Published in final edited form as:
Atherosclerosis. 2013 July ; 229(1): 79–85. doi:10.1016/j.atherosclerosis.2013.03.031.

Hypercholesterolemia and reduced HDL-C promote hematopoietic stem cell proliferation and monocytosis: studies in mice and FH children

Sonia Tolani[1,*], **Tamara A. Pagler**[1,*], **Andrew J. Murphy**[1], **Andrea E. Bochem**[1,2], **Sandra Abramowicz**[1], **Carrie Welch**[1], **Prabhakara R. Nagareddy**[3], **Steve Holleran**[4], **G.K. Hovingh**[2], **Jan Albert Kuivenhoven**[5], and **Alan R. Tall**[1]

[1]Division of Molecular Medicine, Department of Medicine, Columbia University, New York, New York, USA [2]Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands [3]Department of Preventive Medicine and Nutrition, Department of Medicine, Columbia University, New York, New York USA [4]Division of Biomathematics, Department of Pediatrics, Columbia University, New York, New York USA [5]University of Groningen, University Medical Center Groningen, Molecular Genetics, Groningen, The Netherlands

Abstract

Previous studies have shown that mice with defects in cellular cholesterol efflux show hematopoietic stem cell (HSPC) and myeloid proliferation, contributing to atherogenesis. We hypothesized that the combination of hypercholesterolemia and defective cholesterol efflux would promote HSPC expansion and leukocytosis more prominently than either alone.

We crossed *Ldlr*$^{-/-}$ with *Apoa1*$^{-/-}$ mice and found that compared to *Ldlr*$^{-/-}$ mice, *Ldlr*$^{-/-}$/*Apoa1*$^{+/-}$ mice, with similar LDL cholesterol levels but reduced HDL cholesterol (HDL-C) levels, had expansion of HSPCs, monocytosis and neutrophilia. *Ex vivo* studies showed that HSPCs expressed high levels of *Ldlr*, *Scarb1 (Srb1)*, and *Lrp1* and were able to take up both native and oxidized LDL. Native LDL directly stimulated HSPC proliferation, while co-incubation with reconstituted HDL attenuated this effect. We also assessed the impact of HDL-C levels on monocytes in children with familial hypercholesterolemia (FH) (n=49) and found that subjects with the lowest level of HDL-C, had increased monocyte counts compared to the mid and higher HDL-C levels. Overall, HDL-C was inversely correlated with the monocyte count. These data suggest that in mice, a balance of cholesterol uptake and efflux mechanisms may be one factor in driving HSPC proliferation and monocytosis. Higher monocyte counts in children with FH and low HDL cholesterol suggest a similar pattern in humans.

Keywords

Hematopoietic stem cells; atherosclerosis; hypercholesterolemia; HDL-C; apoA1

Corresponding author contact info: Sonia Tolani, MD, Columbia University College of Physicians & Surgeons, 630 W. 168th Street, Room 8-401, New York, New York 10032, st2367@columbia.edu, Phone: (212) 305-5789, Fax: (212) 305-5052.
*These two authors contributed equally to this manuscript

Atherosclerotic cardiovascular disease remains the major cause of morbidity and mortality in industrialized societies despite the advent of potent cholesterol lowering statin drugs and improvements in clinical care [1]. Low HDL-C levels have long been known to be associated with CAD [2–4], especially in the setting of hypercholesterolemia. However, while the association of HDL-C with cardiovascular disease (CVD) is clear, the mechanisms by which HDL exerts its athero-protective effects have not been fully elucidated.

Studies in animals and humans support the hypothesis that HDL prevents atherosclerosis in part via its ability to promote cholesterol efflux from cells [5–7]. Kehra *et al.* recently showed that impaired serum cholesterol efflux potential is associated with an increased burden of atherosclerotic disease [8], suggesting that HDL-mediated cholesterol efflux is a key anti-atherogenic property of HDL. This concept is also supported by bone-marrow transplantation studies showing increased atherosclerosis in hypercholesterolemic mice that have defective cholesterol efflux pathways as a result of knockout of the ATP-binding cassette transporters ABCA1 and ABCG1 in cells of hematopoietic origin. These mice also displayed a marked expansion of hematopoietic stem cells (HSPCs), monocytosis, neutrophilia and systemic foam cell and myeloid cell infiltration of various organs. [7, 9]. Accelerated atherosclerosis and myeloid cell defects were reversed by transgenic overexpression of *Apoa1* [7, 9]. Murphy *et al.* [10] have also recently shown that apoE, in particular, plays an important role in cholesterol efflux in HSPCs. $Apoe^{-/-}$ mice fed a Western-type diet (WTD), develop HSPC and myeloid proliferation, monocytosis and increased numbers of monocyte/macrophages in atherosclerotic plaques [11, 12]. Infusion of reconstituted HDL was able to reverse HSPC and myeloid proliferation.

Surprisingly, while $Apoe^{-/-}$ mice developed HSPC expansion and monocytosis on chow or WTD, $Apoa1^{-/-}$ mice did not [10]. Moreover, humans with Mendelian forms of HDL deficiency (LCAT deficiency, Tangier Disease) also did not display monocytosis [10]. However, in both $Apoa1^{-/-}$ mice and humans with genetically low HDL-C, plasma levels of LDL cholesterol are typically either normal or reduced, and so these findings do not preclude a detrimental effect of low HDL-C on HSPC proliferation in the setting of elevated levels of LDL cholesterol.

We hypothesized that in the presence of hypercholesterolemia, ApoA-1 and HDL would act to reduce HSPC proliferation and monocytosis. We therefore crossed $Apoa1^{-/-}$ mice with $Ldlr^{-/-}$ mice, and showed that the combination of marked hypercholesterolemia and low HDL-C was associated with HSPC proliferation and monocytosis. We also examined the mechanisms and impact of LDL uptake in HSPCs *in vitro*, and showed that proliferative effects of LDL were attenuated by HDL. Finally, we examined the relationship between elevated LDL-C levels and monocytosis in a cohort of children with familial hypercholesterolemia (FH) and confirmed that HDL-C is associated with lower monocyte counts in the setting of elevated LDL-C levels.

Methods

Animals

WT (C57BL/6), $Ldlr^{-/-}$ and $Apoa1^{-/-}$ mice were purchased from The Jackson Laboratory. $Ldlr^{-/-}$ and $Apoa1^{-/-}$ mice were crossed to obtain $Ldlr^{+/-}/Apoa1^{-/-}$ and $Ldlr^{-/-}/Apoa1^{+/-}$ mice. For *in vivo* studies, mice at 8 weeks of age were placed on a Western type diet (21% milk fat, 0.2% cholesterol; catalog no. TD88137; Harlan Teklad) for 6 weeks. For *in vitro* studies, mice were maintained on a normal chow diet. The age of the animals ranged from 8–20 weeks.

Mouse cholesterol assays

Total cholesterol and HDL cholesterol were measured from the plasma of mice using the Cholesterol E or HDL-Cholesterol E kit (from Wako Diagnostics) per the manufacturer's instructions.

Mouse white blood cell counts

Absolute monocytes and neutrophils were determined by applying percentages from flow cytometry to total leukocyte count, which was obtained from freshly drawn blood (via tail bleed) in EDTA tubes and analyzed by automated cell counter (FORCYTE Veterinary Analyzer, Oxford Science Inc.).

Flow cytometry

Blood leukocytes—Blood monocytes and neutrophils were analyzed from whole blood collected in EDTA coated tubes by tail bleed. Blood was placed on ice (all processing on ice), lysed (BS pharm Lyse; BD Biosciences) and washed by centrifuging in HBSS (0.1% BSA, 5mM EDTA). As previously detailed [10], cells were stained with a cocktail of antibodies against CD45-APC-Cy7, Ly6-C/G (Gr-1)-PerCP-Cy5.5 (BD Biosciences-Pharminogen), and CD115-APC (eBiosciences). Monocytes were classified as $CD45^+$, $CD115^+$, and further subdivided into LyC-6^{hi} and LyC-6^{lo} subsets. Neutrophils were classified as $CD45^+$, $CD115^-$, LyC-6^+. Samples were analyzed on an LSR-II (BD Bioscience).

HSPCs—Bone marrow (BM) was harvested from femurs and tibias of mice and lysed to remove RBCs. BM was resuspended in HBSS and incubated with an antibody cocktail which included antibodies to FITC labeled lineage markers (CD45R, CD19, CD11b, CD3e, TER-119, CD2, CD8, CD4, and Ly-6G; all eBioscience), and stem cell markers Sca 1-Pacific Blue and ckit-APC-Cy7. HSPCs were identified as lineage marker negative, Sca1 positive, ckit positive (lin^-, $Sca1^+$, $ckit^+$). Samples were analyzed on an LSR-II or FACSAria, when sorted.

HSPC *in vitro* proliferation

Mice were injected with EdU 18 hours before sacrifice. BM was harvested and processed and stained as described above. Cells were then fixed and permeabilized using BD Cytofix/Cytoperm solution for 20 minutes on ice, washed with BD Perm/Wash buffer, and stained with Alexa Flour-conjugated azides using Click-iT system (Invitrogen). Cells were analyzed on LSR-II and proliferation was quantified as percentage of EdU+ cells.

Real-time PCR analysis of HSPCs

For ex vivo studies, HSPCs were processed and stained in the same manner, and then sorted on FACSAria, directly into RLT lysis buffer (Qiagen). For *in vitro* studies, cultured BM cells (see below for details) were removed from culture plates, washed and then placed in lysis buffer. In both cases, RNA was extracted using RNeasy Micro Kit (Quiagen) and cDNA was synthesized using SuperScript VILO (Invitrogen). Differences in mRNA levels were controlled using the reference gene *m36B4*.

LDL uptake assays

BM cells from chow fed animals were obtained and processed, as described above. BM from 2–3 animals was pooled together and plated onto 24 well plates in IMDM (Gibco) with penicillin, streptomycin and 10% lipoprotein deficient bovine calf serum (Biomedical Technologies Inc.) for 90 minutes to allow for separation of adherent cells. Non-adherent

cells were then removed and cultured for stated time period in the presence of 10% IL-3 supplement media (BD Bioscience), 2ng/mL GM-CSF (R&D) systems and either BODIPY labeled human nLDL (Molecular Probes) or DiI labeled oxLDL (Biomedical Technologies). Cells were then collected, washed of serum and resuspended in HBSS (BSA, EDTA) and stained for HSPCs as described above, with the exception that lineage markers were APC labeled. Cells were analyzed on LSRII and uptake was determined by the mean fluorescent intensity of BODIPY or DiI in lin^{-}, $Sca1^{+}$, $ckit^{+}$ HSPCs.

In vitro HSPC proliferation assays

As with LDL uptake assays, pooled BM cells were plated to remove adherence cells then cultured in IMDM with 10% LPDS, IL-3 and GM-CSF. Cells were then loaded overnight with designated concentration of human native LDL (Biomedical Technologies) +/− reconstituted HDL (rHDL;CSL-111 was provided by CSL Behring AG; CSL-111 is composed of human ApoA-1 and phosphatidylcholine from soybean in a 1:150 ratio). Cells were then collected, washed, fixed and permeablized (as described above) and finally stained with DAPI and analyzed on LSRII.

Human Studies

Heterozygotes children with familial hypercholesterolemia were already enrolled in the Phase III clinical trial NCT01078675, “A Study to Evaluate Rosuvastatin in Children and Adolescents with Familial Hypercholesterolemia,” sponsored by Astra/Zeneca. Blood was collected after overnight fast, and automated blood count was performed to determine WBC counts and differentials. LDL-C, HDL-C and triglyceride levels were fasting values. Data used here are the pre-treatment baseline values. Institutional review boards of participating centers approved the protocol. Statistical analysis for all continuous variables in Table II was done by one-way analysis of variance (ANOVA). For gender, a chi-square test was used, and for statin use, a Fisher’s exact test. Triglycerides are reported as a median and interquartile range since this variable is not normally distributed.

Results

Hypercholesterolemia and reduced HDL-C promote monocytosis, neutrophilia and hematopoietic stem cell proliferation in mice

Previous studies showed that $Ldlr^{-/-}$ mice develop moderate monocytosis and neutrophilia when fed a WTD [10]. To assess if this effect is magnified in the setting of reduced HDL levels we crossed $Ldlr^{-/-}$ mice with $Apoa1^{-/-}$ mice. Given that $Ldlr^{-/-}/Apoa1^{-/-}$ mice have substantially reduced levels of non-HDL cholesterol [13, 14], when compared to $Ldlr^{-/-}$ mice, we bred $Ldlr^{-/-}/Apoa1^{+/-}$ mice to avoid this potentially confounding effect. $Ldlr^{-/-}/Apoa1^{+/-}$ mice had prominent hypercholesterolemia not differing from that in $Ldlr^{-/-}$ controls, as well as approximately 30% reductions in HDL-C levels (Table 1).

Notably, this moderate reduction in HDL-C levels was associated with a prominent increase in blood neutrophils and monocytes after six weeks on a WTD in $Ldlr^{-/-}/Apoa1^{+/-}$ mice when compared to $Apoa1^{-/-}$, $Ldlr^{+/-}/Apoa1^{-/-}$ and $Ldlr^{-/-}$ mice (Figure 1). Ly6-C^{hi} and Ly6-C^{lo} populations of monocytes were both significantly increased in $Ldlr^{-/-}/Apoa1^{+/-}$ mice when compared to other genotypes (Figure 1 B). A lower HDL to total cholesterol ratio across all mice genotypes also significantly correlated with higher blood monocyte percentage (Figure 1C).

The increase in blood leukocytes seen in $Ldl^{-/-}/Apoa1^{+/-}$ mice, paralleled an expansion of the HSPC population in the BM (Figure 2A&B), and increased incorporation of EdU into HSPCs, consistent with increased HSPC proliferation (Figure 2C). While $Ldlr^{-/-}$ mice also

had significantly higher blood monocyte counts (and a trend toward higher neutrophils) when compared with *Apoa1*$^{-/-}$ and *Ldlr*$^{+/-}$/*Apoa1*$^{-/-}$ genotypes, they did not have a corresponding expansion of BM HSPCs. In addition, complete deficiency of *Apoa1* in either *Ldlr*$^{+/+}$ or *Ldlr*$^{+/-}$ mice was not associated with either monocytosis, neutrophilia or expansion of the HSPC population. Of note, there was no difference in total lymphocytes percentages between *Ldlr*$^{+/-}$/*Apoa1*$^{-/-}$ and *Ldlr*$^{-/-}$ mice (data not shown).

This suggests that in the setting of marked hypercholesterolemia as seen in *Ldlr*$^{-/-}$ mice, reduced HDL-C levels is associated with HSPC proliferation and leukocytosis. In addition to increased myeloid proliferation, there was a trend towards increased atherosclerosis in *Ldlr*$^{-/-}$/*Apoa1*$^{+/-}$ mice compared to *Ldlr*$^{-/-}$ mice (P=0.1), as measured by proximal aortic plaque area (Figure 2C,D).

HSPCs have the capacity to take up both native and oxidized LDL

To better understand how HSPCs take up cholesterol we screened FACS-isolated HSPCs from WT and *Ldlr*$^{-/-}$ mice for expression of key receptors for LDL and modified forms of LDL. WT mice predominately express *Ldlr*, *Scarb1 (Srb1)*, and *Lrp1* with virtually no expression of *CD36*, *Sra1* or *Lox1*, and with the exception of *Ldlr*, the *Ldlr*$^{-/-}$ mice had a similar pattern of gene expression (Figure 3A). As expected from this receptor profile, *in vitro* loading with labeled nLDL and oxLDL showed that HSPCs are able to take up both native and modified LDL (Figure 3C). Since SR-BI can mediate uptake of oxLDL [15] this receptor may be mediating oxLDL uptake in these cells. Of note, no significant differences in expression levels of *Abca1*, *Abcg1* or *Apoe* were noted between WT and *Ldlr*$^{-/-}$ mice (Figure 3B). Consistent with a major role of the LDLR in the uptake of LDL, there was saturable high affinity specific uptake of LDL in WT HSPCs that was largely abolished in *Ldlr*$^{-/-}$ HSPCs. Both WT and *Ldlr*$^{-/-}$ HSPCs showed significant levels of non-specific LDL uptake.

LDL directly stimulates hematopoietic stem cell proliferation, which is opposed by HDL

To assess the direct effects of HDL and LDL on HSPC proliferation, we looked at the response of HSPCs to differential loading of cholesterol *in vitro*. BM from *Ldlr*$^{-/-}$ mice was cultured in media with lipoprotein deficient serum in which HDL and LDL cholesterol concentrations were varied. LDL in the absence of HDL, increased HSPC proliferation in a dose dependent fashion (Figure 4A). The addition of rHDL at potentially therapeutic concentrations had a potent effect in reversing proliferation (Figure 4B). Of note, the commercial LDL preparation used in these studies may have contained small amounts of oxidized LDL, which could have contributed to proliferative effects.

HDL deficiency in the setting of hypercholesterolemia is associated with higher monocyte counts in children with Familial Hypercholesterolemia

In order to assess whether the higher monocyte counts observed in *Ldlr*$^{-/-}$/*Apoa1*$^{+/-}$ mice translate into a similar phenotype in humans, we analyzed baseline data previously collected for a trial of rosuvastatin in children with heterozygous familial hypercholesterolemia. The children were statin naïve or underwent a wash-out period. They had an average age of 12 and similar gender distribution (Table 2). Patients were grouped by level of HDL-C, where low HDL-C was defined as ≤ 40mg/dL, mid HDL-C as 40–50mg/dL and high HDL-C as >50mg/dL. LDL cholesterol levels were elevated across the patient population, with an average concentration of 216mg/dL, and importantly were not statistically different among HDL groups. FH children with the lowest HDL-C levels, had significantly higher monocyte counts (Figure 5A). In addition, there was a significant inverse correlation between HDL concentration and monocyte percentage (Figure 5B).

Discussion

In this study, we have shown that the combination of hypercholesterolemia and low HDL levels are associated with increased proliferation and expansion of BM HSPCs and increased monocytosis and neutrophilia in mice. In a parallel translational study, we present evidence that this balance may be important in regulating monocytosis in humans too, as FH children with reduced HDL-C levels had increased monocyte counts compared to FH children with normal or higher HDL-C levels.

In humans, leukocytosis, and in particular, high monocyte counts are clearly associated with cardiovascular disease. In healthy people, increased monocyte count is associated with increased risk for cardiovascular events, ischemic stroke and cardiovascular mortality [16]. Leukocytosis has also been implicated in the development of the accelerated atherosclerosis observed in heterozygous FH patients [17]. In FH adults, low HDL-C levels have been associated with increased atherosclerotic plaque burden [18]. Here we have the benefit of studying leukocytosis in a pediatric population, whose only medical condition is dyslipidemia, so increased monocytes in this group are less likely to be confounded. Thus, unlike studies in adults, leukocytosis is less likely to be reactive to established atherosclerosis, but rather can be considered as a factor contributing to its development.

In addition to leukocytosis and monocytosis, low HDL-C and ApoA-1 are also associated with increased CVD, but previous work in mice has shown an inconsistent relationship with atherosclerosis [14, 19–22]. This may be partly related to the finding of low levels of non-HDL-C in ApoA-1 deficient mice and humans, [23, 24], which is thought to be due to decreased intestinal absorption of cholesterol, and also hypercatabolism of abnormal LDL [14, 25, 26]. This is likely why, paradoxically, heterozygotes for TD and LCAT deficiency have increased atherosclerosis, while findings in homozygotes have been mixed [24, 27, 28]. The trend towards increased atherosclerosis in $Ldlr^{-/-}/Apoa1^{+/-}$ mice in our study is consistent with other studies in mice showing that Apoa-1 deficiency in the setting of hypercholesterolemia contributes to development of atherosclerosis [21, 22]. Of note, in the absence of low HDL, we still see a modest increase in blood monocytes and a trend towards increased blood neutrophils in hypercholesterolemic mice, but in this setting there is no parallel increase in BM HSPCs suggesting that there are also non-stem cell mediated mechanisms at play.

Our study provides some novel insight into the effect circulating lipoproteins have on modulation of HSPCs. We have shown that HSPCs are able to take up both native and oxidized LDL likely in part via LDLR and SR-B1. The relatively high expression of SR-BI in HSPCs, also suggests that it could be participating in cholesterol efflux, as well as ABCA1, ABCG1 and apoE. Previous studies have shown the importance of these latter cholesterol efflux pathways in regulating cholera toxin B binding to the plasma membrane, the cell surface expression of the common beta-subunit of the IL-3/GM-CSF receptor and proliferative responses to IL-3 and GM-CSF [9, 10]. IL-3 is a key growth factor for HSPCs, while GM-CSF promotes differentiation of myeloid progenitors into granulocytes and monocytes. It remains to be seen if a similar pathway is utilized in the current model. We have also showed that LDL loading promotes HSPC proliferation, and that increasing rHDL concentrations to therapeutic levels reverse this effect in cells from $Ldlr^{-/-}$ mice. Of note, we have seen similar proliferative response in HSPCs from WT mice incubated with LDL (data not shown).

Overall, these findings suggest that a balance of uptake and efflux of cholesterol plays a role in HSPC and myeloid proliferation. However, we cannot exclude the possibility that the effects seen in these experiments may be mediated by non-efflux mechanisms. Furthermore,

since we see that uptake of native LDL in *Ldlr*$^{-/-}$ mice is modest when compared to WT mice, we hypothesize that modified LDL may also be playing an important role in HSPC stimulation.

In conclusion, we show that HSPC expansion, and the resultant increase in circulating monocytes and neutrophils, occurs in the setting of combined increases in LDL and low HDL levels. This finding underlines the close link between circulating lipoproteins and leukocytosis, both of which are important contributing factors to the development of atherosclerosis.

Acknowledgments

This work was supported by NIH grant HL107653 and by the Foundation Leducq. Dr. S. Tolani is a Cardiology Post-Graduate Research Fellow supported by the Ruth L. Kirschstein National Research Service Award Intuitional Training Grant (T32). AJM was supported by a post-doctoral fellowship from the American Heart Association (12POST11890019). The authors thank the flow cytometry unit at the Herbert Irving Comprehensive Cancer Center for HSPC sorting and CSL Australia for providing the rHDL used in this study.

References

1. Greenland P, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2010; 56(25):e50–103. [PubMed: 21144964]
2. Jacobs DR Jr, et al. High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the Lipid Research Clinics Prevalence Study. Am J Epidemiol. 1990; 131(1):32–47. [PubMed: 2293751]
3. Gordon DJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989; 79(1):8–15. [PubMed: 2642759]
4. LaRosa JC, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med. 2005; 352(14):1425–35. [PubMed: 15755765]
5. Aiello RJ, et al. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. Arterioscler Thromb Vasc Biol. 2002; 22(4):630–7. [PubMed: 11950702]
6. van Eck M, et al. Leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. Proc Natl Acad Sci U S A. 2002; 99(9):6298–303. [PubMed: 11972062]
7. Yvan-Charvet L, et al. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. J Clin Invest. 2007; 117(12):3900–8. [PubMed: 17992262]
8. Khera AV, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med. 2011; 364(2):127–35. [PubMed: 21226578]
9. Yvan-Charvet L, et al. ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. Science. 2010; 328(5986):1689–93. [PubMed: 20488992]
10. Murphy AJ, et al. ApoE regulates hematopoietic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. J Clin Invest. 2011; 121(10):4138–49. [PubMed: 21968112]
11. Swirski FK, et al. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. J Clin Invest. 2007; 117(1):195–205. [PubMed: 17200719]
12. Tacke F, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest. 2007; 117(1):185–94. [PubMed: 17200718]
13. Zabalawi M, et al. Inflammation and skin cholesterol in LDLr−/−, apoA-I−/− mice: link between cholesterol homeostasis and self-tolerance? J Lipid Res. 2007; 48(1):52–65. [PubMed: 17071966]
14. Zabalawi M, et al. Induction of fatal inflammation in LDL receptor and ApoA-I double-knockout mice fed dietary fat and cholesterol. Am J Pathol. 2003; 163(3):1201–13. [PubMed: 12937162]

15. Gillotte-Taylor K, et al. Scavenger receptor class B type I as a receptor for oxidized low density lipoprotein. J Lipid Res. 2001; 42(9):1474–82. [PubMed: 11518768]
16. Lee CD, et al. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: atherosclerosis risk in communities study. Am J Epidemiol. 2001; 154(8):758–64. [PubMed: 11590089]
17. Junyent M, et al. Influence of HDL cholesterol on preclinical carotid atherosclerosis in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol. 2006; 26(5):1107–13. [PubMed: 16556855]
18. Hausmann D, et al. Angiographically silent atherosclerosis detected by intravascular ultrasound in patients with familial hypercholesterolemia and familial combined hyperlipidemia: correlation with high density lipoproteins. J Am Coll Cardiol. 1996; 27(7):1562–70. [PubMed: 8636537]
19. Li H, Reddick RL, Maeda N. Lack of apoA-I is not associated with increased susceptibility to atherosclerosis in mice. Arterioscler Thromb. 1993; 13(12):1814–21. [PubMed: 8241102]
20. Plummer MR, Hasty AH. Atherosclerotic lesion formation and triglyceride storage in obese apolipoprotein AI-deficient mice. J Nutr Biochem. 2008; 19(10):664–73. [PubMed: 18280133]
21. Moore RE, et al. Apolipoprotein A-I deficiency results in markedly increased atherosclerosis in mice lacking the LDL receptor. Arterioscler Thromb Vasc Biol. 2003; 23(10):1914–20. [PubMed: 12933536]
22. Voyiaziakis E, et al. ApoA-I deficiency causes both hypertriglyceridemia and increased atherosclerosis in human apoB transgenic mice. J Lipid Res. 1998; 39(2):313–21. [PubMed: 9507992]
23. Serfaty-Lacrosniere C, et al. Homozygous Tangier disease and cardiovascular disease. Atherosclerosis. 1994; 107(1):85–98. [PubMed: 7945562]
24. Hovingh GK, et al. Inherited disorders of HDL metabolism and atherosclerosis. Curr Opin Lipidol. 2005; 16(2):139–45. [PubMed: 15767853]
25. Schaefer EJ, et al. Cholesterol and apolipoprotein B metabolism in Tangier disease. Atherosclerosis. 2001; 159(1):231–6. [PubMed: 11689226]
26. Iqbal J, Hussain MM. Evidence for multiple complementary pathways for efficient cholesterol absorption in mice. J Lipid Res. 2005; 46(7):1491–501. [PubMed: 15834127]
27. Ayyobi AF, et al. Lecithin: cholesterol acyltransferase (LCAT) deficiency and risk of vascular disease: 25 year follow-up. Atherosclerosis. 2004; 177(2):361–6. [PubMed: 15530911]
28. Tall AR. Cholesterol efflux pathways and other potential mechanisms involved in the athero-protective effect of high density lipoproteins. J Intern Med. 2008; 263(3):256–73. [PubMed: 18271871]

Highlights

- High LDL and reduced HDL promote monocytosis, neutrophilia and HSPC proliferation in mice
- HSPCs have the capacity to take up both native and oxidized LDL
- LDL directly stimulates HSPC proliferation, which is opposed by HDL
- Lower HDL levels correlate with higher monocyte counts in kids with Familial Hypercholesterolemia

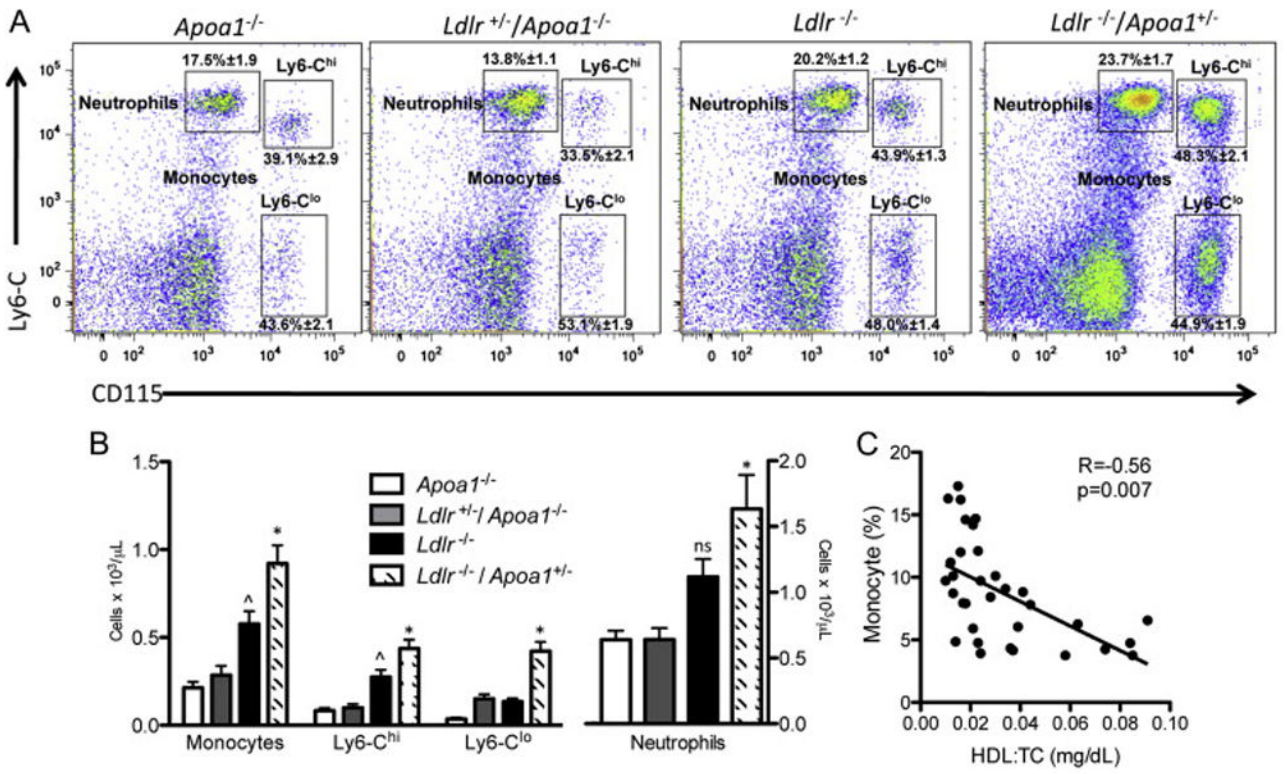

Figure 1.

LdLr$^{-/-}$/ *Apoa1*$^{+/-}$ mice on WTD develop monocytosis, with increases in total, Ly-6 C^{hi} and Ly-6 C^{lo} monocytes, and neutrophilia in the blood. 8-week-old mice were placed on a WTD for 6 weeks and blood was then collected and stained for indicated cell types. (A) Total monocytes, monocyte subsets and neutrophils were analyzed by flow cytometry as seen by representative dot plots with group percent means and (B) expressed as absolute cell counts calculated from percentages applied to total leukocyte count determined by automated cell count. *P<0.05 vs. all other genotypes and ^P<0.05 vs. *Apoa1*$^{-/-}$, *Ldlr*$^{+/-}$/ *Apoa1*$^{-/-}$. Data are mean with SEM, n=10–13. (C) HDL to total cholesterol ratio (ug/mL) is plotted with associated monocyte percentage for all mice in the study.

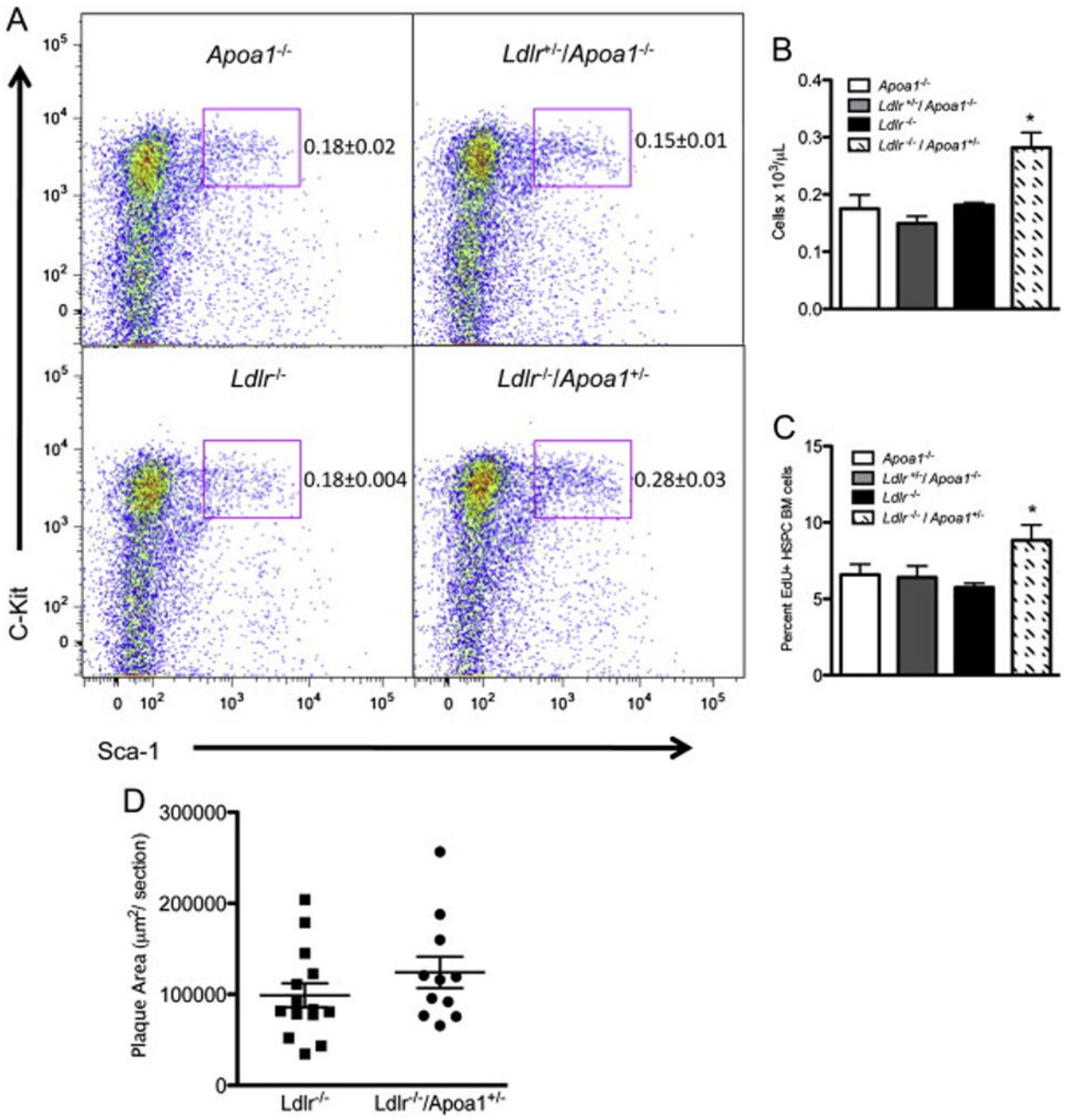

Figure 2.

Ldlr$^{-/-}$/ *Apoa1*$^{+/-}$ mice on a WTD have expansion of HSPCs in the bone marrow and increased proliferation *in* vivo, as well as a trend towards increased atherosclerosis. (A&B) BM cells were isolated and analyzed by flow cytometry to identify percent HSPCs. (C) Prior to sacrifice, mice were injected with EdU. *In vivo* proliferation was determined by EdU incorporation via flow cytometry. *P<0.05 vs. all other genotypes. (D) Hearts were placed in paraffin, stained with H&E and plaque area was determined by averaging plaque at aortic root over serial sections for each animal. P=0.1. Data are mean with SEM, n=10–13.

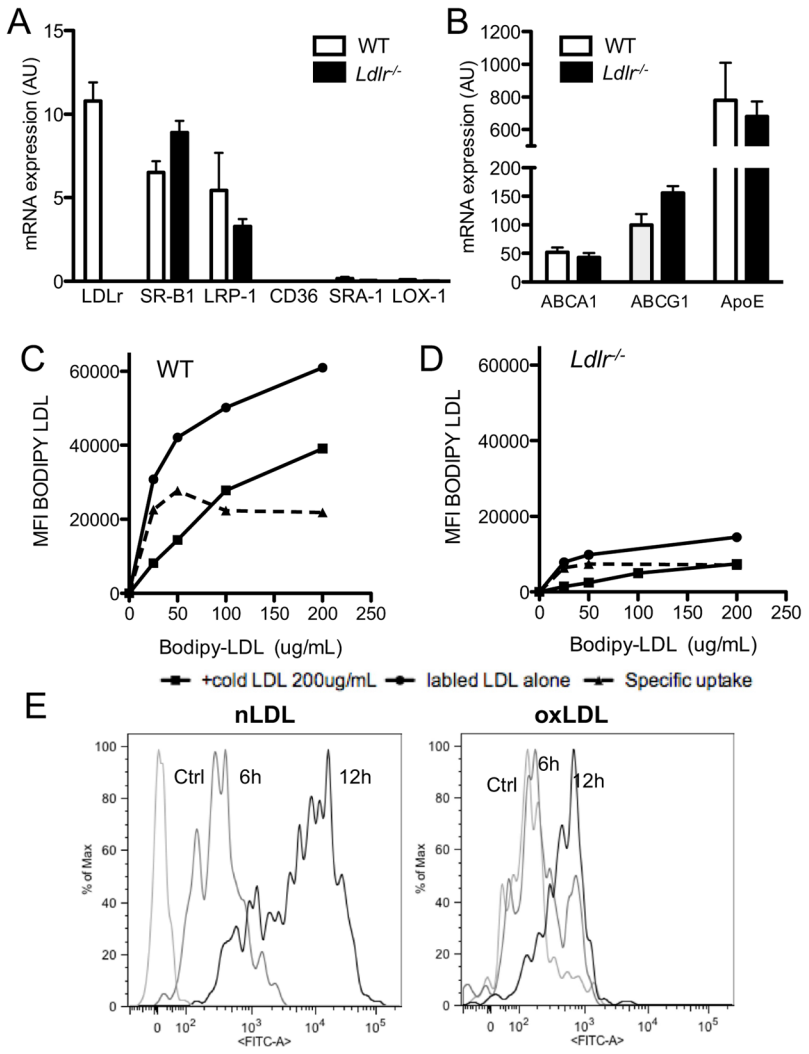

Figure 3.

WT and *LdLr*$^{-/-}$ BM HSPCs take up nLDL and oxLDL. (A&B) HSPCs were isolated from BM by FACS, cDNA was prepared and mRNA expression was quantified by real-time PCR. Data is mean with SEM, *n*=3–5. (C&D) Cultured HSPCs were loaded with increasing concentrations of BODIPY-nLDL alone and in presence of excess unlabeled nLDL (200μg/mL) in WT and *LdLr*$^{-/-}$ BM cells. Uptake was quantified by flow cytometry and data points are averages of mean fluorescence intensity (MFI) of BODIPY in HSPCs. (E) HSPCs were cultured in media with LPDS serum, IL-3 and GM-CSF in the presence of either BODIPY labeled nLDL or DiI labeled oxLDL. Uptake was determined by flow cytometry at 6 and 12 hours.

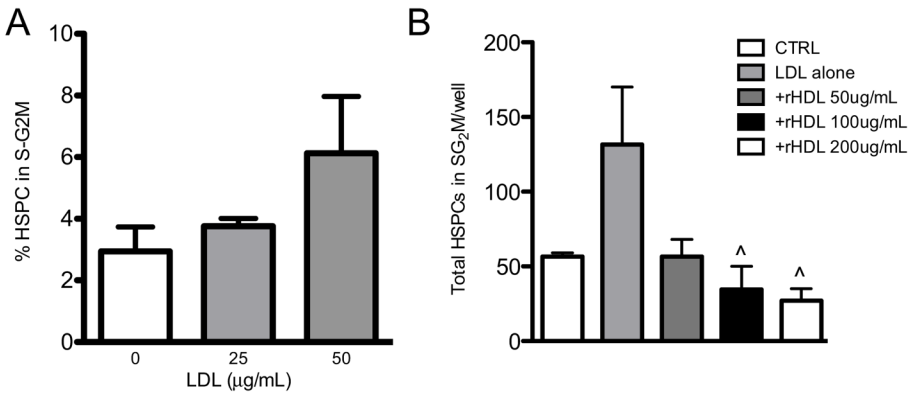

Figure 4.

LDL directly stimulates HSPC proliferation, which can be reversed by reconstituted HDL. (A) BM from *Ldlr*$^{-/-}$ mice was cultured and loaded overnight with increasing nLDL concentration (μg/mL). *In vitro* cell cycling in HSPCs was determined by FACS using DAPI and is expressed by the percent of HSPCs in SG_2M phase. *Ldlr*$^{-/-}$ BM cells co-incubated with nLDL (50μg/mL) and increasing concentrations of rHDL. HSPCs were then sorted by FACS and the average total number of cells per well are shown (B). rHDL concentrations are reflective of protein. ^P<0.05 vs LDL alone.

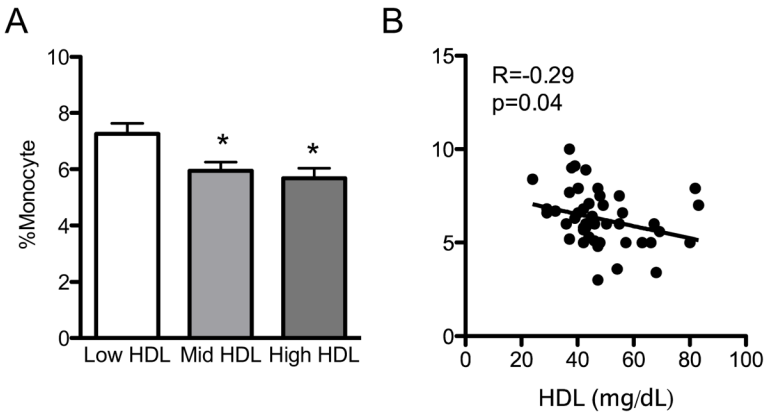

Figure 5.

Children with familial hypercholesterolemia and low HDL have increased circulating blood monocytes. (A) Children with homozygous FH were grouped based on plasma HDL concentration. Low HDL was defined as ≤ 40mg/dL, mid HDL as 40–50mg/dL and high HDL as ≤ 50mg/dL. Percent monocyte was measured by automated blood count. Data is mean with SEM, n=49. *P <0.01 vs low HDL group. (B) HDL plotted with associated monocyte count for all individuals in cohort.

Table 1

Total cholesterol and HDL cholesterol for mice after six weeks of high-fat Western diet.

Genotype	*Apoa1*$^{-/-}$ (n=10)	*Ldlr*$^{+/-}$/*Apoa1*$^{-/-}$ (n=9)	*Ldlr*$^{-/-}$ (n=13)	*Ldlr*$^{-/-}$/*Apoa1*$^{+/-}$ (n=11)
Total Cholesterol (mg/dL)	263.7±18.0 *	569.6±41.9 *	1664.0±82.6	1578±119.3
HDL-C (mg/dL)	16.8±2.7	15.5±2.1	36.0±3.3 *	25.4±1.6

Results expressed as mean ± SEM.

* P≤ 0.05 compared with *Ldlr*$^{-/-}$, *Apoa1*$^{+/-}$.

Table 2

Baseline characteristics of children with familial hypercholesterolemia.

	Low HDL (n=14)	Mid HDL (n=19)	High HDL (n=15)
Age (years)	10.9 ± 4	10.1 ± 3	8.1 ± 3
Male Sex	8 (57%)	8 (42%)	6 (40%)
Statin Use *	5 (36%)	3 (16%)	0
Triglycerides ¶ (mmol/L)	0.94 (0.71–1.12)	0.85 (0.70–1.19)	0.61 (0.55–1.11)
Total Cholesterol (mmol/L)	6.55 ± 1.9	7.24 ± 1.7	7.84 ± 1.0
LDL Cholesterol (mmol/L)	5.18 ± 1.7	5.64 ± 1.6	5.85 ± 0.9
HDL Cholesterol (mmol/L)	0.92 ± 0.13 **	1.16 ± 0.06 **	1.65 ± 0.29 **
Leukocytes (10^9/L)	5.64 ± 1.5	5.81 ± 1.2	5.74 ± 1.0
Neutrophils (percent)	48.5 ± 14	52.0 ± 9.9	47.9 ± 7.5
Lymphocytes (percent)	40.0 ± 14	37.1 ± 9.0	41.1 ± 8.1

* P=0.03 by Fisher's Exact Test,

¶ median and interquartile range,

** P<0.001 vs. other two groups