Autoimmune-mediated glucose intolerance in a mouse model of systemic lupus erythematosus

Curtis L. Gabriel,1 Patricia B. Smith,2 Yanice V. Mendez-Fernandez,2 Ashley J. Wilhelm,2 Audrey Musi Ye,2 and Amy S. Major1,2

1Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee; and 2Department of Medicine/Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, Tennessee

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Autoimmune-mediated glucose intolerance in a mouse model of systemic lupus erythematosus. Am J Physiol Endocrinol Metab 303: E1313–E1324, 2012. First published October 2, 2012; doi:10.1152/ajpendo.00665.2011.—Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies against self-antigens such as double-stranded DNA and phospholipids. Classical comorbidities of SLE include glomerulonephritis, infection, cardiovascular disease, arthritis, skin disorders, and neurological disease. In addition to these classical comorbidities, there is emerging evidence that SLE patients are at higher risk of developing insulin resistance and other components of the metabolic syndrome. Visceral adipose tissue inflammation is a central mediator of insulin resistance in the obese setting, but the mechanism behind the pathogenesis of metabolic disease in the SLE patient population is unclear. We hypothesize that lupus-associated changes in the adaptive immune system are associated with disruption in glucose homeostasis in the context of SLE. To test this hypothesis, we assessed the metabolic and immunological phenotype of SLE-prone B6.SLE mice. B6.SLE mice fed a low-fat diet had significantly worsened glucose tolerance, increased adipose tissue insulin resistance, increased b-cell insulin secretion, and increased adipocyte size compared with their respective B6 controls. Independently of diet, B cells isolated from the white adipose tissue of B6.SLE mice were skewed toward IgG production, and the level of IgG1 was elevated in the serum of SLE-prone mice. These data show that B6.SLE mice develop defects in glucose homeostasis even when fed a low-fat diet and suggest that B cells may play a role in this metabolic dysfunction.

insulin resistance; glucose intolerance; autoimmunity; lupus; autoantibodies

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) is a chronic autoimmune disease in which pathogenic autoantibodies disrupt the functions of various organs and organ systems. Causes of SLE mortality can be clustered into two main groups: early mortality and late mortality (6, 12, 40). The early-mortality group includes glomerulonephritis, infection, and neurological disease, whereas the late-mortality group is mainly composed of cardiovascular disease (CVD) (17, 30). Management of SLE complications has improved over the last several decades, causing a shift in the mortality burden from early causes to late causes. As this burden shifts, it is important to understand the factors that contribute to the development of CVD in the context of SLE. Various studies have shown a higher prevalence of traditional CVD risk factors such as hyperlipidemia or hypertension and nontraditional, SLE-specific risk factors such as autoantibody production or corticosteroid therapy (6). One traditional risk factor, metabolic syndrome, has higher prevalence in the SLE population (8, 11, 22, 33) and may contribute to the increased CVD burden in these patients.

The metabolic syndrome consists of several interrelated disorders, including the “deadly quartet” of truncal obesity, insulin resistance (IR), hypertension, and dyslipidemia (10, 18). IR, a key component of type 2 diabetes mellitus (T2DM), is thought to be caused by chronic inflammatory processes involving complex interactions between cells of the metabolic and immune systems. Several studies have yielded crucial information implicating visceral adipose tissue as a major focus of the chronic inflammation that can contribute to IR and T2DM. Examination of fat tissue has revealed an accumulation of adipose tissue macrophages in obese mice (43), and blocking the accumulation of these cells attenuates IR in these animals (42). More recent studies have implicated the adaptive immune system in the pathogenesis of IR. CD8 T cells exacerbate whereas T regulatory cells ameliorate IR in the context of obesity (13, 27, 45), and B cells have been shown to exacerbate IR through the production of pathogenic immunoglobulins presumably against adipose tissue antigens (44).

There are limited data regarding metabolic syndrome in mouse models of SLE. In the only other study of metabolic syndrome in an SLE model that we are aware of, Ryan et al. (32) have shown that the lupus-prone (NZB/W)F1 strain has increased adiposity, increased macrophage infiltration into adipose tissue, and increased glucose intolerance, but the contributions of the adaptive immune system to this metabolic phenotype was not examined. Therefore, the role of lymphocytes in the pathogenesis of IR in the context of SLE is not known and merits further study. Furthermore, there are no reports on metabolic syndrome in other mouse models of SLE.

We examined the pathogenesis of metabolic syndrome in the B6.SLE mouse model of SLE. The B6.SLE congenic strain is derived from the NZM2410 [(NZB/W)F1 × NZW] model of SLE, which develops severe lupus and nephritis in both sexes (23). Through linkage analyses, Morel et al. (23) identified three major genomic intervals linked to lupus susceptibility in the strain. Using these three chromosomal intervals, termed Sle1, Sle2, and Sle3, the investigators made triple congenic mice on the C57Bl/6 background (24, 25). The B6.SLE strain (originally named B6.Sle123) displays a fully penetrant lupus phenotype similar to that of humans. In the current study, we present evidence that adipose tissue IR and disrupted glucose homeostasis in B6.SLE may be mediated by pathogenic B cells.

Address for reprint requests and other correspondence: A. S. Major, Dept. of Medicine/Div. of Cardiovascular Medicine, Rm. 352, Preston Research Bldg., Vanderbilt Univ. Medical Center, Nashville, TN 37232 (e-mail: amy.major@vanderbilt.edu).

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MATERIALS AND METHODS

Mice. B6.Sle.I, 2,3 mice, hereafter referred to as B6.SLE mice, were a generous gift of Edward Waldman (UTSW, Dallas, TX) and have been previously described (23). C57BL/6 mice (B6) were originally purchased from Jackson Labs (Bar Harbor, ME) and were bred in the animal facility at Vanderbilt University Medical Center. All procedures involving animal subjects received prior approval from the Vanderbilt University Institutional Animal Care and Use Committee.

Diet. B6 and B6.SLE mice were cohoused after weaning and fed a low-fat diet (LFD, 10% kcal from fat, D12450B; Research Diets, New Brunswick, NJ) or high-fat diet (HFD, 45% kcal from fat, D12451; Research Diets) ad libitum.

Glucose tolerance tests. Study mice were fasted for 5–6 h on paper bedding before their tails were nicked under isoflurane anesthesia. Mice were rested for 20 min and then injected intraperitoneally with 1.25 g/kg lean body mass glucose. Blood glucose was measured at baseline and at 15, 30, 45, 60, 90, and 120 min after injection using a One Touch Ultra glucometer.

Anti-dsDNA antibody ELISA. Serum titers of anti-dsDNA antibodies were measured using the protocol described by Shivakumar et al. (35). Nunc MaxiSorp plates were blocked with mBSA in 1× PBS (0.1 mg/ml) at 37°C for 30 min. The plate was then washed with PBS and coated with 50 μg/ml dsDNA in PBS at 37°C for 30 min. The plate was washed twice with PBS and blocked overnight at 4°C with blocking buffer (3% BSA, 3 mM EDTA, and 0.1% gelatin in PBS). Plates were washed twice with PBS. Serum was diluted 1:1,000 in serum diluent (2% BSA, 3 mM EDTA, 0.05% Tween 20 in PBS) and added to the plate and incubated 2 h at room temperature (RT) on an orbital shaker. The plate was washed twice in PBS-Tween and twice in PBS. IgG-HRP (Promega, Madison, WI) was diluted 1:50,000 in secondary diluent (1% BSA and 0.05% Tween in PBS), added to the plate and incubated overnight at 4°C on an orbital shaker. The plate was washed twice with PBS-Tween and twice with PBS, and OptEIA TMB Substrate (BD Biosciences, San Diego, CA) was added to plate and allowed to incubate. Reaction was quenched with 1 M phosphoric acid, and the plate was promptly read at 450 nm.

Islet perfusion assay. Pancreatic islets were isolated and assessed as previously described (5, 41, 46). Briefly, the pancreas was digested in a solution of collagenase P in Hank’s balanced saline solution (HBSS) using a wrist-action shaker. Digestion was quenched with ice-cold 10% FCS-HBSS. The digest was washed, and islets were hand-picked under microscopic guidance. Islet insulin secretion was determined by 10.2±0.3 mg/ml at 37°C for 30 min. The plate was then washed with PBS and coated with 50 μg/ml dsDNA in PBS at 37°C for 30 min. The plate was washed twice with PBS and blocked overnight at 4°C with blocking buffer (3% BSA, 3 mM EDTA, and 0.1% gelatin in PBS). Plates were washed twice with PBS. Serum was diluted 1:1,000 in serum diluent (2% BSA, 3 mM EDTA, 0.05% Tween 20 in PBS) and added to the plate and incubated 2 h at room temperature (RT) on an orbital shaker. The plate was washed twice in PBS-Tween and twice in PBS. IgG-HRP (Promega, Madison, WI) was diluted 1:5,000 in secondary diluent (1% BSA and 0.05% Tween in PBS), added to the plate and incubated overnight at 4°C on an orbital shaker. The plate was washed twice with PBS-Tween and twice with PBS, and OptEIA TMB Substrate (BD Biosciences, San Diego, CA) was added to plate and allowed to incubate. Reaction was quenched with 1 M phosphoric acid, and the plate was promptly read at 450 nm.

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Immunoblotting. Mice were fasted on paper bedding for 4 h and then injected with 1 U/kg body wt insulin. Fifteen minutes later, mice were euthanized, and liver, soleus, gastrocnemius, and perigonadal fat pads were removed and freeze-clamped in liquid nitrogen. Fat was homogenized in a solution of 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 1 mM DTT, 1 mM PMSF, 5 mg/ml protease inhibitor, 50 mM NaF, and 5 mM sodium pyrophosphate and centrifuged for 10 min at 13,000 rpm at 4°C. The lysate was run on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. The membrane was probed with Akt primary rabbit antibody (1:1,000; Cell Signaling, Danvers, MA) or p-Akt primary rabbit antibody (1:1,000; Cell Signaling) and incubated overnight at 4°C. The membrane was then incubated for 1 h at RT with anti-β-actin antibody (1:1,000; Sigma Aldrich) followed by incubation with a goat anti-rabbit 800LT-conjugated secondary antibody (1:5,000; Li-Cor, Lincoln, NE) and goat anti-mouse 680LT-conjugated secondary antibody (1:20,000) and for 1 h at RT. Gels were scanned using an Odyssey Infrared Imaging System (Li-Cor), and bands were quantified using Odyssey version 3.0 software (Li-Cor).

Serum cholesterol, serum triglycerides, and liver triglycerides. Mice were fasted on paper bedding for 4 h, and blood was collected via the retroorbital sinus under isoflurane anesthesia. Blood was allowed to clot for 20 min and was subsequently spun at 13,000 rpm for 20 min at 4°C. Serum was collected, protease inhibitor cocktail was added, and the samples were stored at −80°C. Serum was thawed and diluted 1:100 with 3H2O, and triglyceride and cholesterol were measured using a cholesterol and triglyceride enzymatic assay according to the manufacturer’s protocol (Cliniqa, San Marcos, CA).

Lipids were extracted from the liver by the method of Folch-Lees (14). Individual lipid classes were separated by thin-layer chromatography and visualized by rhodamine 6G. Triglycerides were scraped from the plates and methylated using BF3-methanol as described by Morrison and Smith (26). The methylated fatty acids were extracted and analyzed by gas chromatography. Gas chromatographic analyses were carried out on an Agilent 7890A gas chromatograph equipped with flame ionization detectors and a capillary column (Supelco, Bellefonte, PA). Helium was used as a carrier gas, and fatty acids were identified by comparing the retention times to those of known standards.

Adipocyte size measurement. Perigonadal fat pads were collected and fixed in PBS containing 10% formalin. Fat was embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Adi-
Adiposity was not significantly different between LFD-fed and the samples were stored at 4°C. Serum was collected, protease inhibitor cocktail was added, and clot for 20 min and was subsequently spun at 13,000 rpm for 20 min retroorbital sinus under isoflurane anesthesia. Blood was allowed to clot for 20 min and was subsequently spun at 13,000 rpm for 20 min.

RESULTS

B6.SLE mice are prone to autoimmunity. Increased production of pathogenic anti-dsDNA antibodies and splenomegaly are hallmarks of autoimmune disease in B6.SLE mice. Five-week-old male B6 and B6.SLE mice were placed on LFD or HFD. After 20 wk of diet feeding, both anti-dsDNA antibody titers (Fig. 1A) and spleen weight (Fig. 1B) were significantly increased in B6.SLE mice relative to their respective controls. Interestingly, splenomegaly was more pronounced in HFD-fed B6.SLE mice than in their respective B6 controls in the LFD-fed groups when data were expressed as absolute body glucose level (Fig. 3A) or as area under the curve (Fig. 3B). Interestingly, the glucose curve of LFD-fed B6.SLE mice approached the curve of HFD-fed B6 mice. In addition, female B6.SLE mice fed HFD had significantly higher glucose intolerance than B6 controls after 27–30 wk of feeding (data not shown).

GLUCOSE HOMEOSTASIS AND IR SIGNALING IS ALTERED IN B6.SLE MICE.

Glucose tolerance tests were conducted in male B6.SLE and B6 mice after 20 wk of LFD or HFD feeding (Fig. 3A and B). Glucose intolerance was significantly higher in B6.SLE mice than in their respective B6 controls in the LFD-fed groups when data were expressed as absolute body glucose level (Fig. 3A) or as area under the curve (Fig. 3B). Interestingly, the glucose curve of LFD-fed B6.SLE mice approached the curve of HFD-fed B6 mice. In addition, female B6.SLE mice fed HFD had significantly higher glucose intolerance than B6 controls after 27–30 wk of feeding (data not shown).

To determine whether insulin signaling is disrupted in metabolically active tissues in B6.SLE mice, Western blotting was used to measure the p-Akt-to-Akt ratio in the perigonadal fat pads, liver, soleus muscle, and gastrocnemius muscle. Akt is a critical component of the insulin signaling pathway and becomes phosphorylated after the insulin receptor is ligated, ultimately causing translocation of the GLUT4 glucose transporter to the cell surface. The p-Akt-to-total-Akt ratio was significantly reduced in the adipose tissue of LFD-fed B6.SLE mice relative to B6 controls (Fig. 3C), indicating that insulin signaling is impaired in the adipose tissue of LFD-fed B6 mice.

No significant difference in Akt phosphorylation was seen in LFD-fed strains in the liver or gastrocnemius or soleus muscle, although trends toward lower ratios in B6.SLE mice were observed. No significant differences in p-Akt ratios were seen between strains in any of the tissues in HFD-fed groups.

B6.SLE islets have increased insulin secretion. To determine whether the observed glucose intolerance is due to impaired β-cell function, pancreatic islets were isolated from LFD-fed B6 and B6.SLE mice, and responses to several β-cell secretagogues were measured in an islet perfusion system. Figure 4A shows that insulin secretion was significantly increased in the islets of LFD-fed B6.SLE mice in response to 16.7 mM glucose, 16.7 mM glucose + 50 μM IBMX, and 20 mM KCl, ruling out β-cell defects in glucose sensing, metabolism, or granule exocytosis in LFD-fed B6.SLE mice.

Fig. 1. B6.SLE mice are prone to autoimmunity. A: 5-wk-old B6 (C57Bl/6) and B6.SLE male mice (B6 mice with systemic lupus erythematosus) were fed a low-fat diet (LFD; 10% kcal from fat) or high-fat diet (HFD; 45% kcal from fat) for 20 wk, and blood was collected via retroorbital sinus. Anti-dsDNA antibody titers were measured via ELISA and normalized to stock control serum (n = 6–15/group). B: 5-wk-old B6 and B6.SLE male mice were fed LFD or HFD for 20–25 wk. Spleen weight was measured after death (n = 6–15/group). *P < 0.005 by Student’s t-test.
Circulating adipokines and cytokines are not different in LFD-fed B6.SLE mice. To determine whether circulating adipokines and cytokines contribute to glucose intolerance in B6.SLE mice, fasting levels of leptin, resistin, IL-6, TNFα, and insulin were measured using a cytokine bead array, and fasting glucose was measured using a hand-held glucometer. As seen in Fig. 5A, no differences in fasting insulin or fasting glucose were observed between strains in the LFD-fed groups, and there was a nonsignificant trend toward higher glucose and insulin in HFD-fed B6 mice relative to HFD fed B6.SLE mice. Insulin clearance was estimated by measuring the ratio of fasting serum insulin to fasting serum C-peptide in LFD-fed mice. C-peptide is cleaved from the proinsulin molecule during the formation of insulin (29). C-peptide is cleared in the kidney (47), and insulin is cleared primarily in the liver (9), and the ratio of these two molecules can be used to estimate hepatic clearance of insulin. There was no difference in the C-peptide-to-insulin ratio between B6.SLE and B6 mice, which suggests that SLE disease does not affect the rate of insulin clearance in B6.SLE mice (Fig. 5A, right).

Levels of serum resistin and leptin in LFD-fed groups were indistinguishable, and levels of resistin and leptin were significantly lower in HFD-fed B6.SLE mice relative to HFD fed B6.SLE mice. IL-6 levels were below the level of assay detection in all groups. Collectively, these results suggest that the glucose intolerance and adipose tissue IR observed in LFD-fed mice is not instigated by...
Fig. 3. Glucose homeostasis and adipose tissue insulin receptor signaling is impaired in B6.SLE mice. Five-week-old B6 and B6.SLE male mice were fed LFD or HFD for 20 wk. After 5–6 h of fasting, mice were injected with 1.25 g glucose/kg lean body wt, and blood glucose was measured using a One Touch Ultra glucometer (n = 16–25 mice/group in 4 independent experiments). Data are expressed as blood glucose over time (A) or area under the glucose excursion curve (B). **P < 0.0001 between LFD-fed B6.SLE and B6 mice by two-way ANOVA. ***P < 0.0001 by Student’s t-test.

C: after 20 wk of HFD or LFD feeding, mice were fasted for 4 h and then injected with 1 U/kg lean body wt insulin. Fifteen minutes later, mice were euthanized, and liver, gastrocnemius, soleus, and perigonadal fat were removed and freeze-clamped in liquid nitrogen. Tissue lysate was analyzed by Western blot with Akt and phospho-(p-)Akt antibodies. Data are expressed as integrated intensity of Akt or the ratios of the integrated intensity of p-Akt and Akt (n = 3–5 mice/group). Values are expressed as means ± SE. *P < 0.04 by Student t-test; n.s., not significant.
adipokines or cytokines normally associated with IR and may result from SLE-specific factors.

Adipocyte size is increased in B6.SLE mice. Increased adipocyte size has been correlated with glucose intolerance and adipose tissue insulin resistance. To determine whether B6.SLE mice have increased adipocyte size, we measured adipocyte size in paraffin-embedded adipose tissue. As seen in Fig. 6, average adipocyte size was significantly greater in LFD-fed B6.SLE mice than in B6 controls. There was no difference in average adipocyte size between HFD-fed B6.SLE mice and their controls.
Serum cholesterol, serum triglyceride, and liver triglyceride are significantly reduced in B6.SLE mice. We measured serum cholesterol and triglycerides in study mice to determine whether lipid homeostasis is perturbed in B6.SLE mice. As seen in Fig. 7A, serum cholesterol and triglyceride levels were significantly reduced in HFD- and LFD-fed B6.SLE mice relative to their respective controls. Liver triglyceride content (Fig. 7B) was significantly decreased in HFD-fed B6.SLE mice relative to B6 controls and there was a nonsignificant trend toward reduced triglyceride content in the livers of LFD-fed B6.SLE mice.

Leukocyte content in the stromal vascular fraction varies in SLE mice. Cells of the adaptive and innate immune systems have been associated with adipose tissue dysfunction in mouse models of obesity. To examine the immune compartment in study mice, we used flow cytometry to assess the cell content of the stromal vascular fraction (SVF) in LFD- and HFD-fed B6.SLE mice relative to their respective controls. Liver triglyceride content (Fig. 7B) was significantly decreased in HFD-fed B6.SLE mice relative to B6 controls and there was a nonsignificant trend toward reduced triglyceride content in the livers of LFD-fed B6.SLE mice.

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Additionally, the proportion of CD8+ T cells, but not CD4+ T cells, in LFD-fed B6.SLE mice was significantly increased relative to controls and exceeded the percentages of CD8+ T cells in HFD-fed B6 mice (Fig. 8, D and E). This increase in CD8+ T cell parallels the results of Nishimura et al. (27), in which HFD-feeding caused an increase in CD8+ T cells that correlated with IR in their study.

SVF B cells in B6.SLE mice are skewed toward IgG production. Although no quantitative differences were observed in the CD19+ B cell population in study mice, we hypothesized that B6.SLE mice were predisposed to develop qualitative differences in the SVF resident B cell population. Previous studies by Winer et al. (44) have shown that B cells contribute to adipose tissue IR through production of pathogenic IgG antibodies. We investigated whether SVF resident B cells had undergone class-switch recombination by using flow cytometry to quantify IgD, IgM, and IgG expression on CD19+ B cells (Fig. 9A). The percentage of class-switched IgG+ B cells in B6.SLE mice was significantly higher than that of B6 controls in each diet group. Furthermore, there were significantly higher percentages of IgG+ B cells in the SVF of LFD- and HFD-fed B6.SLE female mice (data not shown). No differences in IgG+ B cell ratios were observed in the liver, indicating that these changes are specific to the gonadal fat pad. To determine whether systemic Ig levels were altered in B6.SLE mice, circulating total IgG, IgG1, IgG2c, and IgM were measured in serum collected from male mice fed HFD or LFD for 20–26 wk (Fig. 9B). LFD- and HFD-fed B6.SLE mice
had significantly higher circulating total IgG, IgG1, IgG2c, and IgM than their respective B6 controls. Female mice also had similarly increased levels of IgG and IgM compared with control mice (data not shown). Perigonadal fat pad lysates were probed for IgG heavy chain to determine whether SLE mice had higher adipose tissue IgG deposition than B6 controls. As seen in Fig. 9C, there was significantly more IgG heavy chain in the perigonadal fat pads of LFD-fed B6.SLE mice compared with LFD-fed B6 controls. Furthermore, there was significantly more IgG deposition in the livers of LFD- and HFD-fed B6.SLE mice relative to their B6 controls. Finally, the expression of the activation marker CD80 was significantly increased in SVF B cells of LFD-fed B6.SLE mice (Fig. 9D). Overall, the data show that SVF B cells are skewed toward a proinflammatory IgG response, that circulating IgG1 levels are elevated in the serum of B6.SLE mice, and that there is increased IgG deposition in the fat pads of B6.SLE mice.

DISCUSSION

In this report, we show for the first time that B6.SLE mice develop more severe glucose intolerance and adipose tissue insulin resistance than B6 controls and that B6.SLE B cells have a diabetogenic immunophenotype characterized by increased circulating IgG, increased IgG expression on B cells, and increased deposition of IgG heavy chain in white adipose tissue (15, 44). These changes were observed in LFD-fed B6.SLE mice, indicating that SLE disrupts glucose metabolism in the context of a LFD. Adiposity did not vary significantly between B6.SLE mice and their controls, indicating that lupus-related immune dysregulation, and not adipose tissue accumulation, causes disruption of glucose homeostasis in mice. Assessment of pancreatic islet function showed increased glucose-stimulated insulin secretion in B6.SLE islets. However, there was not a concomitant increase in fasting serum insulin in B6.SLE mice, which could be explained by either increased insulin clearance or lower islet cell mass. Estimation of insulin clearance with the C-peptide-to-insulin ratio (Fig. 5A, right) showed no difference between mouse strains. Collectively, these data suggest that B6.SLE mice may be compensating for lower islet cell mass by increasing islet secretory capacity. Islet cell mass was not measured, so we cannot confirm that there is a defect in islet cell mass in B6.SLE mice in the present study. These findings contrast with the earlier mouse studies of Ryan et al. (32) in the NZW/F1 model of lupus, in which NZW/F1 mice were shown to have hyperinsulinemia, hyperleptinemia, and higher glucose intolerance than control parental strain mice. Our study differs from that of Ryan et al. in

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In this report, we show for the first time that B6.SLE mice develop more severe glucose intolerance and adipose tissue insulin resistance than B6 controls and that B6.SLE B cells have a diabetogenic immunophenotype characterized by increased circulating IgG, increased IgG expression on B cells, and increased deposition of IgG heavy chain in white adipose tissue (15, 44). These changes were observed in LFD-fed B6.SLE mice, indicating that SLE disrupts glucose metabolism in the context of a LFD. Adiposity did not vary significantly between B6.SLE mice and their controls, indicating that lupus-related immune dysregulation, and not adipose tissue accumulation, causes disruption of glucose homeostasis in mice. Assessment of pancreatic islet function showed increased glucose-stimulated insulin secretion in B6.SLE islets. However, there was not a concomitant increase in fasting serum insulin in B6.SLE mice, which could be explained by either increased insulin clearance or lower islet cell mass. Estimation of insulin clearance with the C-peptide-to-insulin ratio (Fig. 5A, right) showed no difference between mouse strains. Collectively, these data suggest that B6.SLE mice may be compensating for lower islet cell mass by increasing islet secretory capacity. Islet cell mass was not measured, so we cannot confirm that there is a defect in islet cell mass in B6.SLE mice in the present study. These findings contrast with the earlier mouse studies of Ryan et al. (32) in the NZW/F1 model of lupus, in which NZW/F1 mice were shown to have hyperinsulinemia, hyperleptinemia, and higher glucose intolerance than control parental strain mice. Our study differs from that of Ryan et al. in
several key areas. First, the NZW/F1 mice in the Ryan et al. study reported significantly larger white adipose depots than controls. In some cohorts, NZW/F1 mice had twice as much visceral adipose tissue as controls, making it difficult to determine whether the metabolic differences observed were due to changes in body composition or the immune dysregulation observed in the setting of SLE. Our study shows that B6.SLE mice have decreased glucose tolerance and adipose tissue insulin resistance despite having similar adipose tissue mass and increased lean mass relative to controls.

Second, given the newly characterized importance of the adaptive immune system in metabolic syndrome and the critical role of the adaptive immune system in the pathogenesis of SLE, our studies focused on the immunophenotype of the SVF lymphocyte compartment. We observed significantly increased percentages of T cells in the SVF of LFD-fed B6.SLE mice relative to controls. Although no differences in total B cell proportions were detected in B6.SLE mice on either diet, there were several qualitative differences in the B cell compartment. Specifically, the proportion of IgG-positive B cells was significantly increased in both diet groups, and the activation marker CD80 was increased on B cells in LFD-fed B6.SLE mice. Circulating IgG was increased in the periphery of B6.SLE mice on both diets, and there was a significant increase in IgG deposition in the adipose tissue of B6.SLE mice. These results are consistent with the diabetogenic B cell immunophenotype described by Winer et al. (44), in which IgG antibody secreted by B cells in the context of HFD contributed to insulin resistance.

Adipocyte hypertrophy in visceral adipose tissue has been associated with insulin resistance and hyperlipidemia. Although we observed increased adipocyte size in LFD-fed B6.SLE mice relative to controls, there were decreases in serum triglyceride and cholesterol in B6.SLE mice. We have previously observed reduced triglyceride and cholesterol levels in studies of B6.SLE mice (4, 36), and other groups have shown similar changes in serum lipids in patients with rheumatoid arthritis and other autoimmune diseases (16, 21, 31, 34, 37). The mechanism that causes this phenomenon is not known, but it may result from disruption of lipid homeostasis by the chronic inflammation present in autoimmune disease (37).

Fasting levels of glucose, insulin, leptin, resistin, TNFα, and IL-6 were not significantly different between LFD-fed B6.SLE mice and their controls. This may indicate that the metabolic phenotype becomes unmasked only after a challenge with glucose or insulin or that the phenotype is apparent only in the fed state. Levels of glucose, insulin, leptin, and resistin in the fed state were measured in the current study. Furthermore, we observed worsened glucose tolerance and adipose tissue insulin resistance in LFD-fed B6.SLE mice (Fig. 3C), which was not accompanied by significant insulin resistance in the liver or skeletal muscle of B6.SLE mice. Adipose tissue-specific impairment of insulin signaling has been shown to ameliorate glucose intolerance in fat-specific insulin receptor knockout (FIRKO) mice (3). Within this paradigm, we would not anticipate that the LFD-fed B6.SLE mice would have significantly worsened glucose tolerance. However, it is possible that nonclassical, SLE-mediated effects on adipose tissue may be causing the observed metabolic impairment in B6.SLE mice. One molecule associated with both SLE and impairment of adipocyte function is B cell activating factor (BAFF). We observed increased levels of circulating BAFF in B6.SLE
mice and SVF resident B cells in these mice had elevated expression of the BAFF receptor (data not shown). BAFF is a growth factor that promotes proliferation, maturation, and antibody production of B cells. Elevated levels of circulating BAFF have been observed in the context of SLE and is associated with increased disease severity and production of pathogenic autoantibodies (7). In addition, adipocytes have been shown to express the BAFF receptor, and BAFF has been shown to specifically disrupt glucose homeostasis both in vitro and in vivo (15). Future studies will focus on the role of BAFF and IgG in the pathogenesis of metabolic disease in the context of B6.SLE mice.

Fig. 9. SVF B cells in B6.SLE mice are skewed toward IgG production and show higher expression of an activation marker. A: mice were fed LFD or HFD for 20–25 wk before being euthanized. SVF cells and hepatic mononuclear cells were isolated by digesting perigonadal fat pads in collagenase followed by Percoll gradient centrifugation. Cells were stained and analyzed using conventional flow cytometry techniques (n = 6–8 mice/group). *P < 0.05 by Student’s t-test. B: male mice were fed LFD or HFD for 20–26 wk and serum levels of IgG1, IgG2c, IgM, and total Ig were measured using conventional sandwich ELISA. ELISA absorbances were normalized to LFD-fed B6 controls (n = 6–16/group). *P < 0.0005 by Student’s t-test. C: perigonadal fat pads and liver were harvested from male B6 and B6.SLE mice. Total tissue lysate was applied to an SDS-PAGE gel and a Western blot was completed using anti-IgG heavy chain and β-actin antibodies. Data are expressed as the ratio of the integrated intensity of the IgG heavy chain band to the integrated intensity of the β-actin band (n = 6–8/group in fat and 3–6/group in liver). *P < 0.05 by Student’s t-test. D: mice were fed LFD or HFD for 20–25 wk before being euthanized. SVF cells were isolated by digesting perigonadal fat pads in collagenase followed by Percoll gradient centrifugation. Cells were stained for CD80 and analyzed using conventional flow cytometry techniques (n = 5–8 mice/group). *P < 0.05 by Student’s t-test.
Adiposity of B6.SLE mice did not differ significantly from that of B6 mice. Although early studies showed higher BMI in cohorts of lupus patients (19, 20), subsequent studies failed to show significant differences in BMI between SLE patients and healthy controls (22, 28, 38, 39). In one of those studies, Magadmi et al. (22) reported that SLE patients had significantly worsened insulin resistance than healthy control patients, but body composition did not differ significantly between SLE and control groups, and SLE patients were only moderately overweight (BMI 26.6 kg/m²). The observations of Magadmi et al. have been corroborated by several other groups (1, 27, 36). Therefore, we propose that the LFD-fed B6.SLE mice are a very suitable model of SLE-associated metabolic disease. Unexpectedly, reductions in glucose tolerance and insulin signaling were not observed in HFD-fed B6.SLE mice relative to B6 controls. It is possible that HFD feeding “saturates” the phenotype of B6.SLE mice, making it difficult to detect differences in glucose tolerance and insulin signaling between strains. Subsequent studies will focus on LFD-fed B6.SLE mice.

In conclusion, we have shown that B6.SLE mice fed LFD have significantly worsened glucose tolerance and adipose tissue insulin resistance than B6 controls. This B6.SLE glucose intolerance occurs in the presence of a diabetogenic B cell environment which is characterized by the presence of an increased number of IgG-producing B cells and IgG deposits in the white adipose tissue as well as higher levels of circulating IgG. The presence of metabolic dysfunction may exacerbate other SLE comorbidities such as cardiovascular disease. Although it is clear that SLE patients and the B6.SLE mouse model suffer disproportionately from CVD (1, 36), the causes of this disparity have not been completely elucidated. Insulin resistance has higher prevalence in the SLE population, and it is conceivable that it could be a contributing factor in the high CVD burden in these patients. Our current findings suggest potential mechanisms that give rise to metabolic disease in SLE patients and form the foundation for additional studies that could lead to greater understanding of the pathogenesis of SLE comorbidities.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
C.L.G. and A.S.M., conception and design of research; C.L.G., P.B.S., Y.V.M.F., A.J.Y., and A.M.Y., performed experiments; C.L.G. and A.S.M. analyzed data; C.L.G. and A.S.M. interpreted results of experiments; C.L.G. and A.S.M. prepared figures; C.L.G. and A.S.M. drafted manuscript; C.L.G., P.B.S., Y.V.M.F., A.J.Y., A.M.Y, A.S.M. edited and revised manuscript; C.L.G., P.B.S., Y.V.M.F., A.J.Y., A.M.Y. A.S.M. approved final version of manuscript.

REFERENCES


