Tumor necrosis factor receptor 2 (TNFR2) is strongly upregulated on renal tubular epithelial cells by acute cell-mediated rejection (ACR). In human kidney organ culture, TNFR2 signaling both upregulates TNFR2 expression and promotes cell cycle entry of tubular epithelial cells. We find significantly more cells express CD133 mRNA and protein, a putative stem cell marker, in allograft biopsy samples with ACR compared to acute tubular injury without rejection or pretransplant “normal kidney” biopsy samples. Of CD133+ cells, ~85% are within injured tubules and ~15% are interstitial. Both populations express stem cell marker TRA-1-60 and TNFR2, but only tubular CD133+ cells express proximal tubular markers megalin and aquaporin-1. TNFR2+ CD133+ cells in tubules express proliferation marker phospho-histone H3 S10 (pH3 S10). Tubular epithelial cells in normal kidney organ cultures respond to TNFR2 signaling by expressing CD133 mRNA and protein, stem cell marker TRA-1-60, and pH3 S10 within 3 hours of treatment. This rapid response time suggests that CD133+ cells in regenerating tubules of kidneys undergoing ACR represent proliferating tubular epithelial cells with TNFR2-induced stem cell markers rather than expansion of resident stem cells. Infiltrating host mononuclear cells are a likely source of TNF as these changes are absent in acute tubular injury.

**KEYWORDS**

basic (laboratory) research/ science, cellular biology, cytokines/ cytokine receptors, graft survival, immunohistochemistry, kidney disease, pathology/ histopathology, regenerative medicine, rejection: acute
renal function. However, the source of the cells that repopulate the injured nephron is unclear. Some studies support the existence and homing (engraftment) of a stem/progenitor cells in adult kidney for ameliorating injury, whereas other studies support an indirect involvement of circulating cells that mediate paracrine effects on injured epithelial cells to promote repair. If stem/progenitor cells are involved, a key issue is how to identify them. Multiple studies have used CD133 as a marker for progenitor cells in various organs including the human adult kidney. CD133+ cells have been implicated in repair/dedifferentiation of injured proximal and distal tubular epithelia in acute kidney injury (AKI) in human adults and in a mouse model of kidney injury. It is assumed that progenitor cells are in a quiescent state until new stimuli activate their proliferation, differentiation, or migration.

Tumor necrosis factor (TNF) can cause survival and invasion of CD133+ cells in human colon cancer, and a link between TNF and CD133 has been reported in human pancreas. Our laboratory has previously shown that the effects of TNF on graft outcome may vary due to a diverse range of cellular responses that are triggered via the 2 distinct TNF receptors: TNFR1 and TNFR2. In control renal tissue, TNFR1 is expressed primarily on glomerular and microvascular endothelial cells. TNFR2 is minimally expressed, primarily on tubular epithelial cells. In transplant biopsy samples with ACR that show morphological evidence of tubular injury, TNFR1 is lost on the endothelium and this receptor is largely confined to infiltrating leukocytes. TNFR2 is concomitantly upregulated on endothelial cells and much more prominently on tubular epithelium. Furthermore, selective ligation of TNFR2 in a short-term organ culture of human kidney with a mitoneform of TNF (R2-TNF) demonstrates receptor signalling, assessed by phosphorylation of endothelial/epithelial tyrosine kinase, and mediates both upregulation of TNFR2 and entry into cell cycle of tubular epithelial cells. These observations suggest an important role for TNFR2 in tubular regeneration. Our subsequent studies in human renal clear cell carcinoma has revealed a coassociation of TNFR2 with CD133+ cancer stem cell-like cells (CSCs) and CD133+CD45+ small round cells in the interstitium of normal kidney from the same tissues. In both cases, these cells express stem cell markers and TNFR2 ligation initiated cell cycle entry.

The present study was designed to answer the question of whether CD133+ renal stem cells participate in tubular repair associated with ACR. Specifically, we investigated the localization and distribution of CD133 in posttransplant biopsy samples of ACR (histologically categorized as Banff-IA, -IB, -IIA, and -IIB) by immunofluorescence and in situ hybridization, determined their association with TNFR2, and used short-term organ culture of human renal biopsy samples to examine the effect of TNFR2 ligation on CD133+ cells within regenerating renal tubules. Our data here are most consistent with a model in which TNFR2 signaling, likely in response to lymphocyte-derived TNF, stimulates tubular epithelial cells to express stem cell markers as part of the reparative process rather than one in which resident stem cells are activated to repair injured tubules in ACR.

2 METHODS

2.1 Human tissue samples

Cases of renal biopsy ACR with tubular injury (tubulitis) categorized according to Banff 2017 classification (Banff-IA, -IB, -IIA, and -IIB) and cases of acute tubular injury (ATI; without evidence of rejection) were identified by renal pathologists (ST, VBD, and VBR; n = 5 each case) from archives reported between 2015 and 2019. Corresponding normal kidney tissue taken at the time of transplantation (NK) was used for comparative studies with approval of the local research ethics committee. Histological cross sections of 3- to 5-μm thickness were stained with hematoxylin and eosin (H&E) and reviewed by the pathologists. Additional paraffin-wax sections were placed on poly-l-lysine–coated glass slides (BDH Merck Ltd) for IF and in situ hybridization studies.

2.2 Reagents

Antibodies/reagents used included mouse monoclonal anti-CD133 (AC133, cat. W6B3C1; Miltenyi Biotec), rabbit polyclonal anti-CD133 (cat. E90032; Enogene Biotech Ltd.), goat-polyclonal anti-TNFR2 (cat. AP-226-PB; R&D Systems), mouse anti-CD45 (cat. M0701; Dakocytomation), mouse anti-CD24 (cat. MAB248; R&D Systems), goat anti-CD24 (cat. sc-7034; Insight Biotechnology), anti-TRA-1-60 (cat. MA1-023X; ThermoFischer; stem cell markers), and mouse anti–phospho-histone H3 S10 (cat. ab14955; Abcam). Rabbit polyclonal anti-megalin (anti-LRP2; cat. AP6154A-ABG; Stratech), Rabbit anti-aquaporin-1 (AQP-1; cat. Ab134695; Abcam) and Hoechst 33342 (cat. LSH3570; Molecular Probes) were used for nuclei detection.

2.3 Kidney organ cultures

Renal tissue for organ culture was obtained from kidney allograft biopsy samples (n = 5) taken immediately after reperfusion of renal transplants (NK) or from the uninvolved pole of kidney specimens (n = 6) excised because of renal tumors as previously described. To assess the reliability and reproducibility of these assays, multiple samples of cross sections from the medulla through to the cortex were taken from each patient to obtain randomized samples, and all samples were incubated in duplicate. Approximately 1-mm3 fragments of tissue were immersed in M199 (medium containing 10% heat-inactivated FCS, antibiotics, and 2.2 mmol/L glutamine) and incubated for 0, 1.5, 3, 6, and 18 hours at 37°C with either culture media alone (without TNF) or with 10 ng/mL recombinant human TNF (wtTNF; AMS Biotechnology, Europe, Ltd.) or with 1 μg/mL recombinant mutations of the wild-type TNF sequence, which enable the mutated protein (“mutein”) to bind selectively to either of the TNFR subtypes. The specific double mutation of R32W, S86T (here termed R1TNF) allows selective activation of TNFR1,
whereas the D143N, A145R (termed R2TNF) double mutation allows selective activation of the TNFR2 subtype only. Cultures were harvested, fixed in 4% formaldehyde, and processed for paraffin-wax embedding.

### 2.4 | Single-, double-, and triple-IF microscopy

We subjected 5-μm-thick formalin-fixed paraffin-embedded sections of NK, ACR, ATI, and organ cultures to IF as previously described with 1:50; anti-CD133 alone or in combination with stem cell marker; anti–TRA-1-60 or anti-TNFFR2 or anti-CD45 (detects leukocytes), or anti-megalin or anti-aquaporin-1 or anti-phospho-histone-H3S10 (pH3S10; detects replicating cells) or anti-CD24. In addition, parallel sections were subjected to triple-IF staining with anti-CD133, -TNFR2, and -pH3S10. Primary antibodies were then detected using species-specific secondary antibodies; conjugated to Alexa Fluor-488 or -568 (Vector Laboratories) and nuclei detected with Hoechst 33342. No signal was observed when primary antibody was replaced by either nonimmune serum or isotype-matched nonbinding immunoglobulin.

### 2.5 | In situ hybridization

Nonradioactive in situ hybridization was carried out on 5-μm-thick formalin-fixed paraffin-embedded sections of kidney organ cultures as described previously. We first examined the expression of CD133 protein and mRNA in NK, ACR, and ATI using IF and ISH. We found CD133 protein expression in isolated cells in glomeruli and interstitium (<4% mean) but rarely in tubules (Figure 2A). In contrast, a significantly higher number of CD133+ cells was evident in all grades of ACR compared with ATI and NK, and these were mainly confined to tubules with morphological evidence of injury (breach of epithelial membrane and infiltration of lymphocytes). These changes were more pronounced in Banff-IIA/IIB (32.3% ± 0.2%) and Banff-IIB (30.1% ± 0.1%) graded biopsy samples (Figure 2A-E). In contrast, H&E-stained sections of ATI showed tubules with epithelial injury, luminal dilatation, nuclear loss, and nuclear pyknosis with no obvious mononuclear cell infiltration (Figure 1F).

### 3 | RESULTS

#### 3.1 | Histological characterization of NK, ACR, and ATI (without rejection)

H&E-stained sections of NK biopsy samples showed intact tubules lined by epithelial cells and absence of inflammatory cells (Figure 1A). In contrast, biopsy samples with ACR showed either foci of moderate tubulitis (t2; Banff-IA) or severe tubulitis (t3; Banff-IB) with interstitial inflammation involving >25% of nonsclerotic cortical parenchyma with edema (i2/i3) or mild to moderate intimal arteritis (v1/v2; Banff-IIA/IIIB) with tubulitis (Figure 1B-E). Although Banff II grade rejections involved arteries as well as tubules, the extent of tubular injury was at least equivalent to that in grade I biopsy samples. Periodic acid–Schiff silver stain clearly shows marked injury in tubules with lymphocytic infiltration in ACR (insets, Figure 1B-D). In contrast, H&E-stained sections of ATI showed tubules with epithelial injury, luminal dilatation, nuclear loss, and nuclear pyknosis with no obvious mononuclear cell infiltration (Figure 1F).

#### 3.2 | Enumeration and characterization of CD133+ cells protein and mRNA in NK, ACR, and ATI (without rejection)

We first examined the expression of CD133 protein and mRNA in NK, ACR, and ATI using IF and ISH. We found CD133 protein expression in isolated cells in glomeruli and interstitium (<4% mean) but rarely in tubules (Figure 2A). In contrast, a significantly higher number of CD133+ cells was evident in all grades of ACR compared with ATI and NK, and these were mainly confined to tubules with morphological evidence of injury (breach of epithelial membrane and infiltration of lymphocytes). These changes were more pronounced in Banff-IIA/IIB (32.3% ± 0.2%) and Banff-IIB (30.1% ± 0.1%) graded biopsy samples (Figure 2A-E). In contrast, a significantly fewer number of CD133+ cells were seen on sections with ATI (12.6% ± 0.4%; Figure 2F). No signal was seen in sections incubated with nonimmune serum isotype-matched nonbinding immunoglobulin (data not shown). Of note, tubular CD133+ cells in ACR also expressed stem cell–associated marker TRA-1-60 (Figure 2G), which was rare in sections with ATI (data not shown). A majority of the CD133+ tubular cells in ACR were also positive for another stem cell marker CD24, with only a few of these tubular cells not coexpressing CD24 (Figure S1). Costaining for CD133 and CD24 was also seen in a few tubular cells in ATI and in some interstitial cells in NK. These immunostaining results were supported by ISH for mRNA localization. In comparison to sections of NK, which showed only a few CD133+ cells mainly in isolated cells in glomerular, specific strong signal for CD133 mRNA was evident.
in injured tubules and in isolated cells in interstitium (Figure 2H), similar to the distribution of CD133 protein. No signal was observed in negative controls after hybridization with sense probe.

3.3 | CD133\(^+\) cells show colocalization of TNFR2 but not CD45 in NK, ACR, and ATI (without rejection)

We previously reported that TNFR2 protein and mRNA are induced in injured tubular cells in ACR.\(^{25}\) Our subsequent studies in human renal clear cell carcinoma demonstrated localization of TNFR2 to CSCs and that selective engagement of TNFR2 in these cells promoted cell cycle entry and sensitization to killing by a cell cycle–dependent cytotoxic agent.\(^{17}\) Here we examined whether there is an association between CD133 and TNFR2 in ACR and ATI by double-IF. Consistent with our previous findings,\(^{26}\) TNFR2 expression was not detected in NK (Figure 3A). In contrast, a strong colocalization of CD133 and TNFR2 expression was seen in all ACR grades, mainly confined to tubules and in isolated cells within interstitium but rarely in glomerular (Figure 3B-E). In comparison, significantly fewer double-positive cells were detected in sections with ATI (Figure 3F). Table 2 is a quantitative assessment of the double-staining with the highest tubular expression on average, 19.3% ± 0.9% in Banff-IIB and 16.8% ± 0.6% in Banff-IIB compared with ATI 3.6% ± 0.7% and NK 0.3% ± 0.1%. In contrast, the CD133\(^+\) cells lacked leukocyte (leukocyte common antigen [CD45]) marker (Figure 4A-F).

3.4 | Characterization of CD133\(^+\) tubular cells in ACR and ATI

To evaluate whether the normal CD133\(^+\) stem cells in ACR and ATI are in cell cycle, sections were subjected to double-IF for CD133 and for the proliferative marker pH3\(^{310}\). In fact, a majority of the proliferating cells were CD133\(^+\) confined to injured tubules in all ACR grades. Quantitative assessment of the double staining revealed that on average, 21.6% ± 0.5% of the proliferating cells in Banff-I, 29.2% ± 0.3% in Banff-II, 23.7% ± 0.9% in Banff-IIA, and 25.6% ± 0.6% in Banff-IIB and, in comparison, <2.0% in ATI and <1.0% in NK (Figure 5A-F, quantified in 5 g). Using triple IF, we confirmed that most of the proliferating CD133\(^+\) cells in ACR also expressed TNFR2 (Figure 6; Table 3). In contrast, most proliferating cells in ATI did not express CD133. Additional characterization revealed that the CD133\(^+\) tubular cell population also expressed proximal tubular epithelial markers megalin and AQP-1. CD133\(^+\) cells in the renal interstitium lacked these markers (Figure 7).
3.5 \[\text{wtTNF and R2TNF (but not R1TNF) induce CD133 expression, stem cell phenotype, and cell cycle entry in kidney organ culture}\]

Selective ligation of TNFR1 or TNFR2 can differentially regulate gene expression in organ culture of normal human kidney biopsy samples. To specifically examine effects on CD133+ tubular cells, organ cultures of pretransplant biopsy samples (NK) were exposed to wtTNF, R1TNF, and R2TNF treatment for 0, 1.5, 3, 6, and 18 hours and analyzed by IF for megalin, a marker of proximal tubular epithelium. In addition, 3-hour cultures were subjected to ISH. The 0 hour (tissue not subjected to culture) showed

### Table 1: Quantification of the percentage of CD133+ cells in renal tissue from pretransplant (NK), acute cellular rejection grades, and in biopsy samples with acute tubular injury (ATI)

| Tissue type | TEC | INT | GLOM |
|-------------|-----|-----|------|
| NK          | 1.3% ± 0.2% | 3.6% ± 1.2% | 3.1% ± 0.7% |
| Banff-IA    | 26.6% ± 0.3%** | 13.3% ± 0.3%* | 3.1% ± 0.3% |
| Banff-IB    | 32.3% ± 0.2%***,+ | 19.6% ± 0.4%** | 4.2% ± 0.8% |
| Banff-IIA   | 27.1% ± 0.4%**,ψ | 16.6% ± 0.6%* | 2.6% ± 0.4% |
| Banff-IIIB  | 30.1% ± 0.1%** ,ψ | 23.3% ± 0.3%** | 2.3% ± 0.2% |
| ATI         | 12.6% ± 0.4%* | 6.6% ± 0.3% | 2.4% ± 0.7% |

Abbreviations: GLOM, glomerular; INT, isolated cells in interstitium; TEC, tubular epithelial cells.  
Note: All values are mean ± SEM.  
*P < .05 vs NK.  
**P < .001 vs NK.  
***P < .0001 vs NK.  
†P < .01 vs Banff-IA/-IIA/-IIB.  
ψP < .05 vs Banff-IA.
a strong tubular expression for megalin with only a few CD133\(^+\) cells scattered in the interstitium and a rare signal or pH3S10 and TRA-1-60 (Figure S2). UT and R1TNF-treated cultures incubated for 1.5 hours also showed a strong tubular signal for megalin, but an increase in CD133\(^+\) cells was now seen within some tubules, which were also positive for pH3S10 and TRA-1-60 (Figure S3). By 3 hours, compared with UT and R1TNF-treated cultures, which showed only a rare signal in glomeruli and tubules (5.2% ± 1.7% and <2.0 mean), wtTNF and R2TNF treatment dramatically increased the expression of CD133 protein, mainly within tubules (23% ± 1.7% by wtTNF and 18.2% ± 0.9% by R2TNF; Figure 8A). ISH was in concordant with our IF findings and showed wtTNF or R2TNF, but not R1TNF, induced CD133 mRNA in tubular and in isolated cells within interstitium (Figure 8B). No signal was detected in sections hybridized with sense probe. Furthermore, the same treatments induced a strong nuclear signal for pH3S10 in the CD133\(^+\) cells, mainly confined to tubules (wtTNF 12.6% ± 0.3%, R2TNF 9.5% ± 0.1%) compared with R1TNF and UT (4.8% ± 1.2% and <2% mean; Figure 9A, quantified in 9B). There were also a few isolated cells within interstitium that were CD133\(^+\)/pH3\(^+\). Remarkably, although only a rare signal for TRA-1-60 was seen in UT cultures (<2% mean), there was also a significant increase in tubular cell expression in wtTNF and R2TNF-treated cultures (wtTNF 9.2% ± 1.2%, R2TNF 6.5% ± 0.4%) compared with R1TNF-treated cultures (3.2% ± 0.5%; Figure 9C). A similar pattern of wtTNF and R2TNF induction of CD133, pH3S10, and TRA-1-60 expressing tubular cells was evident in organ cultures incubated for 6 and 18 hours. These observations strongly suggest that TNFR2 signaling act on normal tubular epithelial cells to diminish expression of megalin while inducing CD133, TRA-1-60, and cell cycle entry within 3 hours (Figure S3, S4, and S5). Cumulatively, our study supports the interpretation that the regenerating tubular cells in ACR are not stem cells but rather that TNF, acting through TNFR2, induces the expression of stem cell markers. The source of TNF is likely to be the infiltrating mononuclear cells,
that is, lymphocytes and monocyte/macrophages. The paucity of CD133 expression on the regenerating cells of ATI suggests that this process, lacking mononuclear cell infiltrates as a source of TNF, differs from regeneration in ACR in that it does not involve TNFR2 signaling.

4 | DISCUSSION

We have determined the expression of CD133 in renal transplants undergoing ACR and ATI and pretransplant biopsy samples (NK), and determined the effect of TNF receptor ligation on expression...
of CD133 in an organ culture model using pretransplant biopsy samples. We report several novel findings. First, there were an increased number of cells expressing CD133 in ACR, more pronounced in Banff-IB and -IIB rejection compared with ATI or NK, with expression mainly confined to cells within regenerating tubules. Second, the majority of CD133+ cells in ACR upregulate expression of TNFR2 and have entered cell cycle, changes that are most pronounced in Banff-IB and -IIB rejection. In comparison, significantly fewer CD133+TNFR2+ cells were detected in ATI. Third, both tubular and interstitial CD133+ cells are negative for CD45 leukocyte marker but positive for stem cell markers, TRA-1-60, and CD24. Fourth, the majority of CD133+ tubular cells in ACR show coexpression of megalin and AQP-1, markers of proximal tubular epithelium, whereas CD133+ cells in the renal interstitium generally lack these markers. Fifth, in contrast to ACR, most proliferating tubular cells in ATI do not express CD133, TNFR2,

![Figure 6](image-url) **FIGURE 6** Representative panels of high power confocal images of acute cellular rejection graded biopsy Banff-IB triple-immunolabeled for CD133, TNFR2, and pH3. Shows positive staining (arrows) for all the 3 markers in tubular cells (t). Original magnification ×63 [Color figure can be viewed at wileyonlinelibrary.com]

| Tissue type | TEC | INT | GLOM |
|-------------|-----|-----|------|
| NK          | 0.1% ± 0.1% | 0.1% ± 0.1% | 0.1% ± 0.1% |
| Banff-IA    | 10.23% ± 0.6%*** | 4.2% ± 0.1%* | 0.1% ± 0.3% |
| Banff-IB    | 17.33% ± 0.3%*** | 10.4% ± 0.2%** | 2.1% ± 0.1% |
| Banff-IIA   | 11.33% ± 0.4%*** | 5.32% ± 0.4%* | 2.0% ± 0.1% |
| Banff-IIIB  | 14.0.52% ± 0.7%** | 6.6% ± 0.7%** | 1.7% ± 0.2% |
| ATI         | 1.2% ± 0.2%* | 1.0% ± 0.2%* | 0.2% ± 0.3% |

**TABLE 3** Quantification of the percentage of CD133+/TNFR2+/pH3+ cells in pretransplant biopsy samples (NK), acute cellular rejection graded biopsy samples, and acute tubular injury (ATI).

Abbreviations: GLOM, glomerular; INT, isolated cells in interstitium; TEC, tubular epithelial cells. 
**Note:** All values are mean% ± SEM.

*P < .05.
**P < .001.
***P < .0001.

Significant differences were observed between NK, Banff-IA, Banff-IB, Banff-IIA, Banff-IIIB, and ATI for all three markers.
FIGURE 7  Representative confocal images of colocalization of CD133 and megalin (an epithelial cell marker) in sections of acute cellular rejection graded biopsy Banff-IB. A, Left panels are low and high power images showing a majority of the CD133+ tubular cells, also positive for megalin (shaded arrows), with a few CD133+ cells dispersed in interstitium, negative for megalin (unshaded arrows). B, Notably colocalization of CD133 and aquaporin (AQP1) in proximal tubular structures (c). t, tubules. Original magnification (a) ×40 and ×63, (b, c) ×40 [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 8  Representative paraffin section from organ cultures of human kidney pretransplant biopsy either left untreated (UT, in media alone) or treated with wild-type (wt)TNF or R1TNF or R2TNF for 3 h at 37°C and subjected to immunofluorescence (A) or in situ hybridization. B, UT cultures show a rare signal for CD133 protein and mRNA in isolated cells in glomeruli. In contrast, wtTNF and R2TNF (not R1TNF) induce CD133 protein and mRNA expression mainly confined in injured tubular cells (shaded arrows). Inset, zoomed tubule. Notably, cells within interstitium also show signal for CD133 mRNA (red arrows) with absence of signal in negative controls hybridized with a sense probe. Original magnification (A) ×40, (B) ×25, inset ×40 [Color figure can be viewed at wileyonlinelibrary.com]
and TRA-1-60. Sixth, in organ culture, TNFR2 signaling induces TNFR2, CD133, TRA-1-60, and cell cycle entry on normal tubular epithelial cells, with significantly diminished expression of megalin within 3 hours. The earliest changes can be detected within 90 minutes and are even more pronounced at 6 and 18 hours, a time course that is inconsistent with expansion of preexisting resident stem cells.

Injured renal tubules display a remarkable capacity for self-renewal, and the source of cells responsible for repair of injured tubular epithelium has attracted considerable interest. CD133+ cells have been implicated in kidney regenerative processes and could be the ideal cell type for the treatment of AKI.13,20,21,30,31,39 TNFR2 is upregulated in injured tubular cells in ACR,25 and TNFR2 signals promote cell cycle entry in CD133+CSCs and sensitize killing by a cell cycle–dependent cytotoxic agent.17 Our data demonstrate an association between TNFR2 and CD133 in proliferating tubular epithelial cells in ACR compared with ATI, which contain fewer proliferating CD133+ cells and low levels of TNFR2 expression. We do not know if tubular injuries associated with acute antibody-mediated rejection will show patterns of expression of stem cell markers during injury, inducing dedifferentiation and repair. Lindgren et al20 found that regenerating tubular epithelium in ATI also express CD133, but this variance with our results may reflect different injurious stimuli involved in ATI. Several studies have proposed that CD133+ proximal tubular cells in humans represent resident stem cells or progenitors that can form spheres in vitro, clonally expand, ameliorate AKI, and contribute to epithelial lineage.18,40,43 In addition, previous studies by Loverre et al40 reported an increase in proliferating CD133+ cells in repairing tubular damage in human kidney transplant biopsy samples with delayed graft function, but their origin, that is, from stem cells or differentiated tubular cells, was not established.

Our finding of CD133+ cells in the renal interstitium expressing stem cell markers CD133, CD24, and TRA-1-60 is consistent with the existence of renal stem cell–like cells in human kidney.9,10,18,20,30,32,43,44 Several studies propose that tubular cell regeneration might be a result of self-renewal,43,45 and there is direct and indirect evidence in animal models.46–48 Stemness is defined by several factors including self-renewal and multilineage potency. Stem cells have been reported to exist in kidney with multilineage potential ex vivo and an ability to incorporate into tubule segments after injection under renal capsule.9,10,13,18–20,33,49–51 Hematopoietic stem cells can participate in renal regeneration in animal models of AKI,5,52,53 possibly through a paracrine effect rather than participating directly in the repair process.54,55

Our most novel finding is that regenerating tubular cells in ACR express stem cell markers CD133, CD24, and TRA-1-60, likely in
response to TNFR2 signaling, whereas the regenerative process in ATI, which lacks significant mononuclear infiltrates as a source of TNF, shows less CD133 expression, and tubular repair in this setting may not involve TNFR2 signaling. A mechanistic link between TNF and stem cell properties has been previously reported,\(^5^2\) and other studies have implicated endocrine growth factors/cytokines in tubular regeneration after an insult via autocrine and/or paracrine effects.\(^5^5,^5^6\) TNF also increases stem cell phenotype in human dental pulp cells, facilitating clone formation, migration, and differentiation in odontogenic and adipogenic lineages.\(^5^7\)

In conclusion, our data show that proliferating CD133\(^+\) cells expressing TRA-1-60 within the regenerating tubules of renal allografts with ACR appear to be derived from tubular epithelial cells and respond to TNFR2 signaling. This suggests that regeneration in the presence of a source of TNF, for example, a mononuclear infiltrate in ACR, may differ from regeneration in some settings of ATI in which infiltrates and TNF are less prominent.

DISCLOSURE
The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

DATA AVAILABILITY STATEMENT
Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.