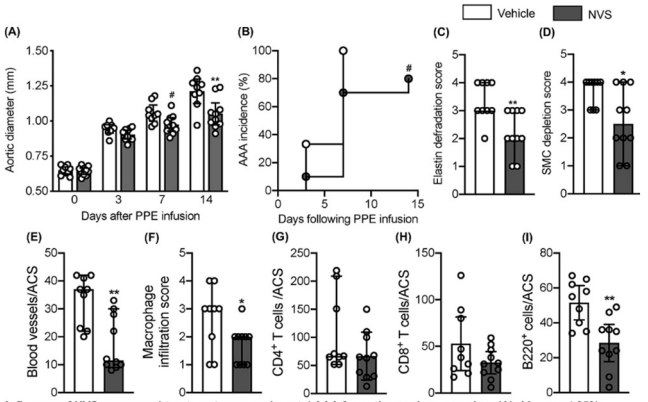
Conclusions: Photochemical linking of extracellular matrix proteins attenuated experimental AAA progression, suggesting a potential translational application for this approach in clinical disease management.



Influence of NVS compound treatment on experimental AAA formation and progression. (A): Mean and 95% confidential interval (CI) of maximal infrarenal aortic diameters for baseline and indicated days following porcine pancreatic elastase (PPE) infusion in vehicle (n=9) and NVS (n=10) treatment groups. ANOVA, 0.05<#P<0.05 and **P<0.01 compared to vehicle treatment. **(B):** AAA incidence. AAA defined as ≥50% increase over the baseline diameter. Kaplan-Meier test, 0.05<#P<0.05 compared to vehicle treatment. (C-I): Median (C-G) or mean (G, H) and 95% CI for elastin (C) and smooth muscle cell (SMC, D) destruction scores, neovessel density (E), macrophage infiltration score (F) and cell counts for individual lymphocyte subsets (G-I). Nonparametric Mann-Whitney test *P<0.05 (F) and Student-t test **P<0.01 compared to vehicle treatment.

Fig.

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22-VIRC-512-AHA-VD

Ccr2 Expression Is Increased in Patients with Symptomatic Carotid Arterial Occlusive Disease

Connor Engel, Mohamed Zaghloul, Rodrigo Meade, Pamela K. Woodard, Robert J. Gropler, Yongjian Liu, Mohamed A. Zayed. Washington University School of Medicine, St. Louis, MO

Background: Chemokines and their receptors play critical roles in the progression of atherosclerosis. The chemokine receptor, C-C chemokine receptor type 2 (CCR2), mediates an essential role in the differentiation of proinflammatory monocytes into macrophages. Recent studies suggest that proinflamatory macrophages in plaques predict plaque progression. While there is no clinical consensus on which patient with asymptomatic carotid artery stenosis would benefit from carotid endarterectomy, we hypothesized that CCR2 plaque content may predict plaque progression.

Methods: Carotid plaque of ten patients undergoing elective carotid endarterectomy were harvested from the operating room, paraffin-embedded, sectioned into 5-μm segments, immunostained for CCR2 and CD68, and imaged. Three 200 × 500-μm regions of interest were randomly selected within 200 μm of the lumen (superficial intima), outside 200 μm of the lumen (deep intima), and the arterial media. Two independent observers evaluated the number of CCR2- and/or CD68-positive cells. Counts in each region of interest were averaged and analyzed with the Student *t* test.

Results: Patients with symptomatic carotid artery stenosis (6/10) demonstrated higher CCR2 and CCR2-CD68 double positive cells within the superficial intima of carotid artery plaques (Figure, *A*; $P < .05$, n = 10). Similarly, patients with symptomatic disease also showed increased CCR2 in the plaque media (Figure, *B*; $P < .05$, n = 10). Interestingly, we also observed a higher number of CCR2 positive cells in the media layer of smokers (6/10) compared with nonsmokers (Figure, *C*; $P < .05$, n = 10).

Conclusions: Our study demonstrates the close association between CCR2 cellular content and the incidence of symptomatic carotid artery disease. Additionally, CCR2 plaque content appears to correlate with risk-factors such as smoking. These studies have important implications regarding the impact of immune modulation on carotid plaque vulnerability in patients with asymptomatic carotid artery stenosis.

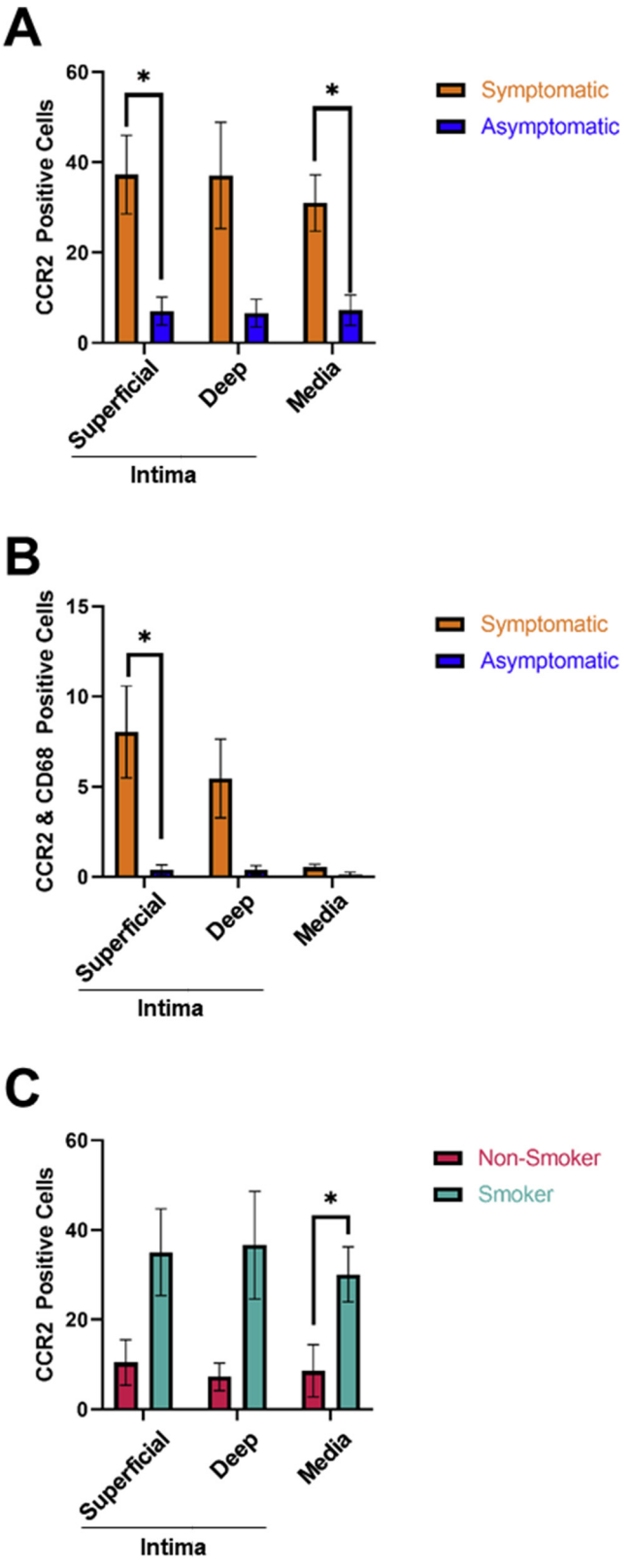

Fig.

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

Christopher O. Audu,[1] Sonya Wolf,[2] William Melvin,[2] Frank Davis,[1] Sriganesh Sharma,[1] Kevin Mangum,[1] Emily Barrett,[1] Amrita Joshi,[1] Andrea T. Obi,[1] Katherine A. Gallagher[3]. [1]University of Michigan, Ann Arbor, MI; [2]Ann Arbor, MI; [3]University of Michigan, Northville, MI

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.

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22-VIRC-463-AHA-VD

Periadventitial Lysyl Oxidase Inhibition Using Electrospun Nanofibers Improves Arteriovenous Fistula Maturation

Brandon C. Applewhite,[1] Yuntao Wei,[1] Fotios M. Andreopoulos,[2] Laisel Martinez,[1] Roberto I. Vazquez-Padron[1]. [1]University of Miami, Miami, FL; [2]University of Miami, Coral Gables, FL

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.

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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

Jackie M. Zhang, Mary Migliorini, Brian Hampton, Fenge Ni, Areck Ucuzian, Dudley Strickland. Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, Baltimore, MD

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.

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