ABSTRACT

Background. Treatment with recombinant human growth hormone (GH) is the standard therapy for short stature in children with chronic kidney disease (CKD). However, concerns have been raised on the potential renal fibrogenic effects of GH. There is no information regarding the renal GH receptor (GHR)-JAK-STAT signaling pathway in CKD.

Methods. Subtotal nephrectomized (CKD) and pair-fed sham-operated control (C) juvenile rats were treated with subcutaneous GH or saline for 2 weeks. A single intravenous GH bolus or vehicle was provided prior to euthanasia.

Results. Reduced body weight in CKD was improved with GH therapy. The remnant kidney showed glomerular hypertrophy and early interstitial fibrosis without inflammatory infiltration. Treatment of CKD rats with GH did not worsen renal function or fibrosis. Kidney GHR mRNA and protein levels were reduced and basal phosphorylation of JAK2 and STAT5 was significantly impaired. However, intravenous GH administration prior to sacrifice normalized STAT5 phosphorylation. Basal renal IL6 mRNA and phosphorylation of its downstream signaling molecule STAT3 were increased as was the product of its action, the suppressor of cytokine signaling 3 (SOCS3) mRNA.

Conclusions. Despite known unaltered circulating GH levels, remnant kidneys of uremic growth retarded juvenile rats show impaired basal signaling along the GH-activated JAK2/STAT5 signaling pathway. This may well be a consequence of the reduced GHR level and the inhibitory effect of the increase in IL-6-mediated SOCS3 expression. This renal GH insensitivity, if present in humans, may protect against the potential adverse renal effects of GH administration in CKD patients.

Keywords: chronic kidney disease, growth hormone, IL-6, SOCS3, STAT5
INTRODUCTION

Growth retardation is a common complication in children with chronic kidney disease (CKD) [1]. According to the North American Pediatric Renal Trials and Collaborative Studies 2005 database, 36.9% of children with CKD had growth impairment [2]. Even though growth failure usually correlates with the degree of renal impairment, those with mild reduction of glomerular filtration rate (GFR) may also exhibit short stature [3]. Despite advances in medical care, growth failure in CKD is associated with increased morbidity and mortality [4]. Growth hormone (GH) is the key endocrine regulator of postnatal growth and mediates its somatotropic actions mainly through insulin-like growth factor I (IGF-I) and to a lesser extent through direct GH activity [5]. Derangements in the GH/IGF-1 axis play a major role in CKD-related growth failure. Random fasting serum levels of GH are normal or increased in children and adults with CKD, depending on the extent of renal failure [1]. A high normal calculated GH secretion rate and amplified number of GH secretory bursts have been reported in pre-pubertal children with end-stage renal disease, likely due to attenuated IGF-1 feedback [5]. This has led to the concept of GH insensitivity or resistance in uremia.

In spite of this GH resistance in uremia, treatment with pharmacologic doses of GH has been shown to be effective for the management of uremic short stature in children [6] and has also been suggested as a therapeutic alternative for uremic muscle wasting in adults [7]. However, concerns with this therapy have been raised because of its potential adverse side effects, including reports of glomerulosclerosis in transgenic mice overexpressing GH or GHRH [8]. However, clinical studies of rhGH therapy for short stature in CKD do not support these findings obtained in animals [6]. The action of GH, a member of the cytokine family, is mediated by the binding of GH to its receptor (GHR), resulting in its dimerization and the auto-phosphorylation of the tyrosine kinase Janus kinase 2 (JAK2) which, in turn, stimulates phosphorylation of signaling proteins that serve both as signal transducers and activators of transcription (STAT) namely STAT1, STAT3 and STAT5 [9]. Upon activation, these STAT proteins translocate to the nucleus and transcribe GH-regulated genes. An intact JAK2-STAT5b signaling pathway is essential for GH stimulation of IGF-1 gene expression [9]. The JAK2/STAT pathway is regulated, amongst other factors, by suppressor of cytokine signaling (SOCS) proteins. These SOCS proteins induced by all members of the cytokine family in different patterns bind to JAK2 and inhibit STAT phosphorylation [10]. The expression of SOCS3 is induced by the translocation of STAT3 into the nucleus after being phosphorylated by JAK2. In addition to GH, phosphorylation of STAT3 is also induced by pro-inflammatory cytokines such as IL-6 [11]. In uremic-related growth retardation and muscle wasting, defects in the post-receptor GH-activated JAK2 and STAT signal transduction pathway could be one of the mechanisms causing GH resistance [12]. In support of this thesis are studies in uremic adult rats that demonstrated impaired GH-stimulated JAK2-STAT5 phosphorylation in the liver, heart and skeletal muscle, despite intact GHR levels [12-14]. However there is no information on the impact of uremia on GH-mediated JAK-STAT signaling in the kidney even though there is concern that chronic elevations of GH may potentially lead to glomerulosclerosis [8]. GH receptor is expressed in kidney tissue, mainly in the tubulointerstitium [15]. Since clinical studies of children with CKD have failed to show a deleterious GH effect on kidney function [6], we postulated that this may be accounted for by CKD suppression of GH-mediated signal transduction. Accordingly we have studied the impact of CKD on GH-mediated signal transduction in the kidneys of young and rapidly growing rats, mimicking the disease in children.

MATERIALS AND METHODS

Animal experimentation

Twenty-day-old male Sprague Dawley rats where used in the experiment (Harlan Laboratories, Jerusalem, Israel). Animal breeding complied with the NIH Guide for the Care and Use of Laboratory Animals. The local institutional review committee approved the study protocol. Animals were housed in standard laboratory cages and pair-fed with normal rat chow (containing 0.8% phosphate) with free access to an unlimited amount of tap water. The animals were 5/6 nephrectomized in two stages, as previously described [16]. Rats were anesthetized using 80 mg/kg of ketamine and 16 mg/kg of xylazine. First, uninephrectomy was performed on the right kidney of CKD rats and sham operation was performed in control group. Two days later 2/3 of the remaining left kidney was removed from the CKD group or sham operation in the control group. Animals had free access to food and water. To assess the impact of GH treatment on the structure and function of the remnant kidney, half of the CKD animals received recombinant human GH, 5 mg/kg body weight (Ferring Pharmaceuticals, Kriyat Malachi, Israel) (CKD-GHsuppl group) or saline (CKDsal group), administered in a single daily subcutaneous dose at the same hour for 14 days. Weight and food consumption measurements were performed daily. Urine was collected and volume measured prior to sacrifice for 24 h, using metabolic cages. To study the impact of CKD on GH-mediated signal transduction in the kidney, control and GH-untreated CKD rats were anesthetized after 14 days with ketamine and xylazine. Then an injection of bovine GH (100 µg/kg) (Monsanto Corp; St. Louis, MO, USA) dissolved in buffer bicarbonate or buffer alone was administered via the inferior vena cava 15 min before euthanasia as follows: control + vehicle (C), control + GH (Cgh), CKD + vehicle (CKD), CKD + GH (CKDgh). The dose of bovine GH used in this experiment is based on preliminary dose–response studies, where the 100 µg/kg/dose was found to exert already maximal STAT5 phosphorylation in the liver. Fifteen animals were examined per group. Blood was drawn from the abdominal vena cava 15 min after the GH bolus. The serum was separated and frozen at ~80°C. A coronal 2 mm slice from the midportion of the remaining kidney was separated and fixed at a 4% paraformaldehyde solution for histomorphological
assessment. The remaining portions were immediately frozen in −80°C for western blot analysis or real-time PCR analysis.

**Immunnoassays**

Serum and urine samples were collected and frozen at −80°C until analysis. Serum and urine biochemistries were analyzed by the Biochemistry laboratory of Soroka Medical Center (Beer-Sheva, Israel). Serum and urine creatinine concentrations were assessed using the standard Jaffe reaction. Urine albumin concentrations were measured using the ‘microalbumin’ method (Beckman-Coulter, CA, USA).

**Histopathological staining**

Kidney tissue was fixed in 4% paraformaldehyde and embedded in Technovit. Sections of 2 µm thickness were cut on a rotation microtome and stained with periodic acid-Schiff (PAS) and hematoxylin. In addition, frozen kidney sections were cut to 6 µm thickness, and then stained using Masson’s trichrome method which turns collagen blue. Photomicrographs were obtained by a light microscope at ×200 magnification (Zeiss Axioplan MR12, Germany). Glomerular volume was measured obtained by a light microscope at ×200 magnification and hematoxylin. In addition, frozen kidney sections were cut to 6 µm thickness, and then stained using Masson’s trichrome method which turns collagen blue. Photomicrographs were obtained by a light microscope at ×200 magnification (Zeiss Axioplan MR12, Germany). Glomerular volume was measured without including the Bowman’s capsule and volume was achieved using the formula: $V_G = (\beta/K) \times (A_G)^{3/2}$, where $A_G$ being the area of a circle, $\beta = 1.32$, the shape of coefficient of spheres and $K = 1.1$, size distribution coefficient [17].

**Immunohistochemistry**

Mouse anti-rat GHR (Santa Cruz Biotechnology, CA, USA) antibody was used in this study. Deparaffinized, rehydrated sections were treated with 3% H₂O₂ for 15 min at room temperature to block endogenous peroxidase activity. The sections were washed with PBS before blocking with 2.5% normal horse serum (Vector laboratories, Inc., Burlingame, CA, USA) for 1 h. Endogenous avidin and biotin were blocked for 15 min using avidin-biotin blocking kit (Vector laboratories, Inc.) and then washed with PBS, followed by 1 h incubation with primary antibody diluted 1:200. After incubation with the primary antibody, sections were washed in PBS, then incubated in appropriately diluted biotinylated secondary antibody for 10 min, washed with PBS, followed by incubation in streptavidin-peroxidase for 10 min and washed in PBS. The sections were then incubated with buffered substrate solution (pH 7.5) containing hydrogen peroxide and 3,3-diaminobenzidine chromogen solution (Vector laboratories, Inc.). Sections were then dehydrated and mounted with permount and examined by light microscope. Image capture was done using the Cellsense Entry software (MATIMOP, Tel Aviv, Israel).

**RNA extraction and real-time PCR**

Total RNA was extracted from the kidney as previously described [18] using the PerfectPure RNA Tissue kit (Genta Systems, Minneapolis, MN, USA). cDNAs were synthesized using high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time PCR (qPCR) assays were performed with power SYBR green PCR master mix (Applied Biosystems) as previously described [18] using the ABI Prism 7300 Sequence detection System (Applied Biosystems). Primers for quantification of TGF-β, collagen type IV, fibronectin GHR, SOCS3, IL-6 and β-actin (Sigma-Aldrich, Rechovot, Israel) are summarized in Table 1. Each sample was analyzed in triplicate in individual assays. The specificity of the reaction is given by the detection of the melting temperatures (Tms) of the amplification products immediately after the last reaction cycle. The target gene expression value was calculated by the ΔΔct method after normalization with a housekeeping gene (β-actin).

**Western immunoblot analysis**

Kidney tissue was homogenized on ice with a polytron (Kinetica, Littau, Switzerland) in lysis buffer (50 mM Tris, pH 7.4, 0.2% Triton X-100) containing 20 mM sodium pyrophosphate, 100 mM NaF, 4 mM EGTA, 4 mM Na₃VO₄, 2 mM PMSF, 0.25% aprotinin and 0.02 mg/mL leupeptine. Extracts were centrifuged for 20 min at 17 000g at 4°C and the supernatants collected and frozen. Antibodies were purchased for the detection of kidney Stat5 and GHR (Santa Cruz Biotechnology), JAK2 and p-JAK2(tyr 1007/tyr1008) (Merk-Millipore, Darmstadt, Germany), STAT3, p-STAT3 (Yyr705), p-STAT5 (Yyr694) (Cell signaling Technology, Inc. Denver, MA, USA) and β-actin (MP Biomedical, Solon, OH, USA). Homogenates were mixed with 5x sample buffer and boiled for 5 min. Then, 100 µg portions of sample protein were loaded in each gel lane and subjected to 7.5–10% SDS polyacrylamide gel, and electroblotted into nitrocellulose membranes. Blots were blocked for 1 h in TBST (0.05% Twin-20) buffer (10 mM tris, pH 7.4, 138 mM NaCl) containing 5% non-fat dehydrated milk, followed by overnight incubation with polyclonal antibody diluted in TBST (0.05% Twin-20) containing 5% dry milk. The phosphorylated antibodies were diluted in TBST (0.05% Twin-20) containing 5% BSA (MP Biomedical). After washing three times for 15 min in TBST (0.05% Twin-20), the blots were incubated with a secondary anti-goat antibody (GHR), anti-mouse antibody (STAT5, STAT3, β-actin) or anti-rabbit antibody (JAK2, p-JAK2, p-STAT5, p-STAT3) conjugated to horseradish peroxidase for exposure to chemiluminescence reagents and developed using X-ray film (Kodak, Rochester, NY, USA).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense</th>
<th>Anti-sense</th>
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<tr>
<td>TGF-β</td>
<td>GTGCTGTAACCAAGGAGACG</td>
<td>CAGGTGTTGAGCCCTTTCAG</td>
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<tr>
<td>Collagen type IV</td>
<td>ATTTCTTGGTAGCACACCCAG</td>
<td>AACGCTGAAGCATCTTGGTGAGTA</td>
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<tr>
<td>Fibronectin</td>
<td>TGAACCTGGCCAGTTGACTG</td>
<td>TACGTTGTTGAGCCCTTTCAG</td>
</tr>
<tr>
<td>GHR</td>
<td>ATCTTTGGGCTGTTTCTT</td>
<td>TAGCTGTTGAGCCCTTTCAG</td>
</tr>
<tr>
<td>SOCS3</td>
<td>CTTTGGAGTGTTCTGGAGCAG</td>
<td>CGTGGACAGCTTCCGGAGACA</td>
</tr>
<tr>
<td>IL-6</td>
<td>GCCAAGTCTAGTCAAGAGCAA</td>
<td>CATTGGAAGTGGGATGAGGA</td>
</tr>
<tr>
<td>β-Actin</td>
<td>CCCGGCAATGACCACTTCTC</td>
<td>CGTATCCATGGCGAACTT</td>
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Table 1. Primers used for real-time PCR

*Impaired GHR signaling in CKD*
1 h at room temperature and then washed again three times. The band antibody was visualized by enhanced chemiluminescence (ECL; Amersham, Life Sciences, Inc.) and exposed to Kodak-BioMax film (Eastman Kodak, Rochester, NY, USA). Protein expression was quantitated densitometrically using Fluorchem software (Alpha-Innotech, CA, USA).

**Statistical analysis**

Two-tailed unpaired Student’s t-tests were applied for comparison of two normally distributed groups. Comparisons between more than two normally distributed groups were made by one-way ANOVA; P-value of <0.05 was considered significant.

**RESULTS**

**Kidney function and damage**

Kidney function was moderately decreased in this subtotal nephrectomy model as reflected by higher levels of serum urea (132.7 ± 7 mg/dL in CKDsal compared with 45.8 ± 3.2 mg/dL in C, P < 0.05) and serum creatinine (0.87 ± 0.05 in CKDsal compared with 0.31 ± 0.06 mg/dL in C, P < 0.001) (Figure 1a). In addition, urinary albumin to creatinine ratio was significantly higher in CKDsal versus C (1.48 ± 0.21 mg/mg in C, P < 0.01) (Figure 1b). Glomerular hypertrophy was seen in CKDsal (22.3 ± 0.06 versus 15 ± 0.034 × 10⁵ µm³ in C, P < 0.05) (Figure 1c). Masson’s trichrome staining (Figure 1d) showed glomerular hypertrophy and a mild increase in fibrosis around the tubules of CKDsal rats. Thus, an early chronic stage is shown. The CKD rats supplemented with rhGH for 2 weeks showed no changes in these parameters in comparison with CKD rats supplemented with saline. Values in the CKDGH-suppl group versus CKDsal were as follows: creatinine: 0.86 ± 0.06 versus 0.87 ± 0.05 mg/dL; albumin-to-creatinine ratio: 1.63 ± 0.3 versus 1.48 ± 0.21 mg/mg; and glomerular volume: 22.6 ± 0.07 versus 22.3 ± 0.06 × 10⁵ µm³ (Figure 1). In addition, mRNA levels of TGF-β, type IV collagen and fibronectin, common markers of renal fibrosis, were increased in CKDsal versus C (TGFβ: 2.1 ± 0.35 versus 1 ± 0.08; type IV collagen: 1.44 ± 0.07 versus 1.0 ± 0.08; and fibronectin: 2.6 ± 0.3 versus 1 ± 0.1 fold of control, P < 0.01). However, there was no significant increase in these markers in the CKDGHsuppl group versus CKDsal after 2 weeks of rhGH treatment (2.6 ± 0.26, 1.8 ± 0.2 and 3.1 ± 0.5 fold of C for TGF-β, type IV collagen and fibronectin, respectively, P = NS) (Figure 2).

**Circulating GH and kidney GHR expression**

Circulating GH levels were unchanged between C and CKD (127 ± 12 ng/mL in CKD compared with 128 ± 15 ng/mL in C, P = NS), as we recently described [19]. However, renal GHR mRNA levels were significantly decreased in CKD and CKDgh compared with C and Cgh (0.14 ± 0.01 and 0.15 ± 0.03 versus 1 ± 0.08 and 1.08 ± 0.2 fold of control, respectively, P < 0.05). No difference was seen between C and Cgh and between CKD and CKDgh (Figure 3a). Similarly, GHR protein (determined by western blot) was also decreased in CKD compared with C (14 ± 4 versus 100 ± 15% of control, respectively, P < 0.05). No difference was seen between C and Cgh and between CKD and CKDgh (Figure 3b). Immunohistochemistry for GHR revealed a predominant tubular expression. Tubular GHR staining was slightly decreased in CKD versus C, but no differences
between C and CKD in glomerular GHR were detected (Figure 3c).

Defects in kidney GHR signal transduction

In order to understand the effect of GH on the kidney in CKD we examined the GHR signaling pathway following a bolus of bovine GH or vehicle, with the latter taken to reflect the basal state. We observed a significant decrease in the basal level of phosphorylated JAK2 in CKD compared with C (34 ± 3 versus 100 ± 7%, respectively) (Figure 4a). Administration of an intravenous single bolus of bGH STAT5 phosphorylation increased significantly in the Cgh and CKDgh groups. Of note despite the lower basal STAT5 phosphorylation in CKD there was no difference in the levels reached in the Cgh compared with CKDgh group (237 ± 41 versus 232 ± 49% of control, P: NS). Thus this intravenous single bolus of bGH was able to overcome the GH-resistant state. Kidney IGF-I mRNA levels were unchanged between groups (0.9 ± 0.1, 1 ± 0.15 and 0.8 ± 0.1 fold of C in Cgh, CKD and CKDgh, respectively).

Kidney SOCS3 mRNA levels were significantly increased in CKD and CKDgh compared with C and Cgh (12.6 ± 1 and 8.3 ± 0.7 versus 1.1 ± 0.1 and 1.4 ± 0.2 fold of control, respectively, P < 0.05) (Figure 5a). There was no significant increase in SOCS3 mRNA levels in response to bGH over the 15 min period of study in either treated group. This may be explained by the time chosen, for it takes 30 min for SOCS3 expression to peak after GH administration [20].

In an attempt to further understand this inhibition of GHR signaling through JAK2, we examined the levels of the inflammatory cytokine IL-6 and phosphorylation of its major signaling protein STAT3, which is involved in the induction of SOCS3. There was a significant increase in basal phosphorylated STAT3 in CKD (504 ± 110% versus 100 ± 21% in the C group, P < 0.05) (Figure 5c). Following GH treatment phosphorylation was essentially unchanged in the CKDgh, 657 ± 73%, and in the Cgh, 132 ± 33%, groups. This may be attributed to the significant, 4-fold, increase in renal IL-6 mRNA in CKD compared with C (3.9 ± 0.7 versus 1 ± 0.1 fold of control, respectively, P < 0.001) (Figure 5b). No differences in any of these parameters were seen in rats treated with a single bolus of bGH.

DISCUSSION

While there is considerable concern that the high doses of GH used to treat short stature in children with CKD may accelerate renal fibrosis, there is no information on the impact of CKD on GH-mediated JAK-STAT signaling in the kidney. This study in juvenile rats with surgically induced CKD addresses this shortcoming. We have recently shown that when juvenile rats are exposed to a state of moderate renal functional impairment over a short period of time, they exhibit many of the same effects seen in CKD patients, including stunted growth, in spite of pair feeding with sham operated controls [19]. This model is not associated with significant anemia or secondary hyperparathyroidism. Morphological examination showed an increase in glomerular volume in association with the early interstitial fibrosis (shown by the Masson’s Trichrome staining) (Figure 1c and d). Together with elevated serum creatinine levels and albuminuria this depicts a model of early stage CKD and for the first time we show evidence for decreased renal GHR signaling, even at this early stage of disease.
The renal effects of GH were clinically identified years ago, including a decreased or increased glomerular filtration rate (GFR) in hypopituitarism and acromegaly, respectively [21]. As a rise in GFR in a patient with impaired renal function may accelerate the progression of renal failure and as transgenic mice which have persistently elevated serum GH levels develop glomerulosclerosis [8, 22], concern arose when recombinant human GH (rhGH) was adopted for the treatment of short stature, particularly in children with underlying renal disease. In our study in juvenile rats we found that the kidney

**FIGURE 3**: GHR expression. Animals received IV bGH (Cgh, CKDgh) or its vehicle (C, CKD) 15 min before euthanasia. (a) Kidney GH receptor (GHR) mRNA. \( n = 10 \) per group. Results are presented as fold of control group. (b) Kidney GHR/\( \beta\)-actin protein ratio. GHR was detected at molecular weight 120 kD, by western blot, \( n = 6 \) per group. The representative gel shows two different animals in each group. Values are mean ± SEM; Different letters above bars indicate a significant difference between groups (\( P < 0.05 \)); groups with common letters are similar. (c) Kidney GHR immunohistochemistry in C and CKD animals. Right panel: results using no specific antibody.

**FIGURE 4**: Defects in JAK/STAT signal transduction. Animals received IV bGH (Cgh, CKDgh) or its vehicle (C, CKD) 15 min before euthanasia. (a) Kidney p-Jak2/Jak2 ratio detected by western blot, \( n = 6 \) per group. (b) Kidney p-STAT5/STAT5 detected by western blot, \( n = 6 \) per group. Bands are shown compared with \( \beta\)-actin. The representative gel shows two different animals in each group. Values are mean ± SEM. Different letters above bars indicate a significant difference between groups (\( P < 0.05 \)); groups with common letters are similar.
tissue may be relatively protected from these potential adverse effects of GH treatment (Figures 1 and 2) because of insensitivity to GH, both at the receptor level as well as GHR JAK2/STAT5 signaling impairment. Circulating GH levels are unchanged in CKD rats [19], but kidney GHR mRNA and protein levels were reduced (Figure 3) and basal phosphorylation of JAK2 and STAT5 were significantly impaired (Figure 4). Of note however, high-dose GH administration normalized STAT5 phosphorylation in keeping with the response described in CKD rats which increase their growth rate when treated with high GH doses [23, 24] and in children with CKD [6, 25].

The decrease in GHR expression as well as the downstream proteins of its signaling pathway in the kidney in association with the increase in the GHR inhibitor SOCS3 shows impaired GHR signaling in the kidneys of CKD rats. SOCS 3 is a member of cytosolic proteins that are induced by cytokines and serve as negative feedback regulators of the JAK-STAT pathway by binding to cytokine receptor-JAK signaling complexes [26]. Among the members of the SOCS family of proteins, GH stimulates SOCS-1, -2, and -3 and CIS expression. In SOCS2-deleted mice, GH-mediated STAT5 activation is enhanced and the animals grow larger than the wild-type mice [27]. The HG mice, a natural SOCS2 knockout, are characterized by the appearance of gigantism post weaning with a 30–50% increase in adult body weight with a proportional increase in muscle mass. Serum GH levels are low while IGF-1 levels are elevated. HG mice have been shown to have an accumulation of collagen, preferentially type III, in dermis, trachea and lungs [28]. SOCS2 knockouts have increased dermal fibrosis and collagen in the pulmonary vasculature, bronchi and bile, pancreatic and salivary ducts [27]. Of note GH transgenic mice develop glomerulosclerosis and treating rats with GH accelerates kidney fibrosis [22, 29]. Pro-inflammatory cytokines also stimulate SOCS expression that, in a negative feedback manner, serve to suppress the cytokines signaling pathway and in this manner can also inhibit GH action [30, 31]. In our study we observed a significant increase in the pro-inflammatory cytokine IL-6, its signaling transducer and activator of transcription STAT3 and its gene product SOCS3 (Figure 5). SOCS3 is a potent negative feedback inhibitor of IL-6 and GH signaling and may well contribute to the GH-resistant state in CKD. Increased basal SOC3 levels have also been noted in the liver of mature CKD rats while in skeletal muscle an increase only occurred after the prolonged administration of GH [13]. In contrast to our previous studies that show an increase in muscle and liver SOCS2 in the uremic rat [12], in this study we have not found significant changes in this suppressing molecule in the remnant kidney tissue.
SOCS and STATs have been implicated in kidney diseases especially diabetic nephropathy. However, the more predominant JAK-STAT pathway in that study was JAK2-STAT3 [32]. In addition, STAT3 activation was shown to play a role in the development of renal fibrosis in a model of unilateral ureteral ligation [33].

The IL-6 system promotes inflammatory events through the activation and proliferation of lymphocytes, differentiation of B cells, leukocyte recruitment and the induction of the acute-phase protein response in the liver [34]. In end-stage renal disease patients, an elevated circulating IL-6 level is a strong predictor of poor outcome [35]. A growing body of evidence supports a novel concept that elevated inflammation contributes to the pathogenesis of CKD [36]. Some hypothesize that the activation of leukocytes and up-regulation of certain cytokines propagate a state of chronic inflammation in CKD patients that likely contributes to progression of the disease [37] and may also induce a GH-resistant state [12]. However, it must be remembered that the JAK-STAT-SOCS pathway is complex and may be associated with the activation of different combinations of its components [32]. Also, apart from the impact of uremia alone on GH-mediated signal transduction, it is conceivable that structural changes in the remnant kidney may also contribute to the GH-resistant state.

In summary, our study supports the hypothesis that activation of pro-inflammatory cytokines in CKD may be the cause for the disruption in the renal GHR signaling pathway, thus preventing the potential side effects of prolonged GH therapy. Despite unaltered circulating GH levels, remnant kidneys of uremic growth-retarded juvenile rats with modest renal failure show impaired basal signaling along the GH-activated JAK2/STAT5 signaling pathway. This is likely a consequence of reduced GHR levels, the inhibitory effect of the increase in IL-6 mediated SOCS3 expression and perhaps the structural changes that occur in the remnant kidney. While extrapolation from rodents to humans must be carried out with caution, this renal GH insensitivity, if present in humans, may be protective against the potential adverse renal effects of modest but not high-dose GH administration in CKD patients.

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CONFLICT OF INTEREST STATEMENT

None declared.

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D. Wiezel et al.
Endothelial-to-mesenchymal transition and renal fibrosis in ischaemia/reperfusion injury are mediated by complement anaphylatoxins and Akt pathway

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ABSTRACT

Background. Increasing evidence demonstrates a phenotypic plasticity of endothelial cells (ECs). Endothelial-to-mesenchymal transition (EndMT) contributes to the development of tissue fibrosis. However, the pathogenic factors and signalling pathways regulating this process in ischaemia/reperfusion (I/R) injury are still poorly understood.

Methods. We investigated the possible role of complement in the induction of this endothelial dysfunction in a swine model of renal I/R injury by using recombinant C1 inhibitor in vivo.

Results. Here, we showed that I/R injury reduced the density of renal peritubular capillaries and induced tissue fibrosis with