Published in final edited form as:

J Clin Lipidol. 2015 ; 9(1): 81–90. doi:10.1016/j.jacl.2014.09.014.

Elevated Interleukin-10: A New Cause of Dyslipidemia Leading to Severe HDL Deficiency:

Moraitis: Elevated IL-10 and Severe HDL Deficiency

Andreas G Moraitis, MD^{1,+}, Lita A Freeman, PhD^{2,+}, Robert D Shamburek, MD², Robert Wesley, PhD³, Wyndham Wilson, MD⁴, Cliona M Grant, MD⁴, Susan Price⁵, Stephen Demosky², Seth G Thacker, PhD², Abdalrahman Zarzour, MD², Ronald L Hornung, PhD⁶, Frank Pucino, PharmD⁷, Gyorgy Csako, MD⁸, Cheryl Yarboro⁷, Iain B McInnes, MD, PhD^{7,9}, Takashi Kuroiwa, MD⁷, Dimitrios Boumpas, MD^{7,10}, V. Koneti Rao, MD⁵, Gabor G Illei, MD⁷, and Alan T Remaley, MD, PhD^{2,8}

¹Metabolism, Endocrinology & Diabetes, Endocrine Oncology Program, University of Michigan, Ann Arbor, MI

²Cardiovascular and Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, NIH, Bethesda, MD

³Hospital Epidemiology, NIH, Bethesda, MD

⁴National Cancer Institute, NIH, Bethesda, MD

⁵ALPS unit, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

⁶Clinical Services Program, Leidos Biomedical Research, Inc. FNLCR, Frederick, MD 21702

⁷National Institutes of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD

⁸Department of Laboratory Medicine, NIH, Bethesda, MD

⁹College of Medicine, Veterinary Medicine and Life Sciences, University of Glasgow

¹⁰Medical School University of Athens, Affiliate Member IMBB, Heraklion and BRFAA Athens, Greece

Abstract

BACKGROUND—Low high-density lipoprotein-cholesterol (HDL-C) is a risk factor for coronary artery disease. Investigating mechanisms underlying acquired severe HDL deficiency in

Corresponding author: Alan T. Remaley, M.D., Ph.D., National Institutes of Health, Bldg.10, Rm. 2C-433, Bethesda, MD 20892-1508, USA, Fax: 301-402-1885, Tel: 301-402-9796, aremaley1@cc.nih.gov.

⁺Drs. Andreas G Moraitis and Lita A Freeman have equally contributed to this article.

Disclosures: None

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

noncritically ill patients (“Disappearing HDL Syndrome”) could provide new insights into HDL metabolism.

OBJECTIVE—To determine the cause of low HDL-C in patients with severe acquired HDL deficiency.

METHODS AND RESULTS—Patients with intravascular large B-cell lymphoma (IVLBCL, n=2), diffuse large B-cell lymphoma (DLBCL, n=1), and autoimmune lymphoproliferative syndrome (ALPS, n=1) presenting with markedly decreased HDL-C, low LDL-C and elevated triglycerides were identified. The abnormal lipoprotein profile returned to normal following therapy in all four cases. All cases were found to have markedly elevated serum interleukin-10 (IL-10) levels that also normalized following therapy. In a cohort of ALPS patients (n=93), IL-10 showed a strong inverse correlation with HDL-C ($R^2=0.3720$, $P<0.0001$). A direct causal role for increased serum IL-10 in inducing the observed changes in lipoproteins was established in a randomized, placebo-controlled clinical trial of recombinant human IL-10 (rhIL-10) in psoriatic arthritis patients (n=18). Within a week of initiating subcutaneous rhIL-10 injections, HDL-C precipitously decreased to near-undetectable levels. LDL-C also decreased by over 50% ($P<0.0001$) and triglycerides increased by approximately 2-fold ($P<0.005$). All values returned to baseline after discontinuing IL-10 therapy.

CONCLUSION—Increased IL-10 causes severe HDL-C deficiency, low LDL-C and elevated triglycerides. IL-10 is thus a potent modulator of lipoprotein levels, a potential new biomarker for B-cell disorders, and a novel cause of Disappearing HDL syndrome.

Keywords

“Disappearing HDL Syndrome”; Severe HDL Deficiency; hypertriglyceridemia; dyslipidemia; B-cell lymphoma; intravascular large B-cell lymphoma; ALPS; psoriasis; IL-10; LCAT

INTRODUCTION

High-density lipoprotein-cholesterol (HDL-C) less than 40 mg/dL (1 mmol/L) is a risk factor for coronary artery disease (CAD).¹ Causes of low HDL-C include rare genetic disorders due to mutations in the ATP-binding cassette transporter A1 (ABCA1), apolipoprotein A-I (apoA-I) and lecithin:cholesterol acyltransferase (LCAT).² These disorders usually present with HDL-C levels below 20 mg/dL. Acquired or secondary causes of low HDL-C account for about half of all cases and can be divided into mild-to-moderate HDL deficiency (HDL-C 20–30 mg/dL) and severe HDL deficiency (HDL-C < 20 mg/dL). Mild-to-moderate HDL deficiency is commonly associated with obesity, hypertriglyceridemia, insulin resistance and sedentary lifestyle, either alone or in combination with metabolic syndrome.³ It is also associated with a wide variety of diseases, such as human immunodeficiency virus (HIV), renal disease, and acute inflammation, as well the use of several medications.³ Reduced HDL-C levels are also seen in patients with chronic inflammatory diseases, such as rheumatoid arthritis^{4, 5} and systemic lupus erythematosus.⁶ The mechanism for lipid abnormalities in these autoimmune and inflammatory disorders is not completely understood, but several cytokines have been implicated to play either a pro- or anti-atherogenic role.⁷

Acquired severe HDL deficiency is relatively uncommon. It may occur after the use of high doses of anabolic steroids or in severe hepatic diseases, such as cholestasis and acute hepatitis, which can lead to low LCAT activity and decreased apoA-I production.^{2,3} “Disappearing HDL Syndrome”, a term first used by Goldberg and Mendez,³ refers to cases of severe HDL deficiency in non-critically ill patients, sometimes long before the clinical or biochemical features of the underlying primary disease become evident. Disappearing HDL Syndrome can also result from an idiosyncratic reaction to medications, such as peroxisome proliferation-activated receptor (PPAR) agonists.³ Additionally, autoantibodies against LCAT in non-Hodgkin lymphoma have also been described as a possible cause.⁸

In this report, we describe several case reports on three related B-cell disorders, namely intravascular large B cell lymphoma (IVLBCL), diffuse large B cell lymphoma (DLBCL), and autoimmune lymphoproliferative syndrome (ALPS), which are all described to be associated with Disappearing HDL Syndrome. Interleukin-10 (IL-10) serum concentrations were markedly elevated in all three disorders at presentation and were inversely related to HDL-C levels, as well as LDL-C, during the course of treatment. Furthermore, we demonstrate from the analysis of samples from a previous clinical trial⁹ that recombinant IL-10 administration leads to profound changes in lipoprotein levels in humans, particularly very low levels of HDL, as well as low LDL, thus mimicking the dyslipidemic lipoprotein pattern observed in our patients with B-cell disorders. These data thus identify IL-10 as an important modulator of lipoprotein metabolism and provide evidence for a direct role of IL-10 in Disappearing HDL Syndrome.

MATERIALS AND METHODS

Subject Description

All patients in this study were seen at the NIH and gave written informed consent under institutional review board (IRB)-approved protocols. Subjects treated with IL-10 therapy in the clinical trial were described in a previous report.⁹

Clinical Laboratory Analysis

Routine laboratory tests were performed by the Department of Laboratory Medicine in the NIH Clinical Center. Serum total cholesterol (TC), direct HDL-C and TG were measured enzymatically on a Siemens Vista Analyzer. Serum LDL-C was calculated using the Friedewald equation. Serum ApoA-I and ApoB were measured nephelometrically on a Siemens Vista Analyzer. % CE in plasma was determined by enzymatically measuring total cholesterol, as well as free cholesterol, in the absence of cholesteryl esterase.

IL-10 measurements

The Human IL-10 ELISA Ready-Set-GO! kit (eBioscience, San Diego, CA) was used according to manufacturer's instructions. 100 μ l of samples diluted 1:4 in 1X Assay Diluent were used per well for all samples except ALPS. ALPS IL-10 plasma levels were determined using Quantikine ELISA kits (R&D Systems, Inc., Minneapolis, MN).

LCAT activity assays

LCAT activity assays were performed as previously described.¹⁰

Statistical analysis

For the relationship between IL-10 and lipoproteins in ALPS, linear regression analysis and Pearson correlation coefficient R^2 was determined, using GraphPad Prism 6 software. For the IL-10 clinical trial results, statistical significance for placebo-adjusted changes from baseline (i.e., before rhIL-10 administration) in lipid parameters were calculated using mixed-model repeated-measures analyses. Unless otherwise indicated, all reported P-values are 2-sided and were considered significant if $P < 0.05$.

CASE REPORTS

CASE 1

A 59-year-old male was admitted as an outpatient with a 3-month history of fever, reduced appetite without nausea or vomiting and a 20 pound weight loss. The patient had a history of hypertension and type 2 diabetes mellitus, which recently improved without any change in his medications. The patient also reported a family history of heart disease. A review of his laboratory tests revealed that his HDL-C had progressively dropped, from a relatively normal level of 40 mg/dL about two years earlier, to 29 mg/dL one year prior to presentation (Fig. 1). At presentation, his HDL-C dropped below detectable limits (<5 mg/dL). Concurrently, low-density lipoprotein-cholesterol (LDL-C) also decreased prior to presentation, although less markedly, and triglycerides (TG) increased during this time period (Fig. 1). His lipid panel results at presentation were as follows: total cholesterol (TC): 86 mg/dL; HDL-C: <5 mg/dL, and TG: 274 mg/dL. Further analysis 2.5 months after presentation revealed the following: TC: 101 mg/dL; HDL-C: <5 mg/dL; TG: 467 mg/dL; ApoA-I: 31 mg/dL (110–205 mg/dL); and ApoB: 118 mg/dL (55–140 mg/dL). Lipoprotein agarose gel electrophoresis showed the absence of any HDL, elevated pre-beta lipoproteins at 41% (2–32%), and increased chylomicrons at 9% (0–2%). Neutral lipid staining of lipoproteins separated by size by non-denaturing polyacrylamide gel electrophoresis also showed very low levels of HDL, which were mostly comprised of small HDL particles (Supplemental Fig. 1S). The patient was also found to be anemic, with a hematocrit of 32.9%, and thrombocytopenic (platelets, 78 K/uL; normal, 150–400 K/uL). Serum lactate dehydrogenase (LDH) was markedly elevated at 2130 U/L (50–150 U/L). The patient subsequently developed sinus pain and a computed tomography (CT) scan of the head revealed a mass in his left maxillary sinus. Upon biopsy, it was diagnosed as IVLBCL, and he was treated with EPOCH-Rituximab chemotherapy.

Twenty-four days after the first cycle of chemotherapy, HDL-C levels recovered to near-normal levels (38 mg/dL) (Fig. 1, Fig. 2A). Western blot analysis confirmed the presence of a normal HDL subpopulation size distribution after chemotherapy compared to the presence of only very low levels of small (α_4 and pre β) HDL particles prior to treatment (Supplemental Fig. 2S). All other lipids also normalized after therapy (TC: 214; TG: 125; LDL-C: 151; apoA-I: 138; and apoB: 115; all mg/dL).

CASE 2

From the Lymphoma Registry of the National Cancer Institute (NCI), a second case of IVLBCL in a 45-year-old woman was identified. Stored serum before and after each cycle of EPOCH-Rituximab chemotherapy was used to measure lipids. HDL-C increased from 4 mg/dL at baseline to 20 mg/dL 38 days post therapy. LDL-C increased from 3.8 mg/dL at day 16 to 55 mg/dL 38 days post therapy, and TG decreased from 775 mg/dL at baseline to 224 mg/dL 38 days post therapy (Fig. 2B). However, unlike the first IVLBCL patient, HDL-C and apoA-I levels never completely normalized, and TG remained elevated. This patient survived only one year after chemotherapy and a sharp decrease in HDL-C and increase in TG occurred shortly before her death (Fig. 2B).

CASE 3

To determine whether HDL disappearance is a unique feature of IVLBCL or is present in other B-cell lymphomas, ten patients with diffuse large B cell lymphoma (five with liver and five without liver involvement) were also tested. Out of these 10, we identified one case of diffuse large B-cell lymphoma in a 58-year-old male with a lipid and lipoprotein profile similar to the IVLBCL cases described above (Fig. 2C). This patient had stage IV diffuse large B cell lymphoma without involvement of the liver. Following EPOCH-Rituximab chemotherapy, his HDL-C increased from below the limit of detection to 30 mg/dL by day 92. Because of his elevated TG, LDL-C was not calculated, but his non-HDL-C increased from 140 to 209 mg/dL and TG decreased from 902 to 392 mg/dL. Further improvements in his lipoprotein profile were observed by day 350 after therapy (Fig. 2C).

CASE 4

A one-year-old girl with a 6-month history of lymphadenopathy, splenomegaly, anemia, thrombocytopenia and failure to thrive was evaluated at the National Institutes of Health (NIH). Peripheral blood flow cytometry revealed the presence of elevated CD3+CD4-CD8-TCR $\alpha\beta$ + DNT cells (47%; normal: <1.5%).^{11, 12} A germline *FAS* (Fas Cell Surface Death Receptor) mutation (535A->T, E98V) was found and a diagnosis of autoimmune lymphoproliferative syndrome (ALPS) was made.^{13, 14} Prednisone therapy (4 mg daily) was initiated at 1 year of age for cytopenias. Her plasma IL-10 level at 15 months of age was noted to be markedly elevated at 400 pg/ml (reference range <9.2 pg/mL). Analysis of a blood sample collected at 14 months of age revealed a total cholesterol level of 104, HDL-C of 3, LDL-C of 66, TG of 175, apoA-I of 40, and apoB of 44 (all mg/dL) (Fig. 2D). Prednisone therapy was supplemented with mycophenolate mofetil (MMF) at age 1.5 years and the patient also received a 9-day pulse therapy of prednisone (15 mg/day) at 2.2 years of age. HDL-C increased slightly but was still very low (Fig. 2D). At 2.5 years of age, a lipid panel showed a total cholesterol level of 139 mg/dL, HDL-C of 9 mg/dL, and triglycerides of 165 mg/dL. Mycophenolate mofetil was then discontinued and sirolimus was started as long term therapy to treat her worsening lymphadenopathy and cytopenias. As the lymphadenopathy and cytopenias improved with immunosuppression therapy, the patient's HDL-C increased to 40 mg/dL and her triglycerides decreased to 80 mg/dL (Fig. 2D). 3 years later, her lymphoproliferation is in remission on sirolimus and her HDL-C has remained normal (Fig. 2D).

RESULTS

Increased levels of several cytokines have been reported in IVLBCL and in other types of B-cell lymphoma,^{15–17} and a wide variety of cytokines are known to affect lipoprotein metabolism.⁷ We, therefore, hypothesized that alterations in cytokine levels may have contributed to the severe HDL deficiency observed in our patients. We first performed a multiplex cytokine assay for patients with IVLBCL (Cases 1 and 2) and found strikingly elevated levels of IL-10 (656 and 642 pg/ml, respectively), which normalized after EPOCH-Rituximab therapy (Supplemental Fig. 3S, A). By comparing lipid and IL-10 profiles for the two IVLBCL patients and the DLBCL patient before and after chemotherapy, we found a close inverse relationship between IL-10 and HDL-C, with IL-10 decreasing and HDL-C increasing during recovery (Figs. 3A–C). A multiplex cytokine assay for the DLBCL patient (case 3) also revealed an elevated IL-10 that normalized after chemotherapy (Supplemental Fig. 3S, B).

Besides their accumulation of abnormal lymphocytes in lymph nodes and spleen, ALPS patients are also known to have elevated IL-10.^{12, 18, 19} Similar to our B-cell lymphoma cases, we observed a decrease in IL-10 and a concurrent increase in HDL-C following treatment of our ALPS patient (Patient 4; Fig. 3D).

We next examined potential causes for decreased HDL in our patients. Native-native 2D gel analysis of Patient 1 showed that the HDL subpopulation distribution was very similar to that of Familial LCAT Deficiency (FLD) patients (Supplemental Fig. 2S;^{20, 21}). The patient primarily had pre β HDL and α 4 HDL particles (Supplemental Fig. 2S). Larger spherical particles (α 1–3) were absent (Supplemental Fig. 2S). The patient also had abnormally low plasma cholesterol esters (CE) (24%), similar to Familial LCAT Deficiency patients.²⁰ Cholesteryl ester levels were also found to be low in the other two patients with B-cell lymphomas and recovered post-chemotherapy in all 3 patients (Fig. 4A). These findings prompted us to measure plasma LCAT activity (Fig. 4B). LCAT activity was found to be markedly decreased prior to treatment compared to healthy controls and recovered post-chemotherapy in Patients 1–3 (Fig. 4B).

Evaluation of a cohort of 93 ALPS patients with a germline *FAS* mutation revealed that 34 (23 children and 11 adults) had HDL-C levels below 10 mg/dL. The majority of these patients (68%) were relatively stable and not on any immunosuppressive treatment at the time of testing. We observed a strong inverse correlation between IL-10 and HDL-C ($R^2 = 0.3720$, $P < 0.0001$) in this ALPS cohort (Fig. 5). Gel electrophoresis of serum lipoproteins also demonstrated that α -lipoprotein levels were inversely related to IL-10 ($R^2 = 0.6651$; $P < 0.02$) (Supplemental Fig. 4S). In addition, TC ($R^2 = 0.2597$; $P < 0.0001$) and LDL-C ($R^2 = 0.1393$; $P = 0.0002$) were also inversely related to IL-10, whereas TG did not significantly correlate with IL-10 (Fig. 5). IL-6 and tumor necrosis factor- α (TNF- α) were also elevated in some of our ALPS patients, but these cytokine values did not correlate with any of the lipoprotein changes during the clinical course of our patients.

Evidence for a causal role of IL-10 in lowering HDL-C was provided by the analysis of serum from a previous randomized, placebo-controlled trial of psoriatic arthritis patients

treated with recombinant human IL-10 (rhIL-10).⁹ In this study, 18 patients with psoriatic arthritis (12 males, 6 females) received either rhIL-10 (5 or 10 µg/kg/day, n=12) or placebo (n=6) subcutaneously for 28 days. In contrast to placebo, treatment with rhIL-10 acutely decreased all measured lipid and lipoprotein parameters except TG, which showed an increase compared to placebo (Fig. 6; Supplemental Table I). After a week of treatment, HDL-C decreased by a mean of 76% ($P<0.0001$, mixed model repeated measures analysis), LDL-C by 46% ($P<0.0001$), apoA-I by 48% ($P<0.0001$), and apoB by 19% ($P<0.05$). Interestingly, lipoprotein(a) [Lp(a)], which is typically relatively invariant in its concentration and also does not usually change in response to most lipid-lowering therapy, decreased significantly, by 57% ($P<0.02$) after rhIL-10 treatment. All rhIL-10 treated subjects had HDL-C concentrations < 20 mg/dL, which was sustained throughout the four-week treatment period (Fig. 6). In contrast, TG levels gradually increased during rhIL-10 therapy and more than doubled by the end of the treatment (Fig. 6). Levels of all lipids and lipoproteins returned to baseline 4 weeks after the rhIL-10 treatment was discontinued.

DISCUSSION

We report here the novel discovery that very high levels of IL-10 are inversely related to HDL-C, as well as LDL-C, in three different disorders of B-cells, namely IVLBCL, DLBCL and ALPS. A direct causal link between IL-10 and lipoprotein levels was established from analysis of samples from a rhIL-10 clinical trial,⁹ which showed that rhIL-10 administration rapidly decreased HDL-C and LDL-C and increased TG, thus recapitulating the observed lipid changes in our patients with B-cell disorders.

IL-10 is produced by a minor subpopulation of B cells, as well as Th1, Th2, Th17 and CD8⁺ T cells, macrophages, and other cell types.^{22–26} It is a well-established anti-inflammatory cytokine, targeting myeloid-derived antigen-presenting cells and B- and T-lymphocytes.^{22–26} Thus, abnormally low IL-10 concentrations are associated with autoimmune and inflammatory disorders, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, and chronic inflammatory bowel disease.^{22–26} However, IL-10 can also have immune stimulatory and proinflammatory effects^{25, 27} and high levels of IL-10 can be a poor prognostic marker in a number of diseases. IL-10 is also a potent growth factor for B-cell lymphomas and, through its anti-inflammatory effect, may decrease tumor surveillance and suppress the antitumor response.²⁴

Interestingly, lipoprotein profiles similar to our cases have also been reported in patients with visceral leishmaniasis,²⁸ a disease also characterized by persistently elevated IL-10 levels.²⁹ Low HDL-C along with low LDL-C and increased TG are also frequently seen in a wide variety of other inflammatory conditions, such as sepsis, rheumatoid arthritis³⁰ and other conditions associated with an acute phase response,⁷ although HDL-C changes in these disorders are usually not as profound as in our cases. High levels of other cytokines besides IL-10 are often present in critically ill patients,³¹ but likely did not significantly contribute to the lipoprotein changes observed in our patients. In Case 1, IL-10 was the only cytokine that was elevated and was inversely related to HDL-C levels. In Case 2, besides IL-10, TNF α and IL-8 were also increased but only moderately and IL-8 increased following chemotherapy. TNF α did not show a significant correlation with HDL-C in our ALPS

cohort, and in a phase I trial³² in cancer patients, recombinant TNF α had only a small, non-dose-dependent effect on TC and HDL-C. Moreover, TNF α production in whole blood cultures decreased and the level of circulating TNF-receptor increased during the treatment of patients with psoriatic arthritis patients.⁹ The ability of IL-10 to decrease HDL-C was confirmed in the clinical trial with rhIL-10, which showed that rhIL-10 treatment markedly decreased HDL-C, lowered LDL-C and raised TG levels, thus producing a similar lipoprotein phenotype as our patients with endogenously elevated IL-10.

The lipid profile (low HDL-C and LDL-C; elevated TG; decreased CE) and the shift in the HDL subpopulation distribution (increased pre β and α 4 HDL subfractions; decreased mature, LCAT-produced α 1–3 HDL particles) in our patients is similar to the lipoprotein profile and other lipid changes found in Familial LCAT Deficiency patients.^{20, 21, 33} While LCAT activity was not as low in our patients as in Familial LCAT Deficiency patients (Fig. 4B), LCAT activity and percent CE in Patients 1–3 prior to chemotherapy was, nevertheless, markedly decreased compared to healthy controls and recovered fully after chemotherapy (Fig. 4). Decreased ABCA1 activity can also lead to lower levels of LCAT activity,^{34, 35} presumably because of low HDL, but the % CE in Tangier disease plasma is usually normal,^{34, 35} unlike in our patients. ApoA-I deficiency can also lead to low HDL and secondarily to low LCAT, but again % CE is relatively normal.³⁶ Overall, the combined data suggest that decreased plasma LCAT activity in our patients is likely a cause rather than a consequence of low HDL-C.

IL-10 treatment in the psoriatic arthritis clinical trial decreased not just HDL-C but LDL-C as well. In Familial LCAT Deficiency, not only HDL-C but also LDL-C is decreased.²⁰ Thus, the low LDL-C observed in our patients could also be due to diminished LCAT activity. Additionally, in mice, it has been previously reported that IL-10 may lead to decreased LDL production.³⁷

The mechanism by which highly elevated IL-10 modulates lipoprotein levels is not clear. Hepatocytes do not express detectable amounts of IL10R1,³⁸ the subunit of the IL-10 receptor that confers specificity for IL-10. Thus, highly elevated IL-10 does not appear to act through the canonical IL-10 receptor and signaling pathway in hepatocytes. The results found in our study and in previous studies³⁷ could possibly be explained by an alternative low-affinity IL-10 receptor in hepatocytes that comes into play only at very high levels of IL-10. *In vivo*, IL-10 could also conceivably interact with canonical IL10 receptors in nonhepatocyte liver cells that do express IL10R1, such as tissue macrophages, endothelial cells and hepatic stellate cells, which could then signal to hepatocytes. Another possibility is decreased apoA-I synthesis and secretion in response to IL-10 in the intestine, as Caco2 cultured intestinal cells and human intestinal epithelial cells have been described to both express IL-10 receptors^{39–41} and synthesize apoA-I.^{42–44} Finally, increased catabolism of HDL in response to highly elevated IL-10 is an important mechanism that could potentially contribute to the observed decrease in HDL-C. It is likely that IL-10 has multiple direct and indirect effects on lipoprotein metabolism and future studies will be needed to establish the relative contribution of decreased LCAT activity to the observed lipoprotein changes in our patients.

Mouse models demonstrate that moderate levels of IL-10 may protect against atherosclerosis mainly through anti-inflammatory effects, but these models do not address the effects of very high systemic IL-10 levels, similar to the levels we observed in our patients, on atherosclerosis. IL-10 is secreted by early and advanced atherosclerotic plaques and, dependent upon the level and site of IL-10 expression, can protect against plaque progression, thrombosis and rupture.^{45, 46} IL-10 knock-out mice had a 3-fold increase in atherosclerotic lesions after a 16-week atherogenic diet, with increased T-cell infiltration, increased IFN γ and decreased collagen in atherosclerotic lesions.⁴⁶ HDL-C decreased significantly in IL-10-deficient mice compared to WT mice,^{46, 47} consistent with the well-characterized effects of systemic inflammation in decreasing HDL-C.⁷ In contrast, mice with moderate (2–4X) overexpression of IL-10 in T lymphocytes had decreased atherosclerosis⁴⁷, attributed to a shift of the T lymphocyte population to a less inflammatory, Th2 cytokine-producing phenotype.⁴⁸ TC and HDL-C also decreased in this model, but not significantly.⁴⁷ Similarly, macrophage-specific IL-10 production also reduced atherosclerosis without changes in circulating cholesterol or triglyceride levels by upregulating the macrophage cholesterol efflux genes PPAR γ , LXR, ABCA1, and ABCG1.⁴⁹ These findings in mouse models demonstrate local anti-inflammatory effects of IL-10 in reducing atherosclerosis, as well as local effects in increasing cholesterol efflux from vessel wall macrophages, but do not provide insight into the effects of very high IL-10 on lowering systemic HDL and LDL levels and their effects on atherosclerosis. In the mouse study most relevant to our work, ApoE-deficient mice were injected with AAV5-mIL10 into muscle. These mice had a 31% reduction in plaque surface area, with decreases in aortic expression of MCP-1 and serum MCP-1 concentration.³⁷ Serum cholesterol concentrations were significantly reduced in AAV5-mIL10-transduced mice, possibly through decreased hepatic production.³⁷ These findings suggest that highly elevated IL-10 may protect against atherosclerosis, at least in mice.

Several human studies have also looked at the association of IL-10 with lipoprotein metabolism and cardiovascular disease, but the level of IL-10 in these studies was much lower than in our study. In a study of 23 patients with acute MI vs. 20 unstable angina patients and 16 healthy controls, moderately elevated IL-10 was associated with acute MI.⁵⁰ In larger studies of patients with acute coronary syndrome, increased IL-10 was associated with increased inflammation and a poorer prognosis⁵¹ and in another study was positively associated with CVD risk.⁵² HDL-C did show a weak inverse correlation with IL-10 in this latter study⁵² but HDL-C and IL-10 levels were not as extreme as in our patients. Low levels of IL-10 have been associated with decreased inflammation, but either did not correlate with HDL-C or showed a slight positive correlation.

Overall, our findings and those of others suggest that similar to its dual role in inflammation,^{25, 27} IL-10 may also have a dual role in determining HDL-C levels. Complete IL-10 deficiency causes an inflammatory state, resulting in a moderate decrease, but not complete disappearance, of HDL-C.^{46, 47} However, in some contexts, highly elevated levels of IL-10 can also exacerbate inflammation,^{25–27} and in our study highly elevated IL-10 was found to be associated with extremely low HDL-C levels. Because markedly elevated IL-10, in our study, also lowered pro-atherogenic LDL particles, its overall impact on CVD risk is uncertain at this time.

In summary, we discovered that elevated IL-10 plays a key role in the linkage between inflammation and lipoprotein metabolism. As evidence for its causality, rhIL-10 treatment in a clinical trial of psoriasis patients acutely lowered HDL-C and LDL-C and raised TG. Finding elevated IL-10 as a cause of “Disappearing HDL Syndrome” in patients with 3 different types of B-cell disorders suggests that IL-10 and low HDL-C could be useful biomarkers for these disorders. Finally, future research focusing on strategies to alter lipoprotein levels by modulating IL-10 levels or its signaling pathways may potentially be useful for developing novel cardiovascular drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Leigh Samsel and Philip McCoy of the Flow Cytometry Core Facility for their assistance with the multiplex assay (Luminex), Cornelio Duarte for excellent technical support and Toshi Sakurai and Boris Vaisman for advice on LCAT activity assays.

Sources of Funding: This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E and from intramural research funds from the National Heart, Lung and Blood Institute, National Cancer Institute, National Institute of Allergy and Infectious Diseases and the National Institute of Arthritis and Musculoskeletal and Skin Diseases. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Non-Standard Abbreviations and Acronyms

ABCA1	ATP-binding cassette transporter A1
ALPS	autoimmune lymphoproliferative syndrome
apoA-I	apolipoprotein A-I
apoB	apolipoprotein B
CAD	coronary artery disease
DLBCL	diffuse large B-cell lymphoma
FAS	Fas Cell Surface Death Receptor
HDL-C	high-density lipoprotein-cholesterol
IL-10	interleukin-10
IVLBCL	intravascular large B-cell lymphoma
LCAT	lecithin:cholesterol acyltransferase
LDL-C	low-density lipoprotein-cholesterol
MMF	mycophenolate mofetil

PsA	psoriatic arthritis
TC	total cholesterol
TG	triglycerides

References

1. Toth PP, Barter PJ, Rosenson RS, Boden WE, Chapman MJ, Cuchel M, D'Agostino RB Sr, Davidson MH, Davidson WS, Heinecke JW, Karas RH, Kontush A, Krauss RM, Miller M, Rader DJ. High-density lipoproteins: A consensus statement from the National Lipid Association. *J Clin Lipidol*. 2013; 7:484–525. [PubMed: 24079290]
2. Rader DJ, deGoma EM. Approach to the patient with extremely low HDL-cholesterol. *J Clin Endocrinol Metab*. 2012; 97:3399–3407. [PubMed: 23043194]
3. Goldberg RB, Mendez AJ. Severe acquired (secondary) high-density lipoprotein deficiency. *J Clin Lipidol*. 2007; 1:41–56. [PubMed: 21291667]
4. Park YB, Lee SK, Lee WK, Suh CH, Lee CW, Lee CH, Song CH, Lee J. Lipid profiles in untreated patients with rheumatoid arthritis. *J Rheumatol*. 1999; 26:1701–1704. [PubMed: 10451065]
5. Myasoedova E, Crowson CS, Kremers HM, Roger VL, Fitz-Gibbon PD, Thorneau TM, Gabriel SE. Lipid paradox in rheumatoid arthritis: The impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease. *Ann Rheum Dis*. 2011; 70:482–487. [PubMed: 21216812]
6. de Carvalho JF, Bonfa E, Borba EF. Systemic lupus erythematosus and “lupus dyslipoproteinemia”. *Autoimmun Rev*. 2008; 7:246–250. [PubMed: 18190886]
7. Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of infection and inflammation on lipid and lipoprotein metabolism: Mechanisms and consequences to the host. *J Lipid Res*. 2004; 45:1169–1196. [PubMed: 15102878]
8. Simonelli S, Gianazza E, Mombelli G, Bondioli A, Ferraro G, Penco S, Sirtori CR, Franceschini G, Calabresi L. Severe high-density lipoprotein deficiency associated with autoantibodies against lecithin:cholesterol acyltransferase in non-Hodgkin lymphoma. *Arch Intern Med*. 2012; 172:179–181. [PubMed: 22271127]
9. McInnes IB, Illei GG, Danning CL, Yarboro CH, Crane M, Kuroiwa T, Schlimgen R, Lee E, Foster B, Flemming D, Prussin C, Fleisher TA, Boumpas DT. IL-10 improves skin disease and modulates endothelial activation and leukocyte effector function in patients with psoriatic arthritis. *Journal of Immunology*. 2001; 167:4075–4082.
10. Vaisman, BL., Remaley, AT. Measurement of lecithin-cholesterol acyltransferase activity with the use of a peptide-proteoliposome substrate. In: Freeman, LA., editor. *Lipoproteins and Cardiovascular Disease: Methods and Protocols*. Methods Mol Biol. New York: Springer; 2013. p. 343–352.
11. Oliveira JB, Bleesing JJ, Dianzani U, Fleisher TA, Jaffe ES, Lenardo MJ, Rieux-Laucat F, Siegel RM, Su HC, Teachey DT, Rao VK. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): Report from the 2009 NIH international workshop. *Blood*. 2010; 116:e35–40. [PubMed: 20538792]
12. Price S, Shaw PA, Seitz A, Joshi G, Davis J, Niemela JE, Perkins K, Hornung RL, Folio L, Rosenberg PS, Puck JM, Hsu AP, Lo B, Pittaluga S, Jaffe ES, Fleisher TA, Rao VK, Lenardo MJ. Natural history of autoimmune lymphoproliferative syndrome associated with *FAS* gene mutations. *Blood*. 2014
13. Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, Puck JM. Dominant interfering *FAS* gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell*. 1995; 81:935–946. [PubMed: 7540117]
14. Sneller MC, Straus SE, Jaffe ES, Jaffe JS, Fleisher TA, Stetler-Stevenson M, Strober W. A novel lymphoproliferative/autoimmune syndrome resembling murine *lpr/gld* disease. *J Clin Invest*. 1992; 90:334–341. [PubMed: 1386609]

15. Ohashi N, Aomatsu M, Mori A, Takahashi M, Shibuya T, Maruyama T, Inoue H, Takegoshi S, Yokoi T, Okuno M. Intravascular lymphoma with extremely low high-density lipoproteinemia. *Intern Med.* 2007; 46:1475–1477. [PubMed: 17827853]
16. Garg A, Hosfield EM, Brickner L. Disseminated intravascular large B-cell lymphoma with slowly decreasing high-density lipoprotein cholesterol. *South Med J.* 2011; 104:53–56. [PubMed: 21119558]
17. Blackman JD, Cabana VG, Mazzone T. The acute-phase response and associated lipoprotein abnormalities accompanying lymphoma. *J Intern Med.* 1993; 233:201–204. [PubMed: 8433082]
18. Magerus-Chatinet A, Stolzenberg MC, Loffredo MS, Neven B, Schaffner C, Ducrot N, Arkwright PD, Bader-Meunier B, Barbot J, Blanche S, Casanova JL, Debre M, Ferster A, Fieschi C, Florkin B, Galambrun C, Hermine O, Lambotte O, Solary E, Thomas C, Le Deist F, Picard C, Fischer A, Rieux-Laucat F. Fas-l, IL-10, and double-negative CD4- CD8- TCR alpha/beta+ T cells are reliable markers of autoimmune lymphoproliferative syndrome (ALPS) associated with FAS loss of function. *Blood.* 2009; 113:3027–3030. [PubMed: 19176318]
19. Caminha I, Fleisher TA, Hornung RL, Dale JK, Niemela JE, Price S, Davis J, Perkins K, Dowdell KC, Brown MR, Rao VK, Oliveira JB. Using biomarkers to predict the presence of *FAS* mutations in patients with features of the autoimmune lymphoproliferative syndrome. *J Allergy Clin Immunol.* 2010; 125:946–949. e946. [PubMed: 20227752]
20. Santos RD, Asztalos BF, Martinez LR, Miname MH, Polisecki E, Schaefer EJ. Clinical presentation, laboratory values, and coronary heart disease risk in marked high-density lipoprotein-deficiency states. *J Clin Lipidol.* 2008; 2:237–247. [PubMed: 21291740]
21. Asztalos BF, Tani M, Schaefer EJ. Metabolic and functional relevance of HDL subspecies. *Curr Opin Lipidol.* 2011; 22:176–185. [PubMed: 21537175]
22. Kalampokis I, Yoshizaki A, Tedder TF. IL-10-producing regulatory B cells (B10 cells) in autoimmune disease. *Arthritis Res Ther.* 2013; 15 (Suppl 1):S1.
23. Yao Y, Simard AR, Shi FD, Hao J. Il-10-producing lymphocytes in inflammatory disease. *Int Rev Immunol.* 2013; 32:324–336. [PubMed: 23617759]
24. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. *Pharmacol Rev.* 2003; 55:241–269. [PubMed: 12773629]
25. Wilke CM, Wei S, Wang L, Kryczek I, Kao J, Zou W. Dual biological effects of the cytokines interleukin-10 and interferon-gamma. *Cancer Immunol Immunother.* 2011; 60:1529–1541. [PubMed: 21918895]
26. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Crit Rev Immunol.* 2012; 32:23–63. [PubMed: 22428854]
27. Muhl H. Pro-inflammatory signaling by IL-10 and IL-22: Bad habit stirred up by interferons? *Front Immunol.* 2013; 4:18. [PubMed: 23382730]
28. Soares NM, Leal TF, Fiuza MC, Reis EA, Souza MA, Dos-Santos WL, Pontes-de-Carvalho L. Plasma lipoproteins in visceral leishmaniasis and their effect on leishmania-infected macrophages. *Parasite Immunol.* 2010; 32:259–266. [PubMed: 20398226]
29. Nylen S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. *Trends Immunol.* 2007; 28:378–384. [PubMed: 17689290]
30. Robertson J, Peters MJ, McInnes IB, Sattar N. Changes in lipid levels with inflammation and therapy in RA: A maturing paradigm. *Nat Rev Rheumatol.* 2013
31. Gordon BR, Parker TS, Levine DM, Saal SD, Wang JC, Sloan BJ, Barie PS, Rubin AL. Relationship of hypolipidemia to cytokine concentrations and outcomes in critically ill surgical patients. *Crit Care Med.* 2001; 29:1563–1568. [PubMed: 11505128]
32. Spriggs DR, Sherman ML, Michie H, et al. Recombinant human tumor necrosis factor administered as a 24-hour intravenous infusion. A phase I and pharmacologic study. *J Natl Cancer Inst.* 1988; 80:1039–1044. [PubMed: 3411618]
33. Ahsan, Lusana, Ossoli, Alica F., Freeman, Lita A., Vaisman, Boris, Amar, Marcelo J., Shamburek, Robert D., Remaley, Alan T. Role of lecithin:cholesterol acyltransferase in HDL metabolism and atherosclerosis. In: Komoda, T., editor. *The HDL Handbook (second edition): Biological Functions and Clinical Implications.*

34. Cheung MC, Wang DB, Lum KD, Albers JJ. Cholesterol esterification by lecithin-cholesterol acyltransferase in A-I-free plasma. *Biochim Biophys Acta*. 1988; 962:258–264. [PubMed: 3139041]
35. Yao JK, Herbert PN, Fredrickson DS, Ellefson RD, Heinen RJ, Forte T, Dyck PJ. Biochemical studies in a patient with a Tangier syndrome. *J Neuropathol Exp Neurol*. 1978; 37:138–154. [PubMed: 632844]
36. Santos RD, Schaefer EJ, Asztalos BF, Polisecki E, Wang J, Hegele RA, Martinez LR, Miname MH, Rochitte CE, Da Luz PL, Maranhao RC. Characterization of high density lipoprotein particles in familial apolipoprotein A-I deficiency. *J Lipid Res*. 2008; 49:349–357. [PubMed: 17991756]
37. Yoshioka T, Okada T, Maeda Y, Ikeda U, Shimpo M, Nomoto T, Takeuchi K, Nonaka-Sarukawa M, Ito T, Takahashi M, Matsushita T, Mizukami H, Hanazono Y, Kume A, Ookawara S, Kawano M, Ishibashi S, Shimada K, Ozawa K. Adeno-associated virus vector-mediated interleukin-10 gene transfer inhibits atherosclerosis in apolipoprotein e-deficient mice. *Gene Ther*. 2004; 11:1772–1779. [PubMed: 15496963]
38. Wolk K, Witte E, Reineke U, Witte K, Friedrich M, Sterry W, Asadullah K, Volk HD, Sabat R. Is there an interaction between interleukin-10 and interleukin-22? *Genes Immun*. 2005; 6:8–18. [PubMed: 15526001]
39. Bourreille A, Segain JP, Raingeard de la Bletiere D, Siavoshian S, Vallette G, Galmiche JP, Blottiere HM. Lack of interleukin 10 regulation of antigen presentation-associated molecules expressed on colonic epithelial cells. *Eur J Clin Invest*. 1999; 29:48–55. [PubMed: 10092989]
40. Denning TL, Campbell NA, Song F, Garofalo RP, Klimpel GR, Reyes VE, Ernst PB. Expression of IL-10 receptors on epithelial cells from the murine small and large intestine. *Int Immunol*. 2000; 12:133–139. [PubMed: 10653848]
41. Latorre E, Mendoza C, Matheus N, Castro M, Grasa L, Mesonero JE, Alcalde AI. IL-10 modulates serotonin transporter activity and molecular expression in intestinal epithelial cells. *Cytokine*. 2013; 61:778–784. [PubMed: 23410504]
42. Green PH, Lefkowitz JH, Glickman RM, Riley JW, Quinet E, Blum CB. Apolipoprotein localization and quantitation in the human intestine. *Gastroenterology*. 1982; 83:1223–1230. [PubMed: 6813184]
43. Magun AM, Brasitus TA, Glickman RM. Isolation of high density lipoproteins from rat intestinal epithelial cells. *J Clin Invest*. 1985; 75:209–218. [PubMed: 3965504]
44. Naganawa S, Ginsberg HN, Glickman RM, Ginsburg GS. Intestinal transcription and synthesis of apolipoprotein AI is regulated by five natural polymorphisms upstream of the apolipoprotein CIII gene. *J Clin Invest*. 1997; 99:1958–1965. [PubMed: 9109440]
45. Mallat Z, Heymes C, Ohan J, Faggin E, Leseche G, Tedgui A. Expression of interleukin-10 in advanced human atherosclerotic plaques: Relation to inducible nitric oxide synthase expression and cell death. *Arterioscler Thromb Vasc Biol*. 1999; 19:611–616. [PubMed: 10073964]
46. Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF, Soubrier F, Esposito B, Duez H, Fievet C, Staels B, Duverger N, Scherman D, Tedgui A. Protective role of interleukin-10 in atherosclerosis. *Circ Res*. 1999; 85:e17–24. [PubMed: 10521249]
47. Pinderski Oslund LJ, Hedrick CC, Olvera T, Hagenbaugh A, Territo M, Berliner JA, Fyfe AI. Interleukin-10 blocks atherosclerotic events in vitro and in vivo. *Arterioscler Thromb Vasc Biol*. 1999; 19:2847–2853. [PubMed: 10591660]
48. Pinderski LJ, Fischbein MP, Subbanagounder G, Fishbein MC, Kubo N, Cheroutre H, Curtiss LK, Berliner JA, Boisvert WA. Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. *Circ Res*. 2002; 90:1064–1071. [PubMed: 12039795]
49. Han X, Kitamoto S, Wang H, Boisvert WA. Interleukin-10 overexpression in macrophages suppresses atherosclerosis in hyperlipidemic mice. *FASEB J*. 2010; 24:2869–2880. [PubMed: 20354139]
50. Tziakas DN, Chalikias GK, Hatzinikolaou HI, Parissis JT, Papadopoulos ED, Trypsianis GA, Papadopoulou E, Tentis IK, Karas SM, Hatseras DI. Anti-inflammatory cytokine profile in acute

coronary syndromes: Behavior of interleukin-10 in association with serum metalloproteinases and proinflammatory cytokines. *Int J Cardiol.* 2003; 92:169–175. [PubMed: 14659849]

51. Malarstig A, Eriksson P, Hamsten A, Lindahl B, Wallentin L, Siegbahn A. Raised interleukin-10 is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome. *Heart.* 2008; 94:724–729. [PubMed: 17690160]
52. Welsh P, Murray HM, Ford I, Trompet S, de Craen AJ, Jukema JW, Stott DJ, McInnes IB, Packard CJ, Westendorp RG, Sattar N. Circulating interleukin-10 and risk of cardiovascular events: A prospective study in the elderly at risk. *Arterioscler Thromb Vasc Biol.* 2011; 31:2338–2344. [PubMed: 21757655]

HIGHLIGHTS

- B-cell lymphoma and ALPS patients with severe HDL deficiency had high serum IL-10.
- IL-10 and lipids normalized coordinately during therapy.
- In 93 ALPS patients, IL-10 showed a strong inverse correlation with HDL-C.
- Injecting recombinant IL-10 into psoriatic arthritis patients decreased HDL-C.
- Elevated IL-10 is a new cause of severe HDL deficiency.

Fig. 1. Time course of lipoprotein profile changes in a patient with IVLBCL

Upper panel: historical lipid values 6.3 years (2303 days) before time of presentation (purple arrow) and subsequent time points (104 days) prior to EPOCH-Rituximab treatment (black arrow). The lower panel highlights the appearance of very low levels of HDL-C 4-5 months prior to presentation (purple arrow) and the recovery of lipid values after initiating EPOCH-Rituximab therapy (black arrow).

Fig. 2. Recovery of HDL-C and other lipoproteins after initiating therapy in patients with B-cell disorders

(AC) Recovery of lipids after EPOCH-Rituximab treatment in (A) Patient 1 (IVBCL); (B) Patient 2 (IVBCL), and (C) Patient 3 (DLBCL). The first time point (“Day 0”) represents the last lipid time point before initiating EPOCH-Rituximab treatment (black arrow). D. Patient 4. HDL-C increase and TG decrease in ALPS patient following treatment. TG, triglycerides; ApoA-I; apolipoprotein A-I. MMF, mycophenolate mofetil; P, prednisone. P* denotes the 9-day increase in prednisone to 15 mg/day.

Fig. 3. IL-10 and HDL-C recover concomitantly during treatment in patients with B-cell disorders

IL-10 and HDL-C time course before and after chemotherapy in: (A, B) 2 IVLBCL and (C) 1 DLBCL patient. As in Fig. 2A, the first time point ("Day 0") represents the last lipid time point before initiating chemotherapy (arrow). D. Recovery of HDL-C and IL-10 in an ALPS patient after treatment. MMF, mycophenolate mofetil; P, prednisone. P* denotes the 9-day increase in prednisone to 15 mg/day.

Fig. 4. Decreased plasma CE and LCAT activity in patients with B-cell disorders

Plasma cholesteryl esters (CE) (A) and LCAT activity (B) were measured before (“Pre”) and after (“Post”) chemotherapy in Patients 1–3. Results from a healthy control (HC) and a Familial LCAT Deficiency (FLD) homozygote and heterozygote patient are also shown. ** indicates P-values <0.001.

Fig. 5. Relationship between IL-10 and lipoproteins in ALPS

Serum from 93 ALPS patients at the time of presentation were used to measure the indicated lipid or lipoprotein and correlated with serum IL-10 levels. R^2 is Pearson correlation coefficient after $\log_{10}$ transformation of IL-10 and lipid values.

Fig. 6. Effect of rhIL-10 treatment on serum lipoproteins

Patients with psoriatic arthritis received daily subcutaneous injections of placebo (n=6), or 5 μ g/kg (n=4) or 10 μ g/kg (n=8) rhIL-10 for 28 days. In contrast to placebo, rhIL-10 had a significant, treatment-related effect on (A) HDL-C ($P < 0.0001$), (B) LDL-C ($P < 0.0001$), (C) apolipoprotein A-I ($P < 0.0001$), (D) apoB ($P < 0.05$), (E) Lp(a) ($P < 0.02$) and (F) TG ($P < 0.005$). The effect on lipids reversed after cessation of treatment (Day 29) (arrow). 2-sided P-values for placebo-adjusted changes in lipid parameters from baseline (i.e., before rhIL-10 administration) to day 29 were calculated using mixed-model repeated-measures analyses and were not adjusted for multiple comparisons.