Renin Promoter SV40 T-Antigen Transgenic Mouse
A Model of Primary Renal Vascular Hyperplasia

Howard J. Jacob, Curt D. Sigmund, Ty R. Shockley, Kenneth W. Gross, and Victor J. Dzau

Transgenic mice containing a ren-2 promoter T-antigen fusion construct (TAG+) develop renal vascular hypertrophy and hyperplasia associated with markedly suppressed renal renin mRNA, renal renin content, and plasma renin concentration. These animals are normotensive. In the present study, the renal and cardiovascular systems are characterized, revealing some surprising findings. Not only are the TAG+ mice normotensive in the face of pronounced renal pathology but also in the presence of an increase in plasma volume. These data raise interesting questions about blood pressure physiology and renal function of the TAG+ mice. Blood nitrogen urea of the TAG+ animal was markedly elevated and plasma creatinine level was in the normal range, indicating prerenal azotemia without renal failure. These findings are consistent with impaired renal perfusion with secondary volume expansion probably as the result of vascular hyperplasia. These transgenic animals provide a unique genetic model for studying the physiology of primarily renal vascular hyperplasia as well as blood pressure control in a low renin state. (Hypertension 1991;17:1167-1172)

Transgenic technology is a powerful tool that can be used to dissect complex physiological processes. It provides the opportunities to examine the integrated physiological consequences of an altered gene or of a normal gene whose expression is suppressed or stimulated. In hypertension, the transgenic approach has resulted in the production of several unique animal models. For example, Mullins et al reported the production of a transgenic rat carrying a ren-2 gene that developed fulminant hypertension. Recently, we obtained a transgenic mouse model of primary diffuse hyperplasia of the intrarenal arteries and arterioles that developed as a result of the expression of a transgene of the SV40 tumor (T) antigen under the control of the regulatory region of the mouse ren-2 gene. We demonstrated that these TAG+ transgenic animals expressed large T-antigen appropriately. Many of the renin-expressing tissues in these mice exhibited hyperplasia or overt neoplasia that had enabled us to establish a renin-expressing cell line. Of interest, the TAG+ mice developed diffuse primary renal vascular hyperplasia/hypertrophy that appeared to encroach on the vessel lumen (see Figure 1). These animals may represent a novel model for the studies of the pathophysiology of renal vascular hyperplasia/hypertrophy and the effect on blood pressure. In this study, we report the initial characterization of the cardiovascular and renal status of these transgenic animals.

Methods

Production of Transgenic Mice

Construction of the transgene and production of the transgenic mice have been described previously. Briefly, the 5' flanking region containing the regulatory sequences, transgene, or construct contains 4.6 kb of the ren-2 gene spliced to the SV40 T-antigen structural gene. Approximately 100 copies of this construct were microinjected into the male pronucleus of a fertilized one cell embryo using a standard method. The embryos were derived from C57BL/10Ros×C3H/HeR (BCF). Transgenic mice were crossed with nontransgenic mice to maintain heterozygosity for the construct.
Figure 1. Photomicrograph of kidneys from TAG– (littermates not carrying transgene) and TAG+ mice (mice carrying the transgene), which were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Panel A: Normal arcuate artery from TAG– mouse. Note single layer of endothelium and 1–2 layers of concentric smooth muscle cells. Panel B: Arcuate artery from TAG+ mouse exhibiting thickening of the arterial wall caused by hyperplasia of medial smooth muscle cells. Severity of this lesion is classified as mild to moderate. More severe lesions (not shown) lead to partial or complete occlusion of the arteriolar lumen.

Southern blot analysis of genomic DNA7 from tail biopsies was used to identify the mice conveying the transgene. Littermates that did not express the T-antigen (TAG–) served as the controls. Genetic and pathological analysis was carried out by investigators (C.S., K.G.) at one site and the physiology experiments and biochemical characterization were performed at another site by investigators (H.J., T.S., V.J.D.) who were blinded to the genetic identities of the mice.

Blood Pressure Determination

Blood pressure was measured in conscious, freely-moving mice using a catheter placed in the abdominal aorta via the femoral artery. For implantation of the catheter, mice were initially anesthetized with ether, followed by an intraperitoneal injection of pentobarbital (30 mg/kg). After the surgery, the mice were allowed to recover for 2 days. On the day of the experiment, arterial pressure was measured using a Cobe CDIIIX (Cobe CV Division, Lakewood, Colo.) transducer connected to a polygraph (Grass Instrument Co., Quincy, Mass.). Mean arterial pressure (MAP) was recorded for 10 minutes. The average MAP was calculated from 10 measurements sampled at 1-minute intervals. After determining the MAP, mice were anesthetized with ether. Blood was collected into tubes containing EDTA. The kidneys were harvested and immediately snap frozen in liquid nitrogen. Plasma was collected after centrifugation. The kidneys and plasma were stored at −70°C.

Plasma Renin Concentration

A radioimmunoassay (RIA) was used to measure PRC. Briefly, this assay measures the amount of angiotensin I generated by plasma renin incubated at pH 7.4 for 1 hour with the plasma of anephric sheep as a source of excess exogenous substrate as described.8 Whole plasma from TAG+ mice and diluted (1:100) plasma from the TAG– mice were incubated with the substrate.

Renal Renin Concentration

One kidney from each mouse was assayed for renin enzymatic activity.9 Each kidney was homogenized in 0.1 M Tris, pH 7.4, 4°C that contained 0.25% Triton X-100, 0.5 mM EDTA, 0.5 mM sodium tetraphosphate, and 0.1 mM phenylmethylsulfonylfluoride, using a sonicator (Heat Systems Ultrasonic Inc., Farmingdale, N.Y.). The diluted (1:100 to 1:1,000) homogenate was then incubated with plasma from anephric sheep as described above.

Renin Messenger RNA Levels

The contralateral kidney (not used for RRC measurement) was used to measure total renin mRNA. Briefly, each kidney was homogenized in guanidine isothiocyanate, and the mRNA was isolated after centrifugation through a cesium chloride pad.10 Renin mRNA levels were determined by Northern blot analysis10 using an α-32P-GTP antisense probe for renin. The mouse ren-2 cDNA was used as the probe.2-4 A probe for chicken B-actin homologous for total mouse actin was used as a control for equal loading of the RNA.2-4 Renin mRNA was quantitated by densitometry of x-ray films using a Helana Quick-Scan (Helana Laboratories, Beaumont, Tex.). Arbitrary units reflecting the hybridization intensity were corrected for RNA loading by normalizing to total actin mRNA. All measurements were made with the linear range of the x-ray film.

Determination of Plasma Volume and Hematocrit

Plasma volume. Mice (male and female) 4–8 weeks old were used for this study. Plasma volume was estimated using bovine 125I-albumin.11 Approximately
Blood Pressure and Renin Status were injected into the tail veins of mice anesthetized with ether. Blood was collected from the orbit of the eye, using graduated microcapillary tubes (5 μl), that were heparin coated. Blood was collected 2 minutes after injection of the radiolabeled albumin. Microcapillary tubes were then centrifuged for 5 minutes and hematocrit measured (Microcapillary Reader, Dannon/IEC Div., Needham Heights, Mass.). The entire microcapillary tube containing both the plasma and red blood cells was measured for radioactive counts (cpm) with a gamma counter. Plasma volume was calculated as follows:

Plasma volume = \( \frac{100 - \text{hematocrit}/100 \times \text{radioactivity (cpm)injected}}{\text{radioactivity/μl blood sample}} \)

**Hematocrit.** Hematocrit was measured in mice aged 1 to 10 weeks. With the exception of the 1-week-old mice, hematocrit was measured as described above. The 1-week-old mice were anesthetized with ether, a laceration was made in the carotid region, and blood was collected.

**Plasma chemistries.** Blood urea nitrogen (BUN), creatinine, and Na⁺ were measured using an Astra-8 clinical analyzer (Beckman Instruments, Inc., Brea, Calif.). BUN was measured using the enzymatic conductivity rate method. Creatinine levels were measured using the Jaffe rate method. The concentration of Na⁺ was measured using an ion-sensitive electrode. These chemistries were performed on the plasma from 9- to 10-week-old TAG+ and TAG- mice.

**Statistics**

Linear regression analysis was first used to determine if there was a significant relation among plasma volume, hematocrit, and age. In addition, a t-test for independent samples was used to compare the pooled data for plasma volume from the different age groups of the TAG+ to the TAG- mice.

**Results**

**Blood Pressure and Renin Status**

At baseline, the conscious blood pressure did not differ between the TAG+ (113±5 mm Hg, n=5) and TAG- (122±4 mm Hg, n=7) animals. However, the renin mRNA and RRC were substantially lower in the TAG+ mice. In fact, the PRC was undetectable in these mice (Table 1). These results confirm our previous findings that TAG+ mice are normotensive and have a markedly suppressed renin-angiotensin system (RAS).

**Plasma Volume and Hematocrit**

On a normal Na⁺ diet, the average plasma volume of the TAG+ animals was significantly higher than that of the TAG- animals (79.1±6.7 versus 58.4±3.6 μl/g, n=6 and n=16, respectively, p<0.009) (Figure 2). For control TAG- mice, the plasma volume was not affected by the age. Interestingly, in the TAG+ mice the plasma volume tended to increase with age (p<0.13). TAG+ mice also exhibited a significantly lower hematocrit as compared with the TAG- controls. TAG- mice showed an increase in hematocrit with age, as anticipated with the known developmental change in normal mice (Figure 3). In contrast, no correlation was seen between the hematocrit and age of the TAG+ animals.

**Renal Function**

Table 2 summarizes the mean value for BUN, serum creatinine, and serum sodium concentration of TAG- and TAG+ mice. As can be seen, the mean BUN of TAG+ animals was approximately fourfold higher than that of TAG- mice. In contrast, the serum creatinine and sodium concentrations were not significantly different between the two groups.

**Discussion**

TAG+ transgenic mice develop diffuse hyperplasia/hypertrophy of the intrarenal arteries and arterioles without other overt cardiovascular pathology. These animals are normotensive and exhibit suppression of renin mRNA, RRC, and PRC. These transgenic mice appear to represent a unique model of primary diffuse renal vascular hyperplasia from the interlobar artery to the afferent arterioles and provide a unique opportunity for studying the pathophysiological consequences of renal vascular hyperplasia. All previous experimental models of renal vascular diseases have concomitant systemic hypertension or renal ischemia. A major problem in hypertension research has been separating out the primary causes and secondary effects of hypertension. For example, it has been difficult to determine if renal vascular hyperplasia is secondary to hypertension or the cause of the hypertension.

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**Table 1. Measurement of Renin in TAG- and TAG+ Mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>PRC</th>
<th>RRC</th>
<th>mRNA density</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG- normal</td>
<td>1,189±505</td>
<td>10,255±1,527</td>
<td>12.8±3.0</td>
</tr>
<tr>
<td>TAG+ normal</td>
<td>ND</td>
<td>121±38*</td>
<td>0.83±0.02*</td>
</tr>
</tbody>
</table>

Values represent mean±SEM. Comparisons were made using an analysis of variance.

*p<0.05, compared with TAG- group. ND, nondetectable; PRC, plasma renin concentration; RRC, renal renin concentration; TAG+, mice carrying the transgene; TAG-, littermates not carrying the transgene.
ally, the RAS has always been activated in the presence of renal vascular hyperplasia.

In this transgenic model, primary renal vascular hyperplasia has been seen in all arteries examined. This was accompanied by the marked suppression of renin and a significant increase in BUN without an increase in creatinine. The exact mechanisms behind this marked prerenal azotemia is not known; however, the marked renal pathology suggests that renal perfusion may be impaired. Since these animals are not hypovolemic, dehydrated, or in cardiac or renal failure, the prerenal azotemia is consistent with the consequences of 1) diffuse anatomic narrowing of the renal vasculature encroaching on the lumen, thereby reducing blood flow and 2) an altered functional response of the renal vasculature (i.e., impaired vasorelaxation or enhanced vasoconstriction in response to vasoactive stimuli). The latter possibility is in accordance with Folkow's hypothesis and is particularly interesting since these animals have suppressed renin angiotensin activity.

The systemic blood pressure of these transgenic animals was normal despite the renal pathology and suppressed RAS. The physiology of blood pressure control in these transgenic mice is interesting and worthy of further investigation. The absence of hypotension in these mice with low renin state suggest that other mechanisms are responsible for the maintenance of blood pressure such as sympathetic nervous system or plasma volume expansion. Indeed, the transgenic animals exhibit an increase in plasma volume. It is equally interesting to note that these
Taken together these data suggest that renal vascular renin expression and that in these transgenic animals, the renin promoter activity of the transgene resulting from this possibility is the observation that the mean renin promoter activity of the transgene resulting from the transgene) were made using a r test for independent samples. Blood urea nitrogen (BUN) and creatinine are expressed as milligrams per deciliter (mg/dl).

transgenic animals are not hypertensive despite renal pathology and plasma volume expansion. This observation provides a good opportunity to test the hypothesis that renal vascular function/structure is essential for the development of hypertension. The complete suppression of PRC (to undetectable levels) may provide a partial explanation for this finding. Indeed, Kreiger et al demonstrated recently that a permissive level of angiotensin is necessary for hypertension to develop during volume expansion. In addition, whether endogenous antihypertensive mechanisms such as atrial natriuretic peptide, renal medullipin, and prostaglandins are activated in these animals remains to be determined.

The findings of low renin mRNA level, RRC, and PRC in these transgenic animals are intriguing; furthermore, we have demonstrated that captopril treatment failed to stimulate the RAS in these mice. These data can be interpreted to be the result of secondary suppression of renin expression as the consequence of plasma volume expansion. Consistent with this possibility is the observation that the mean plasma volume is increased in TAG+ animals. On the other hand, renin expression is suppressed in the adult TAG+ mice but is normal in the neonatal TAG+ mice. Therefore, another possible explanation is that the expression of SV40 T-antigen (driven by the renin promoter) in the developing smooth muscle cells of the renal vasculature represses the renin phenotype of these cells, although current evidence does not support this. Ontogenic studies have demonstrated that renin expression can be seen throughout the renal vasculature during the fetal development. With maturation, renin production becomes limited only to the juxtaglomerular cells at the vascular poles of the glomeruli. Renal ischemia or prolonged sodium depletion plus angiotensin converting enzyme inhibition can recapitulate the fetal pattern of renin expression in the renal vasculature (i.e., the recruitment of renin-producing cells throughout the renal arteries and arterioles).

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The basis of plasma volume expansion is unclear. A likely explanation is that the pregglomerular vasoconstriction as suggested by prerenal azotemia leads to reduced peritubular capillary hydrostatic pressure and enhanced proximal tubular sodium reabsorption and consequently plasma volume expansion.

Another interesting finding is the markedly lower hematocrit of the TAG+ mice as compared with TAG− mice. In the normal developing mouse, hematocrit increases with age and tends to plateau at about 21 days of age. Indeed, this phenomenon is seen in our TAG− control mice. Interestingly, the TAG+ animals did not exhibit this pattern. The reduced hematocrit suggests either hemodilution due to plasma volume expansion or a primary abnormality in erythropoiesis. Since erythropoietin is synthesized by the kidney, it is intriguing to speculate that the renal vascular pathology may in some way result in the impairment of erythropoietin activation. Further research on this issue, including the measurement of erythropoietin levels in these mice, is clearly indicated. Finally, blood loss or hemolysis as the reasons for anemia have not been excluded although these are unlikely causes.

In summary, the renin promoter SV40 T-antigen transgenic mouse is a model of primary renal vascular hyperplasia/hypertrophy. These mice are characterized by normal blood pressure, elevated BUN, normal serum creatinine concentration, suppressed renin, expanded plasma volume, and low hematocrit. Studies elucidating the pathophysiological sequence of events will be most informative for the understanding of the effects of renal vascular hyperplasia on cardiovascular and renal physiology. This model will also offer the opportunity to test the hypothesis of Folkow et al on the altered structural–functional relation of hyperplastic blood vessels in the pathogenesis of hypertension. This report represents the initial characterization of this novel animal model. Further detailed physiological studies are obviously important and are currently in progress.

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References


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**Table 2. Plasma Chemistries for TAG− and TAG+ Mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Na⁺ (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG−(n=7)</td>
<td>19.3±1.6</td>
<td>0.3±0.8</td>
<td>148±1.0</td>
</tr>
<tr>
<td>TAG+(n=7)</td>
<td>75±10.4*</td>
<td>0.4±0.13</td>
<td>148±2.4</td>
</tr>
<tr>
<td>Normal range</td>
<td>13–33</td>
<td>0.19–0.92</td>
<td>110–178</td>
</tr>
</tbody>
</table>

Values represent mean±SEM. Comparisons between TAG− (littermates not carrying the transgene) and TAG+ (mice carrying the transgene) were made using a t test for independent samples.

*p<0.00001.

*To whom correspondence should be addressed. Address correspondence to K. Ellsworth for technical assistance. We also thank Dr. Gauvin and Lisa M. Jacob of Mount Auburn Hospital for conducting the plasma chemistry assays; Chuanzhen Wu and Mary K. Ellsworth for technical assistance. We also thank Melinda Hing for secretarial assistance in preparing this manuscript.


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