CD133+ cells exacerbate AKI

D. Burns1

Methods.

Brain tissue.

Promote endothelial repair in ischemic limb, heart and tubular cell proliferation. Administration of CD133+ cells to vehicle- or CD133+ trophile in derived CD133+ cells unexpectedly exacerbate ischemic injury in mice post-I/R also augmented kidney injury.

Conclusions.


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Human cord blood CD133+ cells exacerbate ischemic acute kidney injury in mice

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Abstract

Background. Acute kidney injury (AKI) in humans has few therapeutic options. In experimental models, administration of progenitor cells facilitates recovery from AKI. Human umbilical cord-derived CD133+ progenitor cells promote endothelial repair in ischemic limb, heart and brain tissue.

Methods. We examined the effects of human CD133+ progenitor cells in bilateral ischemia–reperfusion (I/R) kidney injury in non-obese diabetic severe combined immunodeficient mice. CD133+ cells from human cord blood were injected intravenously at the time of reperfusion and the extent of injury was determined by plasma biochemistry and kidney histology.

Results. In mice with I/R, fluorescently labeled CD133+ cells were detected in blood 2 min after injection but decreased rapidly thereafter with no evidence of homing to the kidneys. In mice subjected to I/R, CD133+ cells significantly increased plasma urea and Cr at 24 h compared to vehicle- or CD133− cell-treated mice. CD133+ cells exacerbated tubular necrosis and apoptosis, increased plasma tumor necrosis factor-α and increased kidney neutrophil infiltration. In contrast, CD133− cells did not affect tubular cell proliferation. Administration of CD133+ cells to FVB/N mice post-I/R also augmented kidney injury.

Conclusions. These data indicate that human cord blood-derived CD133+ cells unexpectedly exacerbate ischemic AKI in mice, possibly through soluble factors. Our study highlights the importance of caution in cell-based therapies for human AKI.

Keywords: acute kidney injury; apoptosis; inflammation; ischemia–reperfusion; stem cells

Introduction

Acute kidney injury (AKI) affects 7–18% of all hospitalized patients and >60% of patients in the critical care setting [1, 2]. Despite advances in our understanding of AKI, mortality still approaches 50% and therapeutic options remain supportive [2]. The major cause of human AKI is ischemic injury, typically resulting from shock/hypovolemia [3]. While the regeneration of tubular epithelium is fundamental to post-AKI recuperation, restoration of endothelial function is a major determinant of recovery. Animal models of ischemic AKI are associated with a 30–50% reduction in vascular density in the kidney, which can persist and impart a risk of future chronic kidney disease [4]. Endothelial permeability in the peritubular capillaries is increased along with the expression of cellular adhesion molecules, resulting in increased leukocyte–endothelial interactions, tissue infiltration and inflammation [5]. Vascular rarefaction and inflammation ultimately prolong hypoperfusion and

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exacerbate tissue injury. Conversely, strategies aimed at inhibiting inflammation, preserving endothelial health and promoting angiogenesis facilitate functional recovery in experimental models of ischemic AKI [6, 7]. Cell therapy has garnered attention as an approach to promote recovery following AKI. In animal models of AKI, cell administration is associated with reductions in renal inflammation, acceleration of tubular regeneration, the promotion of angiogenesis and reductions in fibrosis [8–11]. Beneficial effects have been reported following administration of mesenchymal stromal/stem cells, hematopoietic stem cells, mature endothelial cells and CD34+ progenitor cells [8, 10–13]. However, there is presently no consensus with regard to the most appropriate cell population for potential therapeutic use in human AKI.

CD133, also known as prominin-1, is a human transmembrane glycoprotein and cell surface marker of undifferentiated stem and progenitor populations [14]. CD133 identifies a population of primitive cells expressed by multiple tissues (hematopoietic, endothelial, cardiac, neural, myogenic, pancreatic and renal) and may also identify tumor-initiating cells [14]. CD133+ cells isolated from tissue, peripheral blood or human cord blood have pro-angiogenic properties and are associated with an accelerated repair in ischemic tissue, although effects in renal ischemic models are unknown [15–18]. Accordingly, the purpose of this study was to examine the effects of human umbilical cord blood-derived CD133+ cells on kidney recovery following AKI.

### Materials and methods

#### Animals

Male non-obese diabetic severe combined immunodeficient (NOD-SCID) mice (NOD.CB17-Prkdcscid/J) and FVB/NJ mice (6–8 weeks) were obtained from the Jackson Laboratories (Bar Harbor, ME). NOD-SCID mice lack functional T and B cells and were chosen to avoid any immune-mediated response to human cells. Mice were housed at University of Ottawa animal care facilities and provided free access to food and water. Protocols were approved by the University of Ottawa Animal Ethics Committee and performed according to the recommendations of the Canadian Council for Animal Care.

#### Human umbilical cord blood CD133+ cell purification

Umbilical cord blood units were obtained from The Ottawa Hospital and Queensway Carleton Hospital (Ottawa, Ontario, Canada) following informed written consent and with approval from the institutional research ethics board. Mononuclear cells were isolated using Ficoll density gradient centrifugation and resuspended in 1× phosphate-buffered saline.

### Table 1. Expression of cell surface markers on CD133+ and CD133− cell populations

<table>
<thead>
<tr>
<th></th>
<th>CD133+ (%)</th>
<th>CD133− (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD133</td>
<td>94.20 ± 1.92</td>
<td>Not detected</td>
</tr>
<tr>
<td>CD34</td>
<td>83.22 ± 5.93</td>
<td>1.13 ± 0.15</td>
</tr>
<tr>
<td>CD45</td>
<td>98.57 ± 0.36</td>
<td>95.78 ± 1.68</td>
</tr>
<tr>
<td>KDR/VEGFR2</td>
<td>26.28 ± 2.90</td>
<td>0.49 ± 0.16</td>
</tr>
</tbody>
</table>

*VEGFR2, vascular endothelial growth factor receptor 2. Data are mean ± SEM, n = 3–6.

### Fig. 1. Effect of CD133+ cells in NOD-SCID mice subjected to kidney I/R. Mice were subjected to sham surgery or 60 min of kidney ischemia followed by 24 h of reperfusion. Mice received an intravenous injection of vehicle (I/R), human CD133+ cells (I/R + CD133+, 10⁶ cells per mouse) or CD133− cells (I/R + CD133−, 10⁶ cells per mouse) at the time of reperfusion. Shown are plasma urea (A), Cr (B), K+ (C) and PO4− (D) levels. Values are means ± SEM. *P <0.05, **P <0.001 versus sham, ΔP <0.01 versus I/R, †P <0.05 versus I/R + CD133+, n = 6–12.
Purification of CD133+ cells from mononuclear populations was performed using an EasySep PE selection kit (StemCell Technologies, Vancouver, British Columbia, Canada) following incubation with 1:100 PE-CD133 antibody (293C3; Miltenyi Biotec, Auburn, CA). Cells were eluted from the immunomagnetic column, washed and resuspended in PBS with 2% FBS and stored at −80°C in 90% buffer with 10% dimethyl sulfoxide. Purified cells were characterized as described below. Prior to injection into NOD-SCID mice, cells were thawed and diluted rapidly in sterile PBS. Prior to injection, cell viability was determined by Trypan blue exclusion (0.04% final concentration).

Flow cytometry

Isolated mononuclear cells were characterized for the expression of CD34, CD45 and kinase insert domain receptor (KDR)/vascular endothelial growth factor receptor 2 by flow cytometry. Cells were incubated in the dark with human antibodies to CD34 (fluorescein isothiocyanate-conjugated, 1:100; BD Pharmingen, San Jose, CA), CD45 (pacific blue-conjugated, 1:200; Beckman Coulter, Fullerton, CA) and KDR (allophycocyanin-conjugated, 1:100; R&D Systems, Minneapolis, MN) for 30 min in PBS. Samples were run on an MPL FC 500 cytometer (Beckman Coulter). All data were analyzed using Kaluza software (Beckman Coulter).

Kidney ischemia–reperfusion injury

Bilateral kidney ischemia–reperfusion (I/R) (60 min) was performed on isoflurane-anesthetized mice (male, 6–8 weeks). For experimental details, see online data supplement.

Blood pressure and plasma analysis

Systolic blood pressure was measured in conscious mice using a tail-cuff plethysmography system (BP-2000; Visitech Systems, Apex, NC) as described [19].

Plasma analyte levels were measured using the Synchron CX5 Delta (Beckman Coulter) as described [19].

Histochemistry

At sacrifice, mouse kidneys were fixed in 4% formalin, dehydrated and embedded in paraffin. Tissues were cut into 5 μm sections and stained with hematoxylin and eosin and periodic acid-Schiff. Kidney sections were evaluated for tubular necrosis, neutrophil infiltration, tubular proliferation and apoptosis. For experimental details, please refer to online data supplement.
Myeloperoxidase activity

Myeloperoxidase (MPO) activity was assessed by measuring H2O2-dependent oxidation of O-dianisidine dihydrochloride as described [20, 21]. Kidney, heart, lung, spleen, liver and brain tissues (50 mg) were homogenized in 1 mL of 50 mM potassium phosphate buffer (pH 6.0) containing 0.05% hexadecyltrimethylammonium bromide. After centrifugation (12000 g, 5 min, 4°C), the supernatant was heated for 2 h at 60°C. Absorbance was measured at 460 nm and recorded over 5 min.

Measurement of interleukin-6 and tumor necrosis factor alpha levels in plasma

Plasma samples were examined for the presence of human cytokines (interleukin-6, IL-6; tumor necrosis factor alpha, TNF-α) by enzyme-linked immunosorbent assay (ELISA; Invitrogen, Burlington, Ontario, CA). Levels were determined according to manufacturer’s instructions and expressed as picogram per milliliter of plasma.

Statistical analysis

Data are presented as mean ± SEM. For pairwise comparisons, the Student’s t-test was used. For comparisons involving multiple groups, one- or two-way analysis of variance with a Bonferroni’s post-test was used as appropriate. P <0.05 was considered significant.

Results

Characterization of CD133+ and CD133− cells

Populations of CD133+ and CD133− cells were isolated from human umbilical cord blood samples and cell
surface markers were detected by flow cytometry (Table 1). Approximately $1.5 \times 10^7$ CD133$^+$ cells were isolated from 100 mL of cord blood (94.2% positive for CD133 by flow cytometry). CD133$^+$ cells were enriched in CD34 (83.2 ± 5.9%) and CD45 (98.6 ± 0.4%), and 26.3 ± 2.9% were positive for KDR ($n=3-6$), consistent with previous reports [18, 22]. CD133$^-$ cell populations rarely expressed KDR (0.5 ± 0.2%) and CD34 (1.1 ± 0.2%), while 95.8 ± 1.7% of cells expressed CD45. Prior to injection, cell viability of both CD133$^+$ and CD133$^-$ cells was high, as determined by Trypan blue exclusion (CD133$^+$: 96.5 ± 2.2%; CD133$^-$: 98.8 ± 0.6%, $n=3-4$).

**Distribution of injected cells**

In NOD-SCID mice subjected to kidney ischemia, CD133$^+$ cells labeled with CFMDA were injected via the jugular vein at the time of reperfusion. While cells were readily observed in the blood 2 min following injection (~20% of injected cells), the number of cells in the blood decreased rapidly with only 1.5% of injected cells detectable after 1 h and <1% after 24 h. By histologic analysis, cells were present in tissue immediately after injection, predominantly in the lungs but also in the liver and spleen. However, no cells were detected in any tissue at 1 or 24 h after injection. In particular, no homing of CD133$^+$ cells to the kidney was detected at any time point. Similar results were seen in sham-operated animals receiving CFMDA-labeled cells.

**Effect of CD133$^+$ cells on kidney I/R injury**

In vehicle-treated mice, bilateral kidney I/R was associated with significant increases in plasma urea and Cr at 24 h (urea: $8.1 \pm 0.4$ mM versus $33.9 \pm 5.6$ mM; vehicle versus I/R, respectively, $P<0.01$, Cr: $24.0 \pm 1.0$ μM versus $42.3 \pm 8.0$ μM; vehicle versus I/R, respectively, $P<0.001$, $n=10-11$). There were no changes in plasma K$^+$ or PO$_4^-$ levels at any time point after I/R. At 48 and 72 h, urea and Cr levels returned to baseline.

In sham-operated mice, injection of CD133$^+$ cells ($10^6$ per mouse) had no effect on any plasma biochemical parameters. In contrast, in mice subjected to kidney I/R, administration of CD133$^+$ cells exacerbated increases in plasma urea and Cr levels at 24 h compared with vehicle-treated mice (Figure 1). Significant elevations in plasma K$^+$ and PO$_4^-$ levels were also observed in mice injected with CD133$^+$ cells (Figure 1). Injection of CD133$^-$ cells ($10^6$ per mouse) into mice with I/R had no significant effect on plasma urea, Cr, K$^+$ or PO$_4^-$ compared to vehicle-treated mice with I/R. Interestingly, administration of CD133$^+$ cells to immunocompetent FVB/NJ mice was also associated with increased plasma urea and Cr at 24 h post-I/R compared to vehicle-treated mice (Figure 2).

![Figure 4](https://example.com/fig4.png)

**Fig. 4.** Kidney apoptosis following I/R injury at 24 h. Shown are representative kidney sections from mice subjected to I/R and receiving vehicle (A, I/R), CD133$^+$ (B, I/R + CD133$^+$) or CD133$^-$ (C, I/R + CD133$^-$) cells. Apoptotic cells are indicated by the presence of brown nuclei. Representative positive staining is indicated by arrow. (D): Quantitative analysis expressed as the number of apoptotic nuclei per high-powered field (HPF). Values are means ± SEM. *$P<0.001$ versus I/R, †$P<0.001$ versus I/R + CD133$^+$, $n=3-6$. 
There were no significant differences in systolic blood pressures between mice receiving vehicle, CD133+ or CD133− cells measured 6 h after reperfusion (not shown).

Effect of CD133+ cells on kidney histology

Kidney histologic analysis revealed frank tubular necrosis and accumulation of cast material in tubular lumens at 24 h in all I/R treatment groups (Figure 3A–E). Ischemic injury was regional with the outer medulla being most susceptible and associated with tubular necrosis in the thick ascending limb and proximal tubule, as previously reported in human ischemic AKI [5]. By 48 h, significant tubular injury was no longer evident (not shown). However, compared to both vehicle-treated and CD133− cell-treated mice, administration of CD133+ cells resulted in enhanced tubular injury at 24 h, typified by an increase in the number of necrotic tubules (Figure 3D and F).

Effect of CD133+ cells on apoptosis and cell proliferation

Labeling of apoptotic cells via terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining revealed few apoptotic nuclei throughout the kidney (<1/ high-powered field) in sham-operated animals. Kidney I/R was associated with apoptosis after 24 h, typically in tubular epithelial cells and most frequently in regions adjacent to severe necrotic injury. I/R-induced apoptosis was significantly exacerbated by CD133+ cells, but not by CD133− cells (Figure 4). In all treatment groups, apoptosis was only rarely observed in non-epithelial cells.

Ki-67 staining revealed rare proliferation across all the treatment groups at 24 h after I/R but significant proliferation at 48 h and 72 h (Figure 5). Proliferation was predominantly in tubular epithelial cells (Figure 5A–C). Treatment with CD133+ cells had no effect on the number of proliferating cells compared with vehicle treatment (Figure 5D).

Kidney neutrophil infiltration and MPO activity

In vehicle-treated mice with I/R, neutrophil infiltration was evident in cortico-medullary regions of injury 6 h after I/R (Figure 6A), but disappeared by 24 h. Treatment with CD133+ cells increased the number of neutrophils as compared to vehicle-treated mice (Figure 6A). While no significant difference between CD133+ and CD133−-treated mice was observed, there was a trend toward fewer neutrophils in CD133−-cell treated mice (P = 0.08). On the other hand, activity of MPO was increased in kidneys from mice treated with CD133+ cells compared to vehicle- or CD133−-treated mice (Figure 6B). MPO was significantly elevated in lung following I/R but was
not altered by the administration of CD133+ cells or CD133− cells (Figure 6C). No differences in MPO activity were found in liver or heart across all treatments (Figure 6D and E).

Plasma levels of human pro-inflammatory cytokines

Finally, to examine potential mechanisms by which CD133+ cells achieve their deleterious effects, we measured the plasma levels of human IL-6 and TNF-α by ELISA. Human ELISAs were used in order to specifically identify cytokine production originating from injected cells rather than from an endogenous inflammatory response. Plasma levels of human IL-6 were undetectable in sham-operated mice treated with saline or CD133− cells or in mice subjected to I/R treated with saline or CD133− cells (not shown). In contrast, administration of CD133+ cells significantly increased plasma levels of human TNF-α in mice subjected to I/R compared to all other treatment groups (Figure 7).

Discussion

The present study examined the effects of human umbilical cord blood-derived CD133− cells in a mouse model of ischemic AKI. The principal finding is that the administration of CD133+ cells at reperfusion unexpectedly exacerbates I/R-induced kidney injury in mice. CD133+ cell treatment was associated with an increased inflammation, typified by increases in plasma levels of human TNF-α, neutrophil infiltration into kidneys, exacerbation of tubular injury and augmented kidney dysfunction post-I/R. Administration of a separate population of cord blood-derived CD133− cells had no effect on I/R-induced kidney injury, suggesting a cell-specific effect. Despite having significant effects on I/R-induced kidney injury, there was no evidence that CD133+ cells homed to the kidneys in significant numbers, suggesting that CD133+ cells exert their effects via released factors.

In NOD-SCID mice, clamping of the renal artery and vein was associated with a rapid induction of ischemic injury, apparent at 24 h with complete recovery by 72 h. More sustained kidney injury has been reported in other studies of mouse I/R [12, 23, 24]. Nonetheless, our model induced typical features of AKI including azotemia and tubular necrosis in the cortico-medullary region. Reasons for milder injury seen in our model are unclear, however, T-lymphocyte deficiency is associated with reduced tubular injury and inflammation in mouse ischemic AKI [25]. The lack of T-lymphocytes in NOD-SCID mice may therefore attenuate injury. In addition, the administration of heparin in our model likely limited microvascular congestion and facilitated a more rapid return of kidney perfusion.

The administration of precursor cell populations is associated with accelerated kidney recovery post-AKI [12, 26–28]. Mesenchymal stem cells have been widely reported to have beneficial effects in animal models of AKI.
CXCR4-expressing progenitors may contribute to in
stability [36]. Langwieser administration of endothelial-like progenitors to hyperlipi-
molecular mechanisms are unknown. Additionally,
of vascular endothelial-cadherin antagonism, although
endothelial cells exacerbate ischemic AKI in the setting
and CXCR4 expression levels on CD133+ cells in blood
formation and neointima formation following arterial injury
administration of angiopoietin-1-treated
er in NOD-SCID mice subjected to ischemic AKI [12].
marrow-derived CD133+ cell administration 1 day after reperfusion promotes recov-
ly early outgrowth
pre-clinical studies. The administration of CD133+ cells facilitates recovery in ischemic brain, limb and cardiac injury [16, 17, 32]. Bakondi et al. [17] reported that bone marrow-derived CD33+ cell administration 1 day after ligation reduces infarct volume in cerebral ischemia, poss-
ibly through secretion of pro-survival factors. Similarly, in
hind limb ischemia, CD133+ cells increase capillary density and accelerate recovery of limb perfusion [18, 33, 34]. In the ischemic heart, intra-coronary or intra-myocardial delivery of CD33+ cells increases myocardial perfusion and improves left ventricular performance in humans, albeit in small patient populations [16, 35]. Con-
versely, in the present study, the administration of CD33+ cells immediately following reperfusion was associated with an intensified inflammatory response and increased tubular cell apoptosis, with no effect on tubular cell regen-
eration. This effect was cell-specific as CD33+ cells were without effect on these parameters. The mech-
anism for exacerbation of kidney injury by CD33+ cells are unclear. Patschan et al. [10] have noted that the administration of angioptoeitin-1-treated ‘early outgrowth’ endothelial cells exacerbate ischemic AKI in the setting of vascular endothelial-cadherin antagonism, although molecular mechanisms are unknown. Additionally, administration of endothelial-like progenitors to hyperlipi-
demic mice increases aortic lesions and reduces plaque stability [36]. Langwieser et al. [37] have reported that
CXCR4-expressing progenitors may contribute to inflam-
mation and neointima formation following arterial injury and CXCR4 expression levels on CD133+ cells in blood correlate with plaque instability in patients with carotid artery stenosis [38].

The results of our study were unexpected, particularly, since phenotypically similar human CD34+ hematopoietic cells, administered 24 h after reperfusion, promote recov-
ery in NOD-SCID mice subjected to ischemic AKI [12]. As administration of CD33+ cells in the absence of I/R had no effect on tubular injury or azotemia, it is unlikely that CD33+ cells exert any direct nephrotic effects. An alternative explanation for the exacerbation of ischemic AKI in our study is that the administration of CD33+ cells at reperfusion subjects the injected cells to increased stress due to the burst of reactive oxygen species during reoxygenation [39]. This respiratory burst may, in turn, promote a phenotypic change or the release of pro-inflammatory factors from CD133+ cells, resulting in inflamma-
tory damage to the kidney.

Beneficial effects of cell administration in AKI have been attributed to incorporation into tissue, immunomodu-
lation, physical interaction with resident cells or the secretion of paracrine factors [8, 10]. In our studies, in-
jected CD33+ cells were not detectable in kidneys at any
time point and cells disappeared from the blood rapidly and were undetectable in tissue within 1 h of injection.

The rapid disappearance of cells may occur as a result of phagocytosis by macrophages or by residual natural killer cells in NOD-SCID mice, which have been reported to facilitate mesenchymal stem cell lysis [40]. Given the rapid disappearance of injected cells, the deleterious effects of CD133+ cells are likely due to secreted factor(s) that increase the inflammatory response and tissue injury post-I/R. This is further supported by the observation that CD133+ cell administration exacerbated AKI in immuno-
competent mice. Our results suggest that TNF-α, but not IL-6, may function as one of the secreted factor(s) contributing to pro-inflammatory effects of CD133+ cells in is-
chemic AKI. TNF-α is a potent pro-inflammatory cytokine, which has been implicated in neutrophil infiltration and tubular injury in mouse models of AKI [41, 42]. Accordingly, secretion of human TNF-α by injected
CD133+ cells may contribute to the pro-inflammatory effects seen in our mice. Nevertheless, we cannot rule out the involvement of other, yet, undiscovered factors.

In conclusion, the administration of human umbilical cord-derived CD33+ cells at reperfusion exacerbates I/R-
induced kidney histologic injury, apoptosis and inflam-
mation in a mouse model of ischemic AKI. These effects may be mediated through the release of secreted factors such as TNF-α, but not IL-6. Our study highlights the need for caution in selecting appropriate cell populations for cell therapy in ischemic AKI.

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Conflict of interest statement. None declared.

(See related article by Dragun et al. Expect the unexpected in the cell therapy of renal ischaemia. Nephrol Dial Transplant 2012; 27: 3683–3685.)

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