IL-10 Suppresses Chemokines, Inflammation, and Fibrosis in a Model of Chronic Renal Disease

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IL-10 is a pluripotent cytokine that plays a pivotal role in the regulation of immune and inflammatory responses. Whereas short-term administration of IL-10 has shown benefit in acute glomerulonephritis, no studies have addressed the potential benefits of IL-10 in chronic renal disease. Chronically elevated blood levels of IL-10 in rats were achieved by administration of a recombinant adeno-associated virus serotype 1 IL-10 (rAAV1–IL-10) vector. Control rats were given a similar dose of rAAV1-GFP. Four weeks after injection, IL-10 levels in serum were measured by ELISA, and chronic renal disease was induced by a 5/6 nephrectomy (n = 6 in each group). Eight weeks later, rats were killed and renal tissue was obtained for RNA, protein, and immunohistochemical analysis. Serum levels of IL-10 were 12-fold greater in the rAAV1–IL-10 group by 4 wk after rAAV1–IL-10 administration (345 ± 169 versus 28 ± 15 pg/ml; P = 0.001), and levels were maintained throughout the experiment. rAAV1–IL-10 treatment resulted in less proteinuria (P < 0.05), lower serum creatinine (P < 0.05), and higher creatinine clearances (P < 0.01) compared with rAAV1-GFP–treated rats. Renal interstitial infiltration was significantly attenuated by rAAV1–IL-10 administration as assessed by numbers of CD4+, CD8+, monocyte-macrophages (ED-1+) and dendritic (OX-62+) cells (P < 0.05), and this correlated with reductions in the renal expression of monocyte (renal monocyte chemoattractant protein-1 mRNA and protein) and T cell (RANTES mRNA) chemokines. rAAV1–IL-10 administration decreased mRNA levels of IFN-γ and IL-2 in the kidney. The reduction in inflammatory cells was associated with a significant reduction in glomerulosclerosis and interstitial fibrosis. It is concluded that IL-10 blocks inflammation and improves renal function in this model of chronic renal disease. The feasibility of long-term overexpression of a gene using the AAV serotype 1 vector system in a model of renal disease is also demonstrated.


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W.M. and X.O. contributed equally to this work.

W.W.H. and the University of Florida could be entitled to patent royalties for inventions related to this work, and both own equity in a company that may commercialize some of the technology described herein.

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of animal models, including experimental vascular injury (7,8), autoimmune disease (9), and hepatic ischemia/reperfusion (10). IL-10 has also been reported to reduce inflammation and mesangial cell proliferation in acute glomerulonephritis induced with anti-Thy 1 antibody (11) and to suppress glomerulosclerosis formation in the FGS/Kist, a rat that develops spontaneous focal segmental glomerulosclerosis (12). To date, there have been no studies on the potential benefits of long-term IL-10 therapy in models of chronic renal disease.

In this study, we examined the effect of IL-10 in a model of chronic renal disease induced by 5/6 nephrectomy. Systemic expression of IL-10 was achieved using an adeno-associated virus serotype 1–IL-10 (rAAV1–IL-10) vector. We report a beneficial effect of IL-10 in the remnant kidney model, which is partly mediated by a reduction in the local inflammatory response.

Materials and Methods

Rat IL-10 Expression Vector Construction

A reverse transcriptase–PCR product that contained the rat IL-10 cDNA was provided by F. Cheepsumthorn and L. Watkins (University...
of Colorado at Boulder, Boulder, CO). The rIL-10 cDNA was cloned into a TA cloning vector (pCR-XL-TOPO; Invitrogen Corp., Carlsbad, CA) and sequenced by the UF-ICBR sequencing facility. Only a single silent base variation was found in the rIL-10 cDNA. The rIL-10–AAV1 (pTR2-CB-rIL-10) packageable vector plasmid was made by subcloning rIL-10 into pTR2-CB plasmid (AAV1-ITR–containing vector). The expression of rIL-10 is driven by the hybrid cytomegalovirus enhancer/chicken β-actin promoter/hybrid intron cassette (13).

**Recombinant AAV Virus Production**
rAAV production was performed by co-transfection of the rAAV1-ITR vector construct and a combined Ad/AAV helper construct. Individual helper constructs all express the Ad5 E2a, E4, and VA genes; the AAV Rep gene; and the specific AAV Cap gene desired for pseudotyping (most with AAV1). Transfections were performed by calcium phosphate co-precipitation in an Ad-E1α- and E1β-expressing permissive human cell line (HEK293). rAAV was purified from cells by iodixanol density gradient centrifugation. DNA dot blot assay was performed to quantify the titer of rAAV (14).

**Animals**
The study was approved by the Animal Care Committee at the University of Florida (Gainesville, FL). Male Sprague-Dawley rats (n = 20; Charles River Laboratories, Inc., Wilmington, MA) that weighed between 150 and 175 g received an intramuscular injection into the caudal muscle of the pelvic limb. These injections used 100 μl of saline that contained 1 × 1010 infectious units of either rAAV1-GFP (n = 10) or rAAV1–IL-10 (n = 10) per rat. Four weeks after injection, IL-10 levels in serum were measured by ELISA (see below). Two rats from the rAAV1–IL-10 group did not achieve serum IL-10 levels >100 pg/ml and therefore were excluded from the experiment. The remaining rats underwent the remnant kidney procedure.

Rats underwent a right subcapsular nephrectomy with surgical resection of the upper and lower thirds of the left kidney (n = 20), and tissues were kept for later analysis. To document equivalent reduction in renal mass, the resected kidney was weighed, and the remnant kidney weight was calculated as the weight of the resected whole kidney minus the resected sections of the other kidney. Only rats with a remnant kidney/body weight of 1 to 2% were used, resulting in nine kidney weight was calculated as the weight of the resected whole kidney minus the resected sections of the other kidney. Only rats with a remnant kidney/body weight of 1 to 2% were used, resulting in nine rats in the rAAV1–ITR group and six rats in the rAAV1–IL-10. Three rats in the rAAV1–GFP group died during the experiment; thus, six animals per group were left at the end of the study.

Serum samples were collected at 4, 6, 8, 10, and 12 wk for serum IL-10 measurement. Urine samples were collected at 2, 4, 6, and 8 wk after remnant kidney surgery for urine protein measurement. Eight weeks after nephrectomy, rats were killed for RNA, protein, and immunohistochemical analysis.

**Proteinuria and Renal Function**
Urinary protein excretion (24 h) was measured using the Bio-Rad DC Protein Assay Kit II (Bio-Rad, Hercules, CA), and blood urea nitrogen and creatinine were measured by Alfa Wassermann’s VetACE biochemistry machine (Alfa Wassermann, West Caldwell, NJ); urine creatinine was measured using the Sigma Creatinine kit (Sigma, St. Louis, MO). Creatinine clearance (CcR) rates were calculated as U × V/[P], where U and P denote urinary and serum creatinine concentrations, respectively, and V represents ml urine/min.

**Renal Pathologic and Histologic Studies**
Renal tissues were fixed in Methyl Carnoy’s fixative, and 4-μm paraffin sections were stained with periodic acid-Schiff and hematoxylin. Immunohistochemistry was performed using the following affinity-purified primary antibodies: mAb against CD4, CD8, ED1, and OX62 (BD Pharmingen, San Diego, CA). Horseradish peroxidase–conjugated goat anti-mouse IgG antibodies (Rockland Immunochemicals, Inc., Gilbertsville, PA) were used as secondary antibodies.

For immunohistochemistry, renal tissues were fixed in 4% (wt/vol) buffered parafformaldehyde and frozen at −70°C. Cryosections (4 μm) were incubated with first antibody as described above overnight at 4°C. Sections were washed in PBS, inactivated with endogenous peroxidase in 0.3% H2O2 in methanol, labeled with second antibody as described above followed by mouse peroxidase anti-peroxidase, and developed with DAB substrate kit (Vector Laboratories, Burlingame, CA) to produce a brown color.

For quantification of immunohistochemistry staining, stained sections were imaged using a Axioplan 2 imaging microscope (Carl Zeiss Inc., Thornwood, NY), CR5 digitized color camera, and image-analyzed using Zeiss AutoMeasure software (Axiovision 4.1). The kidney cortex area was evaluated by an investigator without previous knowledge of the experimental group of the animals from which the tissue was taken. Single image frames (700 × 550 μm) were captured at ×400 magnification, and 20 frames per sample were used to count the number of CD4+, CD8+, ED-1+, and OX62+ cells (expressed as cell number/[700 × 550 μm]2).

Glomerulosclerosis and tubulointerstitial fibrosis scores were also measured in all renal biopsies. For glomerulosclerosis, the percentage of glomeruli that exhibited focal or global glomerulosclerosis was determined by evaluation of all glomeruli present in the biopsy. Glomerulosclerosis was defined as segmental increases in the glomerular matrix, segmental collapse, obliteration of capillary lumina, and accumulation of hyaline, often with synechial attachment to Bowman’s capsule (15). Twenty glomeruli per sample were calculated for glomerulosclerosis scores. Tubulointerstitial injury was defined as inflammatory cell infiltration, tubular dilation and/or atrophy, or interstitial fibrosis. Injuries were graded semiquantitatively by a blinded observer, who examined at least 20 cortical fields (magnification ×200) in periodic acid-Schiff–stained biopsies. Only cortical tubules were included in the following scoring system (16): 0 = changes <10% of the cortex; 1+ = changes in up to 20% of the cortex; 2+ = changes in up to 40% of the cortex; 3+ = changes in up to 60% of the cortex; 4+ = changes in up to 80% of the cortex; 5+ = changes in >80% of the cortex sections. Each score per biopsy was based on 20 frames (representing 700 × 550 μm2 tissue areas). Interstitial fibrosis was also assessed by using an Axioplan 2 microscope with Zeiss’s AutoMeasure software to quantify the positive staining areas of interstitial collagen I and collagen III immunostaining on 20 frames per sample tissue area (700 × 550 μm)2.

**RNA Isolation, Reverse Transcription, and Real-Time PCR**
Total RNA was isolated using the SV Total RNA Isolation kit (Promega, Madison, WI) according to the manufacturer’s protocol. The RNA was eluted with 50 μl of RNase-free water. All RNA was quantified by spectrophotometer, and the optical density 260/280 nm ratios were determined. Reverse transcription was performed in a one-step protocol using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer’s protocols. Reactions were incubated at 25°C for 5 min, and 42°C for 30 min, 85°C for 5 min and cooled at 4°C in a Thermocycler (Eppendorf, Hamburg, Germany). Primers (Table 1) were designed by Genetool software (BioTools Inc., Edmonton, Canada), and oligonucleotides were synthesized by Sigma Genosys (Sigma-Genosys Ltd., Woodlands, TX).

Real time PCR analyses were performed using the Optionc PCR machine (MJ Research, Waltham, MA). The SYBR Green master mix kit (BIO-RAD) was used for all reactions with real-time PCR. Briefly, PCR was performed as: 94°C for 2 min followed by 40 cycles of denaturation,
annealing, and extension at 94°C for 15 s, 64°C for 30 s, 72°C for 45 s, respectively, and final extension at 72°C for 10 min. PCR reaction for each sample was done in duplicate for all of the product and for the glyceraldehyde-3-phosphate dehydrogenase control. Ratios for each product/glyceraldehyde-3-phosphate dehydrogenase mRNA were calculated for each sample and expressed as the mean ± SD.

**Measurement of Cytokine and Chemokine by ELISA**

At each time point mentioned above for serum and tissue preparation, IL-10 and monocyte chemoattractant protein-1 (MCP-1) were measured by ELISA (Rat IL-10 BD OptEIA ELISA Set and Rat MCP-1 BD OptEIA ELISA Set; BD Biosciences Pharmingen, San Diego, CA). Briefly, wells of polystyrene microtiter plates (Polysorp F96; Nunc, Glostrup, Denmark) were coated with Capture antibody of IL-10 or MCP-1 in carbonate bicarbonate buffer (pH 9.6) overnight at 4.0°C. The next day, the well was washed three times with PBS that contained 0.5% Tween 20 (PBST) and blocked with PBS that contained 5% BSA for 1 h at room temperature. After three washes, 100 μl of standard and rat serum samples were added into wells. After 2 h of incubation at room temperature and five washes, 100 μl of detection antibody and enzyme reagent mixture were added to each well and the plate was incubated for 1 h at room temperature. Unbound detection antibody and enzyme mixture was removed by seven consecutive washings with PBST. A total of 100 μl of substrate solution was added to each well, and the plate was incubated in dark at room temperature for 30 min. The reaction was stopped by addition of 50 μl of stop solution, and absorbance was measured at 405 nm with a microplate scanning spectrophotometer (Powerwave 200; BIO-TEK Instruments, Winooski, VT).

**Statistical Analyses**

All data are presented as mean ± SD. Differences in the various parameters between groups were evaluated by single-factor ANOVA. Differences in parameters at each time point after remnant kidney surgery were compared by paired t test. Correlation between different factors was evaluated by Pearson correlation, Kendall τ-b, and Spearman ρ correlation. Significance was defined as P < 0.05.

**Results**

**High-Circulating IL-10 Levels in Rats Given an Injection of rAAV1–IL-10**

To confirm overexpression of IL-10 and to evaluate the serum concentration, we collected serum samples at 4, 6, 8, 10, and 12 wk after injection and assayed. IL-10 levels were elevated in all rats that received rAAV1–IL-10, whereas IL-10 remained low in rats that received rAAV1-GFP as control. Levels of IL-10 were 12-fold higher at 4 wk and an average of 10-fold higher than controls at the 8- and 12-wk time points (Figure 1).

**Preservation of Renal Function in rAAV1–IL-10 rats**

The rAAV1–IL-10–treated animals maintained better renal function compared with the rAAV1-GFP group with significantly less proteinuria (P < 0.05), lower mean serum creatinines (P < 0.05), and higher Ccr rates (P < 0.01). Serum blood urea nitrogen levels in rAAV1–IL-10 rats were also lower than that observed in rAAV1-GFP rats, but the difference was NS (Table 2). The proteinuria in both groups gradually increased after remnant kidney surgery, but rAAV1–IL-10–treated rats had less urine protein excretion beginning 4 wk after surgery compared with rAAV1-GFP control rats (Figure 2).

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### Table 1. Real-time PCR primers used in the study

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<th>Gene Symbol</th>
<th>Gene Description</th>
<th>Accession Number</th>
<th>Forward Primer Sequence (5′–3′)</th>
<th>Reverse Primer Sequence (5′–3′)</th>
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<tr>
<td>IL-2</td>
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<td>TTGCTGGCTCATCATCGAATTG</td>
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<td>GCCGACTCATGGGATCATATT</td>
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<tr>
<td>RANTES</td>
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<td>NM_031116</td>
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<td>GCGGTTACTTCTCGAGTCAA</td>
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<tr>
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<tr>
<td>COL III</td>
<td>Collagen, type III, α1</td>
<td>NM_032085</td>
<td>GGTGCGGGTGCGTAGGAG</td>
<td>GGGCACTGTCACCTCTAC</td>
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<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>M32599</td>
<td>CTCACCACCATGGGAGG</td>
<td>GCATGAGCTGTGGGTGAG</td>
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**MCP-1, monocyte chemoattractant protein-1,**
Table 2. Changes of renal function in rAAV1-IL-10 treated group versus rAAV1-GFP control group 8 wk after RK surgery

<table>
<thead>
<tr>
<th></th>
<th>Pre-RK (2 Wk; n = 12)</th>
<th>rAAV1-GFP (n = 6)</th>
<th>rAAV1–IL-10 (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.13 ± 0.05</td>
<td>1.2 ± 0.4</td>
<td>0.9 ± 0.1</td>
<td>0.042b</td>
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<tr>
<td>Serum BUN (mg/dl)</td>
<td>6.4 ± 1.9</td>
<td>56 ± 21</td>
<td>44 ± 6</td>
<td>0.153</td>
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<tr>
<td>Creatinine clearance rate (ml/min)</td>
<td>1.59 ± 0.21</td>
<td>0.9 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>0.047b</td>
</tr>
</tbody>
</table>

*a*rAAV1–IL-10, recombinant adeno-associated virus serotype 1 IL-10; RK, remnant kidney; BUN, blood urea nitrogen. Data are expressed as average ± SD.

**p < 0.05.

Figure 2. Proteinuria. A time-dependent increase of proteinuria (mg/16 h) in both groups is observed after remnant kidney surgery. The progression of proteinuria is significantly inhibited by rAAV1–IL-10 treatment 6 wk after surgery compared with rAAV1-GFP control rats. Shown are mean ± SD; 6 rats are included in each group. *P < 0.05.

Effect of IL-10 on Renal Fibrosis in Rats with Remnant Kidneys

Both groups developed progressive renal disease with focal glomerular scarring and interstitial fibrosis. We found that the differences in glomerulosclerosis and interstitial injury scores between groups were significant (Figure 9; Table 3). Moreover, IL-10–treated rats showed an approximately 50% to 60% reduction in MCP-1 mRNA, and IFN-γ and IL-2 mRNA expression in rAAV1–IL-10–treated rats compared with rAAV1-GFP rats (Figure 5). Kidney tissue lysates were also assayed for MCP-1 (measured by ELISA) and confirmed a lower expression of MCP-1 protein in rAAV1–IL-10–treated rats (Figure 6). The level of MCP-1 protein in individual animals correlated closely with the number of macrophage (ED-1+) cells (r = 0.63), dendritic cells (r = 0.47), CD4+ cells (r = 0.73), and CD8+ cells (r = 0.66) present (Figure 7). Similarly, the level of RANTES mRNA correlated with the number of CD4+ (r = 0.68), CD8+ (0.66), and OX62+ dendritic cells (r = 0.58; P < 0.05 for all comparisons; Figure 7). Serum IL-10 levels also correlated inversely with both renal tissue MCP-1 levels (r = −0.64) and renal RANTES mRNA (r = −0.73; P < 0.05; Figure 8).

Discussion

IL-10 is widely accepted as an anti-inflammatory cytokine (1) and might have beneficial effects on some inflammatory-related diseases (7–10). In kidney disease, short-term expression of IL-10 has been reported to improve glomerulonephritis (11) and glomerulosclerosis (12). We hypothesized that long-term expression of IL-10 by rAAV1 might provide a novel means for treating chronic progressive kidney disease. To study this, we selected the remnant kidney model as it is considered to be a classic model of progressive chronic kidney disease and is characterized by renal inflammation and the slow development of glomerulosclerosis and tubulointerstitial fibrosis (19,20) that resembles human disease.

IL-10 Suppresses Renal Inflammatory Cell Infiltration in Remnant Kidney

Rats that had remnant kidneys and were treated with rAAV1-GFP displayed renal inflammation, as noted by infiltration of macrophages (ED-1+ positive), dendritic cells (OX62 positive), and CD4+ and CD8+ lymphocytes in the interstitial areas of the cortex (Figure 3). rAAV1–IL-10–treated rats showed significantly less infiltration of each of these cell types. The level of serum IL-10 protein in individual animals correlated inversely with the number of macrophage (ED-1+ as cell marker) cells (r = 0.63), dendritic cells (OX62+ as cell marker; r = 0.63), CD4+ cells (r = 0.61), and CD8+ cells (r = 0.69) present (Figure 4).

IL-10 Suppresses Chemokine and Cytokine Expression in Remnant Kidney

To better understand potential mechanisms by which IL-10 reduces renal inflammation, we examined the effect of IL-10 on the expression of the chemokine monocyte chemotactic protein-1 (MCP-1 or CCL2) and RANTES (or CCL5), which are important chemokines that stimulate monocyte and T cell chemotaxis. We also examined mRNA expression of the cytokine IFN-γ which has been reported to augment macrophage-mediated renal injury (17), and mRNA expression of the cytokine IL-2 which has an important role in the activation of T cells (18). Quantitative real-time PCR of whole kidney samples obtained at killing demonstrated a reduction in MCP-1, RANTES mRNA, and IFN-γ and IL-2 mRNA expression in rAAV1–IL-10–treated rats compared with rAAV1-GFP rats (Figure 5). Additionally, the number of macrophage (ED-1+) cells (r = 0.63), dendritic cells (r = 0.47), CD4+ cells (r = 0.73), and CD8+ cells (r = 0.66) present (Figure 7). Similarly, the level of RANTES mRNA correlated with the number of CD4+ (r = 0.68), CD8+ (0.66), and OX62+ dendritic cells (r = 0.58; P < 0.05 for all comparisons; Figure 7). Serum IL-10 levels also correlated inversely with both renal tissue MCP-1 levels (r = −0.64) and renal RANTES mRNA (r = −0.73; P < 0.05; Figure 8).
in that elevated levels of the cytokine of choice can be achieved for the lifetime of the animals (25). Most studies suggest that elevated blood levels of the gene of interest are not achieved until 2 to 4 wk after gene delivery. We therefore waited 4 wk to ensure increased blood levels of IL-10 before initiation of the remnant kidney disease model. At this time point, circulating IL-10 levels were 12-fold higher compared with control rats, and these levels were maintained for all 8 wk after induction of the remnant kidney model. The levels of IL-10 achieved are well within the range of its known biologic effects on cultured cells (26). We also observed no toxicity in our model, and there was no difference in weight or behavior between the IL-10– and GFP-overexpressing rats. We thus could address accurately the effect of chronic IL-10 on the inflammatory cell response in this model.

The primary observation was that IL-10 treatment improved the remnant kidney model, as assessed functionally by better Ccr and lower proteinuria and histologically by less glomerulosclerosis and interstitial fibrosis. The improvement was substantial and resulted in a 30% improvement in renal function and a 50% or greater effect on renal scarring.

The potential mechanism by which IL-10 is renoprotective is likely complex, as IL-10 has multiple actions. Its primary effect is to inhibit activation and effector function of T cells, monocytes, and macrophages. IL-10 binds to its receptor (IL-10R), where it activates several signaling pathways depending on the cell type, including stat 3–dependent and –independent pathways (27), and NF-κB and AP-1 (28,29). A major target of IL-10 is the monocyte, where it blocks its activation and proliferation (30–34). IL-10 potently inhibits production of cytokines, such as IL-1 and TNF, and chemokines, such as MCP-1 and RANTES in activated monocytes/macrophages (33–37). IL-10 also inhibits dendritic cell function (38–40) and T cell activation, the latter in part by its effect on macrophages (41–44). IL-10R1 expression has also been observed on nonhemopoietic cells (45), raising the possibility that some of the actions of IL-10 could be mediated by direct effects on resident renal cell populations.

Because IL-10 acts primarily on monocytes and other immune cell populations, we examined the effect of IL-10 on the monocyte, dendritic cell, and T cell infiltration in our model. Macrophages are known to be present in the remnant kidney model, where they correlate with fibrosis (46). T cells are also known to be present in human and experimental chronic renal disease, although their exact role in the fibrogenic response remains debated (47–50).

Indeed, we confirmed that there was an infiltration of monocyte/macrophages, T cells, and dendritic cells in our model. Macrophages are known to be present in the remnant kidney model, where they correlate with fibrosis (46). T cells are also known to be present in human and experimental chronic renal disease, although their exact role in the fibrogenic response remains debated (47–50).

We further identified a potential mechanism by which IL-10 can block the local inflammatory response. Thus, one of the most important chemokines for macrophages in the kidney is
MCP-1, and MCP-1 is known to be expressed in the remnant kidney model, where it correlates with the monocyte infiltration (20,51,52). Inhibition of MCP-1 has also been found to result in less tubulointerstitial inflammation and fibrosis in several disease models (7,53,54). IL-10 is known to potently suppress MCP-1 in activated macrophages (33); hence, this is a likely mechanism to account for the reduction in monocyte infiltration observed in the IL-10–overexpressing rats. Although MCP-1 is also produced by tubular cells, we were not able to show an effect of IL-10 to suppress MCP-1 in proximal tubular (NRK52E) cells that express the IL-10R (data not shown). Thus, it is likely that the effect of IL-10 is

**Figure 4.** Serum IL-10 level correlates with number of infiltrating inflammatory cells. Serum IL-10 levels correlated inversely with the number of macrophage (ED-1⁺) cells (r = 0.63), dendritic cells (r = 0.63), CD4⁺ cells (r = 0.61), and CD8⁺ cells (r = 0.69) present (P < 0.05 for all). ▲, rAAV1-GAP control group; □ rAAV1-IL-10 treatment group.

**Figure 5.** Cytokine, chemokine, and fibrosis mediators in rAAV1-IL-10 treatment versus rAAV1-GFP control by real-time PCR. The graph shows the fold decrease of mRNA expression of various cytokine, chemokine, and fibrosis mediators by rAAV1–IL-10–treated animals compared with the rAAV1-GFP control animals by real-time PCR. rAAV1–IL-10 treatment significantly reduced mRNA expression of inflammatory cytokine IFN-γ and IL-2, chemokine monocyte chemoattractant-1 (MCP-1) and RANTES, TGF-β, and collagen I (COL I) and collagen III (COL III); *P < 0.05; **P < 0.01. Six rats are included in each group.

**Figure 6.** Kidney tissue MCP-1 levels. rAAV1–IL-10 rats have decreased renal MCP-1 levels compared with rAAV1-GFP control rats by ELISA (corrected for kidney protein concentration); P = 0.028. Six rats are included in each group.
suppression of MCP-1 in the inflammatory cell population and that this slowed the recruitment of monocyte/macrophages into the renal interstitium. It is also possible that some of the protective effects were due to inhibition of RANTES and the T cell infiltration; however, the role of these latter cells in chronic renal disease is still unclear.

Consistent with this hypothesis, we found that serum IL-10 levels correlated inversely with both renal tissue MCP-1 levels and renal RANTES mRNA (Figure 8), and the protein level of MCP-1 protein correlated closely with the number of macrophage, dendritic cells, and T cells. Furthermore, the mRNA level of RANTES correlated closely with the number of dendritic cells and T cells but not with macrophage number (Figure 7), which likely reflects that RANTES works primarily on the T cell population.

The reduction in renal inflammation was associated with significantly less renal fibrosis, as reflected by decreased TGF-β mRNA and decreased collagen mRNA and protein (Figures 5

Figure 7. Correlation of MCP-1 protein and RANTES with inflammatory cell infiltration. The level of renal MCP-1 protein in individual animals correlated closely with the number of macrophages (ED-1+ cells; r = 0.63), dendritic cells (r = 0.47), CD4+ cells (r = 0.73), and CD8+ cells (r = 0.66) present. Similarly, the level of RANTES mRNA correlated with the number of CD4+ (r = 0.68), CD8+ (0.66), and OX62+ dendritic cells (r = 0.58; P < 0.05 for all comparisons). □, rAAV1-IL-10 group; ▲ rAAV1-GFP group.
and 9; Table 3). The reduction in inflammation and fibrosis was also associated with less proteinuria and better preserved renal function. The lower proteinuria likely results from the less severe glomerular injury, which could also potentially result in less tubulointerstitial injury and inflammation because proteinuria is known to activate tubular cells.

Whereas the injection of AAV–IL-10 resulted in high circulating IL-10 levels, IL-10 mRNA levels that were assessed by real-time PCR were actually decreased in the kidneys of IL-10–overexpressing rats (data not shown). This is likely the consequence of the reduction in immune cell infiltration (because these cells are major sources of IL-10). This suggests that the effect that we observed was due to the high circulating levels of IL-10, which could have both systemic and intrarenal effects.

In conclusion, IL-10 treatment slowed renal progression in an animal model of chronic renal disease. It is interesting that the effect may be mediated by suppression of chemokines and reduction in infiltrating inflammatory cells, resulting in a less vigorous fibrotic response. The possibility that IL-10 may have direct suppressive effects on fibrosis also remains possible. Further studies are indicated to elucidate the protective mechanisms by which IL-10 acts in chronic renal disease.
Acknowledgments
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