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Pro-inflammatory high-density lipoproteins and atherosclerosis are induced in lupus-prone mice by a high-fat diet and leptin

BH Hahn, EV Lourenço, M McMahon, B Skaggs, E Le, M Anderson, N Iikuni, CK Lai, and A La Cava

David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA, USA

Abstract

Atherosclerosis is accelerated in people with systemic lupus erythematosus, and the presence of dysfunctional, pro-inflammatory high-density lipoproteins is a marker of increased risk. We developed a mouse model of multigenic lupus exposed to environmental factors known to accelerate atherosclerosis in humans – high-fat diet with or without injections of the adipokine leptin. BWF1 mice were the lupus-prone model; BALB/c were non-autoimmune controls. High-fat diet increased total serum cholesterol in both strains. In BALB/c mice, non-high-density lipoprotein cholesterol levels increased; they did not develop atherosclerosis. In contrast, BWF1 mice on high-fat diets developed increased quantities of high-density lipoproteins as well as elevated high-density lipoprotein scores, indicating pro-inflammatory high-density lipoproteins; they also developed atherosclerosis. In the lupus-prone strain, addition of leptin increased pro-inflammatory high-density lipoprotein scores and atherosclerosis, and accelerated proteinuria. These data suggest that environmental factors associated with obesity and metabolic syndrome can accelerate atherosclerosis and disease in a lupus-prone background.

Keywords

atherosclerosis; murine lupus; pro-inflammatory high-density lipoproteins (piHDL)

Introduction

Accelerated atherosclerosis in patients with systemic lupus erythematosus (SLE) may be associated with dysfunctional high-density lipoproteins (HDL) which are pro-inflammatory (piHDL).¹ These piHDL are defective in several usual functions of HDL, e.g. removing cholesterol from cells and artery walls (reverse cholesterol transport), and protecting low-density lipoproteins (LDL) from oxidation. Oxidized LDL in artery walls activate endothelial cells and attract monocytes, which mature into macrophages, ingest cholesterol and form foam cells. The changes in HDL that convert them to piHDL are reduced quantities of the cholesterol transport protein Apolipoprotein 1 (ApoA1) and the major antioxidant enzyme paraoxonase 1 (PON1), and increased quantities of oxidized lipids, which have pro-oxidant activity. The presence of piHDL in a woman with SLE increases risk for carotid artery plaque 17-fold, and risk for increased intima-media thickness threefold.² Multiple factors other than piHDL participate in SLE-related atherosclerosis, including genetic, metabolic and environmental influences.

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Correspondence to: Bevra H Hahn MD, Professor of Medicine, Division of Rheumatology, UCLA, 1000 Veteran Avenue, Los Angeles, CA 90095, USA. bhahn@mednet.ucla.edu.

In work reported here, mice with multigenic pre-disposition to SLE were exposed to environmental/metabolic factors that accelerate atherosclerosis in humans, i.e. high fat diets and leptin – a hormone associated with metabolic syndrome – to address whether lupus mice could concomitantly develop piHDL and atherosclerosis. Leptin is an adipokine that controls appetite via hypothalamic receptors; its levels are increased in people with obesity, metabolic syndrome, or SLE.^{3–5} Leptin is pro-inflammatory; it activates macrophages to release TNF- α and IL-6, induces endothelial dysfunction, decreases paraoxonase activity in normal human HDL,^{5,6} and increases oxidative stress.⁷ Interventions in this study included a high-fat ‘Western’ diet, with or without injections of leptin. The finding of concomitant atherosclerosis and lupus in a mouse strain with multigenic predisposition to SLE can provide a model for the study of this complication in SLE.

Methods

SLE-prone (NZB $\times$ NZW)F₁ female mice (BWF1), or non-autoimmune BALB/c control females, aged 4 weeks, were divided into two groups and treated for 12 weeks with: 1) standard chow diet (12% fat by weight), or 2) high-fat ‘Western’ diet (21% fat). BWF1 females in an additional group were treated with both high-fat diet and leptin (1 μ g/g intraperitoneally twice a week). BALB/c share two MHC class II molecules with BWF1 mice (I-Ad and I-Ed) and have low susceptibility to atherosclerosis on high-fat diets.⁸ Each group contained 8–12 mice.

Mice were observed daily for survival. Plasma was collected at time 0, 2, 4, 5 and 12 weeks. The following lipids were measured:⁸ total cholesterol, HDL-cholesterol and triglycerides. Non-HDL cholesterol was calculated. Pro-inflammatory HDL were measured as described previously.^{1,2,9} Oxidation of dichlorofluorescein hydrate (DCFH) releases a fluorescent signal. Addition of normal pooled human LDL promotes oxidation of DCFH and a high fluorescent signal, which was set as baseline (defined as ‘1’). Normally functioning human or murine HDL reduce oxidation of human LDL and therefore of DCFH, and reduce the fluorescence signal. In the assay, HDL (100 μ l) purified from test plasmas was added to quench this oxidation of LDL. In human plasma cryopreserved in sucrose, normal HDL gives readings below 1; dysfunctional piHDL gives scores above 1.^{1,2} In mouse assays, HDL from young mice of normal strains reduced the signal below 4; dysfunctional HDL increased the signal above 4. Four mice in each group were sacrificed to obtain aortic tissue. Additional groups of 10 mice in each set were followed for 28 weeks (until age 32 weeks) for measurements of anti-DNA by ELISA at 0, 12 and 28 weeks, and weekly proteinuria using Albustix (Bayer).

Aortic tissue was studied in the UCLA Atherosclerosis Core, using methods previously described.^{8,9} Aortas were removed from the aortic valves to the femoral bifurcation, opened vertically, and stained with Oil Red O. Multiple transverse sections from the aortic valves through 4 cm distal to the valves were examined with computer-assisted microscopy, and the number of lipid deposits were totaled, then reported as number of lipid deposits per μ m² of tissue.

Results

As shown in Figure 1, BWF1 and BALB/c mice on high-fat diets developed significant elevations in plasma levels of total cholesterol (panel A). In both strains, hypercholesterolemia appeared after 5 weeks of high-fat diet and persisted throughout the study (data not shown).

The content of the hypercholesterolemia induced by high-fat diets was quite different in the two strains. Non-HDL cholesterol (containing LDL and very low-density lipoproteins (VLDL)) was significantly higher in BALB/c mice than in BWF1 (panel B), and was not influenced by high-fat diet. In contrast, (Panel C), total quantitative HDL was similar in BALB/c and BWF1 on regular chow, but addition of high-fat diet significantly increased total HDL cholesterol in the BWF1 in both high-fat diet and high-fat-diet-plus-leptin groups. As shown in Panel D, qualitative analysis of HDL showed that exposure to high-fat diet induced dysfunctional HDL in the lupus-prone strain, but not in the controls. BWF1 mice on high-fat diets had significant elevations in their functional HDL scores, indicating the presence of piHDL. Addition of leptin to the high-fat diet further increased the piHDL score in BWF1 mice. Therefore, hypercholesterolemia induced by high-fat diet consists primarily of HDL in BWF1 mice, but not in BALB/c, and the quantitatively increased HDL in BWF1 is qualitatively abnormal. BALB/c HDL are not subject to these changes.

Plasma levels of triglycerides were significantly increased by leptin plus high-fat diet but not by high-fat diet alone in BWF1 mice (data not shown).

At the histological level, the numbers of aortic lipid-containing lesions, i.e. early atherosclerotic lesions, were altered by both high-fat diet and high-fat diet plus leptin in the BWF1 mice, but not in the BALB/c controls (Figure 2, panel A). The numbers of lesions paralleled the increase in piHDL (Figure 1, panel D). Leptin added to high-fat diet produced significantly more lesions in BWF1 than the diet alone (Figure 2, panel A).

Finally, as shown in Figure 2 panel B, the addition of leptin to high-fat diet resulted in acceleration of nephritis in BWF1 mice. For these studies mice were followed for a total of 28 weeks, at which time unmanipulated mice have anti-DNA and a few (<10%) develop proteinuria. The differences in proteinuria were not associated with any differences in serum levels of anti-DNA (data not shown).

Discussion

BWF1 mice on high-fat diets developed dyslipidemia characterized by quantitative increases in HDL, dysfunction of that HDL, and atherosclerosis. The addition of leptin to the high-fat diet further increased piHDL and atherosclerosis. These phenomena were observed only in the lupus-prone strain; the BALB/c controls did not develop significant quantitative or qualitative changes in HDL, or atherosclerosis. BALB/c strains are relatively resistant to induction of atherosclerosis by high-fat diets; several murine strains are known to differ in that aspect and in the effects of diet on various lipid compartments, such as triglycerides, HDL and VLDL.⁸ Methodologies used here cannot determine what proportion of total HDL are normal versus dysfunctional in BALB/c vs BWF1 strains; the methods measure the 'average' function of a population of HDL containing particles of different sizes and functions, depending on their content of cholesterol, cholesterol esters, and proteins (such as PON and Apo1).

Intake of dietary lipid influences autoimmunity. A high-fat diet induces atherosclerosis in MRL/lpr and MRL/n mice.⁸ In C6/Ldlr^{-/-} mice, a short feeding period of high fat (3 days) induced changes in HDL that included addition of lipid peroxides (a feature of piHDL). Thus, in this strain genetically prone to severe atherosclerosis, altered HDL composition occurred quickly with a diet change.¹⁰ Fernandes and colleagues¹¹ showed that increasing omega-3 dietary lipids (fish oil) or restricting dietary calories (and therefore total fat intake) reduced serum levels of total cholesterol, HDL, LDL and triglycerides and delayed onset and severity of autoimmunity, including nephritis, in BWF1 mice. There are other models of atherosclerosis-prone or lupus-prone mice with knock-out of genes that develop both SLE

and atherosclerosis. These include B6.ApoE^{-/-}Fas^{-/-} and MRL/Fas^{-/-}ApoE^{-/-} mice,^{9,12} and chimeras between LDL-R^{-/-} and B6.Sle1.2.3 mice.¹³ The development of atherosclerosis in these models may depend heavily on the gene deficiencies rather than on altered function of HDL. We have reported that the piHDL found in half of SLE patients directly correlated with increased risk of subclinical atherosclerosis.² Here we show that high-fat diet in mice with multigenic predisposition to SLE induces both quantitative increase in and dysfunction of HDL; these changes are increased by leptin and associate with increased development of aortic atherosclerotic lesions. Leptin plays a role in promoting nephritis in other rodent models, including nephrotoxic nephritis from antibodies to glomerular basement membrane,¹⁴ and nephrosclerosis.¹⁵ The mechanisms by which leptin accelerates nephritis in the BWF1 lupus model reported here are under study.

This model may represent a new tool to better understand the link between SLE and atherosclerosis, and to test directly novel therapeutic modalities to prevent accelerated atherosclerosis in SLE.

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Figure 1.

Plasma levels of lipids in BALB/c and BWF1 mice treated with regular or high-fat diet, and in BWF1 mice who in addition to high-fat diets received leptin injections. Each group contains 8–11 mice. Data are shown as mean $\pm$ SD. Panel A: Total cholesterol levels increased significantly in both BALB/c and BWF1 mice on high-fat diets. BWF1 mice on high-fat diet plus leptin had elevated levels similar to those in the same strain on high-fat diet alone. * = $p < 0.01$ compared with BALB/c on regular diet; ** = $p < 0.001$ compared with BWF1 on regular diet, using ANOVA analysis with Tukey's comparison of columns. Panel B: Non-HDL cholesterol levels in each group. Levels are significantly higher in BALB/c mice, on either regular or high-fat diets, compared to BWF1 mice in any group. In BWF1 mice, levels did not increase significantly on high-fat diet with or without leptin. * = $p < 0.05$ compared with same treatment group of BWF1 mice. Panel C: Mean $\pm$ SD for total HDL levels show similar levels in BALB/c and BWF1 mice on regular chow. Significant

increases occurred in BW on high-fat diet with or without leptin, compared with BWF1 on chow. * $p < 0.001$. Differences between high-fat diet in BWF1 and high-fat diet plus leptin were not significant. Panel D: HDL functional scores in each group show significantly higher scores for BWF1 on high-fat or on high-fat diet plus leptin, compared with BWF1 on normal chow. Levels in BALB/c did not differ significantly between the chow and high-fat groups. * $p < 0.001$ for each of the treated BWF1 groups compared with each of the BALB/c groups. ** $p = 0.005$ comparing BWF1 high fat with high fat plus leptin, one-tailed paired t -test.

Figure 2.

Effects of treatments on disease expression. Panel A shows mean $\pm$ SD of lipid-staining aortic lesions per μM^2 for each group (four mice per group). There were few lesions in BALB/c mice on regular or high-fat diets, or in BWF1 mice on regular chow. However, there were significant increases in numbers of lesions in BWF1 on high-fat diets. The numbers were significantly higher in BWF1 mice on high fat plus leptin. * $p < 0.05$ compared with BALB/c and with BWF1 on regular chow. ** $p < 0.03$ comparing BWF1 mice on high fat with high fat plus leptin. Data by ANOVA for all groups and by one-tailed paired t -test for comparison of leptin plus high fat with high fat in BWF1. Panel B. Effect of diets on proteinuria in BWF1 mice. Here mice were studied for 28 weeks after introduction of diets. There were 10 mice in each group. By ANOVA, proteinuria occurred significantly earlier in mice on high-fat-diet-plus-leptin than in the other groups, $p < 0.05$.