Compensatory glomerular growth after unilateral nephrectomy is VEGF dependent

ALLAN FLYVBJERG,1 BIEKE F. SCHRIJVERS,1,2 AN S. DE VRIESE,2 RONALD G. TILTON,3 AND RUTH RASCH4

1Medical Department M, Medical Research Laboratories, Institute of Experimental Clinical Research and 4Department of Cell Biology, Institute of Anatomy, Aarhus University, DK-8000 Aarhus, Denmark; 2Renal Unit, Department of Internal Medicine, Ghent University Hospital, B-9000 Ghent, Belgium; and 3Department of Pharmacology, Texas Biotechnology Corporation, Houston, Texas 77030

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Flyvbjerg, Allan, Bieke F. Schrijvers, An S. De Vriese, Ronald G. Tilton, and Ruth Rasch. Compensatory glomerular growth after unilateral nephrectomy is VEGF dependent. Am J Physiol Endocrinol Metab 283:E362–E366, 2002. First published April 2, 2002; 10.1152/ajpendo.00007.2002.—Various growth factors and cytokines have been implicated in different forms of kidney enlargement. Vascular endothelial growth factor (VEGF) is essential for normal renal development and plays a role in diabetic glomerular enlargement. To explore a possible role for VEGF in compensatory renal changes after uninephrectomy, we examined the effect of a neutralizing VEGF-antibody (VEGF-Ab) on glomerular volume and kidney weight in mice treated for 7 days. Serum and kidney insulin-like growth factor I (IGF-I) levels were measured, since IGF-I has been implicated in the pathogenesis of compensatory renal growth, and VEGF has been suggested to be a downstream mediator of IGF-I. Placebo-treated uninephrectomized mice displayed an early transient increase in kidney IGF-I concentration and an increase in glomerular volume and kidney weight. In VEGF-Ab-treated uninephrectomized animals, increased glomerular volume was abolished, whereas renal hypertrophy was partially blocked. Furthermore, the renal effects of VEGF-Ab administration were seen without affecting the renal IGF-I levels. In conclusion, these results demonstrate that compensatory glomerular growth after uninephrectomy is VEGF dependent.

compensatory renal growth; glomerular volume; insulin-like growth factor I; kidney weight; vascular endothelial growth factor-antibody

VARIOUS GROWTH FACTORS and cytokines have been implicated in different forms of kidney enlargement, such as compensatory renal growth (CRG) after uninephrectomy (9) and renal growth induced by diabetes (7) and by high-protein diets (2, 28). Among these growth factors, growth hormone (GH), insulin-like growth factors (IGFs), and transforming growth factor-β (TGF-β) have been studied the most (2, 7–13), whereas vascular endothelial growth factor (VEGF) has been studied less intensively (5, 28). VEGF is important for normal growth and survival and organ development in neonatal mice (14). In addition, VEGF is essential for normal nephrogenesis and particularly glomerulogenesis (20). Furthermore, VEGF has been implicated in the pathogenesis of early glomerular hypertrophy in experimental diabetes (5). Recently, altered VEGF expression has been implicated in the remodeling and proliferation that occur in renal cortical peritubular capillaries after subtotal nephrectomy (25). Accordingly, the possible role of VEGF in CRG after uninephrectomy was examined in mice using a neutralizing murine VEGF-antibody (VEGF-Ab). Because IGF-I is a mediator of CRG (9) and a relationship between IGF-I and VEGF has been suggested (8, 30), we also examined the effect of VEGF-Ab administration on serum and kidney IGF-I levels.

MATERIALS AND METHODS

Animals. Adult female NMRI mice (Bomholtgaard, Ry, Denmark) with initial body weights of 25–30 g were used. The mice were housed 6–8/cage in a room with a 12:12-h artificial light cycle (7:00 AM to 7:00 PM), a temperature of 21 ± 1°C, and a humidity of 55 ± 5%. The animals had free access to standard chow (no. 1324; Altromin, Lage, Germany) and tap water throughout the experiment. The study complied with Danish regulations for the care and use of laboratory animals.

Study design. The mice were randomized into four groups. Two groups were subjected to a right side nephrectomy for placebo (NP) or VEGF-Ab treatment (NV). The third group was exposed to sham operation (S) by a flank incision with manipulation of the kidney, and the fourth group served as a nonoperated control group (C). All operations were performed under anesthesia with avertin (240 mg/kg body wt ip). One-half of the uninephrectomized mice was treated with intraperitoneal injections of a neutralizing VEGF-Ab. The other one-half, and the sham-operated group, were injected with isotype-matched irrelevant IgG. Animals were injected...
profiles (the capillary tuft omitting the proximal tubular tissue and the Bowman capsule). $V_G$ was calculated as

$$V_G = \frac{\beta}{k} \times (A_G)^{3/2}$$

where $\beta = 1.38$, which is the shape coefficient for spheres (the idealized shape of glomeruli), and $k = 1.1$, which is a size distribution coefficient (24, 32).

**Statistical analysis.** ANOVA for repeated measurements was used to evaluate differences with Student’s $t$-test for unpaired comparisons. A $P$ value $<0.05$ was considered statistically significant. For data not following a normal distribution, the Mann-Whitney Rank Sum test was used. All data are expressed as means $\pm$ SE, with $n$ indicating the number of mice studied. Statistics were performed by the statistical package SPSS for Windows.

**RESULTS**

**Body weight and food consumption.** Body weight and food intake are given in Table 1. No differences in body weight were seen between the different groups on days 2 and 7. Food consumption, presented as the mean value of days 0–2 and days 3–7, respectively, did not differ significantly in any of the groups.

**Kidney weight and glomerular volume and liver weight.** Mean left kidney weight is given in Fig. 1. Sham-operated mice had mean kidney weights at days 2 and 7 that were not different from the control group at day 2 (Fig. 1). The NP group showed an increase in kidney weight of 26% at day 2, and of 21% at day 7 compared with S at days 2 and 7, respectively. At day 2, the VEGF-Ab-treated group showed no renal enlargement compared with S. VEGF-Ab treatment for 7 days tended to reduce the increase in kidney weight after uninephrectomy (Fig. 1). At the same time, kidney weight was signif-

<table>
<thead>
<tr>
<th>Day 2</th>
<th>Day 7</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td>FC, g/24 h</td>
</tr>
<tr>
<td>C</td>
<td>28.2 ± 0.3</td>
</tr>
<tr>
<td>S</td>
<td>27.2 ± 0.5</td>
</tr>
<tr>
<td>NP</td>
<td>27.3 ± 0.5</td>
</tr>
<tr>
<td>NV</td>
<td>26.1 ± 0.3</td>
</tr>
</tbody>
</table>

Data are expressed as means $\pm$ SE; $n = 6–8$ in each group. FC, food consumption; C, control; S, sham-operated, placebo; NP, nephrectomized, placebo; NV, nephrectomized, vascular endothelial growth factor-antibody (VEGF-Ab).
icantly elevated compared with S animals by 14% ($P = 0.004$; Fig. 1).

Mean glomerular volume is given in Fig. 2. The glomerular volume in the NP group was significantly increased by 20% compared with the S group ($P = 0.003$). VEGF-Ab treatment fully prevented the increase in glomerular volume after uninephrectomy at day 7. Mean glomerular volume was not statistically different between control and sham-operated mice.

At day 2, there were no differences in liver weight between the groups (data not shown). However, at day 7, VEGF-Ab treatment reduced liver weight by 10% compared with S ($P < 0.001$), whereas uninephrectomy had no significant effect on liver weight (NP vs. S animals, data not shown).

Serum and kidney IGF-I. Serum and kidney IGF-I levels are given in Table 2. Serum IGF-I was not different in any of the groups at the different time points studied. At day 2, kidney IGF-I was elevated by 32% in NP and 23% in NV compared with S, whereas at day 7, kidney IGF-I was not significantly different among the groups. Accordingly, VEGF-Ab treatment had no influence on kidney or serum IGF-I levels when NP and NV were compared.

**DISCUSSION**

The major new finding of the present study is a specific effect of VEGF-Ab administration on compensatory renal changes in mice subjected to unilateral uninephrectomy. Treatment with VEGF-Ab abolished the glomerular enlargement and partially blocked the renal growth after uninephrectomy without affecting body weight or food consumption. These findings indicate that VEGF plays a major role in the glomerular compensatory response after uninephrectomy.

**Table 2. Mean serum and kidney IGF-I levels at days 2 and 7 in control, sham-operated, and nephrectomized mice treated with placebo or VEGF-Ab**

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<tr>
<th></th>
<th>Day 2</th>
<th>Day 7</th>
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<tbody>
<tr>
<td></td>
<td>s-IGF-I, µg/l</td>
<td>k-IGF-I, ng/g</td>
</tr>
<tr>
<td>C</td>
<td>380 ± 53</td>
<td>128 ± 13</td>
</tr>
<tr>
<td>S</td>
<td>429 ± 18</td>
<td>134 ± 4</td>
</tr>
<tr>
<td>NP</td>
<td>397 ± 37</td>
<td>178 ± 8*</td>
</tr>
<tr>
<td>NV</td>
<td>370 ± 15</td>
<td>165 ± 7*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; $n = 6–8$ in each group. IGF-I, insulin-like growth factor I; s, serum; k, kidney. *$P < 0.01$ vs. S.

CRG after unilateral nephrectomy is a phenomenon well documented in rodents (9, 13, 29) and humans (27). Several growth factors have been suggested to be players in the CRG, e.g., GH and IGF-I (9, 11, 13, 22), TGF-β (18), and epidermal growth factor (EGF; see Refs. 19 and 21). A possible role for the GH-IGF axis in CRG arose initially when unilaterally nephrectomized hypophysectomized rats showed a blunted CRG response (26). Furthermore, the administration of a long-acting somatostatin analog (11), a GH-releasing antagonist (22), or a specific GH-receptor antagonist (9) to uninephrectomized rats has been shown to abolish the early CRG. Interestingly, GH and IGF-I seem to have a different role in adult vs. immature rodents (9, 22). EGF production is increased in the remnant kidney after unilateral nephrectomy (19, 21).

The present results support a pivotal role for VEGF in compensatory glomerular and in part in renal growth. The VEGF system consists of a group of five different isoforms and two VEGF receptors (VEGFR-1 and VEGFR-2; see Ref. 23). VEGF and VEGFRs are essential for normal embryonic development and for normal postnatal growth (14), particularly of glomeruli (20), as shown in knock-out animals (1, 6) and by postnatal VEGF inactivation, respectively. Both VEGF and VEGFRs are expressed in the glomeruli and tubules of normal rat kidney (3). Interestingly, renal expression of VEGF and VEGFRs is increased in experimental diabetes (3), and administration of a neutralizing VEGF-Ab reduces the diabetes-associated increase in glomerular filtration rate, urinary albumin excretion, and glomerular volume in type 1 diabetic rats (5), suggesting a role for VEGF in the pathogenesis of diabetic renal changes (3, 5, 7). Of interest, VEGF-Ab administration in diabetic rats (5) and in high-protein-fed mice (28) abolished glomerular hypertrophy without affecting the diabetes or high protein-associated renal enlargement. In the present study, VEGF-Ab treatment abolished the CRG-associated glomerular hypertrophy, which is in concert with the studies mentioned above. However, in addition to this glomerular effect, VEGF-Ab treatment also reduced the increase in kidney weight after uninephrectomy. Overall, these results support the observation that VEGF, besides being essential for normal glomerulogenesis, is an important growth factor for glomerular changes in physiological and pathophysiological renal changes.
A relationship has been suggested to exist between IGF-I and VEGF, based both on in vitro (15) and in vivo (30) studies. Accordingly, VEGF expression was stimulated by IGF-I in human Saos-2 osteoblast-like cells and murine osteoblasts (15). Furthermore, an IGF-I receptor antagonist (JB3) suppressed retinal neovascularization in a mouse model of proliferative retinopathy along with a reduction in the VEGF-dependent p44/42 mitogen-activated protein kinase pathway (30). In the present study, CRG was associated with the classical early, transient increase in kidney IGF-I (9, 11, 13), whereas serum IGF-I was not influenced. Administration of VEGF for 7 days had no effect on the renal IGF-I changes after uninephrectomy. Theoretically, a compensatory increase in kidney IGF-I, above that of placebo-treated nephrectomized mice, could have been expected in the VEGF-Ab-treated nephrectomized group. This phenomenon, however, was not seen. Accordingly, these results are not contradictory to the hypothesis of an interrelation between VEGF and IGF-I, with VEGF being a downstream mediator of IGF-I (15, 30).

In conclusion, the present study provides direct evidence for an essential role of VEGF in the glomerular changes in CRG.

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