Impact of genetic background on nephropathy in diabetic mice

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Impact of genetic background on nephropathy in diabetic mice. Am J Physiol Renal Physiol 290: F214–F222, 2006. First published August 23, 2005; doi:10.1152/ajprenal.00204.2005.—With the goal of identifying optimal platforms for developing better models of diabetic nephropathy in mice, we compared renal effects of streptozotocin (STZ)-induced diabetes among five common inbred mouse strains (C57BL/6, MRL/Mp, BALB/c, DBA/2, and 129/SvEv). We also evaluated the renal consequences of chemical and genetic diabetes on the same genetic background (C57BL/6). There was a hierarchical response of blood glucose level to the STZ regimen among the strains (DBA/2 > C57BL/6 > MRL/Mp > 129/SvEv > BALB/c). In all five strains, males demonstrated much more robust hyperglycemia with STZ than females. STZ-induced diabetes was associated with modest levels of albuminuria in all of the strains but was greatest in the DBA/2 strain, which also had the most marked hyperglycemia. Renal structural changes on light microscopy were limited to the development of mesangial expansion, and, while there were some apparent differences among strains in susceptibility to renal pathological changes, there was a significant positive correlation between blood glucose and the degree of mesangial expansion, suggesting that most of the variability in renal pathological abnormalities was because of differences in hyperglycemia. Although the general character of renal involvement was similar between chemical and genetic diabetes, Akita mice developed more marked hyperglycemia, elevated blood pressures, and less variability in renal structural responses. Thus, among the strains and models tested, the DBA/2 genetic background and the Akita (Ins2Δcex9v) model may be the most useful platforms for model development.

mouse models of type I diabetes; streptozotocin; Akita mice; albuminuria; strain comparison

Diabetic nephropathy (DN) is a leading cause of end-stage renal disease in humans worldwide (43a). In susceptible individuals, DN follows a predictable tempo and clinical course. The earliest detectable sign of kidney abnormalities in patients with diabetes is the development of low levels of albumin excretion (AE), so-called microalbuminuria, which is associated with histological changes of glomerular mesangial expansion and matrix deposition (11). Patients who progress to clinically overt nephropathy develop more substantial levels of albuminuria, but in patients with type I diabetes, this is not generally observed until at least ten years after the onset of diabetes (14, 33). The development of frank proteinuria is followed by a progressive fall in glomerular filtration rate (GFR). In this more advanced phase, DN is characterized by typical pathological changes, including nodular sclerosis (19), often associated with hypertension. Although tight control of glycemia (16a, 46a) and blood pressure (2), along with the administration of an angiotensin-converting enzyme (ACE) inhibitor (28) or angiotensin receptor blocker (5, 29), can slow the progression of renal disease, there are no known therapies that can prevent the progressive loss of renal function in DN.

The development of animal models of DN has been pursued as an approach to characterize the pathogenesis of DN and to develop new avenues to diagnose and treat this disorder. In this regard, the pancreatic islet cell toxin streptozotocin (STZ) has been widely used to induce diabetes in rodent models (30). Although STZ causes robust hyperglycemia in rats, renal pathological changes are generally mild, resembling only the earliest pathological changes seen in humans (4, 22). Nonetheless, these rat models have been useful in identifying new approaches for treating DN in humans. For example, glomerular hemodynamic studies in rats with STZ-induced diabetes provided the initial rationale for the use of ACE inhibitors in humans with DN (1, 48). Accordingly, generation of animal models that faithfully recapitulate the pathological and physiological features of human DN should be useful to facilitate further advancements in preventing and treating this disorder.

The mouse is an especially attractive platform for developing models of human disease because its genome is tractable for manipulation (10). In this regard, there is a significant published experience in the development and study of both chemical and genetic mouse models of type I diabetes, characterized by insulin deficiency (27, 41a). In addition, several mouse models of type II diabetes, characterized by insulin resistance, have also been described (41). However, characterization of renal complications in mouse models of diabetes has been performed in only a limited number of strains (32, 41, 41a). In general, this work suggests that, similar to rats, mice typically develop only mild proteinuria and modest pathological changes without substantial reductions in GFR. Nonetheless, the capacity to superimpose genetic alterations that could accelerate renal injury provides a potential opportunity for developing mouse models that more closely replicate human DN.

In humans with diabetes, there is a clear-cut genetic susceptibility to the development of nephropathy (15, 37). We therefore reasoned that differences in genetic background might also influence the development of diabetic renal complications in mice. However, there is limited information on whether genetic factors contribute to susceptibility to renal injury in mouse models of diabetes. To test this hypothesis and to take a first step toward the objective of generating better models of DN using the mouse, we characterized renal manifestations of
diabetes in several common strains of laboratory mice using a standardized protocol of STZ-induced diabetes. One purpose of these studies was to determine whether there are differences in susceptibility to kidney injury among genetically distinct mouse lines. A second purpose was to determine whether there are differences in the character of kidney injury between chemical and genetic forms of diabetes; therefore, we also compared albuminuria and renal pathology in C57BL/6 mice with STZ-diabetes to a genetic model of type I diabetes in C57BL/6 mice, the Akita (Insc2+/C96Y) mouse. Our studies suggest that there are significant strain-related differences in renal responses in the STZ model and that the Akita mouse provides a more robust and uniform experimental platform for model development.

MATERIALS AND METHODS

Animals. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and Taconic Farms (Germantown, NY) and were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited animal facility at the Durham Veterans Affairs Medical Center under National Institutes of Health guidelines. The course of STZ diabetes was compared in five inbred mouse lines (C57BL/6, MRL/Mp, BALB/c, DBA/2, and 129/SvEv). We also studied C57BL/6-Insc2+/C96Y (Akita) mice (The Jackson Laboratory), a genetic model of type I diabetes mellitus (47). Throughout the period of study, animals were provided free access to water and standard rodent chow (Lab Diet; Purina Mills, St. Louis, MO). For each strain, 14 male and 14 female mice were studied.

STZ-induced diabetes. Diabetes was induced with intraperitoneal injections of STZ. Immediately before injection, STZ (Sigma, St. Louis, MO) was dissolved in 0.05 M sodium citrate buffer (pH 4.5). Mice received two rounds of injections of 40 mg·kg−1·day−1 STZ for five consecutive days first at 8 and then 15 wk of age. This regimen of repeated, low doses of STZ has been previously shown to induce durable hyperglycemia and longitudinal survival without requirements for exogenous insulin (30, 39). Control animals received intraperitoneal injections of citrate buffer alone.

Blood glucose measurements. Blood glucose was measured 2 wk after the first STZ injection and at monthly intervals thereafter using a Glucometer Elite testing system (Ascensia; Bayer). In the Akita mice, measurements were first performed at 10 wk of age and then at regular intervals as above. After the tarsal area of the left leg was shaved, blood samples were obtained by puncturing the left lateral saphenous vein with a 25-gauge needle (21). Approximately 2 μl blood were collected directly on the testing strip for measurement.

Blood pressure measurements. During the last month of the study, systolic blood pressures were measured in conscious mice using a computerized tail-cuff system (Visitech Systems, Cary, NC) that determines systolic blood pressure using a photoelectric sensor as described (24, 26). This system allows measurements in four mice simultaneously and minimizes the potential for observer bias. Before measurements were initiated, mice were adapted to the apparatus for at least 10 days. Blood pressures were then measured for at least 15 days. The validity of this system has been established previously (24, 26).

Urinary protein measurements. At the end of the study, 24-h urine collections were obtained from mice housed in individual metabolic cages with free access to water and food. Water intake, urine volume, and body weight were monitored to ensure basal conditions. Urinary creatinine concentration was measured using a picric acid assay according to the manufacturer’s instructions (The Creatinine Companion; Exocell, Philadelphia, PA). Urinary AE was measured using an indirect competitive ELISA according to the manufacturer’s instructions (Albuwell M; Exocell).

Renal pathological examination. After 16 wk of diabetes, kidneys were harvested for pathological examination. Before harvest, a cannula was placed in the left ventricle, and the kidneys were perfused in situ with freshly prepared 4% paraformaldehyde using a peristaltic pump (Peristaltic Pump P-1; Amersham Biosciences) at a rate of 7 ml/min. The right kidney was then removed, decapsulated, and weighed. Perfusion-fixed tissue was embedded in paraffin, and sections were stained with periodic acid-Schiff for examination by light microscopy. Glomerular volume was calculated from the mean cross-sectional area of 20 glomerular profiles on each animal using the method of Weibel (44). Glomerular mesangial area was scored from zero to three on 25–40 glomerular profiles from a single section, and the mean was entered as the score for the animal.

Statistical analysis. The values for each parameter within a group are expressed as means ± SE. For comparisons between two experimental groups, an unpaired t-test was used to assess statistical significance. A paired t-test was used for comparisons within a group. For comparisons between multiple groups, ANOVA with Tukey’s multiple-comparisons test was used.

RESULTS

Blood glucose response. As depicted in Fig. 1 and Table 1, this multidose STZ protocol caused significant hyperglycemia in all of the strains of mice tested. Moreover, the hyperglycemia was maintained throughout the study period. However, the magnitude of the blood glucose elevation varied significantly among the different strains. Based on their hyperglycemic responses, the strains can be roughly divided into high and low responders (Fig. 2). The DBA/2 and C57BL/6 strains were both high responders, with blood glucose levels of 459 ± 43 and 334 ± 35 mg/dl, respectively, 16 wk after initial STZ injection (P < 0.005). The three low responder strains (MRL/Mp, BALB/c, and 129/SvEv) developed less hyperglycemia in response to STZ administration, with blood glucose values of 164 ± 29, 184 ± 32, and 201 ± 16 mg/dl, respectively, after 16 wk of diabetes (P < 0.005).

Within each strain, there was also a marked gender difference in blood glucose level at STZ treatment. STZ-treated males uniformly had higher blood glucose levels than females at each time point (Table 1). As early as 8 wk of diabetes (just after the second STZ injection), the difference in blood glucose values between males and females ranged from 49 to 324 mg/dl and was statistically significant for all strains (Fig. 3).

Despite the development of sustained hyperglycemia, most of the STZ-treated animals appeared healthy throughout the study period. An exception to this was the group of diabetic
male DBA/2 mice, which had the most profound hyperglycemia among the STZ-treated groups (P < 0.005). By the end of the study, body weights in these animals were almost 40% lower than the nondiabetic DBA/2 controls (19.4 ± 2 vs. 31.5 ± 0.7 g; P < 0.0003). Several of these male animals appeared lethargic, and two of seven of the diabetic males were killed before completing the study. Although they appeared healthy, diabetic BALB/c and MRL/Mp male animals gained less weight than their respective nondiabetic controls by the end of the study (28.2 ± 0.6 vs. 32.0 ± 0.2 g, P < 0.0003 for BALB/c males and 40.8 ± 1 vs. 44.5 ± 1 g, P < 0.03 for MRL/Mp males). In males from other strains and all female mice, body weights increased during the course of the experiments and were similar between diabetic and control animals.

Blood pressure response. Hypertension is a common accompanying feature of DN in humans (43). Therefore, we monitored blood pressures in all of the groups of mice in this study. As expected, there were differences in baseline blood pressures according to mouse strain (Table 2). Nondiabetic 129SvEv mice had the highest systolic blood pressure of all of the control animals in the study (123 ± 3 mmHg; P < 0.005 compared with all other controls). Baseline gender differences in blood pressure were also observed. For example, both male BALB/c and 129SvEv mice had higher blood pressures than females of the corresponding strain (113 ± 1 vs. 104.2 ± 2 mmHg, P < 0.001 for BALB/c and 129 ± 3 vs. 118 ± 1 mmHg, P < 0.002, for 129SvEv).

STZ diabetes had no effect on blood pressure in female mice. Among male mice in most of the strains studied, blood pressures tended to be lower in diabetic animals compared with controls. However, as shown in Fig. 4, this difference only reached statistical significance for the DBA/2 males. The systolic blood pressure of diabetic DBA/2 males was 99.6 ± 6 mmHg compared with 118 ± 2 mmHg for the nondiabetic DBA/2 males (P = 0.01). In contrast, blood pressures increased significantly in diabetic male MRL/Mp mice (117 ± 2 mmHg) compared with their controls (111 ± 2 mmHg, P = 0.03). The elevated blood pressures in the diabetic MRL/Mp males were accompanied by a reduction in heart rate (573 ± 8 vs. 610 ± 12 beats/min, P = 0.02).

Table 1. Blood glucose response

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Control 2 Weeks</th>
<th>Diabetic 2 Weeks</th>
<th>Control 8 Weeks</th>
<th>Diabetic 8 Weeks</th>
<th>Control 12 Weeks</th>
<th>Diabetic 12 Weeks</th>
<th>Control 16 Weeks</th>
<th>Diabetic 16 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>12</td>
<td>114 ± 4</td>
<td>181 ± 15</td>
<td>114 ± 4</td>
<td>247 ± 22</td>
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<td>286 ± 29</td>
<td>127 ± 4</td>
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</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>115 ± 5</td>
<td>219 ± 14**</td>
<td>119 ± 4</td>
<td>309 ± 22**</td>
<td>110 ± 7</td>
<td>332 ± 43*</td>
<td>134 ± 4</td>
<td>388 ± 50*</td>
</tr>
<tr>
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<td>6</td>
<td>113 ± 6</td>
<td>143 ± 12*</td>
<td>109 ± 6</td>
<td>186 ± 12*</td>
<td>110 ± 6</td>
<td>239 ± 31*</td>
<td>120 ± 6</td>
<td>280 ± 42*</td>
</tr>
<tr>
<td>MRL/Mp</td>
<td>14</td>
<td>91 ± 3</td>
<td>163 ± 29</td>
<td>88 ± 5</td>
<td>260 ± 29</td>
<td>86 ± 3</td>
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<td>331 ± 37**†</td>
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<td>77 ± 5</td>
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<td>77 ± 6</td>
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<td>86 ± 4</td>
<td>200 ± 31*</td>
<td>85 ± 6</td>
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<td>296 ± 18†</td>
<td>109 ± 6†</td>
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<td>336 ± 51*</td>
<td>102 ± 4</td>
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</tr>
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<td>148 ± 9</td>
<td>91 ± 2</td>
<td>166 ± 11</td>
<td>90 ± 3</td>
<td>190 ± 19</td>
<td>105 ± 3</td>
<td>201 ± 16</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>119 ± 8</td>
<td>164 ± 12*</td>
<td>97 ± 2</td>
<td>190 ± 13*†</td>
<td>97 ± 4</td>
<td>217 ± 32*</td>
<td>107 ± 7</td>
<td>220 ± 26*</td>
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<tr>
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<td>99 ± 5</td>
<td>131 ± 11*</td>
<td>85 ± 3</td>
<td>141 ± 13*</td>
<td>83 ± 2</td>
<td>163 ± 15*</td>
<td>104 ± 2</td>
<td>183 ± 18*</td>
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<td>C57BL/6-Ins2Akita</td>
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<td>334 ± 57</td>
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<td>345 ± 52</td>
<td>111 ± 6</td>
<td>282 ± 51</td>
<td>124 ± 5</td>
<td>339 ± 48</td>
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<tr>
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<td>131 ± 6</td>
<td>547 ± 13*†</td>
<td>123 ± 10</td>
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<td>Female</td>
<td>8</td>
<td>97 ± 3</td>
<td>166 ± 17*</td>
<td>114 ± 3</td>
<td>172 ± 8*</td>
<td>99 ± 5</td>
<td>146 ± 8*</td>
<td>115 ± 2</td>
<td>190 ± 13*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. The weeks indicate the duration of diabetes (weeks after initial streptozotocin injection or age-matched C57BL/6-Ins2Akita mice). *P < 0.05 between diabetic and nondiabetic animals. †P < 0.046 between diabetic males and diabetic females.

Fig. 2. High and low responders to STZ. Based on their blood glucose values after 16 wk of STZ diabetes, mice in this study can be divided into two groups: high responders and low responders.

Fig. 3. Effect of gender on blood glucose response to STZ. Male mice were more hyperglycemic than females throughout the study. Significant differences were present as early as 8 wk of diabetes. *P < 0.03 for each comparison between diabetic males and diabetic females.
Table 2. Blood pressure response

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Systolic Blood Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>12</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>104 ± 4</td>
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<tr>
<td>Female</td>
<td>6</td>
<td>103 ± 2</td>
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<tr>
<td>MRL/Mp</td>
<td>14</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>111 ± 2</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>113 ± 5</td>
</tr>
<tr>
<td>BALB/c</td>
<td>14</td>
<td>108 ± 2</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>113 ± 1†</td>
</tr>
<tr>
<td>Female</td>
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<td>DBA/2</td>
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<tr>
<td>Female</td>
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<td>C57BL/6-Ins2Akita</td>
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<tr>
<td>Female</td>
<td>8</td>
<td>107 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. *P = 0.006 between diabetic male and diabetic female mice. †P < 0.04 between diabetic and nondiabetic animals. ‡P < 0.002 between control male and control female mice.

Urinary AE. To screen for evidence of kidney injury associated with diabetes, we measured total 24-h AE for our primary analysis (Table 3). However, we also measured the albumin-to-creatinine ratio (ACR) in each specimen to allow comparison between the two methods. Across the entire study group, there was a significant linear correlation between 24-h AE and ACR (γ = 26.8 ± 0.74x with R² = 0.21; P < 0.005) for all mice. Total 24-h AE (Table 3) in 6-mo-old, nondiabetic mice ranged from 14 to 70 μg·g⁻¹·24 h⁻¹ with no significant differences among the strains (P = 0.08, ANOVA).

However, there were prominent differences in baseline albuminuria between males and females of the same strain. Untreated MRL/Mp males had significantly higher 24-h AE (122.0 ± 47.4 vs. 12.1 ± 2.7 μg·g⁻¹·24 h⁻¹; P = 0.03) than MRL/Mp females. In contrast, nondiabetic female 129SvEv mice had higher total AE than nondiabetic male 129SvEv mice (37.6 ± 7.0 vs. 13.2 ± 3.5 μg·g⁻¹·24 h⁻¹; P < 0.01). Baseline AE was similar in nondiabetic male and female mice from the C57BL/6, BALB/c, and DBA/2 strains.

In general, STZ-diabetes was associated with minimal albuminuria. In this regard, significant increases in albuminuria were only observed in two groups of diabetic animals [BALB/c males: 23.5 ± 10.3 vs. 133.3 ± 43.1 μg albumin·g⁻¹·24 h⁻¹; P = 0.02; DBA/2 males: 37.8 ± 9.5 vs. 130.0 ± 46.5 μg albumin·g⁻¹·24 h⁻¹; P = 0.04 (Fig. 5)].

Renal structure. The effect of STZ-diabetes on kidney weight was variable. Kidney weight in female mice was largely unaffected with one exception, the MRL/Mp female mice that received STZ (279 ± 6 vs. 233 ± 13 mg for left kidney weight, P = 0.01). In male mice, kidney weights tended to be lower in the diabetic animals compared with controls. For example, in the BALB/c, DBA/2, and 129SvEv diabetic males, the left kidney was significantly smaller than the left kidney in the nondiabetic controls (see Table 4).

On evaluation by light microscopy, kidneys of diabetic animals appeared largely normal. The main abnormality noted was modest expansion of the glomerular mesangium. Representative glomeruli, scored from zero to two (scale 0–3), from animals in this study are shown in Fig. 6. Tubular structure was normal except for glycogen nuclei, which are commonly seen in mice with glycosuria. In general, neither interstitial expansion nor vascular changes were observed. However, in kidneys of three of the male diabetic DBA/2/J mice, pathological changes of acute pyelonephritis were seen, including polymorphonuclear infiltration in the tubular lumina and interstitium, which advanced in some foci to frank abscess formation.

Mean mesangial scores for the diabetic and control groups are presented in Table 4. For the pooled groups, mesangial volumes were increased in STZ-treated mice compared with control mice for all strains except MRL/Mp. The greatest absolute increase in mesangial score between control and diabetic mice was seen in the DBA/2 male mice (0.47 ± 0.08 vs. 1.29 ± 0.16; P < 0.005). As discussed above, these mice also had the most marked hyperglycemia and the highest levels of proteinuria. Control wild-type C57BL/6 mice had the lowest average mesangial score among all of the strains studied (0.26 ± 0.04), yet mesangial scores increased more than twofold in the presence of diabetes (0.66 ± 0.09; P = 0.0007).

As with the other parameters that we measured, there were gender differences in the baseline mesangial measurement for the BALB/c strain. For 129SvEv mice, gender differences between males and females developed only after development of STZ diabetes. Mesangial scores correlated with final blood glucose values in all mice with STZ diabetes (Fig. 7). Finally, glomerular volumes tended to be modestly higher in the animals with STZ diabetes, and these differences achieved statistical significance in some of the strains (Table 4).

Genetic vs. chemical diabetes. To examine potential differences in kidney responses in genetic and chemical models of type I diabetes on one inbred background, we compared STZ-treated C57BL/6 animals with C57BL/6 mice heterozygous for the C96Y mutation of the insulin 2 (Ins2) gene. This
mutation causes abnormal processing of insulin, resulting in islet cell dysfunction and death (47). C57BL/6-Ins2Akita (or Akita) mice spontaneously develop profound hyperglycemia in the range that we observed in the strains with vigorous responses to STZ (Fig. 8). As in the STZ-treated mice, there was a significant gender difference in the severity of hyperglycemia between male and female C57BL/6-Ins2+/C96Y mice. However, unlike many of the STZ-treated strains, the magnitude of the blood glucose difference was maintained between males and females throughout the period of study. Compared with C57BL/6 mice that received STZ, the blood glucose values were significantly higher in the male C57BL/6-Ins2+/C96Y mice at 6 mo of age (518 ± 25 vs. 388 ± 50 mg/dl; P = 0.04). Despite their marked and persistent hyperglycemia, male C57BL/6-Ins2+/C96Y mice appeared healthy, and their body weights increased during the period of study (26 ± 0.7 vs. 27 ± 0.6 g; P = 0.35 compared with wild-type controls).

Blood pressure responses also differed between male C57BL/6 mice treated with STZ and male C57BL/6-Ins2+/C96Y animals (Fig. 9). Although C57BL/6 male mice with chemical diabetes tended to have reduced blood pressures, the male C57BL/6-Ins2+/C96Y animals had higher blood pressures (112 ± 4 vs. 103 ± 2 mmHg; P = 0.04) and lower heart rates (595 ± 13 vs. 666 ± 8 beats/min; P < 0.001) than their wild-type littermate controls. On the C57BL/6 background, male mice with the Ins2+/C96Y mutation developed more albuminuria than male mice treated with STZ (AE: 45.2 ± 3.2 vs. 24.9 ± 6.4 µg·30 g body wt−1·24 h−1; P = 0.02; Fig. 10). In contrast to mice with chemical diabetes, Akita male mice developed significant renal hypertrophy compared with wild-type controls (217 ± 12 vs. 162 ± 4 mg for left kidney weight; P < 0.001). Similarly, glomerular volumes increased in diabetic Akita male mice; whereas, in STZ diabetes, glomerular volumes actually decreased in some groups (Table 4), including the markedly hyperglycemic DBA/2 males. Mesangial scores, which increased significantly in Akita male mice compared with wild-type littersmates, also tended to be higher in male C57BL/6-Ins2+/C96Y mice compared with STZ-treated C57BL/6 males, but this difference did not achieve statistical significance (0.94 ± 0.06 vs. 0.49 ± 0.07; P = 0.19). However, the degree of mesangial expansion was much more consistent in the C57BL/6-Ins2+/C96Y mice compared with STZ-treated C57BL/6 mice with a mean SE of only 6% in the genetic model compared with 26% in STZ-treated C57BL/6 male mice. Like the STZ-treated C57BL/6 mice, the Akita males had more mesangial expansion than the Akita females (0.94 ± 0.06 vs. 0.49 ± 0.07; P = 0.0005).

**DISCUSSION**

To examine susceptibility to diabetes-associated renal injury, we have studied the course of chemical diabetes in several inbred strains of mice, using a standardized, low-dose STZ protocol. We selected five common laboratory mouse strains on their demonstrated utility in disease modeling. C57BL/6 mice are among the most widely used inbred mouse strains and have a propensity to develop obesity, type II

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**Table 3. Urinary albumin excretion**

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Control 24-Hour Urine Volume, ml</th>
<th>Diabetic 24-Hour Urine Volume, ml</th>
<th>Control 24-Hour Albumin Excretion, µg/30 g body wt⁻¹·day⁻¹</th>
<th>Diabetic 24-Hour Albumin Excretion, µg/30 g body wt⁻¹·day⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>12</td>
<td>1.5 ± 0.3</td>
<td>5.2 ± 1.2</td>
<td>19.9 ± 3.3</td>
<td>26.3 ± 3.8</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>2.1 ± 0.5</td>
<td>5.6 ± 2.2</td>
<td>19.9 ± 4.6</td>
<td>24.9 ± 6.4</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>1.0 ± 0.2</td>
<td>4.8 ± 1.2*</td>
<td>19.9 ± 5.1</td>
<td>27.3 ± 4.6</td>
</tr>
<tr>
<td>MRL/Mp</td>
<td>14</td>
<td>1.6 ± 0.2</td>
<td>3.1 ± 1.5</td>
<td>72.2 ± 26.6</td>
<td>53.5 ± 17.5</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>2.3 ± 0.2*</td>
<td>4.9 ± 2.6</td>
<td>122.0 ± 47.4†</td>
<td>93.1 ± 24.5‡</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>12.1 ± 2.7</td>
<td>13.9 ± 3.5</td>
</tr>
<tr>
<td>BALB/c</td>
<td>14</td>
<td>1.1 ± 0.2</td>
<td>2.4 ± 0.7</td>
<td>38.6 ± 8.2</td>
<td>100.8 ± 20.4</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>0.7 ± 0.2</td>
<td>3.5 ± 1.6</td>
<td>23.5 ± 10.3</td>
<td>133.3 ± 43.1*</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>1.4 ± 0.3*</td>
<td>1.4 ± 0.1</td>
<td>51.6 ± 13.2</td>
<td>68.4 ± 13.2</td>
</tr>
<tr>
<td>DBA/2</td>
<td>15</td>
<td>1.6 ± 0.2</td>
<td>4.0 ± 1.7</td>
<td>41.2 ± 6.2</td>
<td>59.4 ± 22</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>1.4 ± 0.2</td>
<td>7.9 ± 3.7</td>
<td>37.8 ± 9.5</td>
<td>130.0 ± 46.5‡</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.1</td>
<td>44.2 ± 8.7</td>
<td>19.0 ± 4.6*</td>
</tr>
<tr>
<td>129SvEv</td>
<td>14</td>
<td>1.3 ± 0.1</td>
<td>1.9 ± 0.5</td>
<td>25.4 ± 5.1</td>
<td>30.6 ± 5.1</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>1.1 ± 0.2</td>
<td>2.3 ± 1.0</td>
<td>13.2 ± 3.5</td>
<td>28.2 ± 7.0</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>1.4 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>37.6 ± 7.0</td>
<td>32.9 ± 7.8</td>
</tr>
<tr>
<td>C57BL/6-Ins2ΔIns2/Akita</td>
<td>14</td>
<td>1.1 ± 0.1</td>
<td>9.5 ± 2.9</td>
<td>14.3 ± 1.6</td>
<td>25.7 ± 5.2</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>1.2 ± 0.2</td>
<td>21.2 ± 1.9‡</td>
<td>15.7 ± 2.5</td>
<td>45.2 ± 3.2‡</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>1.1 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>13.3 ± 2.2</td>
<td>11.0 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. *P < 0.03 between diabetic and nondiabetic mice. †P < 0.03 between nondiabetic male and nondiabetic female mice. ‡P < 0.04 between diabetic male and diabetic female mice.
diabetes mellitus, and atherosclerosis (3, 23, 31, 34, 36). MRL/Mp mice have heightened wound healing and a tendency to develop autoimmune disorders (12). BALB/c mice are commonly used in cancer research and have altered inflammatory responses (45). DBA/2 mice, frequently used in cardiovascular research, are susceptible to cochlear and ocular pathology and are resistant to developing atherosclerosis (34). Finally, we also studied 129/SvEv mice, which are the source for most of the embryonic stem cell lines used to generate mice with targeted genetic alterations (42).

To induce diabetes, we used a low-dose STZ protocol (30) to avoid severe hyperglycemia requiring exogenous insulin and to minimize STZ toxicity in other organs. Because of previous reports suggesting that mice treated with certain STZ regimens recover some degree of islet cell function (20, 38), we administered a second course of STZ after 8 wk. Although this regimen generally caused durable hyperglycemia throughout the period of study, there was waning of hyperglycemia in some of the groups, most notably the MRL/Mp females. Among the five stains of laboratory mice, we found significant differences in hyperglycemic responses to this standardized regimen of STZ dosing. Strain differences in the hyperglycemic response to STZ have been reported previously, and they have been variously attributed to differences in the immune response to STZ (38, 46).

Across the strains, our studies show that the severity of albuminuria, as reflected by the 24-h AE, and glomerular mesangial expansion are both proportional to the blood glucose level. Thus these strain differences in susceptibility to STZ will have a major impact on the development of diabetic renal complications. Accordingly, the high responder strains, especially DBA/2, may be the most useful for developing models of DN. In the low responder strains, higher and/or more frequent doses of STZ may be required to achieve more robust hyperglycemia. However, as discussed above, higher doses of STZ may impart greater toxicity and an attendant need for exogenous insulin administration that may complicate model development.

Along with these strain differences, there were marked gender differences in blood glucose responses to STZ in each of the strains that we tested. Females were very resistant to the development of diabetic nephropathy. Differential responses to STZ

Table 4. Renal structure

<table>
<thead>
<tr>
<th>Strain</th>
<th>n</th>
<th>Left Kidney Weight, mg</th>
<th>Glomerular Volume</th>
<th>Mesangial Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Diabetic</td>
<td>Control</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>0.20±0.01</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>MRL/Mp</td>
<td>14</td>
<td>299±21</td>
<td>300±9</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>365±19†</td>
<td>317±14‡</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>233±13</td>
<td>279±6*</td>
<td>0.35±0.02†</td>
</tr>
<tr>
<td>BALB/c</td>
<td>14</td>
<td>215±16</td>
<td>198±13</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>274±2‡</td>
<td>239±11*‡</td>
<td>0.35±0.01†</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>164±7</td>
<td>158±7</td>
<td>0.23±0.005</td>
</tr>
<tr>
<td>DBA/2</td>
<td>15</td>
<td>238±19</td>
<td>212±7</td>
<td>0.30±0.01</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>306±10†</td>
<td>229±13‡</td>
<td>0.32±0.02</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>179±12</td>
<td>200±2</td>
<td>0.30±0.02</td>
</tr>
<tr>
<td>129/SvEv</td>
<td>14</td>
<td>174±9</td>
<td>159±7</td>
<td>0.21±0.01</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>202±5†</td>
<td>184±5*</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>146±6</td>
<td>135±4</td>
<td>0.20±0.009</td>
</tr>
<tr>
<td>C57BL/6-Ins2Akita</td>
<td>14</td>
<td>147±7</td>
<td>178±16</td>
<td>0.23±0.01</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>162±4†</td>
<td>217±12‡</td>
<td>0.26±0.01†</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>130±9</td>
<td>139±18</td>
<td>0.20±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. NA, not available. *P < 0.05 between nondiabetic and diabetic animals. †P < 0.013 between nondiabetic males and nondiabetic females of same strain. ‡P < 0.04 between diabetic males and diabetic females of same strain.
between males and females have been described previously (35, 40) and have been attributed to hormonal effects. These differences are not specific for the STZ model, since blood glucose levels were also significantly lower in female compared with male Akita mice. By contrast, in other models, such as the nonobese diabetic mouse, a model of autoimmune type I diabetes, disease is more severe in females (13). Nonetheless, because the severity of diabetic complications is proportional to the degree of hyperglycemia, the utility of female mice will be limited in this low-dose STZ protocol and in the Akita model of DN.

Hypertension often contributes to the kidney injury in DN, and tight blood pressure control can slow the progression of DN. Consistent with their resistance to hyperglycemia, blood pressures were not significantly altered by STZ treatment in females. In contrast, blood pressures generally tended to be lower in male diabetic animals compared with nondiabetic controls. This reduction in blood pressure was particularly marked in the STZ-treated DBA/2 males that also had the most profound hyperglycemia. Twenty-four hour urine volumes (Table 3) were increased for all diabetic male mice and were predicted by their final blood glucose values ($R^2 = 66\%$; $P < 0.005$). Therefore, we hypothesize that osmotic diuresis and consequent volume depletion may contribute to reduced blood pressures in this circumstance. In contrast, blood pressures increased significantly in MRL/Mp diabetic male mice. Although STZ treatment tended to lower blood pressures in C57BL/6 males, blood pressures were also increased in male C57BL/6-Ins2C96Y mice despite their significantly higher urine volumes. Although the mechanisms underlying the different blood pressure responses in these strains are unknown, the MRL/Mp background and the Akita line may offer advantages for modeling hypertension associated with diabetes.

The development of albuminuria is the earliest detectable sign of kidney involvement in humans with diabetes. Thus we have monitored urinary AE in this study as an index of renal injury, and we used 24-h AE as the “gold standard” for proteinuria in this study. Because 24-h urine collections can be cumbersome, we also measured the ACR in all animals, since...
this can be measured in random urine collections without the
to housing animals in metabolic cages. We found a
significant linear correlation between 24-h AE and ACR.
Significant increases in albuminuria were observed in male,
but not female mice, in some of the strains (BALB/C and DBA/2),
and there was a significant correlation between ACR and blood
levels. Compared with STZ treatment, the Ins2+/C96Y
mutation caused more proteinuria, perhaps reflecting the higher
levels, consistency, and longer duration of hyperglycemia in
these animals. Moreover, among the STZ-treated groups, the
absolute magnitude of albuminuria was greatest in the male
DBA/2 animals that also had the most severe hyperglycemia.
Yet, even these levels were relatively modest compared with
the marked albuminuria observed in humans with overt DN.
Nonetheless, it is possible that proteinuria might be enhanced
by increasing the duration of diabetes and/or superimposing
mutations that accelerate the pace and intensity of kidney
injury.

Consistent with their relatively modest levels of albuminuria,
STZ-treated mice developed only mild mesangial expansion
without other notable structural changes on examination
by light microscopy. This would correspond to the earliest
pathological changes seen in patients with diabetes and microalbuminuria (16, 18). We found no evidence of more
advanced lesions, such as nodular sclerosis, that are characteristic
of overt DN in humans. There were strain-dependent
differences in mesangial volumes at baseline, these differences
were subtle, and there was no direct association between
baseline mesangial volume and degree of expansion with
diabetes. Although there was very little change in mesangial
volumes in diabetic female mice, most STZ-treated male mice
had increases in their mesangial volumes, consistent with their
greater hyperglycemia. However, the extent of mesangial
expansion, even in the most extreme cases, was relatively mod-
est. Once again, DBA/2 mice, which experienced the most
significant hyperglycemia, also had the most marked mesangial
expansion. Similarly, in another model of diabetes using the
D-variant of encephalomyocarditis virus, DBA/2 mice developed
long-lasting hyperglycemia and renal lesions consisting
of mesangial expansion (17). In two of the diabetic male
DBA/2 mice, there was evidence of pyelonephritis, which was
not observed in any other animals. Similar to the relationship
that we observed with proteinuria, there was a significant
correlation between mesangial area and blood glucose levels in
diabetic mice, suggesting a causation association, but the level of
blood glucose could only explain -15% of the variance in this
parameter. Of note, STZ diabetic mice exhibited minimal
glomerular hypertrophy, with glomeruli in diabetic mice aver-
gaging only ~10% larger than those in controls. Our study does
not clearly identify strong strain effects on susceptibility to
renal pathology beyond those explained by differences in
severity of hyperglycemia. However, as discussed above, it is
possible that the relatively short duration of this study may
have limited the severity of renal pathology.

The Ins2+/C96Y Akita mouse model is a genetic model of
induced diabetic males of their parental strain (C57BL/6),
Ins2+/C96Y male mice developed hyperglycemia that was more
pronounced and durable than that observed in the STZ model.
This was associated with higher levels of urine AE, and,
although there was a numerical increase in mesangial scores,
this did not achieve statistical significance with the relatively
small number of animals that were evaluated. Nonetheless, the
extent of these pathological changes was much more consistent
in the Akita animals. Despite their more substantial hypergly-
cemia, body weights were unaffected in the male Akita mice.
Moreover, unlike the STZ-treated animals that tended to have
reduced blood pressures, diabetic Ins2+/C96Y mice develop
hypertension, which is characteristic of humans with DN.
Finally, Akita mice developed renal hypertrophy and increased
glomerular volume, but neither of these abnormalities was seen
in STZ-treated mice. These features of the model suggest that
it may provide a better platform for model development.
Furthermore, the Ins2+/C96Y mutation can be easily inter-
crossed on other transgenic mouse lines to facilitate model
development.

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committee of the AMDCC.

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GENETIC BACKGROUND AND NEPHROPATHY IN DIABETIC MICE


