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Hypertension and impaired vascular function in a female mouse model of systemic lupus erythematosus

Michael J. Ryan and Gerald R. McLemore, Jr.
Department of Physiology, University of Mississippi Medical Center, Jackson, Mississippi

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Ryan MJ, McLemore GR Jr. Hypertension and impaired vascular function in a female mouse model of systemic lupus erythematosus. Am J Physiol Regul Integr Comp Physiol 292: R736–R742, 2007. First published August 3, 2006; doi:10.1152/ajpregu.00168.2006.—Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease that predominantly affects women during their reproductive years. Although women with SLE have hypertension, the underlying mechanisms for this have not been examined. Despite the fact that inflammation is associated with altered endothelial and vascular function, the role of altered vascular function in the development of hypertension during SLE is unclear. In the present study, we tested whether a mouse model of SLE (NZBWF1) develops hypertension and examined whether increased blood pressure was associated with impaired endothelial-dependent relaxation. Female NZBWF1 mice were studied at 8, 20, and 36 wk of age. By 36 wk, urinary albumin and antinuclear antibodies were increased in SLE compared with control mice. Mean arterial pressure, measured by radiotelemetry, was significantly increased in SLE mice (124 ± 3 mmHg, n = 10) compared with control NZW/LacJ mice (111 ± 3 mmHg, n = 7) at 36 wk. Isolated carotid arteries from NZBWF1 mice, precontracted with U-46619 for assessment of endothelial-dependent relaxation, demonstrated a progressively impaired relaxation to ACh with age, although endothelial nitric oxide synthase mRNA expression was not different. Maximal tension generated by 5-hydroxytryptamine was increased in carotid arteries from NZBWF1 mice compared with controls at 8, 20, and 36 wk of age, suggesting a role for altered vascular function early on in the progression of SLE. Taken together, our data support a role for altered endothelial function as a contributing factor to the development of hypertension during SLE.

Systemic lupus erythematosus; autoimmune; hypertension; endothelial; pressure; auto-antibody

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) is a complex genetic autoimmune disorder that leads to the production of antibodies against an individual’s own healthy tissues. Any organ system can be affected during SLE, although individuals often present with skin rashes, joint pain, and renal problems. While no known cure for SLE exists, current immunosuppressive therapies have markedly reduced short-term mortality rates. Long-term mortality rates, on the other hand, are increasingly influenced by cardiovascular complications. Indeed, individuals with SLE are at an alarmingly high risk for stroke, myocardial infarction, atherosclerosis, renal disease, and hypertension.

Interestingly, SLE predominantly affects women (at a ratio of 9:1 women-men) during reproductive years, a period when they are typically thought to be “protected” against the development of cardiovascular disease. During this time, women with SLE have >50 times more likely to develop cardiovascular disease independent of traditional Framingham risk factors (23), with a high prevalence of hypertension, atherosclerosis, and glomerulosclerosis (1, 34, 38). Despite the high incidence of hypertension and peripheral vascular disease, there have been few studies directed at understanding the mechanisms of hypertension during SLE. Although impaired renal function likely contributes to hypertension in latter stages of SLE when nephron loss is severe, factors contributing to hypertension early in the course of the disorder have not been examined. Currently available data regarding endothelial cell biology during SLE have focused more on putative markers of endothelial damage and less on functional vessel changes (9, 15, 28). The scant human studies of endothelial function during SLE are complicated by heterogeneity of populations studied, variation in disease severity, and the long-term consequences of pharmacological therapies (16, 20, 36). Therefore, understanding changes in vessel function under controlled conditions may provide valuable insight toward mechanisms promoting cardiovascular disease during SLE.

Because chronic inflammation is known to affect vascular function, we hypothesized that impaired endothelial function may be an important mechanism contributing to increased blood pressure during SLE. To test this hypothesis, we isolated arteries from a mouse model of SLE (NZBWF1) to determine whether endothelial-dependent relaxation and contraction are altered and whether blood pressure is increased in this model. NZBWF1 is a widely accepted and established mouse model of SLE that has been studied for over 40 years (5, 6). Like humans, SLE affects predominantly the female NZBWF1 mice and is associated with increased urinary albumin, antinuclear antibodies, and inflammatory cytokines (3, 12, 25). To date, there have been no studies examining blood pressure in conscious unrestrained NZBWF1 mice, nor have studies tested whether endothelial dysfunction contributes to changes in blood pressure during SLE.
**METHODS**

**Animals.** Female NZBWF1 (SLE) and NZW/LacJ (control) mice were obtained from Jackson Laboratories (Bar Harbor, ME). We studied mice at 8 (7.9 ± 0.1, n = 26), 20 (20.5 ± 0.3, n = 28), and 36 (37.1 ± 0.2, n = 48) wk of age. All studies were performed with the approval of the University of Mississippi Medical Center Institutional Animal Care and Use Committee and in accordance with National Institutes of Health guidelines.

**Blood pressure measurements.** Radiotelemeters were used to measure mean arterial pressure (MAP; model PAC10 or PAC20; Data Sciences International). Catheters were implanted in the left common carotid artery, and the body of the telemeter was positioned subcutaneously along the right flank of the animal, as previously described (7). Mice were given at least 1 wk to recover before MAP was recorded. Surgery was performed under isoflurane anesthesia. Five second data collections were made every 10 min continuously for three consecutive days. The data are presented as the average pressure over this time for each mouse.

**Vascular ring preparation.** Mouse carotid arteries were removed and prepared for vessel reactivity studies, as previously published (31–33). Resting tension was adjusted stepwise to reach a final tension of 0.25 grams. Endothelium-denuded vessels were created by passing an 8-cm segment of 6 – 0 silk through the lumen of the vessel. For each animal, at least two vessel segments were studied with the averaged response equal to an n of 1.

**Protocols.** Concentration-dependent relaxation (10⁻⁸ to 10⁻⁴ mol/l) to ACh and sodium nitroprusside (SNP) were assessed in carotid vessel segments precontracted with the thromboxane A₂ mimetic U-46619 (0.4 μg/ml), 5-Hydroxytryptamine (5-HT, 10⁻⁸ to 10⁻⁵ mol/l) and U-46619 (0.03–3.0 μg/ml) were used to assess concentration-dependent contraction.

**Urinary proteins.** Mouse urinary albumin was assessed using AlbuStix (Bayer). Data are presented as urinary protein index units based on the following scale (in mg/dl): 0 = negative, 1 = trace, 2 = 30, 3 = 100, 4 = 300, 5 = 2,000.

**Antinuclear antibodies.** Plasma antinuclear antibody (ANA) levels were assessed using a commercial ELISA (USBiological) per the manufacturer’s instructions. Data are expressed as an ANA index (AI = net absorbance sample/net absorbance negative control at 450 nm). An individual sample was considered ANA positive if AI was greater than two (2 × negative control absorbance/negative control absorbance).

**Real-time PCR.** RNA was isolated from the thoracic aorta of control and SLE mice using the RNasy Protect Minikit (Qiagen) per the manufacturer’s instructions. The reverse transcription reaction was carried out using the IScript cDNA synthesis kit (Bio-Rad), and real-time PCR for endothelial nitric oxide synthase (eNOS) was performed using Sybr Green Supermix (Bio-Rad) with the ICycler (Bio-Rad). Each primer for mouse eNOS (sense 5'-CCTTCCGCTAC-CAGCCAGA-3', antisense 5'-CAGAGATCTTCACTGCATTGGC-3') spans the junctions of two exons and has been previously published (8). Primers for 18S ribosomal RNA were used to normalize the data (sense 5'-TAAGTCCCTGCCCTTTGTACACA-3', antisense 5'-GATCCGAGGGCCTCACTAAC-3'). A product-melt curve was generated at the end of the experiment to confirm the presence of a single PCR product. Data are presented as the degree of change relative to control expression levels.

**Statistics.** ANOVA with repeated measures was used to test for statistically significant differences among the concentration-response curves from different mice using Tukey’s post hoc test for all pairwise comparisons. A Student’s t-test was used for experiments comparing only two groups. EC₅₀ values were calculated using Prism GraphPad.
Software for each concentration response. Individual EC$_{50}$ values were averaged for control and SLE mice, and statistical significance was evaluated using Student’s $t$-test. Data are presented as the $-\log$EC$_{50}$. Statistical significance was accepted at $P < 0.05$. 

RESULTS

The NZBWF1 strain (SLE) is an F$_1$ cross derived from New Zealand White (NZW/LacJ) and New Zealand Black (NZB/binJ) mice. Both parental strains have been reported to develop mild autoimmunity, although this does not typically occur in NZW/LacJ parental mice until after 50 wk of age (10, 11). Therefore, female NZW/LacJ (control) mice were used as controls. We measured urinary albumin excretion in control and SLE mice at 8 (1.6 ± 0.3 vs. 1.8 ± 0.2 units), 20 (1.3 ± 0.3 vs. 1.8 ± 0.2 units), and 36 (1.5 ± 0.2 vs. 3.3 ± 0.4 units) wk of age (Fig. 1). These data are consistent with previous reports that NZBWF1 have increased urinary protein with age (10, 18, 29). Urinary albumin did not change in control mice over this time period.

Using radiotelemetry, we measured MAP in conscious unrestrained mice. Data are presented as the mean pressure measured over three consecutive days. Mean pressure was not statistically different at 8 (107 ± 3 vs. 106 ± 2 mmHg) or 20 (109 ± 3 vs. 111 ± 4 mmHg) wk. However, by 36 wk of age, pressure was significantly increased in SLE compared with control mice (111 ± 3 vs. 124 ± 4 mmHg; Fig. 2). We measured ANA in control and SLE mice at each age to assess whether changes in ANA correlate with increased blood pressure. The dotted line in Fig. 3 represents the threshold for an animal to be considered ANA positive, presented as the AI. All SLE mice tested were ANA positive at 36 wk compared with one control. Although AI was greater in SLE mice at 8 and 20 wk, only one NZBWF1 mouse was ANA positive (at 20 wk). No other animals were ANA positive at these ages.

We next asked whether changes in vascular function could be a contributing component to the increased blood pressure during SLE. Using isolated carotid arteries, we assessed endothelial-dependent relaxation with increasing concentrations of ACh. ACh induced concentration-dependent relaxation in vessels precontracted with U-46619. Responses between control and SLE mice were identical at 8 wk of age and were progressively impaired in the SLE mice by 20 and 36 wk of age (Fig. 4). Concentration-dependent relaxation to SNP was not different between control and SLE mice at 8 wk of age but was moderately attenuated at 20 and 36 wk of age (Fig. 5). These data suggest that the impaired response to ACh may be mediated by both impaired endothelial and smooth muscle function.

In addition to examining endothelial-dependent relaxation, we also tested whether there are changes in vessel contractility that might contribute to increased blood pressure. Figure 6 shows the concentration-dependent contraction caused by 5-HT (serotonin). 5-HT-mediated contraction was enhanced in SLE mice compared with controls at 8, 20, and 36 wk of age. We repeated these
experiments in endothelial-denuded (-EC) vessels anticipating that 5-HT-mediated contraction in control animals would be increased to match that of mice with SLE. The data, unexpectedly, show that the enhanced 5-HT response in SLE was attenuated to a level similar to control animals (Fig. 6). To confirm successful denudation, we show that ACh-mediated relaxation is markedly impaired (Fig. 4), whereas smooth muscle responses to SNP were preserved (Fig. 5). Maximal contractile responses to the thromboxane mimetic U-46619 were not different between control and SLE mice (Fig. 7).

EC50 values were calculated for each of the concentration-response curves and presented as the $-\log EC_{50}$ (Table 1). At 36 wk of age, the EC50 for ACh was higher in mice with SLE, and the EC50 for SNP was significantly greater at 20 and 36 wk in mice with SLE. The EC50 for 5-HT was not different at any age despite the significantly enhanced contraction in SLE mice. Finally, although concentration responses to U-46619 were not different, EC50 values were modestly, but significantly, lower in 36-wk-old SLE animals.

Because endothelial-dependent relaxation was progressively impaired in the SLE mice, we tested whether eNOS expression was altered at 8, 20, and 36 wk of age using real-time PCR with RNA isolated from the thoracic aorta of control and SLE mice. The results (Fig. 8) suggest that eNOS expression in the mice examined was not significantly different at any age.

**DISCUSSION**

Studies suggest a high occurrence of hypertension during SLE, with some reporting as many as 74% (1). Several studies also suggest that the increase in blood pressure is not dependent upon glomerulonephritis that is prevalent in individuals with SLE (4, 34, 35). In addition to hypertension, SLE is associated with an accelerated risk for atherosclerosis (38), coronary artery disease (27), and stroke (26). Inflammatory responses in the endothelium are known to contribute to the pathogenesis of each of these, and numerous studies implicate a role for cytokines in the progression of SLE (2, 12, 14, 17, 25, 37). Despite the high incidence of hypertension and vascular disease documented in women with SLE, studies designed to understand the development of hypertension during SLE have been scarce. Similarly, studies have not been performed to directly assess potential changes in vascular function that may contribute to increased blood pressure during SLE. The present study uses an established mouse model to begin to address this. The novel findings are that 1) MAP is increased in the NZBWF1 mouse with age, 2) endothelial-dependent relaxation is progressively impaired with age in this model, and 3) vessel contraction is enhanced in NZBWF1 SLE. The latter two findings occur before the increase in blood pressure, suggesting that endothelial dysfunction occurs early in the course of SLE and may contribute to increased blood pressure.
The NZBWF1 model of SLE has been used for over 40 years to study immunological mechanisms of disease progression. Like human SLE, the females are predominantly affected; they develop immune complex glomerulonephritis and produce autoantibodies, including the diagnostic ANA. Also, like humans, SLE in NZBWF1 mice is genetically complex, with no single candidate gene that causes the disorder. For these reasons, NZBWF1 is widely accepted as a model that closely mimics human SLE.

This is the first study, to our knowledge, to directly measure MAP in conscious freely moving NZBWF1 (SLE) and NZW/LacJ (control) mice. A previous study measured pressure in NZBWF1 mice using the tail cuff method and reported a significantly larger increase in pressure in these mice at a similar age (30). Our data demonstrate a somewhat less robust increase in pressure (13 vs. more than 45 mmHg) than these earlier findings. The discrepancy is likely because of methodological differences for obtaining pressure (13). For example, using radiotelemetry, we can record pressure 24 h a day without disturbing the animals. Tail cuff is only measured at a single point and relies on heating and restraining the mouse to achieve increased blood flow to the tail for measurements of pressure.

To date, few studies have attempted to measure endothelial function in individuals with SLE (16, 20, 36). These studies suggested that flow-mediated dilation is impaired in patients with SLE. However, data in human studies are difficult to interpret because the severity of SLE is markedly influenced by genetic, hormonal, and environmental factors. In one of these previous studies (20), SLE patients with hypertension were completely excluded, and in another (36) pressure was managed with antihypertensive therapies such as angiotensin-converting enzyme inhibitors so there were no significant differences in pressure. In both of these studies, a large percentage of participants were being treated with prednisone. Corticosteroid treatment is common in individuals with SLE because of its immunomodulatory properties; however, prolonged treatment with steroids can have detrimental effects on vascular function and blood pressure (24). Using this mouse model of SLE, we have the advantage of having uniform genetics and environmental conditions without the influence of pharmacological therapies. Therefore, this is the first study to demonstrate a progression of impaired endothelial-dependent relaxation during SLE. Interestingly, the impaired response to ACh begins before the development of proteinuria and increased blood pressure, suggesting that early changes in vessel function may contribute to the development of hypertension during SLE. The early changes in ACh-mediated relaxation do not appear to be related to changes in eNOS mRNA expression. However, a detailed analysis of eNOS protein levels, phosphorylation state, and nitric oxide release will be required to definitively answer this question.

5-HT-mediated contraction can be used as a sensitive and early indicator of altered endothelial function (19). The vascular response to 5-HT results from a balance between its ability to cause smooth muscle contraction and its ability to release nitric oxide from endothelial cells (39). Therefore, although there are no apparent changes in eNOS expression, the enhanced contraction to 5-HT may reflect altered nitric oxide release from endothelial cells. Our data show that, at the earliest age studied, the response to 5-HT is enhanced in the model of SLE. Surprisingly, denuding the endothelium attenuated the enhanced 5-HT contraction in SLE mice and had minimal effect on the control strain. The mechanism behind

Table 1. Log EC50 values for concentration-response curves

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<th>8 Weeks</th>
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<th>36 Weeks</th>
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<td></td>
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<td>ACh</td>
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<tr>
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<tr>
<td>5-HT</td>
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<td>U-46619</td>
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<td>0.60±0.02</td>
<td>0.63±0.04</td>
<td>0.59±0.05</td>
<td>0.51±0.03</td>
<td>0.66±0.05*</td>
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Values are means ± SE. SLE, systemic lupus erythematosus; SNP, sodium nitroprusside; 5-HT, 5-hydroxytryptamine. *P < 0.05 compared with age-matched control.
this finding is not clear; however, these data suggest that a factor or factors released from the endothelium of SLE mice may potentiate 5-HT-mediated contraction. Although enhanced 5-HT contraction may be a contributing factor to the development of hypertension and tissue injury during SLE, consideration should also be given to the possibility that this response reflects a difference in genetic background between the parental control and SLE model.

Because the vascular data are obtained in conduit vessels, our interpretation that impaired vascular function increases pressure during SLE cannot be definitive. However, impaired endothelial function in conduit vessels may reflect increased arterial stiffness that can influence cardiac afterload (21), and recent evidence suggests a direct correlation between impaired carotid artery hemodynamics and hypertension in humans (22). Nonetheless, it will be important to examine vascular function in smaller resistance-type arteries.

In conclusion, SLE is an autoimmune disorder that largely affects young women. These individuals are at increased risk for developing hypertension and vascular disease. To date, the physiological mechanisms underlying hypertension during SLE have not been examined. The present study demonstrates that the NZBWF1 model of SLE develops hypertension that is preceded by altered vascular function. These findings, coupled with previous reports demonstrating NZBWF1 as an appropriate model of human SLE, suggest that these mice may be an important tool for understanding physiological mechanisms that contribute to vascular dysfunction and hypertension during SLE. (31–33)

REFERENCES

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