IL-1 alpha is myristoylated for monocyte surface translocation and promotes atherosclerosis in mice

C. Maeder¹, T. Speer², A. Wirth³, J.N. Boeckel¹, I. Gadi⁴, K. Shahzad⁴, B. Isermann⁴, M. Freichel³, U. Laufs¹, S. Gaul¹

¹University Hospital Leipzig, Klinik und Poliklinik für Kardiologie, Leipzig, Germany
²University Hospital Frankfurt, Department of Nephrology, Frankfurt, Germany
³University of Heidelberg, Institute of Pharmacology, Heidelberg, Germany
⁴University Hospital Leipzig, Department of Diagnostics, Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany

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Background: The role of Interleukin-1 alpha (IL-1α) and beta (IL-1β) in the pathogenesis of atherosclerosis in vivo has been controversial for years. In contrast to IL-1β, which is dependent on the NLR family pyrin domain containing 3 (NLRP3) inflammasome, IL-1α can be tethered to the plasma membrane (cSIL-1α) where it exerts pro-inflammatory effects. We characterised the role of IL-1α and the Nlrp3 inflammasome in a nongenetic hyperlipidemic mouse model and examined the function of cSIL-1α in human monocytes.

Methods: Atherosclerosis was induced by a single injection of mutant PCSK9-AAV8 virus in wild-type (WT, referred to as PCSK9-AAV), Il1a⁻/⁻, Nlrp3⁻/⁻, and Il1b⁻/⁻ mice, in combination with high-fat western diet (21% fat, 0.2% cholesterol) for 12 weeks. Plaque size and lipid content were histologically analysed. The serum proteome was studied using a targeted multiplex PEA (Olink Proteomics). Human primary monocytes and human umbilical vein endothelial cells (HUVECs) were used to examine cSIL-1α-IL1R interaction by analyzing VCAM1 expression and monocyte adhesion. LPS was used to induce IL-1α protein synthesis and membrane translocation in vitro. Myristoylation was measured via flow cytometry.

Results: Il1a⁻/⁻ animals showed significantly reduced atherosclerotic lesion size (-62% vs. PCSK9-AAV) and fat accumulation within the plaque (-64% vs. PCSK9-AAV). Knockout of Nlrp3 and IL-1β did not reduce plaque size or lipid content, indicating an Nlrp3-independent mechanism of the protective effect of Il1a⁻/⁻. Circulating pro-inflammatory cytokines such as IL-1α, IL-1β, and IL-6 were downregulated in serum of Il1a⁻/⁻, Nlrp3⁻/⁻, and Il1b⁻/⁻ mice compared to PCSK9-AAV mice suggesting that the development of atherosclerotic plaques is independent of circulating inflammatory cytokines at an early stage. Therefore, we investigated the role of cSIL-1α on human monocytes. Stimulation with LPS showed enrichment of cSIL-1α by 13.2% (p<0.05) on monocytes without releasing IL-1α. Treatment of HUVECs with LPS-stimulated monocytes resulted in increased VCAM1 expression (+22.2%, p<0.05) and monocyte adhesion (1.9 fold, p<0.05) through a cSIL-1α-IL1R interaction which was abolished by the addition of neutralising antibodies for IL1R1 and IL-1α (p<0.05, vs. LPS-treated monocytes). To investigate whether the IL-1α translocation is mediated by protein myristoylation, human monocytes were pre-incubated with the n-myristoyl-transferase inhibitor IMP-1088 before LPS stimulation, resulting in a reduction of cSIL-1α expression (p<0.05, vs. LPS-treated monocytes).

Conclusion: This study demonstrated a protective effect of Il1a deficiency on the development of atherosclerosis. CSIL-1α mediates monocyte-to-endothelial adhesion by increasing VCAM1 expression through endothelial IL1R1 signaling. The data underscore the importance of the juxtacrine signaling of IL-1α and its role in the development of atherosclerosis independent of circulating cytokine levels.
A: Representative histological images of the aortic root stained with haematoxylin/eosin (H&E) and (B) corresponding quantification of plaque area. PCSK9-AAV served as control of the atherosclerotic model. H&E stainings were imaged with 4 × magnification, scale bar 100 μm. C: Percentage of calcine-labelled monocytes adherent to HUVECs after 4 hours. Monocytes were treated as indicated prior to labelling. Neutralising IL-1α antibody (nIL-1α Ab, 100 ng/ml) was added one hour before treatment with 100 ng/ml LPS. D: VCAM1 expression on HUVECs after 4-hr co-incubation with untreated or LPS-treated human monocytes, measured by flow cytometry. Neutralising IL1R1 antibody (nIL1R1 Ab, 10 μg/ml) was added to HUVECs one hour before the LPS treatment (100 ng/ml) of monocytes. E: Percentage of csIL-1α-positive cells after LPS stimulation (100 ng/ml) with or without 1 μM n-myristoyltransferase inhibitor IMP-1088. CsIL-1α was measured by flow cytometry. Data are presented as mean ± SEM, *p < 0.05.