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Dysfunctional, pro-inflammatory HDL directly upregulate monocyte PDGFR β , chemotaxis and TNF α production

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Abstract

Accelerated atherosclerosis is a major co-morbid condition in autoimmune diseases. Monocytes are the main immune cell involved in atherosclerosis initiation. We hypothesized that dysfunctional, pro-inflammatory HDL (piHDL), which occur in approximately half of SLE patients, might directly influence monocyte gene expression and function. SLE subjects were stratified into three groups: 1) carotid artery plaque+piHDL+ 2) plaque-piHDL+ and 3) plaque-piHDL- (n=18/group). PDGFR β was upregulated in primary monocytes from plaque+piHDL+ patients and in THP-1 cells acutely treated in vitro with piHDL compared to normal HDL. THP-1 chemotaxis was enhanced after treatment with piHDL versus normal HDL. Abnormal migration was restored to normal levels by treatment with imatinib or an apoJ-mimetic peptide. Increased piHDL-mediated TNF α protein levels were reduced with both inhibitors. Dysfunctional piHDL directly influences expression of a small number of transcripts and proteins, and piHDL inhibition through reducing piHDL oxidation or blocking PDGFR β kinase activity restored normal monocyte chemotaxis.

Keywords

high-density lipoproteins; monocyte; PDGFR β ; atherosclerosis; systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) patients have a 5-fold greater risk of myocardial infarction, occurring at a mean age of 49 (versus around 72 in healthy people), leading to a bimodal pattern of death where cardiovascular disease (CVD) is a leading cause of mortality 10 years after diagnosis of SLE [1]. The increase in cardiovascular events observed in SLE is due in part to traditional risk factors [1,2,3,4,5], but SLE itself is an independent factor predisposing to accelerated CVD. Young women (35-44 years old) with SLE are approximately 50 times more likely to have an MI versus age-matched control subjects [4]. Relative risk in a separate SLE cohort for MI or stroke was 10.1 and 7.9, respectively, even after controlling for Framingham factors [6]. Recent studies have also suggested that subclinical atherosclerosis, measured by ultrasound or CT of carotid or coronary arteries, is significantly increased in SLE patients compared to age-matched controls [4,7,8,9].

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High levels of plasma high density lipoprotein (HDL) are widely accepted as being protective against CVD, due to their promotion of reverse cholesterol transport (RCT) and reduction of oxidative species on low density lipoproteins [10,11]. Our group has recently shown that a dysfunctional, pro-inflammatory form of HDL (piHDL) [12,13] is present in 45% of SLE patients versus 4% of controls [9] and its presence highly correlates with the presence of carotid artery plaque, with an OR of 16.1 [14]. Autoantibodies that recognize apoA-I, the main protein component of HDL, are present in SLE patients [15] and recent data suggest that these autoantibodies could contribute to increased disease activity and organ damage through blocking the protective qualities of HDL [16,17,18]. Autoantibodies against oxidized LDL in humans and a mouse model with SLE-like characteristics have also been implicated in increased organ damage and risk for atherosclerosis [19,20,21].

Monocytes are the major immune cell involved in the initiation of atherosclerosis due to their integral roles at many stages of atherogenesis. Activated endothelial cells (EC) attract monocytes by secreting numerous chemokines, including monocyte chemoattractant protein-1 (MCP-1) and TNF α [22]. Monocytes then attach to EC and transmigrate into the arterial intima and secrete factors integral to the formation and ultimate destabilization of plaque [23]. Once in the subendothelial space, monocyte colony stimulating factor (M-CSF) drives monocytes to differentiate into macrophages that ultimately become cholesterol- and lipid-rich foam cells [23].

Studies on the contribution of monocytes to accelerated atherosclerosis and general disease progression in SLE have been limited: increased monocyte recruitment to aortic plaque were noted in a murine model of SLE [24], and TNF-receptor family mediated monocyte apoptosis in SLE-prone mice and humans has been linked to disease progression [25,26].

CD36, a scavenger receptor for oxidized phospholipids implicated in atherogenesis, is upregulated in the human monocyte cell line THP-1 after treatment with SLE patient plasma [27]. In addition, HDL isolated from patients with antiphospholipid syndrome was less effective at inhibiting monocyte binding to cultured EC than HDL isolated from healthy controls [28]. Therefore, we hypothesized that SLE piHDL, in addition to its association with subclinical atherosclerosis, induces a pro-inflammatory phenotype through indirect or direct monocyte interactions.

Materials and Methods

Study population

54 SLE subjects were included in this study. Subjects were drawn from our Biomarkers of Atherosclerosis in SLE cohort study; details of this study protocol are reported elsewhere [14]. In brief, participants were recruited prospectively from the Rheumatology Practices of the University of California Los Angeles (UCLA) and Cedars-Sinai Medical Center, Los Angeles, CA. Eligible participants were women ≥ 18 years of age. Patients with SLE fulfilled at least four of the 1997 revised American College of Rheumatology (ACR) classifications for SLE [29]. Because statins are known to alter HDL inflammatory function [30], subjects were excluded if they had taken statins within the prior three months, or if they had renal failure (defined as creatinine > 2.0), which also alters HDL function [31]. The study was approved by the Institutional Review Boards at UCLA and Cedars-Sinai Medical Center; all participants gave written informed consent.

Eligible women who gave consent provided a blood sample, underwent a carotid ultrasound to assess plaque, and gave consent to provide additional blood samples at two future time points. Monocytes were isolated from samples collected during a regular patient visit as described below. On the day of plasma sampling, the disease activity of SLE subjects was

assessed using the SELINA-SLEDAI validated instrument [32] and Framingham cardiovascular risk factors were assessed as previously described [14]. Only subjects with low disease activity (SLEDAI ≤ 4) were included in this analysis to minimize the influence of SLE activity on monocyte function. HDL function assays were performed on the repeat samples to confirm the presence or absence of piHDL. Patients were stratified into 3 groups: those with plaque and piHDL at the baseline ultrasound, those with piHDL but no plaque at baseline, and those with no plaque and normal HDL function. Transcript expression was examined in eighteen subjects from each study grouping. Clinical data for the final cohort is summarized in Table 1.

Sample collection

30 ml peripheral blood were collected from each subject in green top (heparin) tubes and immediately processed. Plasma was obtained by centrifugation and frozen in EDTA-free 50% sucrose [33]. Peripheral blood mononuclear cells were obtained by Ficoll gradient (Amersham) and the CD14⁺ fraction was isolated by positive separation using anti-CD14 magnetic beads and an AutoMACS (Miltenyi) according to the manufacturer's directions. Average yields were between $1.5\text{--}3 \times 10^6$ monocytes per 30 ml blood, and at least 1.5×10^6 cells were lysed for RNA. HDL cholesterol was isolated with a magnetic bead dextran reagent [30] from cryopreserved plasma collected from SLE subjects. In addition, lipoproteins isolated by magnetic beads then dialyzed against PBS in a high molecular weight cutoff membrane (MWCO, 100 kDa) to remove other plasma factors, as well as HDL purified by ultracentrifugation [34] were used to confirm THP-1 functional assays. HDL purified by ultracentrifugation was also dialyzed (2 kDa MWCO) to remove excess EDTA, as the presence of EDTA completely inhibited chemotaxis. HDL inflammatory score (i.e., whether a subject's HDL was qualified as either piHDL or normal HDL) was determined by a previously described cell-free assay [9,14,30,35]. For all experiments utilizing lipoprotein incubation with cells, piHDL/HDL were isolated from two subjects (Supplementary Figure 1A) and demonstrated similar results. HDL concentrations were measured in the UCLA clinical laboratory using standard methods.

Statistical analysis

Clinical data were analyzed using SPSS 13.0 (SPSS, Inc, Chicago, IL 2005). Skewed continuous variables were logarithmically transformed to attain a normal distribution. For variables that did not attain a normal distribution by logarithmic transformation, nonparametric tests were used. Study groups were compared using analysis of variance for continuous variables and the chi-square test for categorical variables.

RNA purification and quality control

RNA from both primary CD14⁺ cells and the THP-1 cell line was isolated using the RNeasy kit (Qiagen) per the manufacturer's directions, including the optional DNase step to eliminate genomic DNA. Quantitation and 260/280–260/230 ratios were determined on a Nanodrop 1000. RNA used in the real-time PCR array had 260/280 ratios ≥ 1.9 and 260/230 ratios ≥ 1.7 . All RNA was assessed for quality with the Agilent Bioanalyzer 2100 and RNA 6000 Nano chips, and each subject RNA sample used in the real-time PCR assay had a RNA Integrity Number of at least 9.5/10.

RT² Profiler atherosclerosis-focused real-time PCR array

cDNA was made from 810 ng of RNA from each subject and THP-1 sample using the RT2 first strand kit (SABiosciences). Transcript expression was analyzed for each individual subject using an RT2 Profiler real-time PCR atherosclerosis array plate (SABiosciences, PAHS-038) on a Bio-Rad iQ5. Raw data was initially processed using the Bio-Rad iQ5 software (version 2.0) to equalize baseline cycles (2–14) and threshold (40) between each subject's plate for

accurate analysis. Comparison analysis was performed using SABiosciences's web-based PCR array data analysis software (<http://www.sabiosciences.com/pcrarraydataanalysis.php>). One of the five housekeeping genes, B2M, was dropped from the analysis due to inconsistent results across the 54 arrays.

THP-1 cell culture and HDL treatment

The human monocyte cell line THP-1 was obtained from ATCC and was cultured in RPMI plus 10% fetal bovine serum, 2 mM glutamine, 100 U of penicillin/ml, 10 µg streptomycin/ml, and 0.05 mM β-mercaptoethanol at 37°C in 5% CO₂. For THP-1 HDL treatment, cells were counted, washed in RPMI, and resuspended in complete RPMI with 1% FBS (or no FBS and no antibiotics in the migration assays) to minimize effects of serum factors. Cells were then treated with HDL isolated from one SLE subject with either piHDL or from one SLE subject with normal HDL at 20 µg/ml for various time periods at 37°C in 5% CO₂. All experiments with lipoprotein treatments were repeated with piHDL/HDL isolated from one other SLE patient/lipoprotein (Supplementary Figure 1); no significant differences were noted between the two samples. The real-time PCR results reported (Table 3, Supplementary Figure 4) are analyses of three independent incubations of THP-1 with HDL/piHDL from two patients/lipoprotein (six samples/lipoprotein total).

THP-1 migration assay

Cells (1.5×10^5) were treated with HDL/piHDL in triplicate, as described above, for either 2 or 18 hours at 37°C in 5% CO₂. In experiments that utilized imatinib (300 nM, the approximate IC₅₀ for PDGFRβ), the ApoJ mimetic peptide L-[113-122]ApoJ (250 ng/ml), or the ApoA-I mimetic peptide L-4F (1 µg/ml), these treatments were added concurrently with piHDL/HDL to the cells. L-[113-122]ApoJ and L-4F were synthesized from all L-amino acids as described in [36] and [37], respectively. Cells were then added to the upper chamber of the QCM Chemotaxis 96-well cell migration kit (5 µm pore size, Chemicon) and allowed to migrate for 2 hours towards 200 ng/ml MCP-1 (R&D Systems) in RPMI plus glutamine (no FBS/antibiotics) or, in certain experiments, 20 µg/ml of subject HDL/piHDL in the lower well. Per the manufacturer's directions, migrated cells and cells attached to the lower part of the membrane were then lysed, stained with CyQuant GR dye (which binds DNA), and read on a fluorescent plate reader (SpectraMax, Molecular Devices) to quantitate migrated cells. MCP-1 concentrations in the bottom well ranging from 50-300 ng/ml did not promote appreciably different rates of migration. In addition, kinetic assays determined that the vast majority of cells migrated towards MCP-1 within two hours, because 6 and 18 hour timepoints resulted in negligible cell number increases. Results were analyzed with Student's t-test and graphed using Prism 4 (GraphPad). Imatinib, L-[113-122], apoJ and L-4F were generous gifts from Dr. Charles Sawyers (Memorial Sloan-Kettering Cancer Center, New York, NY) and Dr. Mohamad Navab (UCLA).

MCP-1/TNFα ELISAs

THP-1 cells (0.8×10^6 /ml) were treated in duplicate with lipids and therapeutics as described above for 18 hours. Supernatants were cleared of cells by centrifugation and subjected to ELISA per the manufacturer's directions (Quantikine ELISA kits, R&D Systems). Absorbance was read on a plate reader and data were analyzed with Student's t-test and graphed using Prism 4.

Results

Differences in traditional cardiac risk factors in the three SLE patient groups studied

We stratified SLE subjects from the UCLA Biomarkers of Atherosclerosis in SLE cohort into three groups of 18 based on SLEDAI ≤ 4 , the presence of carotid plaque on baseline carotid ultrasound, and HDL inflammatory score (i.e., whether the patient had normal or pro-inflammatory HDL). As shown in Table 1, subjects with plaque and piHDL (PL+piH+) had significantly higher total cholesterol levels and a history of hypertension than subjects with no plaque and piHDL (PL-piH+) as well as subjects without plaque or piHDL (PL-piH-). In addition, PL+piH+ patients were significantly older than PL-piH+ patients. Disease duration was not statistically different between the three groups. Total cholesterol, hypertension, and older age are all established traditional risk factors for atherosclerosis; no other Framingham risk factors were significantly different in the three groups. Notably, no SLE-specific atherosclerosis risk factors, including disease duration, SDI damage, SELENA-SLEDAI, or therapeutics differed significantly between the three groups. These data in the smaller subgroups reconcile well with data from our large cohort [14], with the exception of disease duration and SDI damage, which were both significantly associated with plaque in our larger cohort. Because of the smaller sample size, and because we selected for inactive disease, however, it is expected that the influence and presence of these factors would be minimized.

Pathway-focused real-time PCR studies in primary SLE monocytes identify a small number of plaque- and piHDL-altered transcripts

We utilized an atherosclerosis-focused real-time PCR array to examine mRNA/cDNA from freshly isolated primary monocytes from each patient in our subgroups. Three transcripts – the receptor tyrosine kinase platelet-derived growth factor receptor β (PDGFR β), the antiapoptotic gene Bcl2, and M-CSF – were significantly upregulated in monocytes isolated from PL+piH+ versus PL-piH- (Table 2). A number of adhesion molecules (selectins and integrins), matrix metalloproteinases (MMP-1 and -3), cytokines (IL-1 α and IL-5), and the cholesterol receptor ABCA1 included on the array were not significantly altered (Supplementary Figure 2). MCP-1 and TNF α were significantly upregulated in PL-piH+ versus PL-piH- monocytes (Table 2), raising the possibility that these two transcripts are directly influenced by the presence of piHDL. Only PDGFR β was upregulated in PL+piH+ monocytes when compared to PL-piH+ monocytes. Although T cells have been implicated in the contribution of immune cells to atherosclerosis, identical studies in purified T cell (CD3⁺) populations from the same patient groups (n=14 per group) yielded no significant transcript differences (Supplementary Figure 3).

PDGFR β is upregulated after acute treatment of the human monocyte cell line THP-1 with piHDL

We next tested whether in vitro piHDL treatment could directly influence monocyte gene expression. THP-1 cells were treated with patient-isolated 20 μ g/ml piHDL or normal piHDL and the same real-time PCR array was used to examine atherosclerosis-focused transcripts. PDGFR β transcript levels were 2.84-fold increased, and a number of other genes, including the MCP-1 receptor (CCR2), the PDGFR β ligand PDGF A, and the adhesion molecules tenascin c and CD11c/integrin α x, were upregulated (Table 3, Supplemental Figure 4). Minimal media (1% FBS) was used in the incubation due to non-specific alteration of many transcripts in 10% FBS (not shown). An additional assay examining THP-1 gene expression using piHDL and HDL isolated from two patients different from those in Table 3 yielded similar results (not shown).

piHDL treatment of THP-1 cells accelerates migration

We next asked whether piHDL, in addition to its direct effect on monocyte transcription, could influence monocyte function. As transmigration of monocytes into the subendothelial space is an integral initiating step in atherosclerosis, we examined whether piHDL treatment influences monocyte chemotaxis. THP-1 cells were treated with piHDL, HDL or no lipids for 2 hours, then placed in the top well of a migration chamber and allowed to migrate towards MCP-1 for 2 additional hours. piHDL treatment caused significantly more migration than HDL treatment (Figure 1A). THP-1 cells treated with lipoproteins isolated from an additional 12 subjects (three PL+piH+, three PL-piH+, six PL-piH-) yielded similar results (Supplemental Figure 1B).

ApoJ and apoA-I mimetic peptides decrease piHDL-driven, but not HDL-driven, migration

Previous work has demonstrated that small, orally administered peptide mimetics of two HDL-associated proteins, apoJ and apoA-I, are able to convert piHDL to its anti-inflammatory state and slow or reverse atherosclerosis in mouse models [36,38]. Therefore, we examined whether concomitant apoJ (L-[113-122]apoJ) and apoA-I (L-4F) mimetic peptide and lipid treatment affected THP-1 migration. Both L-[113-122]apoJ and L-4F decreased migration caused by piHDL to equivalent levels of HDL-treated cells (Figure 1B, C). HDL-treated cells were unaffected by the presence of either mimetic peptide.

piHDL itself acts as a mild chemoattractant

We next asked whether properties of piHDL, in a similar manner to oxLDL, increase monocyte motility. Cells were incubated for 2 hours in RPMI (no lipid stimulation) and placed in the migration chamber for 2 hours with 20 µg/ml HDL or piHDL as the chemoattractant instead of MCP-1. piHDL caused a small, but significant, increase in THP-1 migration (Figure 1C).

PDGFRβ inhibition diminishes piHDL-dependent TNFα, but not MCP-1, secretion

Our primary monocyte assays (Table 2) suggested that the presence of piHDL leads to an increase in MCP-1 and TNFα transcripts, and that plaque (and piHDL in THP-1 cells, Table 3) is associated with PDGFRβ upregulation. Therefore, we tested whether neutralization of piHDL by L-[113-122]apoJ and/or inhibition of PDGFRβ with the small molecule inhibitor imatinib led to a decrease in MCP-1 and TNFα protein secretion in THP-1 cells. L-[113-122]apoJ significantly reduced both MCP-1 (Figure 2A) and TNFα secretion (Figure 2B) only in cells treated with piHDL. In contrast, imatinib treatment reduced TNFα production but did not significantly affect MCP-1 secretion in piHDL-treated cells, suggesting that the PDGFRβ signaling axis, when upregulated by piHDL treatment, is disproportionally linked to increased TNFα production. Normal HDL treatment plus either inhibitor did not alter MCP-1 secretion, and only combination treatment of both L-[113-122]apoJ and imatinib in HDL-treated cells led to a decrease of TNFα production that approached statistical significance. Treatment of THP-1 cells with piHDL purified by ultracentrifugation led to significantly higher levels of both TNFα and MCP-1 when compared to cells treated with HDL or piHDL alone (Supplemental Figure 5).

PDGFRβ inhibition in piHDL-1 treated monocytes decreases THP-1 chemotaxis to levels observed in cells treated with normal HDL

We next asked if piHDL-upregulated PDGFRβ levels could play a role in chemotaxis. THP-1 cells were treated with piHDL or HDL in combination with imatinib, L-[113-122]apoJ, or both and allowed to migrate towards MCP-1. Imatinib/piHDL treatment significantly decreased monocyte migration to the levels of both L-[113-122]apoJ/piHDL and imatinib/HDL (Figure 3). HDL-treated cells, as observed with L-[113-122]apoJ, were unaffected by imatinib treatment, and these results were confirmed using piHDL/HDL isolated from six additional subjects (Supplemental Figure 1) as well as using ultracentrifuge-purified and dialyzed

lipoproteins (Supplemental Figure 6). These data suggest that monocytes in the presence of piHDL, either acutely or chronically, upregulate PDGFR β . These cells could become abnormally dependent on PDGFR β -driven signaling pathways that lead to both increased migration and upregulated TNF α production and can be effectively inhibited by a PDGFR β inhibitor that does not affect monocytes in the presence of normal HDL.

Discussion

Accelerated atherosclerosis is a significant cause of morbidity and mortality in SLE patients and is due to a combination of traditional and SLE-specific risk factors. We have previously determined that HDL function is deficient in a much higher percentage of SLE patients versus normal controls [9,39], and this dysfunctional HDL correlates with carotid artery plaque [14]. In addition, dysfunctional HDL predicts atherosclerosis in other autoimmune diseases, such as Crohn's disease [40], antiphospholipid syndrome [28], and systemic sclerosis [41].

In this study, we determined that the presence of piHDL in SLE patients with or without carotid plaque leads to alteration of a small number of transcripts in circulating monocytes, and piHDL directly influences monocyte chemotaxis and secretion of the pro-inflammatory molecules MCP-1 and TNF α . Notably, short-term in vitro exposure of a human monocyte line to piHDL leads to upregulation of PDGFR β mRNA. Either restoration of anti-inflammatory HDL function by L-[113-122]apoJ or inhibition of PDGFR β with imatinib restores migration and TNF α levels to those observed in normal HDL-treated monocytes. These results suggest a novel biologic function of piHDL in human monocytes: induction of PDGFR β -dependent functional pathways that are not observed in cells treated with normal HDL. Therefore, plasma piHDL could induce alternate, abnormal biological networks to drive cellular processes (in this case, PDGFR β -driven monocyte chemotaxis) that are targetable by therapeutic intervention.

Other significantly altered transcripts in monocytes from SLE patients with/without piHDL and plaque could be contributing to atherosclerosis initiation and progression. For example, MCP-1 transcript levels are significantly higher in cells from PL-piH⁺ subjects versus the other two groups, including PL+piH⁺ subjects. These data suggest that MCP-1 secreted from circulating monocytes could play a role in plaque initiation by recruiting other monocytes to the nascent lesion. Because MCP-1 transcript levels in PL+piH⁺ subjects return to base line levels observed in the PL-piH⁻ group, it is possible that the presence of atherosclerotic plaque reduces the necessity of monocyte autocrine-type recruitment via MCP-1 (perhaps through increased MCP-1 secretion by endothelial cells). In addition, Bcl2 is upregulated in PL+piH⁺ subjects, suggesting that the presence of plaque could inhibit monocyte apoptosis through pathways dependent on this antiapoptotic protein. Interestingly, no monocyte transcripts are significantly altered when subjects are stratified by piHDL only (data not shown). These data, along with our previous observation that presence of piHDL increases the odds of atherosclerosis in SLE patients 16-fold [14], could mean that PL-piH⁺ subjects make up a distinct, intermediate population at particular risk for atherosclerosis induction (versus PL+piH⁺ + subjects that, although also having piHDL, have already developed plaque). Therefore, the data presented herein provides additional support for piHDL as an effective biomarker for early identification of SLE patients at risk for accelerated atherosclerosis.

What actually causes HDL to become pro-inflammatory? Unlike discrete proteins, HDL is a complex particle of approximately 400 kDa, made up of 50 or more proteins along with multiple phospholipids, triglycerides and cholesterol. Mass spectrometric experiments to identify protein components of HDL show that, in addition to protective proteins (apoJ, apoA-I, paraoxonase (PON)-1 and -3, hemopexin), other molecule classes, such as complement proteins, endopeptidase inhibitors, and pro-inflammatory molecules (serum amyloid proteins,

apoB-100), are present [34,42]. The ratio of pro-inflammatory:anti-inflammatory proteins could therefore change in HDL, which has been noted with increased levels of serum amyloid A [43]. However, Vaisar et al. observed that HDL levels of anti-inflammatory proteins (PON1, apoA-IV, apoE) actually increase in patients with coronary artery disease [34]. Another possibility is that protective capacities of the anti-inflammatory proteins are compromised or pro-inflammatory proteins become constitutively active through post-translational modification or mutation. Our previous work, however, suggests that PON activity alone or apoA-I levels alone do not correlate with HDL function or carotid artery plaque in SLE patients [14]. A third mechanism for the conversion of HDL to piHDL is that components of piHDL are hyperoxidized [43]. It is probable that all of these mechanisms contribute to the creation and maintenance of piHDL in a complex and as-yet undetermined manner. L-[113-122]apoJ is presumably effective at reversing piHDL function by sequestering lipid hydroperoxides downstream of piHDL [36] and is effective at preventing piHDL-driven functional effects in our studies.

A recent report [42] implicates hemoglobin as a major contributor to piHDL function. Hemoglobin binds the most prevalent HDL lipoprotein, apoA-I, through the hemoglobin scavenger protein haptoglobin and appears to be directly responsible for piHDL activity in humans with coronary heart disease as well as mice on a high fat diet [42]. Hemoglobin-mediated piHDL can be reversed with an apoA-I mimetic peptide with a similar mechanism to L-[113-122]apoJ. Similar results identifying hemoglobin and haptoglobin as causes of piHDL have been observed in rheumatoid arthritis patients [39].

PDGFR signaling has previously been implicated in atherosclerosis, although most studies have focused on SMC or endothelial cells (EC). Monocyte PDGFR β and PDGF-A/B expression are all increased in patients with atherosclerosis [44]. PDGF treatment has been shown to stimulate monocyte migration [45], although other studies suggest that monocytes need to be activated by lymphocytes [44].

Although monocytes play a central role in atherosclerosis initiation, their role in overall atherosclerosis progression could be minimal in comparison to macrophages/foam cells, EC, and SMC. In addition, piHDL could play a greater part in promoting atherosclerosis in these cell types versus monocytes, and our present study does not address this issue. EC and circulating EC clearly play a role in SLE vascular damage, possibly mediated through interferon- α [46,47]. However, it is difficult to study these primary cells by microarray/qPCR array, especially from human atherosclerotic lesions. Regardless, understanding and studying the effects of piHDL on EC, SMC and macrophages are necessary.

In conclusion, the presence of plaque and piHDL in SLE patients causes a small but functionally significant alteration of monocyte atherosclerosis-related transcripts. One of these, PDGFR β , is also upregulated in THP-1 monocytes after short-term incubation with piHDL. Chemotaxis of monocytes treated with piHDL is increased, and this gain of function is effectively blocked with a PDGFR β inhibitor, imatinib, and the piHDL antagonists L-[113-122]apoJ and L-4F. piHDL also causes higher levels of monocyte secretion of MCP-1 and TNF α , and TNF α production is also blocked by imatinib and L-[113-122]apoJ. Our results suggest that dysfunctional piHDL, present in SLE and other autoimmune diseases, might drive a pro-atherosclerotic phenotype in part through its interactions on monocytes through PDGFR β -mediated pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

piHDL increases monocyte chemotaxis and is blocked by an ApoJ-mimetic peptide, L-[113-122]apoJ. THP-1 cells were treated with 20 μ g/ml lipids (treatment shown below bar) and L-[113-122]apo J (if indicated) for 2h and allowed to migrate towards 200 ng/ml MCP-1 (groups A, B) or were left untreated and allowed to migrate towards 20 μ g/ml piHDL or HDL (group C) for 2 additional hours. Relative fluorescence units measured nucleic acids bound to a fluorescent dye after migrated cells were lysed. p values were determined by Student's t-test. Results are representative of four independent experiments. NS=not significant

Figure 2.

piHDL treatment increases monocyte MCP-1 and TNFα secretion, and L-[113-122]apoJ restores secretion of both molecules to HDL-stimulated levels. THP-1 cells were concurrently treated with lipids, L-[113-122]apoJ, and/or imatinib for 18h. Cell supernatants were collected and MCP-1 (A) and TNFα (B) levels were identified by ELISA. Imatinib treatment of piHDL-stimulated cells did not significantly lower MCP-1 levels ($p=0.0818$) but did rescue TNFα levels. p values were determined by Student's t -test. Results are representative of three independent experiments. NS=not significant

Figure 3.

Imatinib treatment rescues piHDL-driven monocyte chemotaxis to normal HDL-treated levels. THP-1 cells were treated with 20 $\mu\text{g/ml}$ lipids (treatment shown below bar) and the inhibitors indicated for 18h and allowed to migrate towards 200 ng/ml MCP-1 (except lane 1 as a negative control). Relative fluorescence units measured nucleic acids bound to a fluorescent dye after migrated cells were lysed. p values were determined by Student's t-test. Results are representative of three independent experiments. NS=not significant

Table 1

Cardiac risk factors, SLE disease measurements and demographic characteristics of subjects studied

	plaque+ piHDL + (PL+piH+) Group 1 n=18	no plaque piHDL + (PL-piH+) Group 2 n=18	no plaque normal HDL (PL-piH-) Group 3 n=18	p-value *
Age (years) (mean $\pm$ SD)	55.2 $\pm$ 8.8 [†]	38.9 $\pm$ 12.9	41.7 $\pm$ 12.6	(1 vs. 2) p=0.04
HDL function (FU)	1.35 $\pm$ 0.46	1.3 $\pm$ 0.4	0.69 $\pm$ 0.19	(1 vs. 3) p<0.001 (2 vs. 3) p<0.001
Total cholesterol (mg/dL) (mean $\pm$ SD)	215.5 $\pm$ 43.5	173.3 $\pm$ 38.4	181.2 $\pm$ 22.9	(1 vs. 2) p=0.001 (1 vs. 3) p=0.007
LDL (mg/dL) (mean $\pm$ SD) [‡]	129.3 $\pm$ 38.6	98.9 $\pm$ 27.9	99.2 $\pm$ 19.7	ns
HDL (mg/dL) (mean $\pm$ SD) [‡]	55.6 $\pm$ 18.7	52.5 $\pm$ 16.0	56.6 $\pm$ 11.2	ns
Triglycerides (mg/dL) (mean $\pm$ SD)	128.2 $\pm$ 68.2	100.2 $\pm$ 47.0	126.5 $\pm$ 93.8	ns
Mean SDI damage ($\pm$ SD) [§]	1.9 $\pm$ 1.8	1.3 $\pm$ 1.9	1.7 $\pm$ 2.1	ns
Mean SELENA-SLEDAI ($\pm$ SD)	2.2 $\pm$ 1.4	2.9 $\pm$ 1.4	3.2 $\pm$ 1.2	ns
Body Mass Index	30.7 $\pm$ 7.5	26.0 $\pm$ 6.0	26.2 $\pm$ 4.7	ns
Mean disease duration (years)($\pm$ SD)	18.2 $\pm$ 9.2	11.2 $\pm$ 6.3	12.9 $\pm$ 9.8	ns
History of Hypertension %(n) [¶]	72.2% (13)	22.2% (4)	33.3% (6)	(1 vs. 2) p=0.003 (1 vs. 3) p=0.02
History of Dyslipidemia %(n) [#]	38.9% (7)	16.7% (3)	33.3% (6)	ns
History of Smoking (ever) %(n) ^{**}	44.4% (8)	27.8% (5)	33.3% (6)	ns
History of Diabetes %(n) ^{††}	11.1% (2)	5.6% (1)	5.6% (1)	ns
Caucasian race %(n)	50% (9)	55.6% (10)	50% (9)	ns
Prednisone Dose (mg/day)	2.7 $\pm$ 4.0	2.9 $\pm$ 0.5	5.8 $\pm$ 8.5	ns
Total Prednisone last 6 months (mg)	638.9 $\pm$ 885.3	565 $\pm$ 692.7	936.9 $\pm$ 1437.2	ns
Hydroxychloroquine use	50% (9)	55.6 % (10)	55.6% (10)	ns

* p-values shown only if statistically significant; calculated using analysis of variance for continuous variables, Chi-Square or Fisher's exact test for dichotomous variables

[†] Values are mean plus/minus standard deviation

[‡] HDL = high density lipoprotein; LDL = low density lipoprotein

[§] SDI = Systemic Lupus Collaborating Clinics/American College of Rheumatology Damage Index

^{||} SELENA-SLEDAI = Safety of Estrogens in Lupus Erythematosus—National Assessment (SELENA) SLE Disease Activity Index

[¶] Hypertension was defined as use of antihypertensive medication or a systolic blood pressure > 140 mm Hg or a diastolic blood pressure > 90 mm Hg.

[#] Dyslipidemia was defined as any one or combination of the following: LDL cholesterol $\geq$ 130mg/dL, Total cholesterol $\geq$ 200 mg/dL, HDL cholesterol $\leq$ 40mg/dL, triglycerides $\geq$ 150 mg/dL

^{**} Smoking was present if subjects had smoked any cigarettes within the last 3 months

^{††} Diabetes mellitus was defined as the presence of a fasting glucose $\geq$ 7.0 mmol/L (126 mg/dL), or in subjects receiving insulin or an oral hypoglycemic

Table 2

PDGFR β upregulation correlates with the presence of plaque and piHDL

Transcript	Avg Ct* group 1 (PL+pi+)	Avg Ct group 2 (PL-pi+)	Avg Ct group 3 (PL-pi-)	Fold [†] 1 vs. 2	p value 1 vs. 2	Fold 1 vs. 3	p value 1 vs. 3	Fold 2 vs. 3	p value 2 vs. 3
PDGFR β	9.66	10.98	11.41	2.49[‡]	0.0194	3.36	0.0074	1.35	0.2939
Bcl2	5.67	6.54	6.88	1.82	0.0444	2.31	0.0332	1.27	0.5937
M-CSF	7.45	8.00	8.90	1.46	0.7017	2.74	0.0385	1.87	0.0782
MCP-1	6.71	4.34	6.43	-5.18	0.5712	-1.21	0.2535	4.26	0.0027
TNF α	2.55	3.43	4.66	1.84	0.1199	4.32	0.0859	2.34	0.0137
GAPDH [§]	-1.28	-1.45	-1.07	-1.12	0.4060	1.16	0.4139	1.30	0.2055

* Average cycle threshold (Ct) is the mean Ct of each transcript for each patient then subtracting the mean Ct of housekeeping transcripts

[†] Fold change is determined by 2⁻(avg Ct group a-avg Ct group b)[‡] Significance in fold change (≥ 2 or ≤ -2) and p-value (≤ 0.05) are **bold**[§] glyceraldehyde 3-phosphate dehydrogenase (GAPDH) represents a housekeeping gene

Table 3

Acute piHDL treatment leads to upregulation of multiple proinflammatory transcripts

Transcript	Avg Ct THP-1 + 20 µg/ml piHDL	Avg Ct THP-1 + 20 µg/ml normal HDL	Fold piHDL vs normal HDL	p-value
PDGFRβ	11.34	12.85	2.84	0.0367
CCR2	3.41	4.66	2.39	0.0326
PDGF A	5.10	6.13	2.04	0.0327
tenascin c	6.50	7.97	2.77	0.0007
CD11c	7.52	8.54	2.03	0.0010
GAPDH	-0.58	-0.80	-1.17	0.6382