mLDL uptake. Uptake of mLDL by macrophages activate LXR target genes and suppresses inflammation via the accumulation of the cholesterol intermediate desmosterol. To test a role of desmosterol in B cells we used transgenic mice overexpressing 3β-Hydroxysterol Δ24-reductase (Dhcr24). The elevated expression of Dhcr24 resulted in reduced desmosterol levels in B cells. There was a significant increase in genes in the cholesterol efflux pathway such as *Abca1* and *Abcg1* in BΔDhcr24 vs BWT cells. BΔDhcr24 cells mobilized more intracellular calcium in response to BCR cross-linking. Functionally, adoptively transferred BΔDhcr24 cells generated a more efficient response to DNP-Ficoll.
Conclusions: Thus, mLDL uptake by B cells likely via desmosterol accumulation plays an anti-inflammatory role through the regulation of B cell activation and functions in atherosclerosis.

Workshop track 1
Workshop: Lipoproteins and Immunity

EAS19-0209.
HYPERCHOLESTEROLEMIA PROMOTES A MAST CELL-CD4+ T-CELL INTERACTION IN ATHEROSCLEROSIS

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Background and Aims: Atherosclerosis development is enhanced by both local and systemic immune responses and mast cells have been shown to accumulate inside atherosclerotic plaques to promote disease progression. Our research focused on pathways via which mast cells can elicit their atherogenic effects and here, we aimed to analyze the interplay between mast cells and CD4+ T-cells in atherosclerosis.

Methods: In LDLr-/- mice fed a Western-type diet (WTD), the MHC-II+/FcεRIα+CD117+ mast cell population in the peritoneum increased significantly (control:30.4±1.7%; WTD:52.5±5.9%, P=0.01). To examine the efficacy of the MHC-II expressing mast cells in vivo, we injected Eα-peptide (1mg/mL), which is specifically presented by MHC-II molecules.
Results: After 24 hours, we detected Eα-peptide fragments on the peritoneal mast cell surface (PBS:2.3±0.2%; Eα:7.4±1.7%; P=0.02). To further investigate the effect of hyperlipidemia on the antigen presentation capacity of mast cells, we co-cultured OVApeptide-pretreated mast cells under either chow or WTD serum conditions with OT-II CD4+ T-cells. CD4+ T-cell proliferation was markedly increased in the WTD condition as measured by [3H]-Thymidine incorporation, (chow-MC: 9033±2222 dpm compared to WTD-MC: 25332±2889 dpm, P<0.0001). Conversely, the aortic CD4+ T cell content of mast cell deficient mice on WTD showed a reduction in ki67+/CD4+ proliferation (apoE-/-: 30.9±5.2% vs apoE/KitW-sh/W-sh: 13.1±1.2%; P=0.006). Of clinical relevance, upon examination of 20 human plaques obtained by endarterectomy surgery, we observed that 18.5±4.4% of human mast cells express HLA-DR, the human equivalent of MHC-II.
Conclusions: In conclusion, we have increasing evidence that mast cells may function as non-classical antigen presenting cells in atherosclerosis.

Workshop track 1
Workshop: Lipoproteins and Immunity

EAS19-0440.
CONTINUOUS TCR SIGNALING IN THE ATHEROSCLEROTIC ENVIRONMENT INDUCES IMMUNOMODULATORY CD8+ T-CELLS EXPRESSING CD39

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Background and Aims: $CD8^+$ T-cells can be atheroprotective in clinically relevant advanced stages of atherosclerosis, as their depletion results in less stable lesions with a more inflammatory phenotype. However, the phenotype and function of these cells in the lesional microenvironment remains to be determined. Here, we address how the atherosclerotic environment affects the functionality of $CD8^+$ T-cells.

Methods: We compared the cytokine production of CD8+ T-cells derived from spleens and aortas of apoE-/- mice with advanced atherosclerosis by flow cytometry.
Results: CD8+ T-cells isolated from atherosclerotic lesions produced lower amounts of IFN-γ and TNF-α than their splenic counterparts. The observed dysfunctional phenotype of the lesion-derived CD8+ T-cells was associated with an increased expression of the ectonucleotidase CD39, which converts inflammatory extracellular ATP into immunomodulatory adenosine. Indeed, pharmacological inhibition of CD39 in apoE-/- mice partly restored cytokine production by CD8+ T-cells. Using a bone-marrow transplantation approach, we showed that induction of CD39 was a consequence of antigen-specific CD8+ T-cell activation via T-cell receptor (TCR) signaling within the lesions. Importantly, analysis of human endarterectomy samples showed a clear microenvironment specific upregulation of CD39 on CD8+ T-cells in the plaques of human patients compared to matched CD8+ T-cells from the blood .
Conclusions: Our results indicate that the continuous TCR signaling in the atherosclerotic plaque induces an immune regulatory CD8+ T-cell phenotype that is associated with decreased cytokine production through increased CD39 expression in both a murine atherosclerotic model and in atherosclerosis patients. This provides a new understanding of atheroprotective immune regulation by CD8+ T-cells.

Workshop track 1
Workshop: Lipoproteins and Immunity

EAS19-0940.
ENGINEERED REGULATORY T CELL ADOPTIVE THERAPY AS A NOVEL TOOL FOR THE TREATMENT OF ATHEROSCLEROSIS

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Background and Aims: Loss of anti-inflammatory activity of Tregs has been associated to immunoinflammatory diseases, including atherosclerosis. Therefore, the use of Treg-Adoptive Cell Therapy(ACT) is emerging as a therapeutic strategy to specifically modulate impaired immune responses. Although ACT has produced encouraging results in animal models, a main limitation remains the possibility to target a selected tissue. Our aim was to develop a plaque-homing selective Treg-ACT in models of atherosclerosis.

Methods: Treg were retrovirally (IRES-EGFP vector) transfected with chemokine receptors or an empty vector and i.v. injected ($2x10^5$ GFP+ cells/mouse) in male 8-week WTD LDLR-KO. Homing of transfected Treg to atherosclerotic plaque, its progression and composition was analysed by flow-cytometry and histology.
Results: The chemokine CX3CL1 is selectively expressed in the aorta, but not in other tissues (lymph nodes, spleen and liver) of 8-week WTD LDLR-KO, contrary to CCL2, usually associated with inflammation during atherosclerosis. Therefore, we compared homing of CCR2- and CX3CR1-transfected Treg to the aorta. While migration of CCR2-Treg was not selective, CX3CR1-Treg showed a specific homing to atherosclerotic plaque ($p<0.05$) with similar homing in lymph nodes and spleen. Next, we investigated whether CX3CR1-Treg reduce atherosclerosis by performing plaque analysis 4 weeks after ACT. Altough levels of plasma cholesterol were similar, CX3CR1- vs control-Treg treated mice showed decreased plaque area and macrophage infiltration, and increased stability($p<0.05$).
Conclusions: CX3CL1/CX3CR1 axis targets selective migration to the atherosclerotic plaque. Overexpressing CX3CR1 appears a promising ACT to promote selective homing of Treg into the plaque thus limiting atherosclerosis progression.

Workshop track 1
Workshop: Lipoproteins and Immunity

EAS19-0453.
ENDOTHELIAL ATYPICAL CHEMOKINE RECEPTOR-3 IS A NOVEL DRIVER OF ATHEROSCLEROSIS

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Background and Aims: Genome wide association studies revealed a strong association between the *CXCL12* gene locus and cardiovascular diseases (CVD), highlighting its receptors CXCR4 and atypical chemokine receptor-3 (ACKR3) as targets for CVD research. Although CXCR4 is well studied in atherosclerosis, ACKR3's particular roles remain to be explicated. The aim of this project is to decipher the role of endothelial ACKR3 in atherosclerosis.

Methods: We studied the role of endothelial ACKR3 through a knockout of ACKR3 in the vascular endothelium (Bmx-cre) of Apolipoprotein-E deficient mice fed with a cholesterol-rich diet. Lesions in the aortic roots and arches were analyzed via hematoxylin and eosin staining. The phenotype was further characterized using *ex-vivo* perfusion and intra-vital microscopy for cell adhesion, Evans-blue injection for endothelial permeability, enzyme linked immunosorbent assay and flow cytometry for inflammation assessment.
Results: Endothelial ACKR3 deficiency resulted in significantly smaller lesion sizes in the aortic roots and arches, accompanied by more stable plaque phenotypes (more lesional collagen and smooth muscle cell content, smaller necrotic cores). Knockout mice revealed less macrophage and intracellular adhesion molecule (ICAM)-positive endothelial cell content within lesions. Moreover, immune cell adhesion on carotid arteries lacking endothelial ACKR3 was decreased whilst endothelial permeability was not affected. Finally, knockout mice showed reduced IL-6 and classical monocyte levels in circulation.
Conclusions: We show that endothelial ACKR3 drives atherosclerosis by supporting leukocyte adhesion onto the endothelium and consequently regulating intra-plaque macrophage accumulation. Endothelial ACKR3 exposes immunomodulatory properties by influencing circulating IL-6 and classical monocyte levels. Furthermore, it plays an important role in plaque stability.

Workshop track 1
Workshop: Lipoproteins and Immunity

EAS19-1075.
MYELOID INTERFERON REGULATORY FACTOR 8 DEFICIENCY PREVENTS THE DEVELOPMENT OF ATHEROSCLEROSIS

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Background and Aims:

Background: IRF8 is a haematopoietic transcription factor crucial for the development of myeloid cells and known to mediate their inflammatory responses. Our group has previously shown IRF8 to selectively modulate the expression of macrophage genes involved in atherosclerotic plaque development.
Aim: To investigate the role of myeloid-IRF8 in atherosclerosis development.
Methods: We have generated a myeloid-IRF8 deficient mouse model, using the LysM directed Cre recombinase, on the LDLR-deficient background ($M\text{-}IRF8KO^{LdlrKO}$). Mice were challenged with a western diet for 12 weeks after which atherosclerotic lesions were quantified in the aortic root by H&E staining respectively.