Bone marrow-derived endothelial progenitor cells confer renal protection in a murine chronic renal failure model

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1Department of Internal Medicine, Seoul National University College of Medicine, 2Kidney Research Institute, Seoul National University, 3Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; and 4Department of Internal Medicine, Chungbuk National University Hospital, Cheongju, Korea

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Sangidorj O, Yang SH, Jang HR, Lee JP, Cha R, Kim SM, Lim CS, Kim YS. Bone marrow-derived endothelial progenitor cells confer renal protection in a murine chronic renal failure model. Am J Physiol Renal Physiol 299: F325–F335, 2010. First published May 19, 2010; doi:10.1152/ajprenal.00019.2010.—Endothelial cell damage and impaired angiogenesis substantially contribute to the progression of chronic renal failure (CRF). The effect of endothelial progenitor cell (EPC) treatment on the progression of CRF is yet to be determined. We performed 5/6 nephrectomy to induce CRF in C57BL/6 mice. EPCs were isolated from bone marrow, grown in conditioned medium, and characterized with surface marker analysis. The serial changes in kidney function and histological features were scrutinized in CRF mice and EPC-treated CRF (EPC-CRF) mice. Adoptively transferred EPCs were present at the glomeruli and the tubulointerstitial area until week 8 after transfer. In CRF mice, renal function deteriorated steadily over time, whereas the EPC-CRF group showed less deterioration of renal function as well as reduced proteinuria along with a relatively preserved kidney structure. Renal expression of proinflammatory cytokines and adhesion molecules was already decreased in the EPC-CRF group at the early stage of disease, at which point the renal function and histology of CRF and EPC-CRF mice were not different. Angiogenic molecules including VEGF, KDR, and thrombospondin-1, which were decreased in the CRF group, were restored by EPC treatment. In conclusion, EPCs trafficked into the injured kidney protected the kidney from the inflammatory condition and consequently resulted in functional and structural renal preservation. Our study suggests EPCs as a potential candidate for a novel therapeutic approach in CRF.

THE GLOBAL BURDEN OF CHRONIC diseases has rapidly increased in the context of an aging population. The number of patients suffering from chronic renal failure (CRF), a prototype of chronic disease, is growing and demand for renal replacement therapy is also increasing (15, 19). Medical efforts have been focused on preventing and slowing functional deterioration following organ damage. The recent discovery of endogenous repair mechanisms adopting hematopoietic stem and progenitor cells has bolstered the concept of tissue repair. In 1997, Asahara et al. (4) demonstrated that CD34-positive hematopoietic progenitor cells derived from peripheral blood mononuclear cells can differentiate into endothelial lineage cells. Since then, many studies have described the endogenous function and therapeutic effect of these cells, termed as endothelial progenitor cells (EPCs). Over the past few years, studies showed the presence of endothelial cell impairment in patients with advanced renal failure and also in patients who underwent renal transplantation (7, 10, 14, 24). The vanishing of glomerular endothelial cells has been reported in several models of progressive renal disease characterized by the development of glomerulosclerosis and tubulointerstitial damage. In addition, renal replacement therapy was reported to improve EPC biology (30).

Mesenchymal stem cells expressing CD29⁺CD73⁺CD14⁻CD34⁻, were evaluated for the potential of structural repair in experimental kidney diseases, but the beneficial effect was limited (8, 16, 21). Among stem cells, CD34-positive cells may be immature cells with endothelial priming and represent putative EPCs (23). By definition, stem cells possess the capability of self-renewal, transforming into dedicated progenitor cells, and differentiating into specialized progeny such as EPCs (29). EPCs can migrate to the ischemic region, where they can differentiate into mature and functioning endothelial cells, thereby preserving proliferative activity (1–4, 22). Several studies have reported that bone marrow (BM)-derived EPCs also have the ability to preserve organ function possibly by vasculogenesis or angiogenesis (9, 34). In recent studies, EPCs mobilized from bone marrow have also contributed to glomerular endothelial cell repair and ameliorated glomerular endothelial injury in experimental glomerulonephritis, supporting that protecting glomerular endothelial cells may attenuate inflammation in glomerulonephritis (18, 25, 30).

Based on recent advances in studies of EPCs in experimental kidney diseases and the lack of treatment strategies that augment repair in CRF, we tested the hypothesis that EPCs enhance repair in chronic renal failure. We analyzed the characteristics of EPCs, transferred these cells into mice that underwent 5/6 nephrectomy and found a significant improvement in renal function and structure. We also evaluated direct and indirect effects of EPCs on the microenvironment of injured kidneys.

MATERIALS AND METHODS

Mice. Male C57BL/6 mice, of approximately similar weights (20–22 g) and 7–8 wk old, were housed under specific pathogen-free conditions. All animal experiments were performed under the approval of the Institutional Animal Care and Use Committee of the Clinical Research Institute at Seoul National University Hospital and according to the Guide for the Care and Use of Laboratory Animals of the National Research Council (NRC).

Isolation and culture of putative EPCs. EPCs were isolated and cultured according to the method used for isolation of EPCs from BM. In brief, BM mononuclear cells were isolated from the femurs and tibias of 7-wk-old male C57BL/6 mice by density gradient centrifu-
gitation with Histopaque-1083 (Sigma-Aldrich, St. Louis, MO). The obtained cells were cultured in EGM-2 MV (Clontech, Mountain View, CA) supplemented with 10% FBS, 1 mg/ml hydrocortisone, 12 mg/ml bovine brain extract, 50 mg/ml gentamycin, 50 mg/ml amphotericin B, and 10 ng/ml epidermal growth factor on fibronectin-coated dishes. After 10 days of culture, detached from the dishes by addition of a 3 mM EDTA solution and a minimal amount of trypsin, cells were passed through a cell strainer. The cells were infused into surgery (5/6 nephrectomy)-induced CRF mice. In some experiments, the cells were labeled with a fluorescence marker, CM-DiI (Molecular Probes, Eugene, OR), by being incubated in PBS that contained 2 μg/ml CM-DiI for 5 min at 37°C and 15 min at 4°C. Labeled EPCs were used for infusion into the mouse. The number of infused cells was determined by intracellular fluorescence intensity. EPCs were infused into surgery (5/6 nephrectomy)-induced CRF mice.

Fig. 1. Characterization of bone marrow-derived cells cultured in endothelial cell growth factor. After 10 days of culture, most of the adherent cells grown in conditioned medium expressed BS1-lectin, CD31, and endothelial nitric oxide synthase (eNOS). Original magnification ×200. DAPI, 4,6-diamidino-2-phenylindole.

Fig. 2. Quantitative evaluation of the expression of cell surface markers. Serial changes in each surface marker occurred when bone marrow-derived cells were cultivated in endothelial cell growth medium. The expression of CD31, CD34, c-kit, Tie-2, Sca-1, KDR, CD133, and BS1-lectin was increased with time. However, the expression of CD29 decreased as cells differentiated into endothelial cells. The values in the graph represent the percentage of each type of marker-positive cells. The plot is representative of at least 6 independent experiments. D, days.
Fig. 3. Localization of transferred endothelial progenitor cells (EPCs) in the kidney. A: CM-Dil-labeled EPCs (red) were intravenously transferred into mice with chronic renal failure (CRF). Kidneys were harvested at 4 and 8 wk following CRF induction. The transferred EPCs were frequently found in the glomeruli where von Willebrand factor (vWF; green) was expressed. Original magnification ×800. B: transferred cells were occasionally found in the tubulointerstitial area. Original magnification ×1,200. Intrarenal localization was evaluated using confocal laser-scanning microscopy. The data shown represent 1 of 3 independent experiments. C and D: EPCs from GFP mice (red) were found along with CD31-expressing cells (green) in the glomerulus (C) as well as in the peritubular capillaries (D). Original magnification ×800.
were transferred with PBS and intravenously injected at a total number of 1 × 10⁶ cells in 300 μl of PBS into each recipient C57BL/6 mouse. To verify the EPC migration and localization, bone marrow cells from green fluorescent protein (GFP) transgenic C57BL/6 mice (kindly donated from Prof. Hyo-Soo Kim, Seoul National University College of Medicine, Seoul, Korea) were isolated and grown in EPC-selective medium. Expression of GFP in EPCs was confirmed by flow cytometry.

Flow cytometry analysis. Flow cytometry analysis (FACs) was performed on freshly isolated cells (at day 0) and on the cells after 10 days of culture (see the online supplementary data, which are available on the journal website).

Dil-Ac-LDL/lectin staining and cell calculation. For Dil-Ac-LDL/lectin staining and cell calculation, see the online supplementary data.

Tube formation on Matrigel plate. In vitro tubelike structure formation was evaluated using Matrigel plates (BD, Franklin Lakes, NJ) as detailed in the online supplementary data.

Induction of experimental CRF and EPC treatment. We developed a mouse CRF model by a modified protocol as described below. The surgery was performed under anesthesia with a mixture of ketamine (100 mg/ml) and xylazine (25 mg/ml). A 1:10 dilution of the stock solution in saline was administered intraperitoneally at 0.02 ml/g of body wt. Bilateral dorsal longitudinal incisions were made to expose both kidneys. The lower branch of the right renal artery was ligated by 9.0 silk suture to produce visible renal ischemia to about one-third the kidney, and the upper pole of the right kidney was amputated using electrocoagulation. Then, the upper branch of the left kidney artery was ligated, and the lower pole was placed back into the renal fossa by cautery. Left nephrectomy was performed 1 wk later (CRF day 0). The left kidney was removed, and the vascular pedicle was ligated at the hilum with the use of 4-0 silk. The time of the left nephrectomy marked the onset of moderate to severe renal failure. For the EPC treatment, in vitro expanded BM-EPCs were administered into CRF mice at day 1 and day 7 (1 × 10⁶ cells each time).

Measurement of blood pressure, proteinuria, serum blood urea nitrogen, and creatinine. For measurement of blood pressure, proteinuria, serum blood urea nitrogen (BUN), and creatinine, see online supplementary data.

Histological and confocal microscopic analysis. Harvested kidneys were fixed in 4% paraformaldehyde, and paraffin-embedded tissue sections (4 μm) were stained with periodic acid-Schiff (PAS) and Masson’s trichrome stain. Detailed analysis methods are described in the online supplementary data.

Quantitative real-time PCR. Renal tissue samples were harvested from mice every 4 wk for 20 wk after CRF induction, and the cytokine expression profile was assessed using real-time PCR as detailed in the online supplementary data.

Bioplex protein array. A panel of cytokines was measured in whole kidney protein extracts with the Bioplex Protein Array system (Bio-Rad, Hercules, CA) according to the manufacturer’s method. This is a multiplexed, particle-based, flow cytometric assay that utilizes anti-cytokine monoclonal antibodies linked to microspheres incorporating distinct properties of two fluorescent dyes. Our assay was designed to detect and quantify IL-1β, IL-6, and IFN-γ. Each cytokine value was normalized by dividing the raw cytokine concentration (pg/ml) with kidney protein concentration (mg/ml) measured by the Bradford method.

Statistical analysis. The results are expressed as means ± SD or means ± SE as appropriate. Statistical analysis was performed using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). To compare more than two groups, a one-way ANOVA using Tukey’s test was performed. A P value <0.05 was considered statistically significant.

RESULTS

Characterization of BM-derived EPCs. After 7 and 10 days of culture, the attached cells were observed as BM-derived EPC (BM-EPC) colony-forming units, which is a central core of rounded cells surrounded by spindle-shaped cells. BM-EPCs grown on Matrigel formed tubelike structures in the presence of endothelial growth factors (Supplementary Fig. S1). More than 90% of the adherent BM-EPCs incorporated acetylated-LDL, and the adherent cells were stained positive for BS-1 lectin (Supplementary Fig. S2). The majority of BM-derived cells cultured in selective medium expressed endothelial nitric oxide synthase (eNOS) and CD31 (Fig. 1). Flow cytometric analysis revealed that endothelial lineage-specific markers such as CD31, CD34, c-kit, Tie-2, Sca-1, KDR, and CD133 increased up to 10 days after plating. But the expression of CD29, which represents mesenchymal stem cells, decreased as the cells were differentiated into EPCs (Fig. 2).

**EPCs trafficked into injured kidneys at the early phase of CRF.** We checked the trafficking of EPCs into the damaged renal tissue with two different methods using confocal microscopy. First, fluorescently tagged EPCs were adoptively transferred into the mice that underwent 5/6 nephrectomy. The transferred EPCs were detected in glomeruli that expressed von Willebrand factor (vWF) at 4 and 8 wk after transfer (Fig. 3A), and a few labeled cells were observed in the tubulointerstitial area at 4 wk after transfer (Fig. 3B). We further confirmed the trafficking of transferred EPCs into the injured kidneys using GFP transgenic mice. The expression of CD31 on BM-EPCs from GFP mice was confirmed using confocal microscopy before transfer (Supplementary Fig. S3). Four weeks after transfer, BM-EPCs from GFP mice were found in both glomerular capillaries (Fig. 3C) and peritubular capillaries (Fig. 3D) where CD31 was expressed together. After 4 wk, the transferred EPCs were also detected in other organs such as the liver and spleen (Supplementary Fig. S4).

**EPCs reduced renal structural damage in CRF mice.** Renal histological examination revealed a marked difference between CRF mice and EPC-CRF mice after 12 wk of disease induction. The glomerulosclerosis index (11, 20) was higher in CRF mice compared with EPC-CRF mice 20 wk after the 5/6 nephrectomy. In CRF mice, most glomeruli showed grade 3+ or 4+ on the scale of the glomerulosclerosis index, whereas EPC-CRF mice showed grade 2+ or 3+ (P < 0.05) (Fig. 4A).

Ki-67-positive tubular cells, which represent proliferation in response to the tubular damage, were increased in CRF mice and reduced by EPC treatment (mean number per ×200 high-power field, age-matched control mice vs. CRF-4 wk vs.
EPC-CRF-4 wk 1.33 vs. 5.00 vs. 3.69, CRF-8 wk vs. EPC-CRF-8 wk 12.65 vs. 3.33).

The degree of renal fibrosis was evaluated with Masson’s trichrome staining. Tubulointerstitial fibrosis was widely observed in CRF mice after 16 wk. However, EPC-CRF mice showed reduced interstitial fibrosis, although mesangial expansion was occasionally observed (Fig. 4B). We compared the expression of transforming growth factor (TGF)-β1 to confirm that in vivo administration of EPCs would prevent the development of glomerular fibrosis. Intraglomerular TGF-β1 expression, assessed by immunohistochemistry and real-time PCR, was increased in CRF mice. However, EPC treatment significantly attenuated TGF-β1 expression (Fig. 5).

**EPCs improved renal function in CRF mice.** After 5/6 nephrectomy, the mice lost weight and maintained a lower body weight throughout the study period. However, EPC-treated CRF mice (EPC-CRF) began regaining their body weight after 12 wk of disease and recovered to the level of age-matched control mice at the end of the experiment (Fig. 6A). Systolic blood pressure was also lower in the EPC-CRF group compared with the CRF group at both 4 and 8 wk (CRF vs. EPC-CRF; 160.9 ± 3.8 vs. 138.5 ± 2.5 mmHg at 4 wk, 159.9 ± 4.03 vs. 135.0 ± 3.0 mmHg at 8 wk, P < 0.05, respectively) (Supplementary Fig. S5). The blood BUN level in CRF mice was three times higher than that of control mice at 4 wk (80 ± 8.2 vs. 23 ± 1.6 mg/dl, P < 0.05) and was consistently elevated throughout the study period. The EPC-CRF mice showed less elevation of BUN at 4 wk of disease (71 ± 9.5 mg/dl), and BUN was maintained at a level lower than CRF mice throughout the study period (P < 0.05). The changes in serum creatinine and BUN were similar (Table 1). Significant proteinuria developed after the induction of CRF and progressed afterward. At 20 wk of disease, the spot urine protein/creatinine was elevated to 35 mg/mg. However, the amount of proteinuria was reduced in EPC-CRF mice, comparable to that of age-matched control mice (urine protein/creatinine (mg/mg) at 16 and 20 wk, CRF vs. EPC-CRF; 29.8 ± 1.2 vs. 11.1 ± 1.7, P < 0.01 and 37.5 ± 1.5 vs. 11.0 ± 1.3, P < 0.01, respectively) (Fig. 6B). We introduced splenocytes into CRF mice to evaluate the unique protective role of EPC treatment. The adoptive transfer of splenocytes into CRF mice did not alter the course of renal impairment both functionally and structurally (Supplementary Fig. S6).

**EPC treatment switched proinflammatory microenvironment of injured kidneys to the anti-inflammatory condition.** Immunohistochemical analysis for cellular infiltration and the quantification of cytokines and adhesion molecules in mRNA and protein levels were performed to evaluate the cellular and molecular mechanisms of the renoprotective effect of EPCs. The histological findings were similar between the CRF and the EPC-CRF groups at 4 and 8 wk of disease. However, we hypothesized that the favorable functions of EPCs for protecting the renal impairment may begin in the early period of disease.

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**Fig. 5.** Transforming growth factor (TGF)-β1 expression was attenuated by the EPC treatment. A: intraglomerular expression of TGF-β1 was evident in CRF mice; however, it was markedly attenuated in EPC-CRF mice. B and C: the number of glomeruli containing TGF-β1-positive cells (B) and TGF-β1 mRNA expression (C) were significantly decreased by EPC treatment. *P < 0.05. Representative pictures were taken at 20 wk of disease. Values are means ± SE and represent 1 of 3 independent experiments (3 mice in each group for each experiment). Original magnification ×400.
thus determining the course of disease progression afterward. At 4 wk of disease, T cells expressing CD3, neutrophils, and macrophages/monocytes were present at peritubular areas in CRF mice. However, the EPC-CRF group had significantly less infiltration of inflammatory cells at 4 wk (Fig. 7). The mRNA expression of proinflammatory cytokines and adhesion molecules such as IL-1β, TNF-α, IL-6, MCP-1, ICAM-1, and VCAM-1, was significantly increased in CRF mice compared with that of control mice at 4 and 8 wk. However, the expression of proinflammatory cytokines and adhesion molecules was significantly decreased with EPC transfer at 4 and 8 wk (Fig. 8A). At 4 wk, CRF mice showed increased expression of IL-1β, IL-6, and IFN-γ in protein level, and the increment of these cytokines was attenuated by EPC treatment (Fig. 8B).

We further confirmed the expression profiles of VEGF and E-selectin in protein levels. VEGF was expressed in glomerular and peritubular capillaries in control mice. In CRF mice, very little VEGF expression was detected at 4 wk of disease. Although mRNA expression was similar at 4 wk of disease between CRF and EPC-CRF mice (Fig. 8A), VEGF expression was restored in the EPC-CRF group and was found in peritubular capillaries at 4 wk of disease (Fig. 9A). E-selectin was detected in the peritubular capillaries of CRF mice after 4 wk of disease but not in control or EPC-CRF mice at 4 wk (Fig. 9B).

Finally, we assessed the expression patterns of molecules that are associated with endothelial cell migration and neovascularization. After 5/6 nephrectomy, KDR and thrombospondin-1 expression significantly decreased at 4 and 20 wk of CRF compared with control mice. The adoptive transfer of EPCs restored the expression of these molecules to the levels comparable to those of age-matched control mice (Fig. 9C).

### DISCUSSION

In this study, we characterized the ex vivo commitment of BM-derived EPCs in terms of angiogenic phenotype and lineage potential. Our study revealed that the adoptive transfer of BM-EPCs prevented the progression of CRF by migrating into the site of damage and reducing inflammation. These findings suggest that BM-EPCs have a therapeutic potential for cell therapy.

There have been many studies investigating the characteristics of EPCs. Schatteman and colleagues (26) identified a subset of BM-EPCs that expressed CD31 or Tie-2 after 7 days in culture on fibronectin and showed upregulation of CD34 and VEGFR-2 thereafter. These cells take up acetylated LDL (ac-LDL), bind Ulex lectin, and form tubelike structures in vitro (26). EPCs were also reported to perform phagocytosis on ac-LDL, while BS-1-lectin staining identifies mouse endothelial cells (35). EPCs have been thought to improve endothelial function and angiogenesis. Ischemia of limb muscle or myocardium may trigger EPCs to migrate from the BM, whereas EPC levels remain low under normal conditions. EPCs are conventionally characterized by hematopoietic stem cell markers such as CD34 or CD133, expression of an endothelial surface marker, uptake of Dil-acetylated lipoprotein, and lectin binding (33). The characteristics of BM-EPCs in our study were consistent with the common features of EPCs reported in past studies. Analysis of BM-EPCs in vitro demonstrated their ability to form colonies that were positive for several mature EC markers as well as progenitor markers. We also described the localization of an endothelial-specific marker, eNOS expression, in the BM-EPCs of mice using immunohistochemistry. Uptake of LDL and a lectin binding prop-

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### Table 1. Serial changes in renal function by administration of endothelial progenitor cells

<table>
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<tr>
<th>Week of Disease</th>
<th>Control</th>
<th>CRF</th>
<th>EPC-CRF</th>
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<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>Creatinine (mg/dl)</td>
<td>BUN (mg/dl)</td>
<td>Creatinine (mg/dl)</td>
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<tr>
<td>Week 4 (n = 20/group)</td>
<td>23 ± 1.6</td>
<td>0.4 ± 0.05</td>
<td>80 ± 8.2</td>
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<tr>
<td>Week 8 (n = 16/group)</td>
<td>28 ± 1.7</td>
<td>0.4 ± 0.05</td>
<td>82 ± 6.6</td>
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<tr>
<td>Week 12 (n = 12/group)</td>
<td>31 ± 3.2</td>
<td>0.4 ± 0.06</td>
<td>83 ± 11.9</td>
</tr>
<tr>
<td>Week 16 (n = 8/group)</td>
<td>28 ± 2.0</td>
<td>0.4 ± 0.07</td>
<td>106 ± 15.0</td>
</tr>
<tr>
<td>Week 20 (n = 4/group)</td>
<td>27 ± 3.2</td>
<td>0.7 ± 0.05</td>
<td>104 ± 15.3</td>
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Values are means ± SD for control blood urea nitrogen (BUN) and creatinine. All others are means ± SE. CRF, chronic renal failure; EPC, epithelial progenitor cells. *P < 0.05 compared with CRF. †P < 0.01 compared with CRF.
We revealed the role of BM-EPCs in a technically challenging mice CRF model. Some studies have suggested that C57BL/6 mice have one specific gene (Ren-1) that partially accounts for the milder glomerulosclerosis compared with other mouse strains (17). We induced CRF in C57BL/6 mice utilizing 5/6 nephrectomy with an additional protocol (6, 17). The consequences of CRF were manifest as kidney lesions typical of chronic changes and were distinct compared with control mice at all ages examined. Mice suffered from moderate CRF at least up to 8–12 wk after 5/6 nephrectomy, which was indicated by elevated BUN values and reduced body weight. Consistent with the impaired kidney function, the histopathological lesions were progressively aggravated in these mice. Thus we successfully developed a murine CRF model in C57BL/6 mice following surgery.

BM-EPCs have been reported to ameliorate the dysfunction of ischemic organs, possibly by inducing vasculogenesis or angiogenesis in tissues by stimulating the reendothelialization of injured blood vessels (9, 34), during wound healing (13), as well as of blood vessels damaged by limb ischemia (3, 4) and postmyocardial infarction (27). However, fewer data are available regarding the role of BM-EPCs in renal diseases, especially in CRF. Administration of BM-EPCs significantly improved renal function, and immunofluorescence analysis revealed that fluorescent-labeled EPCs accumulated at glomeruli as well as at interstitia. EPCs can differentiate into mature cells in vivo after 4 wk (12). Our results suggest that EPCs may migrate into some capillary networks of glomeruli. Interestingly, after transfer of BM-EPCs into CRF mice, fluorescent-tagged cells were primarily found in glomerulosclerotic lesions. These results apparently correspond to the results obtained by Rookmaaker et al. (25), who showed a relative contribution of BM-derived cells in glomerular repair when

Fig. 7. Changes in cellular infiltrations depending on the EPC treatment. At 4 wk after CRF induction, CD3⁺ T cells infiltrated into periarteriolar areas (arrows). Similarly, neutrophils (MPO⁺) and monocytes (F4/80⁺) were detected in periarteriolar and tubulointerstitial areas (arrows). With EPC transfer, however, the cellular infiltration was significantly attenuated. Original magnification ×400. PAS, periodic acid-Schiff.
endothelial progenitors were transferred into anti-Thy-1.1 nephritis mice. We further confirmed the presence of transferred cells at the site of damage using BM-EPCs from GFP transgenic mice.

Considering the high angiogenic efficiency of EPCs (32) and a significant anti-inflammatory effect in vivo, even small numbers of EPCs may alter the basic pathophysiology of CRF, i.e., endothelial cell loss and dysregulation (24, 31). The anti-inflammatory effect of BM-EPCs has been addressed in our experiment. Endothelial cells play an important role in inflammatory diseases by recruiting inflammatory cells. EPCs are active responders that stimulate leukocytes and proinflammatory cytokines (28, 32). Differential expression of TGF-β1 and KDR between CRF mice and EPC-CRF mice suggested EPCs have anti-inflammatory activity. Again, EPC administration decreased the expression of adhesion molecules and proinflammatory cytokines. To further confirm the anti-inflammatory activity of BM-EPCs, we transferred splenocytes into CRF mice instead of BM-EPCs. The adoptive transfer of splenocytes into CRF mice did not alter the course of renal impairment both functionally and structurally. Conversely, EPC treatment normalized the expression of some factors that may preserve vascular integrity. Anti-inflammatory effects of BM-EPCs at the early phase of CRF seem to suppress the progression of renal structural damage and consequently result in renal functional preservation at the advanced phase of CRF. Favorable effects of EPC treatment in maintaining body weight and attenuating hypertension may also improve the outcome of EPC-treated CRF mice. There are two clinical reports that support our results showing favorable effects of EPCs on systolic blood pressure and renal function. In CRF patients, systolic blood pressure was inversely correlated with the number of peripheral EPCs, and the number of EPCs was partially restored when the adequacy of dialysis improved (5, 7).

There are several limitations in our study. Most importantly, we did not delineate the interactions between transferred cells and renal cells. Although we showed that the transferred cells were localized at capillaries expressing endothelial antigens, the exact function of transferred cells per se was not evaluated. The precise mechanisms of how the transferred cells are incorporated into the damaged structure should be evaluated in future experiments. Another drawback was that we could not evaluate the effect of cell dosage and the therapeutic window. Although we found that cell transfer after 4 wk of disease did not prevent progression, we should look for the time window

![Fig. 8. Differential expression of proinflammatory cytokines and adhesion molecules depending on the EPC treatment.](image)

A: transcript levels of various cytokines and adhesion molecules were analyzed by real-time PCR at 4 wk of disease. The results for each cytokine were normalized with respect to GAPDH expression. Each protein expression was differentially regulated in response to EPC treatment after the induction of CRF. Values are means ± SE and represent 1 of 3 independent experiments (4 mice in each group for each experiment). *P < 0.05. B: at 4 wk of disease, the protein expression of IL-1β, IL-6, and IFN-γ was increased in CRF mice. EPC treatment attenuated the increment of these proinflammatory cytokines. Values are means ± SE and represent 1 of 3 independent experiments (3 mice in each group). *P < 0.05 in CRF vs. EPC-CRF for IL-1β and IL-6. P = 0.08 for IFN-γ.
Fig. 9. Preservation of angiogenic proteins by EPC treatment. A: at 4 wk of disease, VEGF expression was barely detectable in CRF mice; however, EPC treatment preserved VEGF expression in the peritubular capillaries. Arrow indicates peritubular expression of VEGF. Original magnification ×400. B: E-selectin was detected in the peritubular capillaries in CRF mice after 4 wk of disease, but was barely detectable after EPC treatment. Arrow indicates peritubular capillaries. Original magnification ×200. C: expression of KDR and thrombospondin-1 was significantly decreased at 4 and 20 wk in CRF mice compared with control mice. However, the adoptive transfer of EPCs normalized the expression of these factors comparable to control mice. This graph represents 1 of 3 independent experiments (4 mice in each group for each experiment). *P < 0.05.
for effective treatment since many patients already have established disease at the time of their first visit to the clinic.

In conclusion, the administration of BM-EPCs may be helpful, not only for the design of novel therapies to prevent or retard the progression of renal failure but also for the manipulation of postnatal stem cells for the treatment of chronic diseases. Future work should be focused on the molecular interactions between the transferred EPCs and stromal cells of the kidney, where the primary insult may be initiated.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


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