Grant, Roche; **R. J. Gropler:** Other, ERT, Research Grant, NIH, GE; **Y. Liu:** Nothing to disclose; **M. A. Zayed:** Nothing to disclose

22-VIRC-484-AHA-VD

Epigenetically Altered Diabetic Wound Plasmacytoid Dendritic Cells Direct Wound CD4 T-cells toward a Th17 Phenotype

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Background: Plasmacytoid dendritic cells (pDCs) are first responders to tissue damage and interact with wound CD4 T cells to orchestrate tissue repair. In diabetes, the role of pDCs and their subsequent influence on wound CD4 T cells and inflammation during tissue repair is unknown.

Methods: Using human diabetic wound single cell sequencing, we identified that human wound CD4 T cells are primarily the Th17 phenotype. Thus, we hypothesized that pDCs in the wounds may regulate this inflammatory T-cell phenotype, contributing to chronic inflammation. Although pDCs exist in low numbers in the wounds, we isolated wound pDCs from diabetic and control mice and examined them for inflammatory cytokine expression.

Results: We found that diabetic pDC produced significantly more IL6 (among other inflammatory cytokines) compared with controls. This is important as interleukin (IL)6 has been shown to skew CD4 T cells toward a Th17 phenotype in tissues. Upon co-culture of diabetic pDCs with naïve CD4 T-cells, a significant increase in Th17 cells was observed by intracellular flow cytometry analysis. To examine the increased IL6 from diabetic pDCs, we isolated diabetic and control wound pDCs and performed an epigenetic superarray. We identified that histone demethylase Jarid1C was significantly decreased in diabetic pDCs compared with controls. We then isolated diabetic pDCs and performed a chromatin immunoprecipitation analysis on the IL6 nuclear factor-κB binding sites in the promoter and identified an increase in trimethylated lysine 4 on histone 3, a marker regulated by Jarid 1C and consistent with increased transcription of IL6.

Conclusions: Taken together, these data suggest that, in diabetes, pDCs are epigenetically altered to produce increased IL6 and contribute to increased Th17 and inflammation in diabetic wounds.

Author Disclosures: C. O. Audu: Nothing to disclose; **K. A. Gallagher:** Nothing to disclose; **S. Wolf:** Nothing to disclose; **W. Melvin:** Nothing to disclose; **F. Davis:** Nothing to disclose; **S. Sharma:** Nothing to disclose; **K. Mangum:** Nothing to disclose; **E. Barrett:** Nothing to disclose; **A. Joshi:** Nothing to disclose; **A. T. Obi:** Nothing to disclose

22-VIRC-463-AHA-VD

Periadventitial Lysyl Oxidase Inhibition Using Electrospun Nanofibers Improves Arteriovenous Fistula Maturation

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Background: Arteriovenous fistula (AVF) nonmaturation is associated with postoperative fibrosis and an increase in lysyl oxidase expression. The purpose of this study was to determine whether unidirectional periadventitial delivery of β-aminoproprionitrile (BAPN) using bilayer poly lactic-co-glycolic acid nanofiber scaffolds is a feasible approach to reduce AVF fibrosis and encourage adaptive vascular remodeling.

Methods: AVFs were created in 30 Sprague Dawley rats (200-350 g) of both sexes by an end-to-side anastomosis of the epigastric vein to the femoral artery. BAPN-loaded (b-BAPN) (n = 10) or vehicle (b-VEH) (n = 10) scaffolds (5 mm × 5 mm) were wrapped around the juxta-anastomotic zone of the epigastric vein immediately after anastomosis. AVFs without treatment were used as an additional control (n = 10). Changes in lumen diameter were followed-up weekly using ultrasound examinations. Flow was calculated using the pulse wave velocity and lumen diameter. AVFs were collected at 21 days for histomorphometric analysis and to assess fibrosis.

Results: b-BAPN significantly increased dilation of AVFs at day 14 (185.9%) compared with b-VEH (135.5%; $P = .0006$) and control AVFs (113.6%; $P < .0001$). The treatment (214.2%) had a significant difference compared with b-VEH (135.5%; $P < .0001$) and AVF controls (122%; $P < .0001$) at day 21 as well. In addition, blood flow was significantly enhanced in the treatment group (38.13 mL/min) versus b-VEH (9.436 mL/min; $P = .0002$) and AVF controls (16.11 mL/min; $P < .0001$). Histomorphometric analysis confirmed the ultrasound findings. Treatment with b-BAPN significantly increased lumen area compared with control AVFs (354,127 μm^2 vs 95,580 μm^2; $P = .0048$). While there was no difference in intimal growth ($P = .5460$), b-BAPN significantly reduced luminal occlusion with respect to control AVFs (0.0611 vs 0.2067; $P = .0002$). Finally, b-BAPN (49.48%) significantly reduced the fibrosis percent area compared with b-VEH (72.14%; $P = .0016$) and control AVFs (74.5%; $P = .0007$).

Conclusions: Periadventitial lysyl oxidase inhibition prevents AVF postoperative fibrosis and promotes adaptive vascular remodeling.

Author Disclosures: B. C. Applewhite: Nothing to disclose; **Y. Wei:** Nothing to disclose; **F. M. Andreopoulos:** Nothing to disclose; **L. Martinez:** Nothing to disclose; **R. I. Vazquez-Padron:** Nothing to disclose

22-VIRC-536-AHA-VD

Low-density Lipoprotein Receptor-related Protein 1 Prevents Aortic and Superior Mesenteric Artery Aneurysms by Modulating Excessive Angiotensin II Signaling

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Background: Low-density lipoprotein receptor-related protein 1 (LRP1) is known to contribute to vascular homeostasis and plays a protective role against the development of aneurysms. LRP1 has specifically been associated with abdominal aortic aneurysms in genome-wide association studies. We and others have also previously reported that mice with a conditional smooth muscle cell knockout of LRP1 ($smLRP1^{-/-}$) exhibit vascular wall dysfunction and aortic dilation. In this study, we sought to investigate the molecular mechanisms by which LRP1 regulates vessel wall integrity in hopes of identifying potential therapeutic targets against aneurysm progression.

Methods: Analysis of proteomic data of the descending thoracic aorta, abdominal aorta, and superior mesenteric artery (SMA) of $smLRP1^{-/-}$ mice confirmed excess activation of the angiotensin signaling pathway. To verify these observations, we designed experiments to determine if losartan, an angiotensin II receptor blocker, could prevent aneurysm progression in $smLRP1^{-/-}$ mice. Following treatment, we analyzed the aortic vasculature from LRP1+/+ and $smLRP1^{-/-}$ adult mice (16 weeks of age) with or without treatment.

Results: Without losartan, $smLRP1^{-/-}$ mice developed spontaneous aortic and SMA aneurysms as demonstrated by increased vessel dilation, vessel medial thickening, and significant fragmentation of the elastic lamina. We found that when treated with losartan, $smLRP1^{-/-}$ mice had a complete restoration of medial layer thickness and a partial decrease of elastin fragmentation compared with untreated $smLRP1^{-/-}$ mice. Furthermore, losartan completely blocked SMA aneurysm formation in $smLRP1^{-/-}$ mice.

Conclusions: Our findings suggest a critical role for LRP1 in maintaining vascular homeostasis and preventing aneurysm formation through its regulation of the angiotensin signaling pathway. Further studies will need to investigate the connection between LRP1 and the angiotensin signaling pathway in aneurysm formation to further understand the complex pathophysiology and potentially develop effective therapeutic targets for this disease.

Author Disclosures: J. M. Zhang: Nothing to disclose; **M. Migliorini:** Nothing to disclose; **B. Hampton:** Nothing to disclose; **F. Ni:** Nothing to

disclose; **A. Ucuzian:** Nothing to disclose; **D. Strickland:** Nothing to disclose

22-VIRC-542-AHA-VD

Lack of Mitogen-activated Kinase Phosphatase-5 in Macrophages Protects Ldlr-null Mice against Atherogenesis

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Background: Mitogen-activated protein kinases, including JNK, ERK, and p38 mitogen-activated protein kinase, are critical in regulating the expression of various proinflammatory and anti-inflammatory cytokines and chemokines. Previous work has suggested that the absence of MAP kinase phosphatase-5 (MKP-5) inhibits oxidized low-density lipoprotein (oxLDL)-induced macrophage foam cell formation without influencing the MAKP activation. The current study is to determine the role of macrophage MKP-5 in the pathogenesis of atherosclerosis and underlying mechanisms.

Methods: Nine-week-old male congenic MKP-5 deficient (MKP-$5^{-/-}$) and C57Bl/6J control (WT) mice on an low-density lipoprotein (LDL) receptor knock-out (LDL$R^{-/-}$) background were fed with a high-fat diet containing 1.25% cholesterol for 14 weeks. Global deficiency of MKP-5 attenuated atherosclerotic plaque formation without altering the lipid profile in vivo. To further elucidate the macrophage-specific effect of MKP-5 in atherogenesis, lethally irradiated LDL$R^{-/-}$ mice were transplanted with wild-type or MKP-$5^{-/-}$ bone marrow and subjected to high-fat feeding.

Results: Mice transplanted with MKP-$5^{-/-}$ bone marrow developed smaller atherosclerotic lesions accompanied by decreased lipid deposition and macrophage content compared with wild type. Lack of MKP-5 in macrophages reduced plasma levels of interleukin-1α (IL-1α) and IL-7, elevated anti-inflammatory cytokines IL-1 receptor antagonist (IL-1rn), and IL-4. Mechanistically, lack of MKP-5 in macrophages inhibited ox-LDL-induced foam cell formation through enhanced cholesterol efflux mediated by increased expression of ATP-binding cassette transporters ABCA1 and ABCG1.

Conclusions: These data suggest that the myeloid MKP-5 deficiency reduces atherosclerosis progression and foam cell formation by ameliorating cholesterol efflux and inhibiting inflammation.

Author Disclosures: X. Zhang: Nothing to disclose; **Z. Zhao:** Nothing to disclose; **M. Baldini:** Nothing to disclose; **C. Zhang:** Nothing to disclose; **B. Tao:** Nothing to disclose; **L. Zhang:** Nothing to disclose; **A. M. Bennett:** Nothing to disclose; **J. Yu:** Nothing to disclose

22-VIRC-517-AHA-VD

Impaired Ischemic Myocutaneous Wound Revascularization and Transdermal H_2S Emissions in Diabetic Rats

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Background: Hydrogen sulfide (H_2S) has been recognized as an important signaling molecule in cellular O_2 sensing, wound healing and angiogenesis. Studies have shown abnormal H_2S levels in diabetic patients with cardiovascular disease. Diminished H_2S signaling may play a causative role in diabetic foot wounds. The Transdermal Arterial Gasotransmitter Sensor (TAGS) device measures real-time H_2S emissions through the skin. In this work, we used the novel TAGS device to characterize transdermal H_2S emissions during diabetic and nondiabetic wound healing for the first time.

Methods: Dorsal peninsular-shaped myocutaneous ischemic flap wounds were created under anesthesia. Sprague-Dawley (SD) and Zucker Diabetic Fatty (ZDF) rats (n = 10 each) were compared. Transdermal H_2S emissions, laser speckle contrast images and planimetric photos were serially taken from the wound flap area over 14 days. After humane killing, healed flap tissue was collected for histologic (hematoxylin and eosin) analysis of panniculus carnosus (skin muscle) viability as a proxy for degree of ischemic insult.

Results: ZDF rats were significantly hyperglycemic (mean 516 mg/dL vs 201 mg/dL for SD; $P = .002$). Similar mean baseline (preoperative) H_2S emissions were observed in SD (16 ppb) and ZDF (12 ppb) rats ($P = .25$). During revascularization and healing, ZDF wounds emitted significantly less H_2S (10 ppb at day 14) as compared with SD (28 ppb at day 14; $P < .01$). ZDF wounds demonstrated impaired flap engraftment and revascularization by laser speckle contrast images (mean 65.6 perfusion units for ZDF vs 188.0 perfusion units for SD at day 14; $P < .01$) and planimetric analysis (mean 16.6% necrosis for ZDF vs 5.3% necrosis for SD at day 14; $P = .01$). Panniculus carnosus mean myofibril count, myofibril diameter, and layer thickness were significantly decreased ($P < .01$) in the ZDF cohort, suggesting greater tissue ischemic insult and muscle loss.

Conclusions: Diabetic rats have impaired wound H_2S production and poor revascularization. These physiologic alterations are accompanied by greater wound necrosis and histologic ischemic insult. This finding suggests H_2S abnormalities in diabetes may play a role in the pathogenesis of impaired wound healing and could represent a potential future therapeutic target for these difficult wounds.

Author Disclosures: J. Giacolone: Nothing to disclose; **B. Matheson:** Nothing to disclose; **R. Shekarriz:** Nothing to disclose; **N. Kanagy:** Nothing to disclose; **R. Clark:** Nothing to disclose

22-VIRC-472-AHA-VD

Imatinib Promotes Reverse Cholesterol Transport and Elevates Scavenger Receptor B1

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Background: Dyslipidemia is a cardiovascular risk factor for coronary artery disease and atherosclerosis that is characterized by elevated serum cholesterol and lipid levels. Although high-density lipoprotein-associated cholesterol (HDL-C) is associated with a reduced risk of cardiovascular events, targeted therapy to increase HDL-C levels have been unsuccessful in altering outcomes of associated atherosclerotic disease. Single nucleotide polymorphisms in *SCARB1*, the gene that encodes HDL receptor scavenger receptor B1 (SR-BI), are associated with dyslipidemia and atherosclerotic cardiovascular disease. We were the first to identify inherited mutations in *SCARB1* that segregate with disease in a family with severe coronary artery disease and dyslipidemia, including elevated HDL. Our findings suggest that HDL function (vs HDL-C concentration) may be a promising target for cholesterol-based therapy.

Methods: Here, we performed an unbiased high throughput drug screen with 788 US Food and Drug Administration-approved compounds, using HepG2 cells to measure endogenous HDL binding. We identified five compounds that significantly increased endogenous HDL binding: imatinib, trimethoprim, eszopiclone, clemastine, and mepenzolate, of which, imatinib was the only compound to increase SR-BI expression. Imatinib is a tyrosine kinase inhibitor that is a chemotherapeutic agent designed to treat individuals with chronic myeloid leukemia.

Results: Limited clinical evidence suggests a reduction in total cholesterol with 400 mg/day imatinib. Additionally, imatinib treatment (150 mg/kg) in mouse models of atherosclerosis reduces total cholesterol. Yet, no data are available on the effects of imatinib on HDL and reverse cholesterol transport. We have found that imatinib promotes HDL binding and SR-BI expression in vitro. Furthermore, in wildtype C57Bl/6 mice on a high-fat, high cholesterol diet, imatinib treatment (50 mg/kg) was sufficient to decrease plasma total cholesterol, HDL-C and triglyceride levels and elevate hepatic SR-BI expression.

Conclusions: Our data support the exploration of imatinib-mediated SR-BI regulation, HDL metabolism, and RCT pathway to identify new therapeutic targets for dyslipidemia.

Author Disclosures: H. C. Sucharski: Nothing to disclose; **E. K. Dudley:** Nothing to disclose; **J. Williams:** Nothing to disclose; **R. Dewal:** Nothing to disclose; **K. I. Stanford:** Nothing to disclose; **P. J. Mohler:** Nothing to disclose; **S. N. Koenig:** Nothing to disclose