Published in final edited form as:

Lupus. 2008 December ; 17(12): 1070–1078. doi:10.1177/0961203308093551.

Accelerated atherosclerosis is independent of feeding high fat diet in systemic lupus erythematosus–susceptible LDLr^{−/−} mice

NA Braun¹, NS Wade¹, EK Wakeland², and AS Major^{1,3}

¹Department of Pathology, Vanderbilt University Medical Center, Nashville, Tennessee, USA

²Department of Immunology, University of Texas Southwestern Medical Center, Dallas, Texas, USA

³Department of Medicine, Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Abstract

Individuals suffering from systemic lupus erythematosus (SLE) are predisposed to accelerate cardiovascular disease. Our laboratory has recently developed an animal model of SLE-accelerated atherosclerosis. We have shown that, following 8 weeks feeding high fat Western diet, radiation chimeras consisting of SLE-derived haematopoietic cells transferred to low-density lipoprotein (LDL)r^{−/−} mice (LDLr.Sle) have increased atherosclerosis compared with C57Bl/6 bone marrow recipients (LDLr.B6). However, this feeding regimen resulted in significant mortality in SLE-susceptible mice compared with controls with surviving animals having extremely elevated serum cholesterol (>500 mg/dL) and increased serum markers of kidney pathology. To test the hypothesis that SLE-associated autoimmune dysregulation can exacerbate atherosclerosis under more mild serum cholesterol conditions (approximately 200 mg/dL), we examined SLE and lesion development in radiation chimeras fed either a normal chow or high fat Western diet for 8 weeks. High fat fed LDLr.Sle mice exhibited increased mortality and were significantly more hypertensive. LDLr.Sle mice had greater titres of antibodies against dsDNA, oxLDL and phospholipid compared with controls. Lupus-susceptibility increased the atherosclerotic lesions and the percentage of CD4⁺ T cells in the lesions of proximal aortas, independent of diet. These data show that increased dyslipidemia resulting from high-fat feeding can exacerbate autoimmunity and associated vascular complications. Conversely, they also show that autoimmune dysregulation can accelerate atherosclerosis in LDLr-deficient animals independent of feeding high fat diet. Collectively this study provides additional evidence that the accelerated atherosclerosis observed in SLE is autoimmune associated.

Keywords

atherosclerosis; autoimmunity; high fat diet; lupus

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease characterized by the production of a wide range of autoantibodies. Clinical complications

Correspondence to: Amy S Major, PhD (Assistant Professor), Department of Medicine, Division of Cardiovascular Medicine, Vanderbilt University, Medical Center, 2220 Pierce Avenue, Room 383 PRB, Nashville, TN, 37232-6300, USA. Email: amy.major@vanderbilt.edu. Nicole A Braun and Nekeithia S Wade contributed equally to this article.

Reprints: <http://www.sagepub.com/journalsReprints.nav>

because of SLE usually result in end-organ disease such as glomerulonephritis, arthritis, vasculitis and various neurological disorders.¹ First recognized as a serious complication in lupus over 30 years ago, atherosclerosis has gained interest as a major cause of mortality in patients with lupus.¹⁻⁴ In fact, with all other risk factors being equal, including hypertension, hyperlipidemia, diabetes and obesity, the risk of coronary events in patients with SLE is approximately eight times greater when compared with non-SLE controls and approximately 30% of deaths in SLE are atherosclerosis related.^{3,4} Therefore, understanding how the presence of SLE exacerbates the atherosclerotic condition is essential to optimize risk reduction for cardiovascular disease (CVD) while treating the SLE-associated inflammation.

Atherosclerosis, like SLE, is a disease involving many cellular processes, and has classically been associated with hypercholesterolemia. A large body of evidence also supports inflammation and immunity in the pathogenesis of CVD. It is well known that macrophages and T cells are present in all stages of atherosclerotic lesions and promote inflammation by producing various cytokines, attracting smooth muscle cells and other lymphocytes and increasing plaque vulnerability.⁵ B-cell responses are also thought to be involved in the pathogenesis of atherosclerosis and for the most part are thought to be protective. Although the involvement of acquired immunity in atherosclerosis is strongly supported by these studies, mechanisms appear to be cell type dependent and multifaceted.

A recent study by our laboratory described the development of an animal model for accelerated atherosclerosis in the face of SLE. We made low-density lipoprotein (LDL)^r mice susceptible to SLE by transferring haematopoietic cells from the congenic B6.*Sle1.2.3* mouse strain. This unique animal model of human SLE was developed by placing three lupus-susceptibility gene intervals identified in NZM2410 mouse strain on the C57Bl/6 background.⁶ Using this approach, we showed that making LDL^r mice susceptible to lupus increased atherosclerosis in the aortic root and increased inflammatory cell accumulation in lesions. However, in general, patients with lupus do not suffer from the severe hypercholesterolemia observed in LDL^r mice fed a high fat diet (e.g., cholesterol levels >500 mg/dL). Therefore, the current study was conducted to show that exacerbation of atherosclerosis in lupus-susceptible mice occurs under conditions of more moderate dyslipidemia as that observed in LDL^r mice on a normal chow diet (total cholesterol of approximately 200 mg/dL) and that overt accumulation of atherogenic lipoproteins (i.e., VLDL and LDL) can enhance SLE disease.

Methods

Mice

All mice used in these studies have been backcrossed onto the C57Bl/6 background. C57Bl/6 and LDL^r-deficient mice were originally obtained from The Jackson Laboratory and are maintained in our colony. The lupus congenic B6.*Sle1.2.3* strain has been described and characterized extensively.⁶⁻¹³ The B6.*Sle1.2.3* mice are essentially 97% genetically homologous to the C57Bl/6 strain with the NZM2410-derived lupus susceptibility loci accounting for approximately 3% of the genome. All mice are maintained in microisolator cages and used according to the guidelines and the approval of the Vanderbilt University Institutional Animal Care and Use Committee.

Production of radiation chimeras

Transfer of the wild type or lupus-susceptible bone marrow has been previously described.¹⁴

Atherosclerosis studies

LDLr-deficient animals received either C57Bl/6 or B6.*Sle1.2.3* bone marrow. Sixteen weeks following transplantation, one half of the animals in each group were started on a high fat Western diet (21% milk fat and 0.15% cholesterol) for 8 weeks. The remaining mice were kept on chow diet for the same period of time. At the end of this time, animals were killed and analysed for the extent of atherosclerosis and the presence and severity of symptoms of SLE.

Immunohistochemistry

Staining for macrophages (Moma-2) and CD4⁺ T cells was performed as previously described.¹⁴ CD11c staining for dendritic cells was conducted using a rat anti-CD11c primary antibody (BD Biosciences, San Jose, CA USA) followed by incubation with Texas red-conjugated anti-rat IgG (Vector Labs, Burlingame, CA, USA). Cells were visualized by fluorescent microscopy and quantified by counting the number of positive cells in lesions.

ELISAs

Serum titres for antibodies against oxLDL and dsDNA were conducted as previously described.¹⁴ ELISAs for antibodies against β 2-glycoprotein I (β 2-GPI) was performed by coating a 96-well Maxisorb plate with 10 μ g/mL of purified β 2-GPI in PBS. Plates were blocked and mouse serum was added at a dilution between 1:1000 and 1:5000 and incubated overnight at 4 °C. Plates were washed with 0.5% Tween/PBS and incubated with HRP conjugated goat anti-mouse IgG (Promega, Madison, WI, USA) for 1 h at RT. Reactions were developed using the TMB substrate (BD Biosciences).

Serum lipoprotein analyses

Total serum cholesterol and triglyceride were measured in fasted mice using a colorimetric assay as previously described.¹⁴ Fast performance liquid chromatography (FPLC) was conducted by separating lipoproteins on a Superose 6 column (Amersham Promega, Piscataway, NJ, USA) followed by cholesterol measurement in each fraction as described.^{14,15}

Measurement of systolic blood pressure

Systolic blood pressure was measured using a tail cuff BP-2000 instrument (Visitech Systems, Apex, NC, USA) on conscious, preconditioned mice as described.¹⁴

Purification and activation of CD4⁺ T cells

CD4⁺ T cells from the spleens of C57Bl/6 and B6.*Sle1.2.3* congenic mice were isolated by positive selection using magnetic beads conjugated to anti-CD4 antibodies according to the manufacture's protocol (Miltenyi Biotec, Auburn, CA, USA). Cells were then stimulated with Phorbol myristate acid (PMA) and ionomycin (1 μ g/mL) for 2 h at 37 °C and 5% CO₂. Cells were then washed, stained with anti-CD40L antibody (BD Biosciences) and analysed by flow cytometry.

Statistical analyses

Statistical analyses were conducted using PRISM 5.0 software (GraphPad Software Inc., La Jolla, CA, USA). For data showing a normal distribution, significant differences were calculated using an ANOVA with a Bonferroni post-test. For data that require a nonparametric test, a Kruskal–Wallis test was conducted with a Dunns post-test.

Results

High fat Western diet increases dsDNA antibody titres and increases mortality in lupus-susceptible LDLr^{-/-} mice

Lethally irradiated LDLr^{-/-} mice received either C57Bl/6 or lupus-susceptible B6.*Sle1.2.3* congenic bone marrow. Recipient mice are hereafter referred to as LDLr.B6 and LDLr.*Sle*, respectively. Sixteen weeks following transplantation, mice were either fed a high fat Western diet for eight additional weeks or left on normal rodent chow. LDLr.*Sle* mice fed with high fat Western diet had a 37% mortality rate, whereas LDLr.*Sle* mice on a chow diet only showed a 10% mortality rate (Figure 1A). Control LDLr.B6 mice tolerated both diets with a 100% survival rate at the end of the 24-week study. At the time of killing, the LDLr.*Sle* mice had significantly higher titres of anti-double stranded DNA (anti-dsDNA) serum antibodies compared with LDLr.B6 controls (Figure 1B). This was evident independent of diet. In addition, the LDLr.*Sle* mice on a Western diet had significantly increased anti-dsDNA antibodies compared with chow fed LDLr.*Sle* mice. Body weight at the time of killing did not differ between the LDLr.B6 and LDLr.*Sle* mice in either diet group (Figure 1C). The data confirm that the SLE phenotype was transferred to the LDLr-deficient mice. In addition, they show that feeding lupus-susceptible mice a high fat Western diet increases mortality and disease severity as determined by dsDNA autoantibody titre.

Increased immunoglobulin deposition in kidneys correlated to the dsDNA antibody titre in that LDLr.B6 mice on chow diet had the least staining and LDLr.*Sle* mice on Western diet had the most staining (Figure 2A). Similarly, urine protein was the greatest in LDLr.*Sle* mice on Western diet (Figure 2B). Finally, systolic blood pressure in LDLr.*Sle* mice on a Western diet was significantly greater than LDLr.*Sle* mice on chow or LDLr.B6 mice on either Western or chow diet (Figure 2C). Collectively, these data suggest that renal function was decreased in LDLr.*Sle* mice on high fat diet.

Lupus-susceptibility increases atherosclerosis in LDLr-deficient mice in the absence of overt dyslipidemia

Our previous study showed that transfer of lupus susceptibility increases atherosclerosis in LDLr-deficient mice on a Western diet.¹⁴ However, feeding high fat diet in this animal model results in extreme elevations in circulating cholesterol; often greater than 1000 mg/dL. This level of cholesterol would be considered physiologically irrelevant to the human patient with lupus. We hypothesized that SLE could exacerbate atherosclerosis under more physiologic levels of serum lipoproteins (i.e., approximately 200 mg/dL). Measurement of atherosclerotic lesions in the proximal aorta showed that LDLr.*Sle* mice had increased atherosclerosis compared with LDLr.B6 controls independent of diet (Figure 3A). In fact, the increase in atherosclerosis in LDLr.*Sle* mice on chow compared with controls was actually greater (approximately 2.0-fold) than the difference between the mice-fed Western type diet (approximately 1.3-fold). Analysis of the cellular composition of plaques by immunohistochemistry showed similar macrophage (Moma-2) and dendritic cell (CD11c) content among all groups of mice (data not shown). However, CD4⁺ T-cell content was increased approximately threefold in the LDLr.*Sle* mice compared with LDLr.B6 animals (Figure 3B). This increase was independent of diet because both chow and Western diet fed animals showed similar percentages of CD4⁺ T cells in lesions. Stimulation with PMA and ionomycin showed an increase in CD40L expression in *Sle* primary CD4⁺ T cells compared with wild type C57Bl/6 mice (Figure 3C).

The increase in atherosclerosis in LDLr.*Sle* mice fed a chow diet occurred in the absence of changes in serum cholesterol or triglyceride levels (Table 1) or cholesterol-containing lipoprotein distribution as determined by FPLC (Figure 4, left panel). Mice fed a Western diet

showed increased serum cholesterol and triglycerides compared with chow fed mice (Table 1). In addition, as reported in our previous study,¹⁴ serum cholesterol and triglycerides were slightly, but significantly, decreased in LDLr.*Sle* mice. The difference in total cholesterol was associated with decreased non-high density lipoprotein (HDL) cholesterol (Figure 4, right panel). These data show that transfer of SLE to LDLr-deficient mice can increase atherosclerosis independent of large increases in total serum cholesterol or triglycerides associated with high fat diet feeding. In addition, they show that increased numbers of CD4⁺ T cells in the lesions of LDLr.*Sle* mice is also independent of severe hyperlipidemia.

Lupus susceptibility increases oxLDL and phospholipid antibodies in LDLr-deficient mice

To examine the effect of diet on production of immunoglobulin against modified LDL and phospholipid, we measured titres of oxLDL- and β 2-glycoprotein I (β 2-GPI)-specific antibodies in sera of control and SLE-susceptible LDLr-deficient mice. Table 2 contains the anti-oxLDL total antibody and IgG isotype titres from chow and Western diet fed mice. In general, the LDLr.*Sle* mice had higher total antibody, IgG₁ and IgG_{2a} titres in serum compared with LDLr.B6 mice. This increase in oxLDL-specific IgG was independent of diet feeding (Table 2). In addition, chow fed LDLr.*Sle* mice had significantly higher anti-oxLDL IgM levels compared with LDLr.B6 mice. The LDLr.*Sle* mice fed a Western diet had increased, but not significantly higher, levels of anti-oxLDL IgM.

To determine whether the antibody response to oxLDL was associated with a Th2 or a Th1 T helper phenotype, we calculated the IgG₁ (Th2) to IgG_{2a} (Th1) isotype ratio. Independent of diet, the LDLr.*Sle* mice had an increased IgG₁/IgG_{2a} ratio indicating that the immune response of these animals was skewed toward a Th2 phenotype. Additionally, the LDLr.B6 mice fed a Western diet appeared to have more of a Th1 type phenotype than LDLr.B6 mice on chow.

Antibodies to the phospholipid β 2-GPI are present in patients with SLE and the anti-phospholipid syndrome.^{16,17} These antibodies are thought to be associated with increased risk of CVD. Therefore, we determined the serum titre of β 2-GPI antibodies in our chow and Western diet fed animals. LDLr.*Sle* mice fed with chow diet had significantly higher total antibody, IgM and IgG₁ specific for β 2GPI compared with chow fed LDLr.B6 mice (Table 3). IgG_{2a} showed a trend toward increased levels in LDLr.*Sle* mice, but did not reach statistical significance. In mice fed with Western diet, β 2-GPI-specific IgG₁ and IgG_{2a} were significantly increased in LDLr.*Sle* mice compared with control LDLr.B6 mice. Analysis of the IgG₁/IgG_{2a} ratio showed similar trends as seen with the oxLDL antibody titres. In general, the LDLr.*Sle* mice had more of a Th2 phenotype compared with LDLr.B6 mice, independent of the diet fed. Additionally, LDLr.B6 mice fed a high fat diet appeared to have more of a Th1 phenotype. Collectively, these data indicate that there are quantitative and qualitative differences in the immune response to vascular disease-associated antigens between the LDLr.B6 and LDLr.*Sle* mice.

Discussion

Individuals suffering with SLE are at increased risk for developing accelerated forms of atherosclerosis and vascular disease. Because many patients with SLE do not fall into the traditional risk group for atherosclerosis, the underlying aetiology for its acceleration remains largely unknown. Until recently, lupus and atherosclerosis studies have been hampered by the lack of an appropriate animal model that simultaneously develops both diseases. However, recent studies by our laboratory¹⁴ and others^{18–20} have reported that transfer of the lupus phenotype to atherosclerosis-susceptible mouse strains (e.g., LDLr- or apoE-deficient mice) results in dysregulated immunity, chronic inflammation and increased atherosclerotic lesions. However, we also observed that when placed on the traditional atherosclerosis-inducing Western diet, the LDLr.*Sle* animals exhibited increased mortality compared with the LDLr.B6

controls with the first animals dying as early as 2 weeks following diet initiation. Mortality by the end of the 8-week feeding period was approximately 40%. Whether this increased death was due to renal failure, heart failure or both is currently unknown. However, in the surviving LDLr. *Sle* mice, urine protein was elevated indicating the presence of some degree of kidney disease.

In general, patients with lupus do not develop the severe dyslipidemia observed in LDLr-deficient mice fed with Western diet (cholesterol ≥ 600 mg/dL). In addition, increased serum cholesterol is not predictive of accelerated atherosclerosis in patients with lupus.^{21,22} Therefore, we decided to test the hypothesis that the transfer of lupus is sufficient to exacerbate atherosclerosis in the presence of more physiological levels of serum cholesterol (approximately 200 mg/dL). To test this hypothesis, we transferred lupus-susceptible or resistant bone marrow to LDLr-deficient mice and fed the mice either chow or high fat Western diet. In line with our previous study, we observed a 37% mortality rate at the end of 8 weeks in LDLr. *Sle* mice fed with Western diet compared with LDLr. *Sle* mice fed with chow diet or LDLr. B6 mice fed either diet (Figure 1A). In addition, LDLr. *Sle* mice fed a high fat diet had higher serum titres of dsDNA antibodies compared with LDLr. B6 mice on high fat and LDLr. *Sle* mice on chow diet. These data are in line with previous work reporting that lupus-susceptible NZB/W F1 mice fed a high fat diet develop increased anti-dsDNA and cardiolipin antibody titres,^{23,24} increased MHC class II expression on accessory cells,²⁵ increased cytokine production and more severe lupus nephritis.²⁶ However, the dietary effects in these studies were observed over a period of 2–9 months of feeding. In the current study, the LDLr. *Sle* mice were only fed diet for 2 weeks when the animals started dying. In two separate studies, Lin, et al.^{24,25} reported a decreased life span in the NZB/W F1 mice fed with high fat diet. The average life span of these animals was 285 days compared with the low fat fed controls, which lived for an average of 389 days. Because we were interested in measuring atherosclerosis, we did not allow the mice to proceed past 8 weeks of diet feeding. However, by 6 weeks of diet feeding, the LDLr. *Sle* mice on high fat diet had already suffered significant mortality compared with the other three groups indicating that the life span of these animals was greatly decreased. Because the LDLr. *Sle* mice fed a high fat diet had dsDNA titres even greater than the chow fed LDLr. *Sle* mice, we were not surprised to see that the immunoglobulin deposition, urine protein grade and systolic blood pressure were also elevated in these animals (Figure 2). Although most individuals with SLE do not exhibit total serum cholesterol levels greater than 200 mg/dL, the fact that the LDLr. *Sle* mice fed high fat diet have even greater disease symptoms compared with the LDLr. *Sle* mice on chow diet suggests that severe dyslipidemia can further exacerbate lupus disease in these animals. Certainly, these data are consistent with the clinical evidence showing that elevated serum total cholesterol in patients with SLE is associated with increased kidney pathology and death.²⁷

Examination of atherosclerosis in the proximal aorta of the mice showed that increased atherosclerosis in LDLr. *Sle* mice compared with control LDLr. B6 controls was independent of high fat diet feeding (Figure 3). In fact, the LDLr. *Sle* mice fed with chow diet showed an even greater increase over controls compared with their high fat fed counterparts. Additionally, the average serum total cholesterol and triglyceride levels did not differ between the LDLr. B6 and LDLr. *Sle* mice on chow diet and the serum lipoprotein distribution of cholesterol was similar (Figure 4). However, the LDLr. *Sle* mice fed a high fat diet had decreased serum cholesterol and triglyceride with decreased non-HDL cholesterol. The accumulation of CD4⁺ T cells in the atherosclerotic lesions of LDLr. *Sle* was similarly not dependent of high fat diet feeding and CD4⁺ T cells isolated from chow fed B6. *Sle* triple congenic mice show increased CD40L expression upon stimulation with PMA and ionomycin compared with C57Bl/6 controls. Collectively, these data suggest that the autoimmune dysregulation and perhaps chronic inflammation have a greater influence on atherosclerosis than serum cholesterol; a more traditional risk factor for atherosclerosis. More importantly, the data show that non-

physiologically high serum cholesterol levels are not necessary to exacerbate atherosclerosis in the face of lupus.

Although the lipoprotein distribution of cholesterol did not differ between LDLr-deficient mice receiving either normal or lupus-susceptible bone marrow, we cannot exclude the possibility that the LDLr.*Sle* mice harbour dysfunctional HDL. HDL functions not only in reverse cholesterol transport but also acts to prevent oxidation of LDL.²⁸ When inflammation becomes chronic, HDL appears to lose its capacity to prevent the formation of oxLDL. In SLE, it has been shown that HDL function is abnormal in 45% of patients compared with only 4% of control.²⁹ Therefore, in the lupus mice, it will be interesting to determine whether we see similar decreases in HDL's anti-oxidative abilities compared with normal control animals.

Patients with lupus are known to develop autoantibodies to many atherosclerosis-associated antigens, such as oxLDL, β 2-glycoprotein I, cardiolipin³⁰ and antibodies to atheroprotective proteins such as the HDL-associated apolipoprotein AI.³¹ A recent study by Svenungsson, *et al.*³² reported a strong correlation between plasma concentrations of oxLDL and anti-oxLDL antibodies in SLE patients with coronary heart disease (CHD) complications. The authors suggest using atherosclerosis-associated antibodies as a screen for identifying SLE patients with increased risk for the development of atherosclerosis. However, whether the autoantibody production in these studies was the cause of enhanced atherosclerosis or a secondary effect of other immune responses remains to be determined. In the current study, we observed an increase in the anti-oxLDL and β 2-GPI serum IgG levels in LDLr.*Sle* mice compared with control LDLr.B6 animals. This increase appeared to be diet independent as the LDLr.*Sle* mice fed a Western diet did not show increased levels of antibodies compared with LDLr.*Sle* mice fed with chow diet. Interestingly, the LDLr.*Sle* mice show an IgG₁ bias for both anti-oxLDL and anti- β 2-GPI antibodies compared with LDLr.B6 mice independent of diet. This suggests that high fat feeding in LDLr.*Sle* mice skews the immune response toward a Th2-like phenotype. It has been shown in apoE-deficient mice that atherosclerotic lesion progression is accompanied by an increase in the oxLDL IgG₁ serum titres³³ and a bias toward Th2 immune responses. Therefore, these data support the hypothesis that the atherosclerotic disease in the LDLr.*Sle* mice is more advanced than those seen in the LDLr.B6 controls.

Although titres of oxLDL antibodies are shown to correlate directly with severity of disease and are often used as markers of CHD risk,^{34–37} their role in the initiation and/or progression of atherosclerosis is not yet conclusive. In addition, it has been shown that in patients with SLE, there is extensive antibody cross-reactivity between antiphospholipid (e.g., β 2-GPI) and oxLDL. Therefore, in the current study, it cannot be concluded that the oxLDL antibodies are unique from the β 2-GPI antibodies. However, a recent report by Kobayashi, *et al.*³⁸ suggested that anti- β 2-GPI IgG significantly increases oxLDL/ β 2-GPI complex binding to macrophages and uptake via Fc γ receptors and, thus, may contribute to the atherosclerotic process. Therefore, one may hypothesize that the increase in β 2-GPI antibody titres seen in the LDLr.*Sle* mice accelerates uptake of modified atherogenic lipoproteins such as oxLDL/ β 2GPI complexes and potentiates foam cell formation in these animals. Interestingly, although increased anti- β 2-GPI antibodies are associated with increased risk of atherosclerosis, one study in mice showed that β 2-GPI reactive T cells, and not antibodies, may be pathogenic.³⁹ The authors reported that adoptive transfer of whole splenocytes, but not T-depleted splenocytes, from mice immunized with β 2-GPI increased atherosclerosis in LDLr-deficient animals in the absence of detectable antigen-specific antibody. These data would argue that antibodies against β 2-GPI are a useful marker for cardiovascular risk but are not pathological. However, the current studies do not directly address either of these possibilities and ongoing studies in our laboratory are aimed at examining these hypotheses.

In conclusion, in our current study, we have reported that severe dyslipidemia, as that seen in LDLr-deficient mice fed with Western diet, can exacerbate the lupus phenotype and accelerate mortality beyond that previously reported in the NZB/W F1 mice fed with high fat diets.²⁵ This suggests that perhaps the lipoprotein profile (i.e., increased VLDL and LDL cholesterol) can adversely affect the lupus disease process. Additionally, we also provide strong evidence that the accelerated atherosclerosis observed in LDLr.*Sle* mice is more closely associated with immune hyperactivity and not a secondary effect of autoimmune-mediated renal pathology associated with SLE. Planned future studies will allow us to delineate which specific immune cell type and function facilitates accelerated atherogenesis, ultimately leading to the identification of novel therapeutics that target both lupus and CVD.

Acknowledgements

We thank Adam C.Morgan for expert technical assistance. This work was supported by a grant from the Lupus Research Institute, New York, NY, the National Institutes of Health (NIH) BIRCWH Grant 5 K12 HD043483-04 (to A.S.M.), the NIH NRSA 5 T32HL07751 (year 15) Training Grant in Mechanisms of Vascular Disease (to N.A.B.) and the NIH ST32 HL007411-29 Training Grant in Cardiovascular Mechanisms: Training in Investigation (to N.S.W.).

References

1. Ward MM, Pyun E, Studenski S. Causes of death in systemic lupus erythematosus. Long-term followup of an inception cohort. *Arthritis Rheum* 1995;8:1492–1499. [PubMed: 7575699]
2. Aranow C, Ginzle EM. Epidemiology of cardiovascular disease in systemic lupus erythematosus. *Lupus* 2000;9:166–169. [PubMed: 10805482]
3. Lockshin MD, Salmon JE, Roman MJ. Atherosclerosis and lupus: a work in progress. *Arthritis Rheum* 2001;44:2215–2217. [PubMed: 11665959]
4. Chiu YH, Jayawardena J, Weiss A, et al. Distinct subsets of CD1d-restricted T cells recognize self-antigens loaded in different cellular compartments. *J Exp Med* 1999;189:103–110. [PubMed: 9874567]
5. Glass C, Witzaum J. Atherosclerosis. the road ahead. *Cell* 2001;104:503–516. [PubMed: 11239408]
6. Morel L, Rudofsky UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1994;1:219–229. [PubMed: 7889410]
7. Mohan C, Morel L, Yang P, Wakeland EK. Genetic dissection of systemic lupus erythematosus pathogenesis: Sle2 on murine chromosome 4 leads to B cell hyperactivity. *J Immunol* 1997;159:454–465. [PubMed: 9200486]
8. Mohan C, Alas E, Morel L, Yang P, Wakeland EK. Genetic dissection of SLE pathogenesis. Sle1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J Clin Invest* 1998;101:1362–1372. [PubMed: 9502778]
9. Mohan C, Yu Y, Morel L, Yang P, Wakeland EK. Genetic dissection of Sle pathogenesis: Sle3 on murine chromosome 7 impacts T cell activation, differentiation, and cell death. *J Immunol* 1999;162:6492–6502. [PubMed: 10352264]
10. Morel L, Mohan C, Yu Y, et al. Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J Immunol* 1997;158:6019–6028. [PubMed: 9190957]
11. Morel L, Yu Y, Blenman KR, Caldwell RA, Wakeland EK. Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain. *Mamm Genome* 1996;7:335–339. [PubMed: 8661718]
12. Sobel ES, Mohan C, Morel L, Schiffenbauer J, Wakeland EK. Genetic dissection of SLE pathogenesis: adoptive transfer of Sle1 mediates the loss of tolerance by bone marrow-derived B cells. *J Immunol* 1999;162:2415–2421. [PubMed: 9973523]
13. Sobel ES, Morel L, Baert R, Mohan C, Schiffenbauer J, Wakeland EK. Genetic dissection of systemic lupus erythematosus pathogenesis: evidence for functional expression of Sle3/5 by non-T cells. *J Immunol* 2002;169:4025–4032. [PubMed: 12244205]

14. Stanic AK, Stein CM, Morgan AC, et al. Immune dysregulation accelerates atherosclerosis and modulates plaque composition in systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 2006;103:7018–7023. [PubMed: 16636270]
15. Major AS, Fazio S, Linton MF. B-lymphocyte deficiency increases atherosclerosis in LDL receptor-null mice. *Arterioscler Thromb Vasc Biol* 2002;22:1892–1898. [PubMed: 12426221]
16. Delgado Alves J, Ames PR, Donohue S, et al. Antibodies to high-density lipoprotein and beta2-glycoprotein I are inversely correlated with paraoxonase activity in systemic lupus erythematosus and primary antiphospholipid syndrome. *Arthritis Rheum* 2002;46:2686–2694. [PubMed: 12384928]
17. Delgado Alves J, Kumar S, Isenberg DA. Cross-reactivity between anti-cardiolipin, anti-high-density lipoprotein and anti-apolipoprotein A-I IgG antibodies in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. *Rheumatology (Oxford)* 2003;42:893–899. [PubMed: 12730551]
18. Aprahamian T, Bonegio R, Rizzo J, et al. Simvastatin treatment ameliorates autoimmune disease associated with accelerated atherosclerosis in a murine lupus model. *J Immunol* 2006;177:3028–3034. [PubMed: 16920939]
19. Gautier EL, Huby T, Ouzilleau B, et al. Enhanced immune system activation and arterial inflammation accelerates atherosclerosis in lupus-prone mice. *Arterioscler Thromb Vasc Biol* 2007;27:1625–1631. [PubMed: 17446440]
20. Aprahamian T, Rifkin I, Bonegio R, et al. Impaired clearance of apoptotic cells promotes synergy between atherogenesis and autoimmune disease. *J Exp Med* 2004;199:1121–1131. [PubMed: 15096538]
21. Roman MJ, Shanker BA, Davis A, et al. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N Engl J Med* 2003;349:2399–2406.
22. Roman MJ, Salmon JE, Sobel R, et al. Prevalence and relation to risk factors of carotid atherosclerosis and left ventricular hypertrophy in systemic lupus erythematosus and antiphospholipid antibody syndrome. *Am J Cardiol* 2001;87:663–666–A611. [PubMed: 11230862]
23. Morrow WJ, Ohashi Y, Hall J, et al. Dietary fat and immune function. I. Antibody responses, lymphocyte and accessory cell function in (NZB × NZW)F1 mice. *J Immunol* 1985;135:3857–3863. [PubMed: 2415589]
24. Lin BF, Jeng SJ, Chiang BL, Huang CC. Dietary fat affects lipids and anti-cardiolipin antibody levels in autoimmune-prone NZB/WF1 mice. *Br J Nutr* 1997;77:657–669. [PubMed: 9155512]
25. Lin BF, Huang CH, Chiang BL, Jeng SJ. Dietary fat influences Ia antigen expression, cytokines and prostaglandin E2 production of immune cells in autoimmune-prone NZB × NZW F1 mice. *Br J Nutr* 1996;75:711–722. [PubMed: 8695598]
26. Yumura W, Hattori S, Morrow WJ, Mayes DC, Levy JA, Shirai T. Dietary fat and immune function. II. Effects on immune complex nephritis in (NZB × NZW)F1 mice. *J Immunol* 1985;135:3864–3868. [PubMed: 2933453]
27. Tisseverasinghe A, Lim S, Greenwood C, Urowitz M, Gladman D, Fortin PR. Association between serum total cholesterol level and renal outcome in systemic lupus erythematosus. *Arthritis Rheum* 2006;54:2211–2219. [PubMed: 16802357]
28. McMahon M, Hahn BH. Atherosclerosis and systemic lupus erythematosus: mechanistic basis of the association. *Curr Opin Immunol* 2007;19:633–639. [PubMed: 18083018]
29. McMahon M, Grossman J, FitzGerald J, et al. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum* 2006;54:2541–2549. [PubMed: 16868975]
30. Thiagarajan P. Atherosclerosis, autoimmunity, and systemic lupus erythematosus. *Circulation* 2001;104:1876–1877. [PubMed: 11602485]
31. Vuilleumier N, Reber G, James R, et al. Presence of autoantibodies to apolipoprotein A-1 in patients with acute coronary syndrome further links autoimmunity to cardiovascular disease. *J Autoimmun* 2004;23:353–360. [PubMed: 15571929]
32. Svenungsson E, Jensen-Ustad K, Heimburger M, et al. Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation* 2001;104:1887–1893. [PubMed: 11602489]

33. Zhou X, Paulsson G, Stemme S, Hansson GK. Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apoE-knockout mice. *J Clin Invest* 1998;101:1717–1725. [PubMed: 9541503]
34. de Geest B, Collen D. Antibodies against oxidized LDL for non-invasive diagnosis of atherosclerotic vascular disease. *Eur Heart J* 2001;22:1517–1518. [PubMed: 11492979]
35. Wu JT, Wu LL. Autoantibodies against oxidized LDL. A potential marker for atherosclerosis. *Clin Lab Med* 1997;17:595–604. [PubMed: 9316776]
36. Steinerova A, Racek J, Stozicky F, Zima T, Fialova L, Lapin A. Antibodies against oxidized LDL—theory and clinical use. *Physiol Res* 2001;50:131–141. [PubMed: 11522041]
37. Paiker JE, Raal FJ, von Arb M. Auto-antibodies against oxidized LDL as a marker of coronary artery disease in patients with familial hypercholesterolaemia. *Ann Clin Biochem* 2000;37:174–178. [PubMed: 10735360]
38. Kobayashi K, Tada K, Itabe H, et al. Distinguished effects of anti-phospholipid antibodies and anti-oxidized LDL antibodies on oxidized LDL uptake by macrophages. *Lupus* 2007;16:929–938. [PubMed: 18042586]
39. George J, Harats D, Gilburd B, et al. Adoptive transfer of beta(2)-glycoprotein I-reactive lymphocytes enhances early atherosclerosis in LDL receptor-deficient mice. *Circulation* 2000;102:1822–1827. [PubMed: 11023938]

Figure 1. Severe dyslipidemia increases mortality and serum titres of dsDNA antibodies

(A) Percentage of mice (total of 9–13 mice per group) surviving following feeding chow diet (squares) or Western diet (circles) for 8 weeks. (B) Serum titres for anti-dsDNA antibodies in LDLr.B6 (solid symbols) and LDLr.Sle (open symbols) mice fed a chow or Western diet. (C) Body weights for LDLr.B6 (solid symbols) and LDLr.Sle (open symbols) mice fed a chow or Western diet. * $P < 0.05$ as determined by one-way ANOVA. Data represent two identical experiments.

Figure 2. Immune complex deposition, urine protein and blood pressure are increased in LDLr.*Sle* mice fed high fat diet

(A) LDLr.*Sle* mice fed high fat diet exhibit increased immune complex deposition in glomeruli as detected by immunohistochemistry. Shown is one representative mouse per group. Kidney sections from a total of five mice per group were analysed with similar results. (B) Urine protein grade in mice (3–7 mice per group) was determined by Chemstix at the time of killing.

LDLr.*Sle* mice (open bars) have increase urine protein compared with LDLr.B6 controls (closed bars). (C) LDLr.*Sle* mice (open circles) on Western diet have increased systolic blood pressure compared with LDLr.*Sle* mice on chow diet (open squares) and LDLr.B6 mice fed chow (closed squares) or Western diet (closed circles). * $P < 0.05$ as determined by one-way ANOVA. Data represent two identical experiments.

Figure 3. Transfer of lupus-susceptibility to LDLr-deficient mice increases atherosclerosis independent of diet

(A) Average lesion area as determined by oil-red-O staining in LDLr.B6 (closed bars) and LDLr.Sle (open bars) mice. Lesion quantitation was performed on 9–11 mice in each group. Shown is data from two identical experiments. (B) Detection of CD4⁺ T cells in lesions of LDLr.B6 (closed bars) and LDLr.Sle (open bars) on chow or Western diet. Positive cells are expressed as a percent of all lesion cells as determined by DAPI staining. Four sections from 5–10 mice per group were analysed. (C) Primary CD4⁺ T cells were isolated from C57Bl/6 (B6) and B6.Sle1.2.3 (Sle) mice and stimulated for 2 h with PMA and ionomycin. Shown is one of three experiments with similar results.

* $P < 0.05$ as determined by Kruskal–Wallis test.

Figure 4. FPLC analyses of serum cholesterol lipoprotein distribution

Lipoproteins were separated by size-exclusion chromatography and assayed for cholesterol as described in 'Methods'. Serum from LDLr.B6 (closed circles) and LDLr.S/e (open circles) was pooled before undergoing separation and analysis (9–11 mice per group). Similar profiles were obtained from individual mice.

Table 1

Serum cholesterol and triglyceride

Group	N	Cholesterol mg/dL (±SEM)	Triglyceride mg/dL (±SEM)
Chow diet			
LDLr.B6	11	230.4 (11.3)	76.6 (13.0)
LDLr. <i>Sle</i>	12	239.4 (10.7)	86.2 (12.5)
Western diet			
LDLr.B6	10	971.4 (57.4)	311.6 (10.6)
LDLr. <i>Sle</i>	10	767.1 (73.3) ^a	201.8 (26.6) ^a

^a $P < 0.05$ compared with LDLr.B6 mice on Western diet.

Table 2
Serum titres of oxLDL-specific antibodies

Group	Total Ig ($\pm$ SEM) ^a	IgM ($\pm$ SEM)	IgG ₁ ($\pm$ SEM)	IgG _{2a} ($\pm$ SEM)	IgG ₁ /IgG _{2a} ($\pm$ SEM)
Chow					
LDLr.B6	0.402 (0.104)	0.770 (0.114)	0.302 (0.093)	0.431 (0.097)	0.661 (0.020)
LDLr.Sle	0.900 (0.128) ^b	1.267 (0.102) ^b	0.956 (0.095) ^b	0.743 (0.069) ^b	1.307 (0.101) ^b
Western					
LDLr.B6	0.398 (0.108)	1.01 (0.180)	0.236 (0.090)	0.527 (0.131)	0.399 (0.059) ^c
LDLr.Sle	0.839 (0.090) ^b	1.335 (0.069)	1.058 (0.024) ^b	0.944 (0.006) ^b	1.119 (0.022) ^b

^a Average OD_{450nm} $\pm$ SEM.

^b $P < 0.05$ compared with LDLr.B6 mice on the same diet as determined by one-way analysis of variance.

^c $P < 0.05$ compared with LDLr.B6 mice on chow diet as determined by one-way analysis of variance.

Table 3
Serum titres of β 2-GPI-specific antibodies

Group	Total ($\pm$ SEM) ^a	IgM ($\pm$ SEM)	IgG ₁ ($\pm$ SEM)	IgG _{2a} ($\pm$ SEM)	IgG ₁ /IgG _{2a} ($\pm$ SEM)
Chow					
LDLr.B6	0.149 (0.033)	0.120 (0.030)	0.128 (0.061)	0.158 (0.065)	0.981 (0.187)
LDLr.Sle	0.690 (0.180) ^b	0.543 (0.172) ^b	0.588 (0.115) ^b	0.370 (0.122)	2.040 (0.304) ^b
Western					
LDLr.B6	0.467 (0.186) ^c	0.253 (0.101)	0.069 (0.024)	0.179 (0.063)	0.474 (0.084)
LDLr.Sle	0.514 (0.140)	0.402 (0.089)	0.722 (0.051) ^b	0.635 (0.093) ^b	1.270 (0.219) ^b

^a Average OD450nm $\pm$ SEM.

^b $P < 0.05$ compared with LDLr.B6 mice on the same diet as determined by one-way analysis of variance.

^c $P < 0.05$ compared with LDLr.B6 mice on chow diet as determined by one-way analysis of variance.