# CHOLESTEROL EFFLUX PATHWAYS SUPPRESS INFLAMMASOME ACTIVATION, NEUTROPHIL EXTRACELLULAR TRAP FORMATION, AND ATHEROSCLEROSIS

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## Immune cell crosstalk in atherosclerosis

### W1:3.
### CHOLESTEROL EFFLUX PATHWAYS SUPPRESS INFLAMMASOME ACTIVATION, NEUTROPHIL EXTRACELLULAR TRAP FORMATION, AND ATHEROSCLEROSIS

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**Aim:** The CANTOS trial indicated that pathways required for IL-1β secretion increase cardiovascular risk in humans. IL-1β and IL-18 are produced via the NLRP3 inflammasome in myeloid cells in response to excessive cholesterol accumulation, but mechanisms linking NLRP3 inflammasome activation to atherosclerosis are unclear. ATP Binding Cassette A1 and G1 (ABCA1/G1) mediate cholesterol efflux to HDL and Abca1/g1 deficiency in myeloid cells leads to excessive cholesterol accumulation. We aimed to obtain new insights into mechanisms linking NLRP3 inflammasome activation to atherogenesis.
**Methods:** We generated myeloid Abca1/g1 deficient mice with or without deficiency of the inflammasome components Nlrp3 or Caspase-1, transplanted their bone marrow into Ldlr-/- mice, and fed them Western-type diet.
**Results:** Myeloid Abca1/g1 deficiency increased plasma IL-18 levels in Ldlr-/- mice, with reversal by Nlrp3 or Caspase-1 deficiency, indicating NLRP3 inflammasome activation. Myeloid Abca1/g1 deficiency enhanced Caspase-1 cleavage not only in splenic monocytes and macrophages, but also in neutrophils, indicating a previously unrecognized role for neutrophil cholesterol accumulation in inflammasome activation in vivo. Myeloid Abca1/g1 deficiency dramatically enhanced neutrophil accumulation and neutrophil extracellular trap (NET) formation in atherosclerotic plaques, which was NLRP3 inflammasome dependent. Nlrp3 or Caspase-1 deficiency also decreased atherosclerotic lesion size in myeloid Abca1/g1 deficient Ldlr-/- mice. Tangier Disease patients, who carry a homozygous loss-of-function mutation for ABCA1 and have increased myeloid cholesterol content, showed marked increases in plasma IL-1β and IL-18 levels compared to controls, suggesting human relevance.
**Conclusions:** Increased cholesterol content in myeloid cells, and notably neutrophils, activates the NLRP3 inflammasome, enhancing neutrophil accumulation and NETosis in atherosclerotic plaques.

### W1:4.
### GLUCAGON-LIKE PEPTIDE 1 RECEPTOR AGONIST LIRAGLUTIDE IMPACTS IMMUNE CELL PHENOTYPES IN APOLIPOPROTEIN E DEFICIENT MICE DURING PROGRESSION AND REGRESSION OF PRE-ESTABLISHED ATHEROSCLEROSIS

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**Aim:** Biologically active macrophages and dendritic cells are the main immune cell subsets which drive atherosclerosis development. Recent evidence suggests liraglutide, the glucagon-like peptide-1 receptor (GLP-1R) agonist, can alter macrophage phenotypes. We hypothesized that liraglutide could limit progression and induce regression of atherosclerosis in vivo via modulation of the inflammatory response.
**Methods:** Bone marrow derived macrophages (BMDMs) from wild-type C57BL/6 mice were treated with liraglutide to define it's effect on macrophage phenotypes. In parallel, apolipoprotein E deficient (ApoE-/-) mice were fed a high-fat (60%) high-cholesterol (1%) diet for 8-12 weeks to induce atherosclerotic disease with 300μg/Kg daily liraglutide from weeks 3-8 or weeks 8-12 to investigate disease progression and regression, respectively. Macrophages were analysed for M1 and M2 markers by gene expression analysis, ELISA and flow cytometry. Monocytes and dendritic cells were analysed from splenic and lymphoid tissues by flow cytometry. Atherosclerotic lesions in aortae from ApoE-/- mice were quantified by en face analysis.
**Results:** Liraglutide halted atherosclerotic lesion formation in ApoE-/- mice coincident with decreased M1 marker and significantly increased M2 marker expression in BMDMs. There was also significantly reduced pro-inflammatory and increased anti-inflammatory monocyte/macrophage populations and dendritic cells in lymphoid tissues in vivo. This coincided with a significant reduction in total lesion formation in ApoE-/- mice suggesting liraglutide inhibits initiation and development of atherosclerosis.
**Conclusions:** This data supports a therapeutic role for liraglutide as an atheroprotective agent in both halting progression and inducing regression of atheroscelrosis, via modulating immune cells towards a pro-resolving phenotype.