Postobstructive regeneration of kidney is derailed when surge in renal stem cells during course of unilateral ureteral obstruction is halted

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Postobstructive regeneration of kidney is derailed when surge in renal stem cells during course of unilateral ureteral obstruction is halted. Am J Physiol Renal Physiol 298: F357–F364, 2010. First published November 11, 2009; doi:10.1152/ajprenal.00542.2009.—Unilateral ureteral obstruction (UUO), a model of tubulointerstitial scarring (TIS), has a propensity toward regeneration of renal parenchyma after release of obstruction (RUUO). No information exists on the contribution of stem cells to this process. We performed UUO in FVB/N mice, reversed it after 10 days, and examined kidneys 3 wk after RUUO. UUO resulted in attenuation of renal parenchyma. FACS analysis of endothelial progenitor (EPC), mesenchymal stem (MSC) and hematopoietic stem (HSC) cells obtained from UUO kidneys by collagenase-dispersed single-cell suspension showed significant increase in EPC, MSC, and HSC compared with control. After RUUO cortical parenchyma was nearly restored, and TIS score improved by 3 wk. This reversal process was associated with return of stem cells toward baseline level. When animals were chronically treated with nitric oxide synthase (NOS) inhibitor at a dose that did not induce hypertension but resulted in endothelial dysfunction, TIS scores were not different from control RUUO, but EPC number in the kidney decreased significantly; however, parenchymal regeneration in these mice was similar to control. Blockade of CXCR4-mediated engraftment resulted in dramatic worsening of UUO and RUUO. Similar results were obtained in caveolin-1-deficient but not -overexpressing mice, reflecting the fact that activation of CXCR4 occurs in caveolae. The present data show increase in EPC, MSC, and HSC population during UUO and a tendency for these cells to decrease to control level during RUUO. These processes are minimally affected by chronic NOS inhibition. Blockade of CXCR4-stromal cell-derived factor-1 (SDF-1) interaction by AMD3100 or caveolin-1 deficiency significantly reduced the UUO-associated surge in stem cells and prevented parenchymal regeneration after RUUO. We conclude that the surge in stem cell accumulation during UUO is a prerequisite for regeneration of renal parenchyma.

fibrosis; caveolin; AMD3100; mesenchymal stem cells

GLOMERULOSCLEROSIS and tubulointerstitial scarring (TIS)—the main processes governing progression of chronic renal diseases—are well studied and described (3, 7–9, 11, 15). Unilateral ureteral obstruction (UUO) has been consistently utilized as a convenient model of TIS, although this process is much less pronounced in mice than in rats, rabbits, and dogs (5). This model has provided valuable insights into the molecular and cellular mechanisms of TIS progression. Specifically, the role of various subsets of infiltrating leukocytes, hypoxia, angiotensin II, transforming growth factor (TGF)-β, TNF-α, plasminogen activator inhibitor-1, and reactive oxygen species, to name a few, has been established (reviewed in Refs. 3, 5, 7–9, 11, 15). Notwithstanding these advances, significant controversies exist. For instance, the role of stem cells in progression of fibrosis has not been unequivocally established. In a UUO model, Roufosse et al. (17) showed no contribution of bone marrow-derived cells to progression of fibrosis. On the other hand, Yamashita et al. (26) provided evidence that renal resident label-retaining cells undergo transition to myofibroblastic phenotype, thus potentially contributing to the progression of fibrosis. Most recent studies from Weinberg’s laboratory (12) have challenged this dogma by demonstrating the ability of epithelial cells that have undergone epithelial-mesenchymal transition to generate stemlike cells. Hence, in general and in this particular case of UUO, the role of stem cells in the progression of TIS remains unresolved.

In this vein, an impressive ability of obstructed shrunken kidneys to structurally regenerate renal parenchyma may be instructive. Clinical experience suggests that the release of UUO (RUUO) not only does not lead to progressive deterioration but is rather associated with improved renal function (2, 16). In experimental RUUO, Cochrane et al. (6) demonstrated partial functional recovery, reduced macrophage infiltration, and decreased proline and collagen content compared with the obstructed state. Could this model provide insights into the role of stem cells during the obstruction, but most importantly, during the postobstructive remodeling of the kidney? Could other factors recently implicated in tipping the balance between fibrosis and regenerative processes, such as the presence of endothelial dysfunction and expression of caveolin-1, modulate the outcome? These questions were addressed in the present study, the results of which demonstrate that preventing the accumulation of hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) during RUUO disrupts post-RUUO regeneration of the renal parenchyma.

MATERIALS AND METHODS

Animal studies. Adult male FVB/N mice (10–12 wk old) were obtained from the Jackson Laboratory (Bar Harbor, ME). Caveolin-1−/− and caveolin-1-overexpressing transgenic male mice of the same age were generated by M. P. Lisanti’s laboratory. The animal study protocol was in accordance with the National Institutes of...
Diego, CA; 0.3 mg/ml) in drinking water throughout the study period. The animals in groups 4–6 received N\textsuperscript{\textbf{N}}-monomethyl-l-arginine (l-NMMA, Axxora, San Diego, CA; 0.3 mg/ml) in drinking water throughout the study period. In a separate group of experiments, caveolin-1 and caveolin-1-overexpressing transgenic male mice underwent unilateral ureteral obstruction (UUO) for 10 days.

Assessment of renal morphology and interstitial fibrosis. Kidneys were collected from mice after 10 days of UUO or 3 wk after RUUO for structural analysis (n = 4–6 for each group). At harvest, each kidney was washed with saline, blotted dry on gauze, and weighed. Whole kidney weight was expressed as a percentage of body weight at the time when mice were euthanized. Midcoronal kidney sections were fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. Paraffin sections (4 μm thick) were stained with Sirius red and scored on the basis of pixel intensity. Changes in renal pelvis and papillary area were also assessed by scores ranging from 0 to 3 as determined as follows: 0, normal kidney; 1, mild change; 2, moderate change; 3, severe change. Alternatively, kidney sections were stained with Sirius red and scored on the basis of pixel intensity. Changes in renal pelvis and papillary area were also assessed by scores ranging from 0 to 3 as determined as follows: 0, normal kidney; 1, mild change; 2, moderate change; 3, severe change. Alternatively, kidney sections were stained with Sirius red and scored on the basis of pixel intensity. Changes in renal pelvis and papillary area were also assessed by scores ranging from 0 to 3 as determined as follows: 0, normal kidney; 1, mild change; 2, moderate change; 3, severe change.

Table 1. Kidney weight and renal cortical thickness after UUO and 3 wk after relief of UUO

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment</th>
<th>Percent Kidney Weight, %body wt</th>
<th>Cortical Thickness, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Unilateral control</td>
<td>0.91 ± 0.02</td>
<td>Contr</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10-day UUO</td>
<td>1.02 ± 0.05</td>
<td>Obstr</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>10-day UUO + 3-wk relief</td>
<td>0.86 ± 0.04</td>
<td>Contr</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>l-NMMA only</td>
<td>0.83 ± 0.07</td>
<td>Obstr</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>10-day UUO + l-NMMA</td>
<td>0.77 ± 0.07</td>
<td>Contr</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10-day UUO + l-NMMA</td>
<td>0.71 ± 0.02</td>
<td>Obstr</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>AMD3100 only</td>
<td>0.80 ± 0.03</td>
<td>Contr</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>AMD3100 only</td>
<td>0.78 ± 0.04</td>
<td>Obstr</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10-day UUO + AMD3100</td>
<td>0.91 ± 0.02</td>
<td>Contr</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>10-day UUO + AMD3100</td>
<td>0.70 ± 0.02*</td>
<td>Obstr</td>
</tr>
</tbody>
</table>

Values are means ± SE for n mice. UUO, unilateral ureteral obstruction; Contr, contralateral nonobstructed kidney; Obstr, UUO; l-NMMA, N\textsuperscript{\textbf{N}}-monomethyl-l-arginine. *P < 0.05 compared with control kidney.
Renal morphology and tubulointerstitial damage were examined with H & E staining and Masson’s trichrome or Sirius red, respectively. The CK showed normal histarchitecture, with distinct cortex, medulla, and renal papilla. In contrast, UUO kidney displayed conspicuous dilated proximal tubules and increased collagen deposition in tubulointerstitial areas (Fig. 1). UUO was associated with a marked attenuation of renal papilla and thinning of outer medulla and cortex (25% reduction from CK). However, after 3 wk of RUUO, the cortex and the medulla showed a remarkable restoration of renal parenchyma. Cortical thickness was recovered almost to control level (14% increased from UUO).

Stem cell accumulation during UUO. One of the possible mechanisms to account for the rapid restoration of the renal parenchyma after the relief of obstruction could be stem cell dependent. To determine the possible participation of stem cells during UUO or during regenerative processes after RUUO, we performed flow cytometric analysis of cells isolated from whole kidneys. Compared with CK, flow cytometric analysis of renal cells isolated from UUO kidney demonstrated a significant increase in CD34/Flk-1 (3-fold vs. control; approximate number of these cells in the kidney averages $3 \times 10^4$ in control and $9.9 \times 10^4$ in UUO) and CD150/c-kit (9-fold vs. control; approximate number of these cells in the kidney averages $21 \times 10^4$ in control and $134 \times 10^4$ in UUO) double-positive cells, indicative of EPC and HSC accumulation, respectively. Nestin (4-fold vs. control) and vimentin (15-fold vs. control)-positive cells, as well as CD29/CD44 (5-fold vs. control; approximate number of these cells in the kidney averages $13 \times 10^4$ in control and $78 \times 10^4$ in UUO) double-positive cells were also significantly increased after UUO, suggesting an increase in cells of the mesenchymal lineage during UUO. Ten-day UUO followed by 3 wk of RUUO was characterized by a significant drop in EPC, HSC, and MSC almost to baseline control levels (Figs. 2 and 3).

Effect of nitric oxide synthase inhibition on natural course of UUO and its relief. There is accumulating, albeit controversial, evidence linking stem cell mobilization with nitric oxide synthase (NOS) function (1, 13). Therefore, we addressed the next question: Does inhibition of NOS with nonhypertensinogenic doses of L-NMMA affect stem cell recruitment by the obstructed kidney? To avoid any contribution of elevation in blood pressure to the results of UUO, we employed subpressor doses of L-NMMA that have been shown to induce a mild endothelial dysfunction but no proteinuria or other functional changes in the kidney (19). UUO kidneys from L-NMMA-treated mice showed a similar reduction of KW/BW compared with CK or RUO kidney of nontreated mice. The data demonstrated that L-NMMA-treated CK showed normal histarchitecture except for a slight increase in perivascular fibrosis and UUO kidneys displayed marked tubular dilatation with increased interstitial collagen deposition. After 3 wk of RUUO, L-NMMA-treated kidneys showed a significant restoration of tubular dilatation and decline in interstitial collagen deposition (Fig. 1, D–F).

Subpressor doses of L-NMMA (0.3 mg/ml) also showed a similar pattern of increase in EPC, HSC, and MSC during UUO. Such increase in EPC, HSC, and nestin- and vimentin-positive cells during UUO was attenuated after RUUO, as in the control group. Of interest, the increase in EPC was significantly less pronounced in the L-NMMA-treated mice compared with the nontreated control group. On the other hand, the CD150/c-kit-positive cells and nestin- and vimentin-positive cells showed comparable increase after UUO in control and L-NMMA-treated mice. These data indicate that subpressor doses of L-NMMA, previously shown to be associated with mild endothelial dysfunction (19), did not significantly alter the course of UUO or RUUO, despite the curtailed surge in renal EPC. Hence, it appears that mild nitric oxide deficiency does not alter fibrotic and regenerative processes at least in the present experimental setting (Fig. 3).

Blockade of stem cell recruitment to obstructed kidney and its effect on postobstructive regeneration. To elucidate the role accumulating stem cells during UUO play in post-RUUO regeneration, we next attempted to prevent engraftment of kidney by stem/progenitor cells. Blockade of CXCR4 receptor by AMD3100 treatment significantly blunted the increase of EPC, HSC, and MSC during UUO (group 2 vs. group 8), suggesting possible attenuation of stem cell engraftment in UUO kidney. Late increase in EPC, HSC, and MSC was noted after discontinuation of AMD3100 treatment in RUO kidney (Fig. 4).

AMD3100-treated mice showed a significant reduction of KW/BW in UUO kidneys compared with CK. Furthermore,

![Fig. 1. Representative images of the kidneys obtained from mice with unilateral ureteral obstruction (UUO) and after its release (RUUO): A: control nonobstructed kidney showing normal distinct cortex, medulla, and renal papilla without interstitial fibrosis. B: marked attenuation of renal papilla and outer medullary area with thinning of renal cortex. Markedly dilated tubules and increased collagen deposition are seen with 10-day UUO. C: mild dilatation of tubules with partial restoration of renal parenchyma are noted 3 wk after RUUO. D: L-NMMA-monomethyl-L-arginine (L-NMMA)-treated control mice show normal histarchitecture except for a minimal increase in perivascular fibrosis. E: UUO on the L-NMMA background demonstrates marked tubular dilatation with increased collagen deposition. F: RUUO on the L-NMMA background displays significant restoration of histarchitecture and reduced collagen deposition. Masson’s trichrome stain: top, panoramic views at magnification ×40; bottom, magnification ×400. n = 4–6 in each group.](https://ajprenal.physiology.org/doi/fig/10.1210/ajprenal.198.2.F359)
RUUO kidneys showed further decrease in KW/BW compared with control kidneys (Table 1). CK in AMD3100-treated mice showed a near normal histoarchitecture, whereas UUO kidney showed a significant increase in tubular injury and interstitial fibrosis that was accompanied by marked attenuation of renal papilla with thinning of outer medulla and cortex (Table 2). In contrast to nontreated control mice subjected to the analogous treatments, 3 wk of RUUO in AMD3100-treated mice showed further aggravation in the loss of renal parenchyma with persisting papillary attenuation and renal pelvis dilatation (Fig. 5).

Role of caveolin-1 expression level in progression of fibrosis. Another way to manipulate stem cell engraftment of the kidney is through regulation of caveolin-1 expression. Caveolin-1 expression is a prerequisite for stem cell engraftment because CXCR4 dimerization induced by stromal cell-derived factor-1 (SDF-1) occurs in caveolar lipid-rich domains (24). It has been shown that incorporation of CXCR4 into membrane lipid rafts is necessary for SDF-1-induced homing of HSC and EPC (25). These data raised the question of the role played by CXCR4-SDF-1 interaction in the observed surge of stem cells in the obstructed kidney.

RUUO and RUUO kidneys showed further decrease in KW/BW compared with control kidneys (Table 1). CK in AMD3100-treated mice showed a near normal histoarchitecture, whereas UUO kidney showed a significant increase in tubular injury and interstitial fibrosis that was accompanied by marked attenuation of renal papilla with thinning of outer medulla and cortex (Table 2). In contrast to nontreated control mice subjected to the analogous treatments, 3 wk of RUUO in AMD3100-treated mice showed further aggravation in the loss of renal parenchyma with persisting papillary attenuation and renal pelvis dilatation (Fig. 5).

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To address this question, we examined the course of UUO in caveolin-1-deficient and caveolin-1-overexpressing mice. Genetic manipulation of caveolin-1 expression level resulted in strikingly divergent responses to UUO. In caveolin-1−/− mice, the degree of developing fibrosis was dramatically increased compared with wild-type and caveolin-1-overexpressing transgenic animals (Fig. 6). At the level of the microvasculature, however, the highest increase in neointimal formation, as judged by the media-to-lumen ratio, was detected in caveolin-1-overexpressing mice (Fig. 7).

The abundance of stem cells in the kidney was examined next. As shown in Fig. 8, only the postobstructive surge of MSC in the kidney was dramatically curtailed in caveolin-1−/− mice, whereas EPC and HSC, although slightly reduced, did not change significantly. The fact that caveolin-1 deficiency was associated with the worsening of interstitial fibrosis and reduction in renal MSC, compared with obstruction in wild-type mice, suggests that accumulation of MSC does not necessarily lead to aggravation of fibrosis; rather it is the reduction of their surge that is associated with worsening of interstitial fibrosis.

**DISCUSSION**

UUO is associated with progressive renal fibrosis and scarring and a decline in renal function. RUUO is characterized by a variable recovery of renal function and structural repair of renal parenchyma. Although numerous studies have examined the events that lead to renal fibrosis, less is known about the inherent mechanisms that promote tissue remodeling and regeneration. A mouse model of UUO and RUUO that results in development of renal fibrosis and loss of parenchyma was established by Cochrane et al. (6) and reexamined here, along with several recently discovered modulators of fibrosis and/or regeneration.

The obstructed kidney from 10-day UUO demonstrated profound ablation of renal cortex and outer medulla and was accompanied by marked increase in EPC, HSC, and MSC in the renal parenchyma. After 3 wk RUUO, cortical parenchymal thickness was nearly restored, along with decrease of EPC, HSC, and vimentin- or nestin-positive cells. Treatment with AMD3100, a specific antagonist of CXCR4, markedly attenuated observed stem cell mobilization during 10-day UUO and was associated with the failure to regenerate renal parenchyma after 3 wk RUUO. Flow cytometric analysis of renal parenchymal cells in AMD3100-treated mice after 3 wk RUUO showed delayed marked increase in EPC, HSC, and vimentin- and nestin-positive, as well as CD29- and CD44-positive cells. These results suggest that a wave of stem cell accumulation in the kidney during the period of obstruction, but not after its

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Tubular Injury</th>
<th>Fibrosis Score</th>
<th>Pelvis Dilatation</th>
<th>Papillary Attenuation</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>2.89 ± 0.07</td>
<td>2.56 ± 0.17</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>3.90 ± 0.87</td>
<td>2.34 ± 0.53</td>
<td>1.56 ± 0.34</td>
<td>0.50 ± 0.29</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.81 ± 0.22</td>
<td>&lt;0.01</td>
<td>0.31 ± 0.22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0.98</td>
<td>&lt;0.01</td>
<td>0.08</td>
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</tr>
<tr>
<td>5</td>
<td>4</td>
<td>4.07 ± 1.35</td>
<td>1.78 ± 0.49</td>
<td>2.29 ± 0.86</td>
<td>2.25 ± 0.31</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.63 ± 0.38</td>
<td>&lt;0.01</td>
<td>0.63 ± 0.38</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>0.1 ± 0.18</td>
<td>&lt;0.01</td>
<td>0.1 ± 0.18</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>3.63 ± 1.2</td>
<td>1.84 ± 0.62</td>
<td>1.79 ± 0.58</td>
<td>2.14 ± 0.49</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>5.17 ± 0.83</td>
<td>2.78 ± 0.35</td>
<td>2.39 ± 0.48</td>
<td>2.86 ± 0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE for n mice. TIS, composite tubular injury score (= tubular injury + fibrosis score).
release, may serve as a mechanism of renal structural repair following RUUO.

Owing to the fact that all studied cells, EPC, HSC, and MSC, showed a surge in the obstructed kidneys, it is difficult to dissociate the contribution of each cell type to the postrelief structural recovery. In this vein, the results of L-NMMA pretreatment may shed some light. Under these experimental conditions, only the surge in EPC was found to be inhibited, whereas the numbers of HSC and MSC residing in the obstructed kidneys were not different from nontreated controls. The fact that this did not affect the regenerative outcome after the release of obstruction may be indicative of the leading role played by HSC and/or MSC in the positive remodeling. It must be acknowledged, however, that a two-parameter assignment of cells to different stem cell populations is tenuous. Despite the fact that traditional markers have been utilized in this study, some overlap with nonstem cells is inevitable and interpretation of findings requires caution.

Prior experience with stem cell mobilization or transplantation in UUO brought about conflicting results. Stem cell factor (SCF)- or granulocyte-colony stimulating factor (G-CSF)-induced mobilization of bone marrow stem cells did not reduce tubulointerstitial fibrosis in UUO (20). This finding may be difficult to interpret as it could represent the net result of opposing trends due to the multicellular nature of mobilizing effects elicited by either of these agents, which could have canceled each other. For instance, increased influx of classically activated macrophages could potentially aggravate fibrosis, in fact reversing any possible beneficial effect observed upon macrophage depletion in UUO (21). In accord with our findings, however, label-retaining cells were found in increased numbers after UUO (26), where they reportedly participated in epithelial-mesenchymal transition. The role played by these cells in postrelief remodeling and tissue regeneration was not studied. Our data suggest that the accumulated stem cells could have participated in this process. This conclusion is based on

Fig. 5. Effect of AMD3100 on postobstructive kidney regeneration: representative images of kidneys obtained from mice with 10-day UUO and 3 wk after its release. AMD3100-treated control mice kidney appears grossly normal (A). UUO in AMD3100 treated mice demonstrated marked tubular dilatation with increased collagen deposition. Moreover, marked attenuation of renal papilla and thinning of outer medullar and cortical areas are noted (B). After 3 wk of RUUO, AMD3100-treated kidneys display further shrinkage of renal parenchyma, especially the outer medulla and the cortex (C). Masson’s trichrome stain: top, panoramic views at magnification ×40; bottom, magnification ×400; n = 4–6 in each group.

Fig. 6. Fibrosis is prevalent in caveolin-1/-/- obstructed kidneys. Quantitative assessment of the degree of fibrosis, as assessed with Sirius red (SR) staining, in Cav-1/-/- and Cav-1-overexpressing mice compared with wild-type (WT) counterparts subjected to UUO (top) and representative images of obstructed kidneys (bottom). Values are means ± SE; n = 4–6.

*p<0.05 vs WT UUO
**p<0.05 vs UUO
p=0.08 vs Cav-/- UUO

WT, UUO  Cav-/- UUO  Cav-/-+/- UUO  WT, Co  Cav-/-, Co  Cav-/-+/-, Co

WT-UUO  cav-/- UUO  cav-/-+/- UUO  WT  cav-/-  cav-/-+/-
the demonstration of halted and even reversed regeneration in mice treated with AMD3100, which curtailed the accumulation of stem cells in the kidney, possibly via the reduced engraftment. Indeed, we have no data to support or reject the possibility of engraftment versus proliferation of resident stem cells in the obstructed kidney; such an analysis would require cell fate tracing experiments. EPC did not appear to be of critical importance for the regenerative processes, insofar as prevention of their accumulation in the obstructed kidney did not palpably affect the postrelief outcome.

It can be argued that the detectable population of CD34-positive/Flk-1-positive cells may incorporate mature endothelial cells. Unfortunately, this is an unavoidable limitation of the techniques used. However, considering the opposite trends in the shrinking size of population of vascular endothelial cells in the obstructed kidney vis-à-vis the growing number of detectable CD34+/Flk-1+ cells, it is reasonable to assume that the difference reflects the increased contribution by EPC.

The data obtained in caveolin-1 knockout and transgenic mice may emphasize the contribution of enhanced stem cell mobilization and impaired engraftment: Apart from its role in the activation of CXCR4, caveolin-1 may also play an important role in stem cell mobilization. Sbaa et al. (18) have demonstrated that neovascularization in Cav-1−/− mice impaired because of the defective mobilization of EPC from the bone marrow after stimulation with SDF-1. This was due to the blockade of CXCR4 internalization in the absence of caveolin-1. At the same time, adhesion of Cav-1−/− EPC to SDF-1-presenting endothelial cells was enhanced, a property that could be reproduced in wild-type mice by small interfering RNA inhibition of caveolin-1 expression. These data emphasize the existing caveolin-dependent dualism in SDF-1-induced actions on CXCR4: Caveolin-1 deficiency causes both reduced mobilization and enhanced homing, a paradox that requires further in-depth studies. Using fluorescence resonance energy transfer, Toth et al. (22) demonstrated that SDF-1 enhanced CXCR4 receptor dimerization, an effect that was inhibited by the CXCR4 antagonist AMD3100. In a model of vascular injury, however, caveolin-1 deficiency enhances neointimal formation (10). Caveolin-1 deficiency has been found to increase cell proliferation and collagen synthesis, thus leading to myocardial hypertrophy and pulmonary hypertension; both were normalized in Cav-1−/− mice by reexpressing caveolin-1 in endothelial cells (14). In accord with this, administration of caveolin-1 scaffolding domain peptide (Cavtratin) affords an antifibrotic effect in lung injury (23).

In addition, our data suggest that the timing of stem cell accumulation in the renal parenchyma of the obstructed kidney is of essence: Despite the fact that cells reaccumulate after the relief of obstruction and cessation of AMD treatment, regeneration does not occur and fibrosis progresses.

In conclusion, the data presented here extend previous observations by Cochrane et al. (6), as well as the existing clinical experience on the regenerative capacity of the kidney after obstruction has been relieved. One of the proposed mechanisms for positive kidney remodeling under these conditions has been identified here as the accumulation of stem cells and the derailment of parenchymal regeneration when the surge in stem cells was halted. The data presented allow us to rule out the major role of EPC in this process and suggest the potential contribution of MSC and HSC, although they do not distinguish between them.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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