Bone marrow cell infusion ameliorates progressive glomerulosclerosis in an experimental rat model

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Bone marrow (BM) cells contribute to the maintenance and repair of several compartments of the kidney, including the endothelium, interstitium, epithelium, and the mesangium. The aim of this study was to explore the therapeutic use of bone marrow-derived cells (BMDC) that can differentiate into endothelial and mesangial cells in a model of progressive glomerulosclerosis. To investigate the involvement of BMDC in glomerular repair, progressive glomerulosclerosis was induced in enhanced green fluorescent protein BM chimeric rats by a one-shot injection of anti-Thy-1.1 monoclonal antibody, followed by unilateral nephrectomy. Subsequently, these rats were treated with either a BM cell infusion or phosphate-buffered saline. Renal function, intravital glomerular hemodynamics, and histological alterations were examined 12 weeks after anti-Thy-1.1 monoclonal antibody injection. Inflammatory infiltration of macrophages in the kidneys was evaluated by immunofluorescence of ED-1. We also determined whether BMDC contributed to repair and regeneration of endothelial and mesangial cells by immunofluorescence monitoring. As a result, BM cell infusion improved renal function and glomerular hemodynamics, and histological alterations with reduced glomerular infiltration of macrophages, leading to dramatically reduced mortality in this model of progressive glomerulosclerosis. We also demonstrated that, in the BM cell infusion group, more BMDC contributed to repair and regeneration of endothelial and mesangial cells than in the untreated group. The present study provides us with a conceptual basis for the development of therapeutic stem cell strategies aimed at enhancing recovery from progressive glomerulosclerosis.

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In the normal glomerulus, the endocapillary region is a closed space surrounded by the glomerular basement membrane and is functionally separated by the hydrostatic pressure across the glomerular capillary wall. For the maintenance of normal glomerular function, construction of the physiological structure composed of endothelial cells, mesangial cells, and mesangial matrices in a well-balanced way is necessary.[1] Once glomerular damage occurs, glomerular function may be disturbed before effective repair of glomerular cell and matrix components occurs. There is accumulating evidence that persistent disturbances of the regenerative process of glomerular capillary tufts may lead to irreversible progressive glomerulosclerosis.[2–6]

There is increasing evidence that bone marrow (BM) cells can differentiate into a wide range of specialized cells, such as hepatocytes,[7,8] cardiomyocytes,[9] and skeletal muscle cells.[10] BM cells contribute to the maintenance and repair of several compartments of the kidney, including the endothelium,[11] interstitium,[12] epithelium,[13] and the mesangium.[14,15] This plasticity of stem cells may be useful in therapeutic strategies designed to enhance tissue regeneration after severe organ injury. Bone marrow transplantation has been used as a tool to investigate the various roles of BM cells in glomerular diseases.

Very recently, our work using the enhanced green fluorescent protein (EGFP) transgenic rat[16] supported the idea, proposed by Rookmaaker *et al.*,[11] that BM-derived endothelial precursor cells were involved in the recruitment of glomerular endothelial cells in anti-Thy-1 antibody (Ab)-induced glomerular injury. This line of approach enabled us to undertake trials of treatment for regeneration using bone marrow transplantation, as highlighted in stem cell administration therapy of other organs. To explore therapeutic strategies of BM cell infusion, we selected the experimental model of progressive glomerulosclerosis, which can be induced in the rat by a single injection of anti-Thy-1.1 monoclonal Ab, followed by unilateral nephrectomy.[2] In particular, irreversible glomerular sclerosis in BM chimeric rats, transplanted with EGFP (+) BM cells, is much more severe.[16] If no treatment was given, most rats died by 12 weeks after injection of anti-Thy-1.1 Ab.

Here, we focus on the therapeutic effect of BM cell infusion on the prognosis of fatal, progressive glomerulosclerosis

in terms of histopathological and glomerular hemodynamics analysis.

RESULTS

Infusion of BM cells markedly decreased mortality in the progressive glomerulosclerosis rat model

As reported in our recent study,[16] this experimental rat model was chosen, as it is easier to induce progressive glomerulosclerosis and death from chronic renal failure than in other rat systems. In our present study, only two of eight untreated rats survived. The other six rats died at 2, 3, 5, 7 and 8 weeks after 1-22-3 Ab injection. However, five of six BM cell-infused rats survived till 12 weeks, only one died at 9 weeks (Figure 1), suggesting that bone marrow-derived cells (BMDC) were a useful therapeutic strategy after progressive glomerulosclerosis.

Chimeric rats display no evidence of radiation-induced renal dysfunction

Five weeks after irradiation, we determined renal function and other general characteristics in chimeric rats. As shown in Table 1, serum creatinine (0.27 ± 0.15 mg/dl), blood urea nitrogen (17.9 ± 3.7 mg/dl) and 24-h protein excretion (2.37 ± 4.65 mg/24 h) in chimeric rats 5 weeks after irradiation were similar to those observed in control rats (0.30 ± 0.21, 18.4 ± 5.5 mg/dl and 2.15 ± 1.94 mg/24 h, respectively). In addition, we also found no significant differences in serum total protein, albumin and total cholesterol between control and chimeric rats (Table 1).

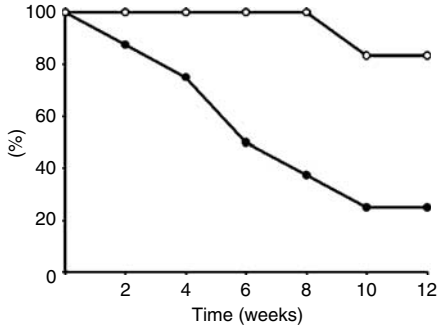

Figure 1 | Survival rate. ●, Untreated group ($n = 8$); ○, BM cell infusion group ($n = 6$).

BM cell infusion ameliorated renal dysfunction

We also examined whether BMDC have an impact on renal function. Following Ab injection and Nx, all rats had renal dysfunction with significant increases in serum creatinine from levels of 0.27 ± 0.15 to 2.59 ± 1.87 mg/dl in the untreated group and 1.56 ± 0.87 mg/dl in the BM cell infusion group. All rats had a significant increase in the serum blood urea nitrogen from levels of 18.0 ± 3.7 to $217.1.3 \pm 185.5$ mg/dl in the untreated group and 121.1 ± 81.2 mg/dl in the BM cell infusion group. However, renal function in animals with BM cell infusion was significantly better than in untreated animals throughout the study (Table 1).

Urinary protein excretion was determined on days 3, 7, 14, 28, 42, 56, 70, and 84 after anti-Thy-1.1 monoclonal Ab injection. Up to 42 days after Thy-1 Ab injection, urinary protein excretion in both BM cell infusion-treated and -untreated groups were apparently higher than that in controls, but no significant differences between the two groups was seen. In the untreated group, urinary protein excretion sharply increased with time, up to 229.3 ± 102.8 mg/24 h. In the BM cell infusion group, urinary protein excretion also increased with time. However, it was significantly lower than in the untreated group from days 56 to 84 after injection of Thy-1 Ab (Figure 2), suggesting that BMDC could partly repair renal injury and lower urinary protein excretion.

Light microscopy

Glomerular alterations were examined on week 12 after anti-Thy-1 Ab injection by light microscopy. Sequential renal histology during course the of disease, as described recently in the same experimental schedule,[16] was abbreviated here. On day 84, in the untreated group, severe mesangial cell proliferation with matrix expansion was observed in the majority of the glomeruli. Many glomeruli showed crescentic lesions and diffuse tubular atrophic changes with interstitial cell infiltration (Figure 3a and b). Only moderate mesangial cell proliferation with mesangial matrix and mild crescentic lesions were observed in the BM cell infusion group (Figure 3c and d). No pathological findings were observed in the chimera group (Figure 3e). The mesangial expansion index and glomerular sclerosis index in the untreated group were

Table 1 | Serum biochemical analysis and urinary protein excretion

	N	TP (g/dl)	Alb (g/dl)	TC (mg/dl)	BUN (mg/dl)	Cr (mg/dl)	UP (mg/day)
Control	14	6.3 ± 0.5	4.2 ± 0.3	99.8 ± 26.4	18.4 ± 5.5	0.30 ± 0.21	2.15 ± 1.94
BM-chimera	14	6.7 ± 0.3	4.4 ± 0.2	103.6 ± 15.7	18.0 ± 3.7	0.27 ± 0.15	2.37 ± 4.65
Untreated	4	5.5 ± 0.9^{a}	3.1 ± 0.6^{a}	192.8 ± 34.1^{b}	217.1 ± 185.5^{b}	2.60 ± 1.87^{b}	229.3 ± 102.8^{b}
BM cell infusion	5	5.8 ± 0.9^{a}	3.5 ± 0.7^{a}	174.8 ± 27.5^{b}	$121.1 \pm 81.2^{b,c}$	$1.56 \pm 0.87^{b,c}$	$138.8 \pm 45.2^{b,c}$

TP=total protein; Alb=albumin; TC=total cholesterol; BUN=blood urea nitrogen; Cr=creatinine; UP=urinary protein excretion.
Each value is expressed as mean ± s.d. Data in BM-chimeric rats were obtained at 5 weeks after irradiation. Data in untreated group and BM cell infusion group were obtained at 7 and 12 weeks, respectively, after Thy 1 antibody injection.
[a]$P < 0.05$ vs control $P < 0.01$.
[b]$P < 0.01$ vs control rats.
[c]$P < 0.01$ BM cell infusion group vs untreated group.