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Kidney Injury in Murine Models of Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Acute graft-versus-host disease (GVHD) affects different organs, including the skin, liver, and gastrointestinal tract. Although kidneys are not among the organs commonly known to be the target of acute GVHD, kidney damage is frequently reported after allogeneic hematopoietic stem cell transplantation (allo-HSCT). We have studied the effect of bone marrow transplantation (BMT) on the kidneys in different murine models of GVHD. We found that glomerular damage in the kidneys is a common pathological finding in mice after BMT. The histopathological features of glomeruli damage included mesengiolysis, mesangial proliferation and edema, subendothelial and endothelial thickening, splitting of capillary walls in glomeruli, narrowing and collapsing of capillary lumens, fibrinoid necrosis of afferent arterioles, intimal hyperplasia, and microthrombi. These pathological features are similar to those detected in kidneys of patients with thrombotic microangiopathy (TMA) after allo-HSCT. We previously showed that activation of the complement system plays a role in GVHD-induced tissue injury in mice. Here we report the presence of complement activation products in the kidney specimens of mice after BMT. We also report that complement deficiency reduced the extent and severity of post-BMT glomerular damage in mice. We conclude that BMT in mice is associated with glomerular injury and tubulointerstitial nephritis, and that kidney damage is at least partially mediated by activation of the complement system.

INTRODUCTION

Allogenic hematopoietic stem cell transplantation (allo-HSCT) can cause acute graft-versus-host disease (GVHD) [1]. Acute GVHD is an alloimmune response elicited by donor T cells against epithelial cells, resulting in tissue injury in various organs of the recipient. Organs commonly targeted by acute GVHD include the skin, liver, and gastrointestinal tract; however, tissue injury in other organs, including the kidneys, frequently occurs after allo-HSCT [2]. Acute kidney injury is reported in 50% to 73% of allo-HSCT recipients [2]. Although infection, total body irradiation, and chemotoxicity are known to contribute to kidney injury, whether acute GVHD damages the kidneys has not been well characterized. We investigated the histological changes in kidneys in murine models of bone marrow transplantation (BMT). We found that in addition to interstitial nephritis and tubulitis [3,4], glomerular injury is a common manifestation of kidney injury after BMT in mice. Many of the pathological changes in the kidneys after BMT are similar to the histopathological changes detected in the kidneys of patients with post-BMT thrombotic microangiopathy (TMA) [5,6].

We previously showed that the complement system has a role in the GVHD-induced organ injury occurring after BMT in mice [7,8]. In the present study, we investigated whether the complement system is also involved in the BMT-induced damage to the kidneys. We detected deposition of complement proteins in kidney glomeruli after BMT. The extent of glomerular injury in the kidneys was less in C3-deficient mice compared with wild-type (WT) recipient mice. We concluded that kidney damage is common after BMT, and that the complement system plays a role in the kidney injury after BMT.

METHODS

Murine Models of Acute GVHD

All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Texas MD Anderson Cancer Center. All recipient mice were age-matched females age 2 to 6 months at the time of BMT. We used 2 different murine models of GVHD in our study. In the first model, GVHD was induced by the disparity in MHC class I and II antigens between BALB/c (H-2^d) donor mice and C57BL/6 (H-2^b) recipient mice. To generate BMT chimeras, WT B6 or C3$^{-/-}$ mice (C57BL/6 background; The Jackson Laboratory, Bar Harbor, ME) mice received 12 Gy TBI (^{137}Cs source split into 2 doses) on day -1, followed by 10×10^6 T cell-depleted (TCD) bone

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marrow (BM) cells plus 15×10^6 splenocytes from BALB/c mice (National Cancer Institute, Frederick, MD) mice on day 0 [9]. The WT control mice received only TCD BM cells. Mice were monitored for clinical signs of GVHD (ie, hair loss, hunched back, and diarrhea) and weighed twice weekly. Serum concentrations of blood urea nitrogen (BUN) and creatinine were measured in blood samples collected via the retro-orbital route on days 7 and 10 post-transplantation. For histopathological studies, tissue samples were collected from the skin, liver, intestine, lung, and kidney and then fixed in 10% formalin. The tissue samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissue slides were graded by a pathologist according to the published GVHD scoring system [10].

In the second model GVHD model (parent to F1), 50×10^6 splenocytes from female WT C5BL/6 (H-2b) donors were infused to B6D2F1 (H-2b/d) recipient mice. In this model, GVHD was manifested in the recipient mice within 2 weeks. The blood and tissue samples from GVHD and control mice were assessed at 2, 4, and 8 weeks post-transplantation.

Tissue Preparation and Histological Staining

Fixed tissues were processed and embedded in paraffin following routine protocols. Tissues were sectioned at 4-μm thickness for routine staining with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and immunohistochemistry. Thin sections (2 μm thickness) were used for periodic acid methenamine (PAM) silver stain. Morphometric analysis was performed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Fifteen random glomerular cross-sections per experimental animal were photographed using a digital camera (DP11; Olympus America, Melville, NY). H&E-stained sections were used for the assessment of glomerular cellularity, and the amount of extracellular matrix was evaluated using PAM silver stains. Mesangial area, glomerular area, and capillary wall thickness were quantified by Image J analysis [11] of silver-stained slides.

Statistical Analysis

Statistical analyses were performed the SPSS (IBM, Armonk, NY). Means between groups were compared using t-test without assuming equal variances. A P value of $<.05$ was considered statistically significant. All data are expressed as mean $\pm$ SEM.

RESULTS

Kidney Pathology after BMT in Mice

WT B6 and C3$^{-/-}$ mice were lethally irradiated and infused with BM cells and splenocytes from BALB/c donors to induce GVHD. Within 8 weeks post-transplantation, 75% of C3-deficient recipients survived, compared with only 20% of WT mice. Whereas WT recipients had severe GVHD in the skin, intestine, liver, and lung, C3-deficient mice exhibited only mild changes in these organs, as reflected in their significantly lower GVHD scores [7].

We examined the histology of kidneys resected from recipient mice, using H&E, PAM silver, and PAS staining and found widespread tubulointerstitial nephritis and widespread glomerular damage (Fig. 1). Tubulitis and interstitial nephritis have previously been reported in murine and rat BMT models [3,4], but we found that >60% of glomeruli in kidneys of recipient mice showed morphological evidence of injury.

Serum concentrations of BUN and creatinine in blood samples collected on days 7 and 10 post-transplantation remained within normal limits. At 7 days after BMT, the mean BUN concentration was 19.6 $\pm$ 1.7 mg/dL in C3-deficient mice and 27.5 $\pm$ 9.6 mg/dL in WT mice (P = .30, t test), and mean creatinine concentration was $\leq$.2 mg/dL in both groups (n = 8 mice). At 10 days after BMT, mean BUN was 19.1 $\pm$ 1.2 mg/dL in C3-deficient mice and 19.9 $\pm$ 1 mg/dL in WT mice (P = .64, t test), and mean creatinine was $\leq$.2 mg/dL in both groups (n = 6 mice).

The histopathological features of glomeruli damage included mesengiolysis, mesangial proliferation and edema, subendothelial and endothelial thickening, splitting of capillary walls in glomeruli, narrowing and collapsing of capillary lumens, fibrinoid necrosis of afferent arterioles, intimal hyperplasia, and microthrombi (Figure 1). In addition to tubilitis, inflammatory cells also infiltrated subendothelium in glomeruli, mainly macrophages (Supplementary Figure 1). Many of the pathological findings in the kidneys after BMT in our murine models were similar to those identified in patients with thrombotic microangiopathy after BMT [12,13].

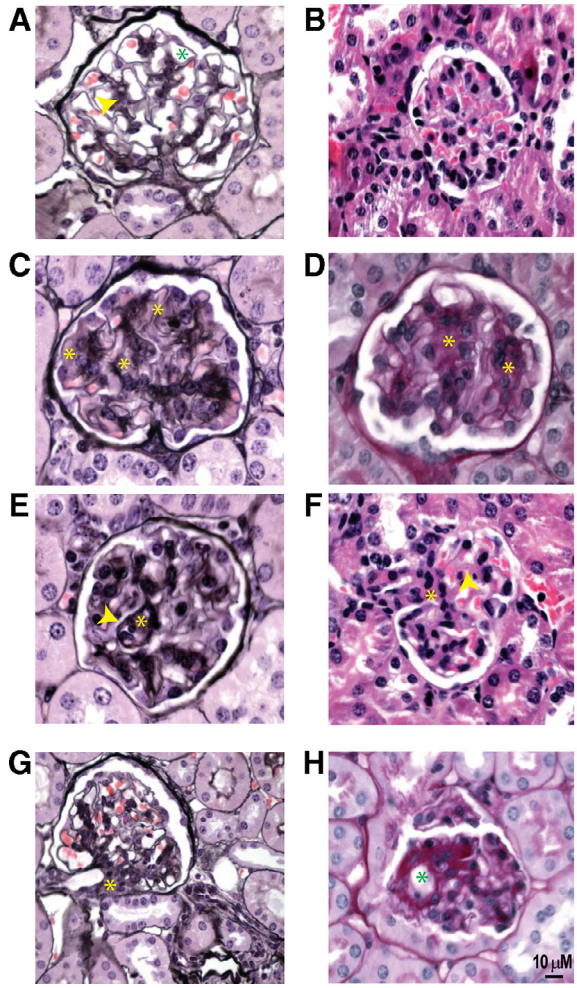

Figure 1. Glomerular injury after BMT in mice. Irradiated recipient C57BL/6 mice received 10×10^6 TCD BM cells plus 15×10^6 splenocytes from BALB/c mice. Kidney specimens were collected at 7 days after BMT, embedded in paraffin, sectioned, and then stained with PAS and PAM silver stains. (A) Normal glomerulus in mice (silver stain). *Glomerular capillary lumen; the arrowhead indicates mesangial cells. (B) Normal glomerulus in mice (H&E stain). (C) Subendothelial and mesangial swelling resulted in narrowing and collapsing capillary lumens (PAM silver stain). (D) Mesangial proliferation and swelling (PAS stain). (E) Thickening and splitting (arrowhead) of capillary walls in glomeruli. *Occlusion of capillary lumen. (F) Thickening and splitting of capillary walls (arrowhead) and occlusion of afferent arteriole lumen (*) by a hyaline microthrombus (PAS stain). (G) Mesangial proliferation (PAM silver stain). (H) mesengiolysis (PAS stain).

We also examined the deposition of complement proteins in murine kidneys after BMT. Immunostaining of kidney specimens for C3 and its degradation products showed evidence of increased complement deposition in the majority of kidney glomeruli after BMT (Figure 2).

Complement Deficiency Reduces the Severity of Kidney Injury after BMT

We previously showed that complement deficiency reduces GVHD-associated injury in the skin, liver, and intestine of transplantation recipients [7]. To investigate the role of complement activation in kidney injury after BALB/c to C57BL/6 BMT, we compared the kidneys of WT and C3-deficient recipient mice. Lack of C3 in the recipient mice significantly reduced the severity of kidney injury after BMT. While

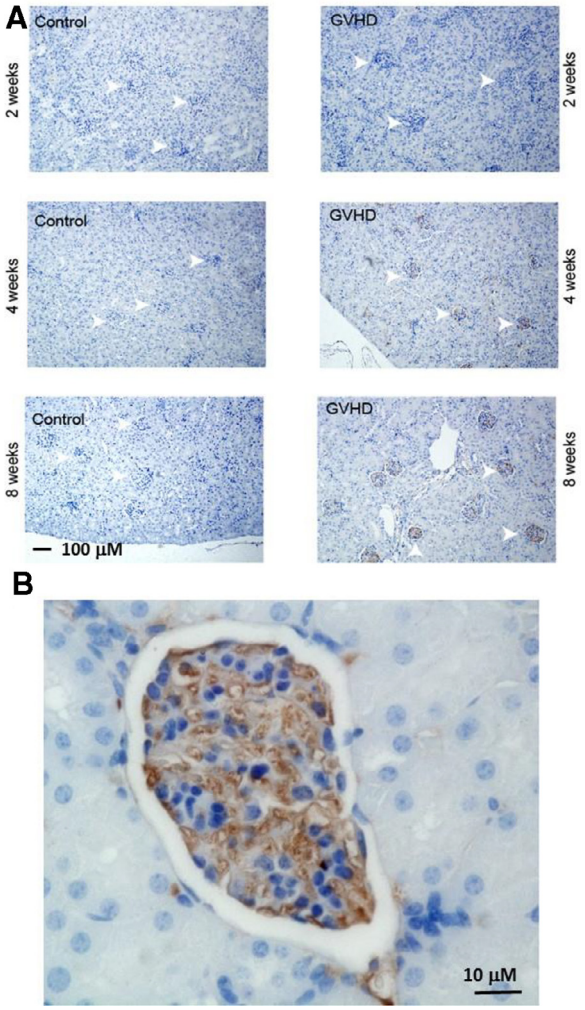

Figure 2. Deposition of complement in kidney glomeruli after bone marrow transplant (parent to F1) in mice. Splenocytes (50×10^6) from female C57BL/6 (H-2b) donors were infused to B6D2F1 (H-2b/d) recipient mice (GVHD group) or to C57BL/6 (H-2b) mice (control group). The kidney samples from GVHD and control mice were collected at 2, 4, and 8 weeks post-transplantation. (A) Immunostaining of kidney specimens from control and GVHD mice using C3 antibody showed a progressive deposition of C3 in glomeruli. Arrowheads point to glomeruli in kidney sections (10 × magnification). (B) C3 deposition in kidney glomerulus examined at a higher magnification (40× magnification).

histopathological evidence of glomerular damage (as summarized above) was present in 65% of glomeruli in WT C57BL/6, the percentage of damaged glomeruli in C3-deficient mice was reduced to 46% (PAS stain; n = 4 mice per group; 70 to 80 glomeruli examined per mice) ($P = .01$, t test) (Figure 3A). PAM silver staining showed similar reductions in the percentage of damaged glomeruli in C3-deficient mice and WT mice (62% versus 45% respectively; $P = .001$, t test).

Further quantification of the pathological changes by image analysis of the PAM silver-stained slides from kidney specimens demonstrated a significantly larger mesangial area in WT glomeruli compared with C3-deficient glomeruli (mean mesangial area/glomerular area, $.16 \pm .04$ in WT mice versus $.12 \pm .03$ in C3-deficient mice). The average capillary wall in the glomeruli was significantly thicker in the WT mice compared with the C3 KO mice (16.5 ± 3.1 pixels versus 14.5 ± 3.5 pixels) (Figure 3B).

DISCUSSION

A large percentage of patients undergoing allo-HSCT develop acute kidney injury within 100 days after transplantation [2]. This percentage can be as high as 73% in patients who received myeloablative conditioning before allo-HSCT. Patients who develop acute kidney injury early after transplantation are at increased risk of progression to chronic kidney failure later in the post-transplantation course [14,15]. Although clinical and laboratory abnormalities consistent with acute kidney injury may be related to the toxicity of the preparatory regimen (chemotherapy or total body irradiation), use of calcineurin inhibitors, infections, and hepatic sinusoidal obstruction syndrome; however, the occurrence of acute GVHD is also an important risk factor for acute kidney failure [2,16–18]. We used murine models of BMT [19] to investigate kidney injury after allo-HSCT. Studying kidney injury in different murine models of BMT provided us with the opportunity to monitor kidney histopathology over time after BMT and to dissect the pathogenesis of BMT-associated acute kidney injury. Two previous studies reported kidney injury after BMT in mice and rats [3,4]. Both of these studies identified tubulitis and interstitial nephritis, as evidenced by infiltration of T cells, as the main pathological findings in the kidneys after BMT. In our study, in addition to tubulointerstitial nephritis, we detected extensive glomerular injury including mesengiolysis, the proliferation of mesangial cells, endothelial and subendothelial swelling, narrowing and collapsing of capillaries, and microthrombi in kidneys of recipient mice after BMT. Many of these findings are similar to the findings in thrombotic microangiopathy in the kidneys.

Despite the pathological changes in the kidneys after BMT in mice, serum markers of kidney function remained within normal limits, consistent with the resistance of mice to immune and nonimmune kidney injuries [20].

We previously reported that the complement system has a role in the pathogenesis of GVHD in mice and that complement deficiency reduces the mortality and severity of GVHD [7]. In the mixed lymphocyte reaction assays, a complement inhibitor reduced the proliferation and differentiation of alloreactive T cells [8]. In the present study, we examined whether complement activation also plays a role in kidney injury after BMT. We found the progressive deposition of complement proteins in kidneys after BMT in both C5BL/6 to B6D2F1 (parent to F1) and BALB-c to C5BL/6 models of HSCT.

There are 3 complement pathways: the classical, alternative, and lectin pathways. These pathways differ in their initiation steps but converge on the formation of a C3 convertase that propagates complement activation. The C3 convertase complex cleaves plasma C3 to generate C3b that is deposited on the target cell membrane. The membrane-bound C3b participates in the serial activation of complement proteins (C5, C6, C7, C8, and C9) that eventually form the C5b-9 complex (ie, membrane attack complex). Other active end-products of complement activation are the anaphylatoxins (C3a and C5a) that mediate several biological functions. Unregulated activation of the complement system in atypical hemolytic uremic syndrome (aHUS) results in kidney injury. There are many clinical and pathological similarities between aHUS and transplantation-associated thrombotic microangiopathy (TA-TMA) [12,13], and even mutations in genes encoding complement protein have been reported in patients with TA-TMA [21]. Pathological findings in the kidneys in our murine models were similar to those reported in kidneys of patients with BMT-associated thrombotic microangiopathy.

A

		Glomeruli examined	Glomeruli damaged	ratio
PAS Stain	WT	81	52	0.65
	C3KO	82.75	38.5	0.46
				p=0.001
Silver stain	WT	83.5	51.5	0.62
	C3KO	75.5	34.5	0.45
				p=0.001

B

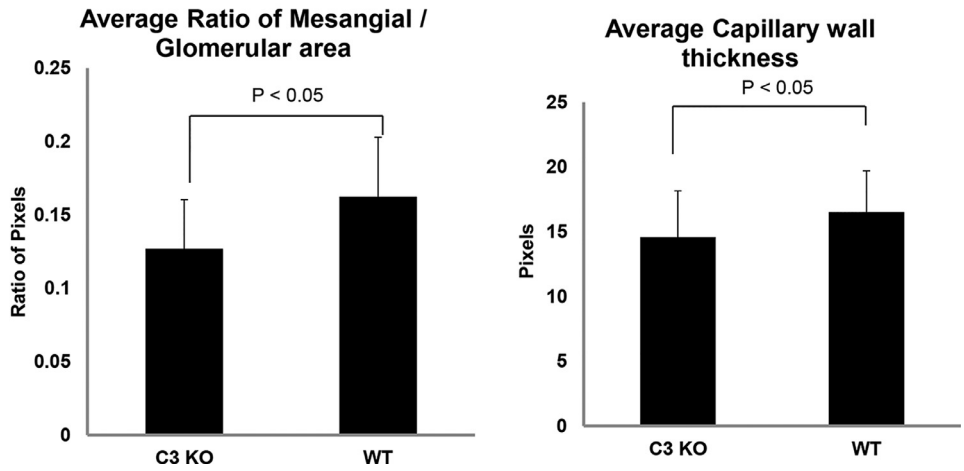

Figure 3. Complement and kidney injury after BMT in mice. (A) The kidney specimens collected from recipient mice and stained with PAS or PAM silver stain were examined for evidence of glomerular injury (n = 4 mice per group). For each specimen, 70 to 90 glomeruli were examined, and the percentage of injured glomeruli was compared between WT mice (n = 4) and C3-deficient recipient mice (n = 4). Statistical significance was calculated using the *t* test. (B) Mesangial area, glomerular area, and capillary wall thickness were quantified in kidneys of WT mice and C3- deficient mice after allo-HSCT. At least 10 glomeruli from each PAM silver-stained kidney slide were imaged at 60× magnification, and 5 areas of each glomerulus were selected for analysis with ImageJ. Area statistics were calculated in square pixels.

Based on our findings, we conclude that the complement system is involved in the pathogenesis of kidney injury after BMT, and that the lack of a functional complement system (C3 deficiency in recipient mice) reduces the severity of kidney injury. Whether inhibition of the complement system reduces the severity or frequency of acute kidney failure after BMT remains to be investigated in future studies.

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Authorship statement: Q.M. designed research, analyzed data, and wrote the paper.

D.L. performed research and analyzed data.

H.G.V. performed research.

M.J.Y. performed research and analyzed data.

V.A-K. designed research, analyzed data, and wrote the paper.

SUPPLEMENTARY MATERIALS

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.06.027.

REFERENCES

1. Shlomchik WD. Graft-versus-host disease. *Nat Rev Immunol*. 2007;7:340–352.
2. Hingorani S. Renal complications of hematopoietic-cell transplantation. *N Engl J Med*. 2016;374:2256–2267.
3. Higo S, Shimizu A, Masuda Y, et al. Acute graft-versus-host disease of the kidney in allogeneic rat bone marrow transplantation. *PLoS One*. 2014;9: e115399.
4. Schmid PM, Bouazzaoui A, Schmid K, et al. Acute renal graft-versus-host disease in a murine model of allogeneic bone marrow transplantation. *Cell Transplant*. 2017;26:1428–1440.
5. Changsirikulchai S, Myerson D, Guthrie KA, McDonald GB, Alpers CE, Hingorani SR. Renal thrombotic microangiopathy after hematopoietic cell transplant: role of GVHD in pathogenesis. *Clin J Am Soc Nephrol*. 2009;4:345–353.
6. Mii A, Shimizu A, Kaneko T, et al. Renal thrombotic microangiopathy associated with chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Pathol Int*. 2011;61:518–527.
7. Ma Q, Li D, Nurieva R, et al. Reduced graft-versus-host disease in C3-deficient mice is associated with decreased donor Th1/Th17 differentiation. *Biol Blood Marrow Transplant*. 2012;18:1174–1181.
8. Ma Q, Li D, Carreño R, et al. Complement component C3 mediates Th1/Th17 polarization in human T-cell activation and cutaneous GVHD. *Bone Marrow Transplant*. 2014;49:972–976.
9. Taylor PA, Ehrhardt MJ, Lees CJ, et al. Insights into the mechanism of FTY720 and compatibility with regulatory T cells for the inhibition of graft-versus-host disease (GVHD). *Blood*. 2007;110:3480–3488.
10. Blazar BR, Taylor PA, McElmurry R, et al. Engraftment of severe combined immune deficient mice receiving allogeneic bone marrow via in utero or postnatal transfer. *Blood*. 1998;92:3949–3959.
11. Rangan GK, Tesch GH. Quantification of renal pathology by image analysis. *Nephrology (Carlton)*. 2007;12:553–558.
12. Batts ED, Lazarus HM. Diagnosis and treatment of transplantation-associated thrombotic microangiopathy: real progress or are we still waiting. *Bone Marrow Transplant*. 2007;40:709–719.
13. Masias C, Vasu S, Cataland SR. None of the above: thrombotic microangiopathy beyond TTP and HUS. *Blood*. 2017;129:2857–2863.
14. Jo T, Arai Y, Kondo T, et al. Chronic kidney disease in long-term survivors after allogeneic hematopoietic stem cell transplantation: retrospective analysis at a single institute. *Biol Blood Marrow Transplant*. 2017;23:2159–2165.
15. Sakellari I, Barbouti A, Bamichas G, et al. GVHD-associated chronic kidney disease after allogeneic haematopoietic cell transplantation. *Bone Marrow Transplant*. 2013;48:1329–1334.

16. Singh N, McNeely J, Parikh S, Bhinder A, Rovin BH, Shidham G. Kidney complications of hematopoietic stem cell transplantation. *Am J Kidney Dis*. 2013;61:809–821.
17. Krishnappa V, Gupta M, Manu G, Kwatra S, Owusu OT, Raina R. Acute kidney injury in hematopoietic stem cell transplantation: a review. *Int J Nephrol*. 2016;2016: 5163789.
18. Mori J, Ohashi K, Yamaguchi T, et al. Risk assessment for acute kidney injury after allogeneic hematopoietic stem cell transplantation based on Acute Kidney Injury Network criteria. *Intern Med*. 2012;51:2105–2110.
19. Reddy P, Negrin R, Hill GR. Mouse models of bone marrow transplantation. *Biol Blood Marrow Transplant*. 2008;14(1 suppl 1):129–135.
20. Yang HC, Zuo Y, Fogo AB. Models of chronic kidney disease. *Drug Discov Today Dis Models*. 2010;7:13–19.
21. Jodele S, Zhang K, Zou F, et al. The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. *Blood*. 2016;127:989–996.