Myeloid Cell PKM2 Deletion Enhances Efferocytosis and Reduces Atherosclerosis

Prakash Doddpattar,1 Rishabh Dev,1 Madankumar Chattge,1 Rakesh Kumar Patel,1 Manish Jain,1 Niran Dhanesha,1 Steven R. Lentz,1 Anil K. Chauhan,1, 2 University of Iowa, Iowa City, IA; 3 Iowa City, IA

Background: The glycolytic enzyme pyruvate kinase muscle 2 (PKM2) is upregulated in monocytes/macrophages of patients with atherosclerotic coronary artery disease. However, the role of cell type-specific PKM2 in the setting of atherosclerosis remains to be defined. We determined whether myeloid cell-specific PKM2 regulates efferocytosis and atherosclerosis.

Methods: We generated novel myeloid cell-specific PKM2−/− mice on Ldlr−/− background (PKM2m−/−/Ldlr−/−). Controls were littermate PKM2m+/−/Ldlr−/− mice. To rule out sex-based differences, male and female mice were placed on a high-fat Western diet for 14 weeks, starting at 8 weeks.

Results: PKM2 was upregulated in macrophages of Ldlr−/− mice fed the Western diet compared with a control Chow diet. Myeloid cell-specific deletion of PKM2 led to a significant reduction in lesions in the whole aorta and aortic sinus despite high cholesterol and triglyceride levels. Furthermore, we found decreased macrophage content in the lesions of myeloid cell-specific PKM2−/− mice associated with decreased MCP-1 levels in plasma, reduced transmigration of macrophages in response to MCP-1, and an impaired glycolytic rate. Macrophages isolated from myeloid-specific PKM2−/− mice fed the Western diet exhibited reduced expression of proinflammatory genes, including MCP-1, interleukin-1β, and interleukin-12. Myeloid cell-specific PKM2−/− mice exhibited reduced apoptosis concomitant with enhanced macrophage efferocytosis and upregulation of LRPI in macrophages in vitro and atherosclerotic lesions in vivo. Silencing LRPI in PKM2-deficient macrophages restored inflammatory gene expression and reduced efferocytosis. As a therapeutic intervention, inhibiting PKM2 nuclear translocation using a small molecule reduced glycolytic rate, enhanced efferocytosis, and reduced atherosclerosis in Ldlr−/− mice.

Conclusions: Genetic deletion or limiting PKM2 nuclear translocation in myeloid cells reduces atherosclerosis by suppressing inflammation and enhancing efferocytosis.
**Conclusions:** Photochemical linking of extracellular matrix proteins attenuated experimental AAA progression, suggesting a potential translational application for this approach in clinical disease management.

**Author Disclosures:** B. Xu: Nothing to disclose; T. Ikezoe: Nothing to disclose; J. Guo: Nothing to disclose; T. Shoji: Nothing to disclose; K. S. Warner: Employment, Alucent Biomedical; K. Kauser: Employment, Alucent Biomedical; R. L. Dalman: Nothing to disclose

---

**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 proinflammatory 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 x 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 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.

**Author Disclosures:** C. Engel: Nothing to disclose; M. Zaghloul: Nothing to disclose; R. Meade: Nothing to disclose; P. K. Woodard: Other.