Citrullination of histoneH3 in neutrophil via CXCL1 enhances neutrophil adhesion to femoral artery of LDLR−/− mice fed HFD

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Background: Vascular inflammation plays an important role in the development of atherosclerosis. Previously we have shown that a high-fat diet (HFD) increased neutrophil adhesion to the vascular intima in wild-type (wt) mice (Osaka M. Sci Rep. 2016). However, the involvement of neutrophils in atherosclerosis-related vascular inflammation is not well known.

Purpose: This study examined that neutrophil extracellular trap (NETs) or the hypercitrullination of histone H3 in neutrophils enhances neutrophil adhesion to atheroprone-arteries in LDL receptor null (LDLR−/−) mice.

Methods: We observed leukocyte adhesion in the femoral artery of LDLR−/− mice fed normal chow (NC) or HFD, and determined leukocyte subtype that adhered on vascular endothelium under neutrophil or monocyte depletion using intravital microscopy. Importantly, neutrophil adhesion was examined under the administration of TDFA which inhibits NETs and citrullination of histone H3, in LDLR−/− mice fed HFD. Furthermore, immunohistochemistry for citrullinated histone H3 in peripheral neutrophils of mice was examined. Comprehensive cytokine/chemokine analysis for a plasma of mice was performed to determine the factors citrullinating histone H3 in LDLR−/− mice. Moreover, these mice were treated with a novel specific PPARα agonist, to reduce the elevation of plasma triglyceride levels.

Results: Leukocyte adhesion in LDLR−/− mice fed HFD significantly increased compared to NC. More interestingly, it significantly enhanced compared to wt mice fed HFD. Furthermore, neutrophil depletion rather than monocyte depletion diminished leukocyte adhesion, suggesting that the leukocyte subtype that adhered in LDLR−/− mice fed HFD was neutrophil. Neutrophil adhesion in these mice significantly was reduced by the administration of TDFA, suggesting a pivotal role for histone H3 citrullination in neutrophil adhesion. Moreover, citrullination of histone H3 in neutrophils from LDLR−/− mice fed HFD but not from those without HFD was significantly enhanced. In addition, comprehensive cytokine/chemokine analysis revealed an increase of CXCL1 in plasma of LDLR−/− mice fed HFD. CXCL1 enhanced neutrophil adhesion to HUVECs, and the adhesion significantly decreased by the treatment of TDFA to neutrophil in vitro non-static adhesion assay. These results showed that CXCL1 enhanced neutrophil adhesion in LDLR−/− mice fed HFD through citrullination. Furthermore, when these mice were treated with PPARα agonist, observed histone citrullination, as well as neutrophil adhesion, was significantly reduced.

Conclusion: These results suggest that HFD induced histone citrullination in neutrophils in LDLR−/− mice and PPARα agonist plays a role during hypertriglyceridemia-mediated vascular inflammation in atherosclerosis.