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Augmenting podocyte injury promotes advanced diabetic kidney disease in Akita mice

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ABSTRACT

To determine if augmenting podocyte injury promotes the development of advanced diabetic nephropathy (DN), we created mice that expressed the enzyme cytosine deaminase (CD) specifically in podocytes of diabetic Akita mice (Akita-CD mice). In these mice, treatment with the prodrug 5-flucytosine (5-FC) causes podocyte injury as a result of conversion to the toxic metabolite 5-fluorouracil (5-FU). We found that treatment of 4–5 week old Akita mice with 5-FC for 5 days caused robust albuminuria at 16 and 20 weeks of age compared to 5-FC treated Akita controls, which do not express CD (Akita CTLs). By 20 weeks of age, there was a significant increase in mesangial expansion in Akita-CD mice compared to Akita CTLs, which was associated with a variable increase in glomerular basement membrane (GBM) width and interstitial fibrosis. At 20 weeks of age, podocyte number was similarly reduced in both groups of Akita mice, and was inversely correlated with the albuminuria and mesangial expansion. Thus, enhancing podocyte injury early in the disease process promotes the development of prominent mesangial expansion, interstitial fibrosis, increased GBM thickness and robust albuminuria. These data suggest that podocytes play a key role in the development of advanced features of diabetic kidney disease.

1. Introduction

Diabetic nephropathy (DN) is a serious complication of diabetes mellitus [1]. The economic consequences of this disorder are significant because the incidence of both diabetes mellitus and DN has reached epidemic proportions in developed countries [2,3]. Indeed, DN is the most common cause of end-stage renal disease (ESRD) in the United States [1]. While current strategies slow disease progression [4,5], approximately 20–30% of patients with diabetes ultimately develop ESRD requiring renal replacement therapy [3]. As a result, much effort has been devoted to understanding the mechanisms that promote glomerular damage in diabetic kidney disease with the hope of identifying new therapeutic targets and treatment strategies.

While mesangial cells were the initial focus of research into the molecular mechanisms of DN, more recent studies have concentrated on glomerular podocytes in disease pathogenesis [6]. These highly differentiated cells are important for maintaining the integrity of the glomerular filtration barrier [7,8]. In diabetes, podocyte injury is a common feature of both experimental and human diabetic kidney disease [6,7]. For example, foot process widening and loss of glomerular nephrin expression are observed in the early stages of diabetic kidney disease [6,7]. In the later stages of the disease, a reduction in the number of glomerular podocytes is characteristic of both human diabetic nephropathy and animal models of diabetic kidney disease [9–11]. Because podocytes are terminally differentiated cells with a limited capacity for replication [8,12], sufficient loss of podocytes leads to instability of the glomerular tuft and glomerulosclerosis [8]. In support of this hypothesis, urinary albumin excretion rates correlate negatively with podocyte number in patients with type 1 diabetes mellitus [11]. Similarly, podocyte number is a strong predictor of progressive renal disease in diabetic Pima Indians with microalbuminuria [10]. Podocyte injury might, therefore, promote the development of

Abbreviations: DN, diabetic nephropathy; ESRD, end-stage renal disease; SBP, systolic blood pressure; H&E, hematoxylin and eosin; PAS, periodic acid Schiff; SEM, error of the mean; ANOVA, one way analysis of variance; tetO, tet operator sequence; PminCMV, minimal CMV promoter; BP, blood pressure; CTLs, controls; UAE, urinary albumin excretion; CD, cytosine deaminase; Nv(P/Glom), podocyte density; N(P,Glom), podocytes per glomerulus; Vglom, glomerular volume; TEM, transmission electron microscopy; GBM, glomerular basement membrane; TG, transgenic; rtTA, reverse tetracycline transactivator; tTA, tetracycline transactivator; GFR, glomerular filtration rate.

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the characteristic functional and histopathologic features of both type 1 and type 2 diabetic kidney disease.

To investigate the role of glomerular podocytes in the pathogenesis of DN, we examined the effect of augmenting podocyte injury on the severity of diabetic kidney disease using the FVB/NJ Akita model of diabetes mellitus [13] and transgenic (TG) mice developed in our laboratory [14]. Akita mice are a genetic model of type 1 diabetes mellitus commonly utilized to study diabetic kidney disease [13,15,16]. These mice develop sustained and durable hyperglycemia associated with early features of DN in humans [13,15,16]. To selectively induce podocyte injury, we crossed Akita mice with TG mice expressing the yeast enzyme CD specifically in glomerular podocytes [14]. CD catalyzes the conversion of the prodrug 5-FC to 5-FU [17], a metabolite that inhibits both DNA and RNA synthesis and promotes death in cells that are not actively dividing. After targeting CD to podocytes, we selectively enhanced podocyte injury by treating mice with a short course of 5-FC. We found that augmenting podocyte injury at the onset of hyperglycemia promoted the development of some features of advanced diabetic kidney disease later in the disease process. These data support the notion that podocyte injury plays a critical role in the pathogenesis of diabetic kidney disease.

2. Materials and methods

See Supplementary data.

3. Results

3.1. Development of the diabetic CD model

In previous studies [14], we developed a doxycycline inducible approach to target expression of CD to renal or extrarenal tissues using available Tet-On or Tet-Off mice [18]. For podocyte specific expression, two TG mice are required. The first TG animal expresses the rtTA under the control of the human podocin promoter [19]. The second TG mouse expresses CD under the control of tet operator sequence (tetO) and a minimal CMV promoter (PminCMV) [18]. By breeding the two TG mice, animals are obtained that express both transgenes. In these "double" TG mice, treatment with doxycycline induces CD expression. For the studies, we utilized "double" TG Akita mice, which express CD in the presence of doxycycline (Akita CD mice) as well as "single" TG and non-TG Akita controls (Akita CTLs), which do not express CD in the presence of doxycycline. Additional CTLs included wild type "single" TG and non-TG mice. At 4 weeks of age, mice were treated with doxycycline for 1 week and then received 5 doses of 500 mg/kg 5-FC for 5 consecutive days while the doxycycline was continued, as previously described [14]. Mice were then studied as outlined in the Section 2.

3.2. Effect of the diabetes on blood glucose levels, kidney weight, systemic BP and heart weight

Hyperglycemia and systemic blood pressure are important determinants of the severity of kidney disease in diabetes mellitus [3]. We, therefore, first examined blood glucose levels in the Akita mice. As shown in Fig. 1A, blood glucose levels were similarly elevated in Akita-CD mice and Akita CTLs at 12, 16 and 20 weeks of age. Hyperglycemia was associated with a significant and similar increase in urine output in both groups of Akita mice (Supplementary Fig. 1A). We next evaluated the diabetic milieu on systemic blood pressure (BP), heart weight and kidney weight. As shown in Fig. 1B, BP tended to be elevated in Akita-CD mice and Akita CTLs compared to CTLs at 12- and 20-weeks of age, but these differences

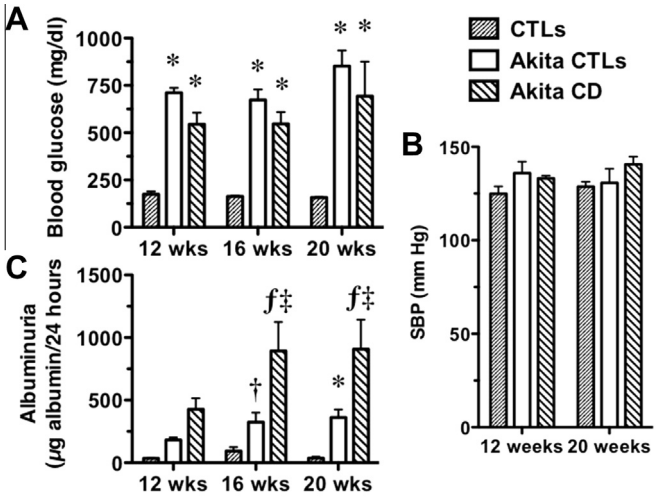

Fig. 1. Fasting blood glucose levels, systolic BP (SBP) and urinary albumin excretion (UAE) in Akita mice. (A) Akita CD and Akita CTLs mice developed sustained hyperglycemia, which was significantly increased in both groups of Akita amice compared to CTLs and persisted throughout the duration of the study. (B) SBP tended to be elevated in both groups of Akita mice compared to controls at 12 and 20 weeks of age but these differences were not statistically significant. (C) UAE was significantly elevated in both groups of Akita mice compared with CTL animals at 16 and 20 weeks of age. UAE was also elevated in Akita CD mice compared to Akita CTLs at 16 and 20 weeks of age. Eight Akita CTLs, 9 CTLs and 5–6 Akita CD mice were studied (one mouse died during the study). $^{*}P < 0.05$, $^{\dagger}P < 0.01$ $^{f}P < 0.005$ vs CTLs, $^{\ddagger}P < 0.01$ vs Akita CTLs.

did not reach statistical significance. Heart weight was also increased in both groups of Akita mice compared to CTLs at 20 weeks of age, but these differences were only significant for the Akita CTLs compared to CTL animals (Supplementary Fig. 1B). Lastly, consistent with the known effects of the diabetic milieu on kidney hypertrophy, kidney weight was similarly enhanced in both Akita-CD mice and Akita CTLs compared to CTL animals at 20 weeks of age (Supplementary Fig. 1C).

3.3. Effect of the diabetes on albuminuria and serum creatinine levels

Fig. 1C shows the effects of diabetes mellitus on urinary albumin excretion (UAE). UAE was not significantly different at the 12-week time point but, was significantly increased in both groups of Akita mice compared to CTLs at 16- and 20-weeks of age. Moreover, UAE was significantly increased in Akita CD mice compared to Akita CTLs at these same time points. The findings were similar when data was expressed as micrograms albumin per milligram creatinine (Table 1). To evaluate renal function, we measured serum creatinine levels as described in the Section 2. There was a modest but statistically insignificant increase in Akita CD mice (0.32 ± 0.08 mg/dl) and Akita CTLs (0.31 ± 0.11 mg/dl compared to CTL animals (0.20 ± 0.04 mg/dl).

Table 1
Urinary albumin excretion (μg albumin/mg creatinine).

	Age		
	12 weeks	16 weeks	20 weeks
CTLs	34 ± 2.9	51 ± 11	36 ± 11
Akita CTLs	262 ± 44	358 ± 86†	312 ± 67*
Akita CD	902 ± 406	1552 ± 944‡	1576 ± 137‡

* $P < 0.05$.
† $P < 0.01$ vs Akita CD.
‡ $P < 0.01$ vs CTLs.