Stromal Cells Protect against Acute Tubular Injury via an Endocrine Effect

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ABSTRACT

Emerging evidence suggests that the intravenous injection of bone marrow–derived stromal cells (BMSC) improves renal function after acute tubular injury, but the mechanism of this effect is controversial. In this article, we confirm that intravenous infusion of male BMSC reduced the severity of cisplatin-induced acute renal failure in adult female mice. This effect was also seen when BMSC (or adipocyte-derived stromal cells (AdSC)), were given by intraperitoneal injection. Infusion of BMSC enhanced tubular cell proliferation after injury and decreased tubular cell apoptosis. Using the Y chromosome as a marker of donor stromal cells, examination of multiple kidney sections at one or four days after cell infusion failed to reveal any examples of stromal cells within the tubules, and only rare examples of stromal cells within the renal interstitium. Furthermore, conditioned media from cultured stromal cells induced migration and proliferation of kidney-derived epithelial cells and significantly diminished cisplatin-induced proximal tubule cell death in vitro. Intraperitoneal administration of this conditioned medium to mice injected with cisplatin diminished tubular cell apoptosis, increased survival, and limited renal injury. Thus, marrow stromal cells protect the kidney from toxic injury by secreting factors that limit apoptosis and enhance proliferation of the endogenous tubular cells, suggesting that transplantation of the cells themselves is not necessary. Identification of the stromal cell–derived protective factors may provide new therapeutic options to explore in humans with acute kidney injury.


Recent studies of injury in organs such as the heart, liver, brain, and pancreas have introduced the controversial concept that adult bone marrow cells can provide a source of organ protection, organ repair, or both.1–4 Multiple mechanisms for these effects have been proposed, including differentiation of bone marrow–derived cells into organ-specific phenotypes, fusion of bone marrow–derived cells with existing differentiated cells, protection of existing cells by either paracrine or endocrine actions of the bone marrow cells, and/or inhibition of inflammatory responses associated with the organ injury. The bone marrow contains multiple cell types, with the majority of cells belonging to the hematopoietic lineages (hematopoietic stem cells [HSC] and their lineage-positive derivatives) and the supporting stromal cells (marrow stromal cells [MSC], which are believed to provide the niche for HSC to survive for long periods under adverse conditions). Although still controversial, differentiation into organ-specific cell types has been proposed for both HSC and MSC, whereas fusion has been predominately associated with HSC and organ protection and suppression of inflammation have been proposed as features of MSC action.

HSC are well characterized and can be purified on the basis of the surface expression of c-Kit and Sca-1 and the absence of the lineage markers that are found on their downstream derivatives (thus...
HSC are lin–cKit+/Sca1+). In contrast, the MSC population is not well characterized and historically has been defined as bone marrow–derived cells that adhere to tissue culture dishes and proliferate extensively in vitro. These cells lack lineage marker expression but may express other surface proteins such as CD133 and Stro1. As noted, MSC are believed to play a supportive role in maintaining the viability of HSC, but these cells have also been found to be capable of differentiating into adipocytes, chondrocytes, and osteocytes in vitro and therefore are alternatively called “mesenchymal stem cells.” Furthermore, a rare population of MSC termed multipotent adult progenitor cells have been cultured in vitro and found to differentiate into other cell types, including neurons and myocytes.

A possible role of bone marrow–derived cells in the kidney’s response to injury was first proposed when studies of human female kidneys that were transplanted into male recipients demonstrated Y chromosome–positive cells within the tubules. Because bone marrow cells can be mobilized into the circulation after hypoxia or ischemia, these studies supported the concept that bone marrow–derived cells might directly participate in kidney tubule repair. In conceptual agreement with this possibility, studies in which whole bone marrow from a donor mouse was infused into a recipient mouse that had been subjected to renal injury have confirmed that bone marrow–derived cells enter the injured kidney. However, the distinct majority of these cells are present in the renal interstitium and express markers of inflammatory cells such as leukocytes and macrophages, thus suggesting that these cells might worsen the acute renal injury as a result of their proinflammatory effects rather than improve repair.

Previous work by our laboratory demonstrated that ablation of bone marrow by irradiation worsens the course of ischemia/reperfusion–induced acute renal failure and that infusion of lineage-depleted bone marrow cells (containing both bone marrow–derived stromal cells [BMSC] and HSC) can restore the repair process to normal, again supporting the idea that bone marrow cells normally play a role in either preventing renal injury or improving renal repair.

Further insight into the importance of bone marrow–derived cells in modulating renal injury was provided by the work of Morigi et al When they separated whole bone marrow into HSC and MSC fractions and injected them separately, they found that the MSC fraction, not the HSC fraction, provided protection against acute tubular injury. This general observation has been reproduced in several models of acute kidney injury, including glycerol injection and ischemia/reperfusion. However, the mechanism of the MSC effect remains controversial because some groups reported that the injected BMSC infiltrate the kidney and directly populate the injured renal tubule, whereas others have found only transient evidence for injected MSC in the renal vasculature and no evidence for direct BMSC incorporation into tubules during the repair process. These transiently present MSC have been shown to provide paracrine support for vascular endothelial cells in the injured kidney.

Understanding the mechanism by which bone marrow cells protect against acute tubular damage is a critical aspect of developing therapeutic interventions for patients on the basis of these cells. If the cells act by engrafting the tubules long term, then either they will need to be host-derived (to prevent rejection) or the patient will require immunosuppressive therapy. In contrast, if the cells merely transit through the kidney and act in a paracrine manner to protect or stimulate the endogenous renal cells, then they might only need to survive for a few days and thus could be expanded from a single donor for use by multiple recipients. Finally, if the protective effect is mediated in an endocrine manner, then injection of the cells themselves would not be required but rather the factors that those cells secrete could be provided immediately at the time of kidney injury.

To address these disparate possibilities, we compared intravenous and intraperitoneal injection of bone marrow–and adipocyte-derived MSC and found that they provided indistinguishable levels of protection in a model of cisplatin-induced acute tubular injury. Furthermore, we found only rare examples of these transplanted cells in the renal parenchyma but did find that animals that received these cells displayed a decrease in endogenous tubular cell apoptosis. Conditioned medium produced by cultured MSC was found to induce epithelial cell growth and survival in vitro and to protect mice from acute kidney injury when injected intraperitoneally.

RESULTS

In Vivo Effects of BMSC

For determination of the mechanism by which BMSC improve the outcome of acute kidney injury, mice were administered injections of two doses of cisplatin to induce acute tubular injury followed 24 h later by intravenous infusion of either 2 × 10° BMSC or vehicle control (200 μl volume). Both groups of mice became systemically ill with evidence of dehydration and anorexia (weight loss), although mice that received BMSC demonstrated greater survival as compared with control mice (Figure 1A). Evaluation of renal function in mice that were treated with cisplatin alone demonstrated a marked rise in blood urea nitrogen (BUN) and moderate rise in creatinine by day 3, whereas mice that received BMSC exhibited less of an initial decline in renal function (Figure 1, B and C). All surviving mice were killed after BUN and creatinine determination on day 6. Of note, the apparent improvement in renal function parameters on day 6 in the cisplatin control group was primarily due to the previous death of mice that exhibited the highest BUN and creatinine values on day 3. Examination of renal histology on day 6 demonstrated diffuse tubular injury in the cisplatin control group, primarily in the cortical proximal tubules (Figure 1D). Kidneys from mice that received intravenous BMSC infusion revealed fewer numbers of necrotic tubules and fewer tubular casts.

In the kidney as well as other organs, MSC have been pro-
posed in some cases to contribute directly to tissue repair, whereas other reports suggested a paracrine or endocrine action. To determine whether injected MSC must transit through the kidney to provide their protective effect, we examined the ability of stromal cells injected intraperitoneally to protect against acute kidney injury. For these experiments, we compared BMSC with a similar population of cells derived from adipose tissue (AdSC). Mice were again subjected to two doses of cisplatin followed 24 h later by intraperitoneal injection of either $2 \times 10^6$ BMSC or $1 \times 10^5$ AdSC or vehicle control (500 μL of PBS). Similar to the results with intravenous BMSC, mice that received cisplatin followed by either intraperitoneal BMSC or AdSC demonstrated improved survival as compared with control mice (Figure 2A). BUN values were less elevated in both groups of treated mice, with no statistical difference between the BMSC and AdSC groups (Figure 2B). Renal pathology on day 6 again showed more severe tubular damage in the cisplatin control group as compared with the mice that received intraperitoneal BMSC or AdSC (Figure 2C).

Because cells that were injected into the peritoneum could slowly enter the circulation and thus transit through the kidney, we used Y chromosome tracing to determine whether injected stromal cells could ever be detected in the renal parenchyma of cisplatin-treated mice. For these experiments, we used male BMSC or AdSC injected either intravenously or intraperitoneally into cisplatin-treated female mice. Fluorescence in situ hybridization for the Y chromosome was performed on multiple sections from two to three mice in each group. This approach generally results in the detection of the Y chromosome in approximately 60 to 80% of cells from a male kidney, depending on the thickness of the section. In our experiments, 65% of tubular and nontubular cells were found to be Y chromosome positive in our male control kidneys, whereas 0 cells were positive in female control kidneys (Figure 3A, quantified in 3D). We found no examples of Y chromosome–positive tubular cells in any kidney section examined at either 24 or 96 h after cell injection (Figure 3, B and C). A single Y chromosome–positive nontubular cell was detected in the

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**Figure 1.** Effects of intravenous bone marrow–derived stromal cells (BMSC). Mice were given intraperitoneal injections of cisplatin (10 mg/kg) on day 0 and day 1, followed by intravenous injection of BMSC or vehicle control on day 2. (A) Survival curve of cisplatin-treated mice with or without BMSC (the numbers in parentheses are surviving mice on day 6 per total mice in that group). (B and C) Blood urea nitrogen (BUN) and creatinine values at the beginning of the experiment and on days 3 and 6. *P < 0.05 versus cisplatin alone; **P < 0.01 versus cisplatin alone. (D) Renal histology on day 6 (images shown are representative areas of the cortex). Magnification, ×40 (hematoxylin and eosin [H&E] stain).
interstitium of one of the mice 24 h after receiving BMSC intravenously (data not shown). To determine where the injected cells localized, we killed cisplatin-treated female mice 1 h after injection of male BMSC and examined the liver, spleen, lungs, and kidneys for the presence of Y chromosome–positive cells. Numerous Y chromosome–positive cells were found in the vasculature of the lung at this early time point, with no Y chromosome–positive cells detected in the liver, spleen, or kidneys (data not shown).

The failure to detect injected stromal cells in the kidney led us to investigate the hypothesis that the primary effect of infused BMSC or AdSC is on endogenous tubular cell function. For examination of this question, mice that were treated with cisplatin with or without BMSC were killed on day 4 to determine the degree of tubular cell apoptosis. Examination of the renal cortex revealed approximately 30 terminal deoxynucleotidyl transferase–mediated digoxigenin-deoxyuridine nick-end labeling (TUNEL)-positive cells per high-power field in cisplatin control mice, whereas mice that received BMSC had fewer than five TUNEL-positive cells per high-power field (Figure 4, A and B, quantified in C). Both tubular and nontubular cells were found to be TUNEL positive, although the majority of the cells were in the tubular compartment.

Renal tubular repair after acute injury is believed to require proliferation of the surviving endogenous tubular cells. Immunostaining for proliferating cell nuclear antigen (PCNA) demonstrated that kidneys from mice that had received BMSC after the cisplatin injection had a greater number of PCNA-positive cells as compared with cisplatin control mice (Figure 4, D and E, quantified in F). Because PCNA expression is also upregulated during the process of DNA repair and therefore might be increased as a result of cisplatin-mediated DNA damage independent of cell proliferation, a separate group of mice were administered and injection of bromodeoxyuridine (BrdU) for 3 d before being killed, and proliferating cells were detected by BrdU uptake. Again, a significantly larger number of BrdU-positive cells were detected in the BMSC-treated mice, with the majority of these cells lying within the tubular basement membrane (Figure 4, G and H, quantified in I).

**In Vitro Effects of BMSC**

In a separate series of experiments, isolated BMSC were cultured under various conditions in an attempt to induce differentiation toward an epithelial lineage. Culture of BMSC in the presence of growth factor combinations including hepatocyte...
growth factor (HGF), EGF, retinoic acid, and erythropoietin, as well as culture in serum from mice that had been subjected to renal ischemic or toxic injury, failed to result in expression of any epithelial markers by the cultured cells (data not shown). However, co-culture of fragments of kidney with the BMSC, even in medium that lacked FCS, resulted in the appearance of small islands of cells with an epithelial appearance. These cells expressed the epithelial markers cytokeratin, zonula occludens-1, and E-cadherin (Figure 5, A and B, and Supplemental Figure 2) and proliferated in culture for more than 10 d. Co-culturing male BMSC with female kidney fragments or GFP-positive BMSC with wild-type kidney fragments demonstrated that these islands of epithelial cells were derived from the kidney fragment and not from the BMSC (Figure 5C). Even in cases in which the BMSC were entirely surrounded by an epithelial island, there was no detectable expression of epithelial markers by the BMSC (Supplemental Figure 2).

To determine whether direct contact between the BMSC and the kidney fragment was required for migration and proliferation of the renal epithelial cells, we performed separation of the BMSC from the kidney fragment using a Transwell filter apparatus (Becton Dickinson Labware, Franklin Lakes, NJ),
Injury via an Endocrine Effect

BMSC Protect against Cisplatin-Mediated Tubular Cell Injury via an Endocrine Effect

The ability of BMSC to secrete factors that stimulate renal epithelial cell survival and proliferation led us to investigate the possibility that these same endocrine effects could inhibit tubular cell death. Immortalized mouse proximal tubule (MPT) cells were cultured in the presence of cisplatin with or without BMSC-CM. Sustained cisplatin exposure resulted in the detachment and death of many MPT cells at 24 h and most cells at 48 h (Figure 6, A and B). However, the addition of BMSC-CM significantly inhibited cell detachment at 24 h (Figure 6C, quantified in D) and decreased cisplatin-induced cell death after 48 h (Figure 6E, quantified in F). In contrast, conditioned medium from cultured MPT cells failed to provide protection against cisplatin-induced cell death (data not shown).

Cumulatively, these results fail to demonstrate that BMSC or AdSC directly contribute to renal epithelial cell protection or repair but rather support the model that these cells primarily act in an endocrine manner to secrete factors that can prevent tubular epithelial cell death and stimulate proliferation. This finding suggests that in vivo injection of the cells may not be necessary to obtain the protective effect.

For addressing this possibility, mice were subjected to cisplatin injection with or without administration of BMSC-CM or AdSC-CM intraperitoneally. On the basis of the prediction that the half-life of many of the factors present in CM would be short, mice were given 1 ml of conditioned medium twice daily beginning at the time of the first cisplatin injection. Control mice received the cisplatin with an equal volume of IMDM that had not been used for BMSC or AdSC culture. Even though the large volume of fluid prevented dehydration in these mice, six of 15 of those in the cisplatin control group had died by day 6, whereas no mice died in the CM-injected groups (Figure 7A). Furthermore, BUN values were markedly improved in both BMSC-CM– and AdSC-CM–treated mice at 3 and 5 d after cisplatin injection (Figure 7B). Consistent with the results obtained with injection of the BMSC themselves, the rate of apoptosis was diminished in the BMSC-CM group (Figure 7C), and renal pathology was significantly better in the two groups of mice that received CM as compared with the cisplatin controls (Figure 7D).

**DISCUSSION**

In agreement with the studies of Duffield et al. and Togel et al., our experiments provide no evidence that injected stromal cells entered the renal parenchyma in significant numbers or directly contributed to tubular cell repopulation. In fact, the complete absence of injected MSC in the renal parenchyma argues that the bone marrow–derived cells that are found in the renal parenchyma after either whole bone marrow infusion or lineage-negative bone marrow infusion must be derived...
from HSC and their derivatives. Instead, our data suggest that BMSC as well as AdSC secrete a factor or factors that can inhibit cisplatin-induced renal epithelial cell apoptosis in vitro and in vivo and thereby limit the renal injury induced by this toxin as well as improve the survival of mice that are subjected to cisplatin injection.

MSC have been shown to secrete a variety of factors, including growth factors such as HGF, vascular endothelial growth factor, IGF-1, and EGF; prostaglandins such as PGE2; and cytokines including G-CSF, stem cell factor, and M-CSF. Of these, HGF and IGF-1 have been shown to reduce tubular injury when given to mice subjected to either toxic or ischemic acute kidney injury whereas vascular endothelial growth factor can mediate endothelial as well as epithelial cell proliferation and survival after injury. Finally, MSC-secreted factors such as TGF-β and PGE2 can inhibit lymphocyte activation and thereby suppress the inflammatory responses that might otherwise augment the tubular injury and increase rates of apoptosis. Therefore, it is likely that multiple factors that are secreted by the BMSC are acting in concert to limit the acute injury associated with cisplatin exposure.

To date, no successful therapies that alter the outcome of acute kidney injury in patients have been developed. Several individual growth factors have shown promise in mouse models but have not proved effective in the limited human trials attempted. This study demonstrates excellent protection against cisplatin-induced injury in the mouse using conditioned medium from cultured stromal cells. Identification of the protective factors present in this medium should provide us with new therapeutic avenues to consider in humans with acute kidney injury.

CONCISE METHODS

Reagents
Antibodies for FACS analysis and immunocytochemistry were obtained from BD Biosciences (San Jose, CA) unless otherwise indicated. Anti-mouse CD105/endoglin-FITC was purchased from R&D Systems (Minneapolis, MN). IMDM and FCS were purchased from Invitrogen (Carlsbad, CA). Cisplatin was obtained from Sigma Chemical Co. (St. Louis, MO). MPT cells are a line derived from the ImmortoMouse and were a gift of Dr. Jon Schwartz (Boston University, Boston, MA).

Stromal Cell Isolation and Expansion
BMSC were isolated and cultured from bone marrow of 6- to 8-wk-old male C57/B6 mice by the method of Peister et al. Whole bone marrow cells were plated at 1 to 2 × 10^7/10-cm dish in IMDM that was supplemented with 10% FCS, and the nonadherent cells were removed by a medium change at 48 to 72 h and every 4 d thereafter. When the cells reached near confluence, they were trypsinized and passaged at low density for further expansion. At the end of the second passage or at 5 to 8 wk of expansion, BMSC were used for transplantation or generation of conditioned medium (Supplementary Figure 1).
AdSC were purified from abdominal adipose samples by the method of Meyerrose et al. Briefly, abdominal adipose tissue was harvested at the same time as the bone marrow, and samples were washed thoroughly with PBS followed by digestion in 0.075% collagenase (Sigma) for 30 min at 37°C. Digestion was stopped with IMDM media (Invitrogen) containing 10% fetal bovine serum (FBS). The digested adipose tissue was centrifuged at 1200 g for 10 min to obtain a cell pellet. The pellet was then resuspended in PBS and filtered through a 70-μm nylon screen. Cells were plated at a density of 1 × 10^7 cells/10-cm dish, and nonadherent cells were removed by washing. Adherent cells were maintained at subconfluence in IMDM with 10% FBS, 50 U/ml penicillin, 50 μg/ml streptomycin sulfate, and 2 mM l-glutamine (Invitrogen). Twenty-four hours after initial plating, nonadherent cells were removed by washing and the cells were refed with fresh medium. Cells were maintained at subconfluence by dissociation with 0.25% trypsin-EDTA (Sigma-Aldrich) and replating under the same culture conditions at a 1:4 dilution.

Figure 7. In vivo effects of BMSC-CM on cisplatin injury. Mice were given intraperitoneal injections of cisplatin (10 mg/kg) on day 0 and day 1. All mice received twice-daily intraperitoneal injections of either 1 ml of IMDM (control) or 1 ml of BMSC-CM or AdSC-CM. (A) Survival curve of cisplatin-treated mice with or without BMSC-CM or AdSC-CM (the numbers in parentheses are surviving mice on day 6 per total mice in that group). (B) BUN values at the beginning of the experiment and on days 3 and 6. *P < 0.05 versus cisplatin alone; **P < 0.01 versus cisplatin alone. (C) TUNEL staining was performed on day 3 in mice that received cisplatin with or without BMSC-CM. n = 3 mice from separate experiments; *P < 0.01. (D) Renal histology on day 6 from a mouse in each group (images shown are representative areas of the cortex). Magnification, ×40 (H&E stain).
Characterization of Adult Stromal Cells

Surface marker expression of BMSC and AdSC was determined by FACS analysis. Briefly, cells were detached using trypsin/EDTA for 5 min, immediately washed with PBS to remove trypsin, and resuspended at 10^6/ml. Of note, no difference in marker expression (including MSC-related and hematopoietic molecules) was observed when cells were detached using a cell scraper rather than trypsinization. The cell suspension (100 μl) was incubated at 4°C for 15 to 30 min with 10% FCS, followed by incubation with the specific antibody at 4°C for 30 min. The cells were washed with PBS and interrogated by flow cytometry (FACS Calibur; Becton Dickinson). Negative controls were performed using isotype control antibodies (BD Bioscience, San Jose, CA) and the number of positive cells per 10,000 events was determined using the Cell Quest software (BD Bioscience).

Cisplatin-Induced Acute Tubular Injury

Experiments were performed using 10- to 12-wk-old male C57BL/6 mice weighing approximately 18 to 20 g. For induction of acute renal failure, mice were given an intraperitoneal injection of cisplatin (10 mg/kg body wt) on 2 successive days (day 0 and day 1, total dosage 20 mg/kg). Twenty-four hours after the second cisplatin dose (day 2), BMSC (2 × 10^6 cells/mouse), AdSC (1 × 10^5 cells/mouse), or vehicle (control) was injected either via tail vein or intraperitoneally. Blood for BUN and creatinine was obtained before the first cisplatin dose (baseline) and in surviving mice on days 3 and 6 of the experiment.

For TUNEL staining, mice were killed 48 h after the second cisplatin dose and kidneys were harvested. For detection of proliferation, mice were administered an injection of BrdU (100 mg/kg intraperitoneally; BD Bioscience) for 3 successive days before being killed. Kidney tissues were processed for histology (hematoxylin and eosin), TUNEL, or immunostaining as described. All surviving mice were killed 6 d after the first cisplatin injection, and kidneys were collected for histology.

Immunocytochemistry

Kidney sections were subjected to antigen retrieval, and slides were blocked and labeled with a 1:50 dilution of monoclonal anti-BrdU antibody (Sigma) and Alexa488-conjugated goat anti-mouse secondary antibody (Invitrogen). For PCNA detection, a monoclonal mouse anti-rat PC12 antibody was used at dilution 1:40 (Calbiochem, San Diego, CA) and developed using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Apoptotic cells were identified using a TUNEL assay using the In Situ Cell Death Detection Kit (Roche, Mannheim, Germany). Accordingly, kidney sections were deparaffinized and rehydrated, and antigen retrieval was performed with BD Pharmingen Retrievalen A kit (BD Biosciences) and labeled with the TUNEL reaction mixture for 60 min at 37°C. Scoring for BrdU-, PCNA-, or TUNEL-positive cells was carried out by counting the number of positive nuclei per field in 10 randomly chosen sections of kidney cortex and outer medulla using ×40 magnification. Approximately 1000 to 1500 nuclei were screened per each field. For each condition, all data from three to five separate mice obtained from separate experiments were pooled to obtain final cell numbers.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization was performed as described previously. Briefly, sections were deparaffinized and rehydrated as described previously, antigen retrieval was performed in BD Retrievalen A solution (BD Pharmingen) followed by washing in 2× SSC, and HCl and postfixation were performed with 4% paraformaldehyde. Air-dried sections were incubated with the mouse Y chromosome–specific probe overnight, washed in 2× SSC, and then incubated with anti-rhodamine (1:20; Roche Diagnostics, Indianapolis, IN) in 4× SSC/1% BSA/0.1% Tween20 at 37°C for 45 min. Slides were finally mounted with VECTASHIELD/DAPI (Vector Laboratories) and sealed before viewing.

Measurement of Serum Creatinine and BUN

Serum BUN values were performed by the Mouse Metabolic Pheno-typing Center at Yale University using the COBAS Integra system (Roche). Creatinine measurements were performed using HPLC as per the method of Yuen et al. Experiments Using BMSC-CM and AdSC-CM

The CM was generated as follows: 1 × 10^5 BMSC or AdSC at 5 to 8 wk of culture were washed and refed with serum-free IMDM for 4 d. The medium was harvested, cell debris was removed using centrifugation, and the supernatant (CM) was used immediately for in vitro infection or in vitro co-culture. A total volume of 1 ml of CM or control IMDM was injected in the peritoneal space two times each day for the 6 d of the experiment.

BMSC Co-Culture Experiments

BMSC were isolated and maintained as described previously, followed by co-culture in the absence of FCS with either kidney fragments or microdissected tubule segments. Individual tubule segments were identified using the criteria of Chabardes et al. In some experiments, BMSC were harvested from enhanced green fluorescent protein–expressing mice, and kidney fragments were obtained from enhanced green fluorescent protein–negative mice to allow determination of the source of the epithelial colonies. Immunocytochemistry of the cultured cells was performed by fixation with 4% paraformaldehyde/PBS followed by primary antibody addition (anti-pan-cytokeratin; 1:200; Sigma) or anti-E-cadherin (1:100; BD transduction). In some experiments, BMSC were cultured in the presence of HGF (40 ng/ml), erythropoietin (10 IU/ml), EGF (20 ng/ml), retinoic acid (0.1 to 1 μM), serum from mice that had undergone previous ischemia/reperfusion (10% vol/vol), or combinations of these in an attempt to induce mesenchymal-epithelial transformation of the cultured BMSC.

In Vitro Assay for Cell Death

Immortalized MPT cells at 60 to 70% confluence were exposed to IMDM with or without cisplatin (5 μg/ml) and with or without BMSC-CM for 24 to 48 h. BMSC-CM was obtained as described pre-
viously and then diluted 1:1 with fresh medium containing 10% FCS (final concentration 50% CM, 5% FCS). Control cells received fresh IMDM with 5% FCS. After 24 h, adherent cells were harvested by trypsinization and viable cell numbers were determined by manual counting of cells that had excluded trypan blue. Cell death was determined in both adherent and floating nonpermeabilized cells by using flow cytometry to detect cells with surface phosphatidylserine expression (annexin V binding) and propidium iodide (BD Bioscience) uptake using a commercial kit (BD Pharmingen).

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DISCLOSURES

None.

REFERENCES

11. Miller SB, Martin DR, Kissane J, Hamerman MR: Insulin-like growth factor I accelerates recovery from ischemic acute tubular necrosis in
33. Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ: Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood 103: 1662–1668

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