High density lipoproteins exert pro-inflammatory effects on macrophages via passive cholesterol depletion and PKC-NF-κB/STAT1-IRF1 signaling.

**Methods & Results:** Pre-incubation of human and murine macrophages in vitro with human reconstituted (apolipoproteinA-I/phosphatidylcholine) or native HDL significantly decreased LPS-induced anti-inflammatory IL-10 production, while the opposite was observed for the pro-inflammatory mediators IL-12 and TNF. We found that these effects are mediated by passive cholesterol depletion and lipid raft disruption, without involvement of ABCA1, ABCG1, SR-B1 or CD36. These pro-inflammatory effects are confirmed in vivo in peritoneal macrophages from ApoA1 transgenic mice, which have high circulating HDL levels. In line, innate response required for clearance of P. aeruginosa bacterial infection in lung were compromised in mice with low HDL levels. Native and reconstituted HDL enhances Toll Like Receptor-induced signaling by activating protein kinase C (PKC), since inhibition of PKC ablated the observed HDL effects. Using microarray analysis and macrophages from NF-κB luciferase mice, we observed that HDL induces NF-κB activation. Western blot and ChIP-PCR analyses showed that in particular the p65 subunit was activated. Using specific knock-out mice for the upstream activation pathways, we show that the observed HDL effects are independent from the upstream kinases IKK, NIK and CKII. Furthermore, using STAT1 knock-out mice we observed that also STAT1 is involved in the pro-inflammatory HDL effects on IL-10 and IL-12 secretion. On the other hand, using pharmacological inhibitors, we show that HDL enhances ADAM protease activity, thereby mediating TNF release.

**Conclusion and Clinical Relevance:** HDL exerts pro-inflammatory effects on macrophages via passive cholesterol depletion by activation of PKC, NF-κB and STAT1. These pro-inflammatory activities on macrophages could at least partly underlie the disappearing therapeutic potential of HDL raising therapy in current cardiovascular clinical trials.

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Homocysteine accelerated the formation of THP-1 macrophages-derived foam cells and cholesterol disorder via regulating the expressions of LXRα, ABCA1 and ABCG1

**Purpose:** To evaluate the potential effects of Hcy on cholesterol efflux of THP-1 macrophage-derived foam cells and the expressions of LXRα, ABCA1 and ABCG1 and verify the cholesterol efflux by using LXRα agonist to clarify the underlying mechanisms.

**Methods:** THP-1 monocyes were cultured and differentiated into macrophages with PMA. Then macrophages were induced by Hcy at 0.50, 100, 200 μmol/L with ox-LDL at 100 μmol/L for 24h to become foam cells. Positive CD14 was detected by flow cytometry to examine the percentage of macrophages.

**Results:** Compared with control group, the CD14 positive result showed that Hcy groups had more foam cells (P<0.05). Increased Hcy promoted the cholesterol accumulation in foam cells. Large quantities of red lipid droplets appeared in foam cells. The result of foam cells counting showed statistical difference between control and Hcy groups (P<0.05). And CE/TC in Hcy groups were higher than the control group (P<0.05). Besides, the mRNA and protein of LXRα, ABCA1 and ABCG1 were lower than the control group (P<0.01). And Hcy at 100μmol/L had most significant difference in the above results (P<0.01). Finally the LXRα agonist group(5μg/ml T0901317+100μmol/L) reversed the effects of Hcy on cholesterol efflux (P<0.05).

**Conclusions:** Hcy can increase the accumulation and reduce the efflux of cholesterol in foam cells. Inhibition of LXRα-ABCA1/ABCG1 pathway may be a potential mechanism of Hcy induced disorder of cholesterol metabolism, which can provide a new insight to the scientific research and clinical work of AS.