Myeloid Progenitors: A Radiation Countermeasure that is Effective when Initiated Days after Irradiation

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INTRODUCTION

There is an ever-increasing risk of military, civilian, and emergency responders being exposed to potentially lethal doses of ionizing radiation. Events involving release of radioactive material, either intended or unintended, are a real possibility and would be potentially catastrophic in nature (1), which highlights the need to develop therapies to counter the effects of ionizing radiation exposure on human subjects. Acute radiation syndrome (ARS) occurs in humans after whole-body or significant partial-body irradiation of 1 Gy or more delivered at a high dose rate. Adverse clinical sequelae of ARS include hematopoietic (2–6 Gy), gastrointestinal (GI: ≥10 Gy), and cerebrovascular (~100 Gy) syndromes (2). While it is not considered feasible to mitigate cerebrovascular syndrome, individuals receiving lower radiation doses that result in hematopoietic and GI syndromes may be amenable to radiation countermeasures that are in development. While the hematopoietic syndrome is characterized by a massive loss of hematopoietic stem cells and early progenitors resulting in cytopenias, the GI syndrome is characterized by extensive apoptotic cell death in the crypt cell progenitor compartment and upper reaches of the intestinal microvilli. This extensive cell death is followed by disintegration of the intestinal wall and loss of barrier function, ultimately resulting in death from fluid and electrolyte imbalance, intestinal bleeding and sepsis. Although a broad range of radiation doses can cause hematopoietic, GI injury and multiorgan failure, their relevant impact on radiation-induced mortality is dose- and dose-rate-dependent, and the lowest lethal doses cause severe bone marrow damage, but only slight GI damage. Because multi-organ dysfunction is involved (3–5), radiation countermeasures that can prevent or mitigate overlapping syndromes would greatly benefit the affected population.

Radiation countermeasures can be grouped into three categories: (1) radioprotectors or radioprotectants are prophylactic agents and are administered before exposure to prevent radiation-induced damage, (2) radiomitigators are drugs administered during or after radiation exposure with the aim of preventing or reducing the action of radiation on
tissue before the appearance of symptoms, and (3) radiation therapeutics are agents given after overt symptoms appear to treat or facilitate recovery from ARS and delayed effects of radiation exposure (6). Numerous candidate radiation countermeasures, mostly radioprotectors, have been identified and investigated. Despite promising observations with various agents to date, none has been approved by the U.S. Food and Drug Administration as a radiation countermeasure for ARS. The majority of studied radiation countermeasures primarily influence hematopoietic stem and progenitor cells, while providing little benefit against the higher doses that induce GI syndrome. Effective radiomitigators, which would be needed in a large radiation incident, are rare (7).

Hematopoietic stem and progenitor cells are functionally heterogeneous cells with varying degrees of self-renewal capacity. Long-term reconstituting hematopoietic stem cells (HSC) provide life-long hematopoiesis, giving rise to mature cells of all blood forming lineages (8). This is the only hematopoietic cell that durably engrains, and it is the primary functional component in bone marrow transplantation. Transiently reconstituting, or short-term HSCs and multipotent progenitors (MPP) are downstream of long-term HSCs in the hematopoietic maturation pathway, and possess identical multilineage potential, but little or no self-renewal capacity (9). The earliest branch points between the lymphoid and myeloid lineages are defined as the common lymphoid progenitor (CLP), and the common myeloid progenitor (CMP). The CMP further differentiates into the granulocyte/macrophage progenitor (GMP), and into the megakaryocyte/erythrocyte progenitor (MEP). None of these cells (CLP, CMP and their progeny) possess extended self-renewal ability (10, 11), but are capable of partially restoring functional hematopoiesis for a limited time (12–14). A combination of CMP/GMP has been shown to protect mice from lethal doses of pathogenic fungus or bacteria (15, 16). Furthermore, purified populations of HSCs, MPPs, CMP, or MEP, but not GMP, protect lethally irradiated congenic mice (9, 14). A key aspect of these findings is that committed progenitors can produce the preferred cell type for a limited time that can be of benefit in lethal irradiation victims.

Cellerant Therapeutics has previously developed culture conditions to produce large numbers of mouse myeloid progenitors (mMPC) from HSC as a radiomitigator. mMPC primarily consist of lineage-\(^{-}\)low c-Kit\(^{+}\) myeloid progenitors (CMP, GMP and MEP), expresses varying levels of CD11b\(^{+}\) and Gr1\(^{-}\) cells indicates a commitment to the myeloid lineage (17, 18). In contrast to whole bone marrow grafts, T and B cells are not present in detectable levels among mMPC and the risk of graft-versus-host disease (GVHD) is negligible. In vivo studies demonstrate that allogeneic myeloid progenitors give rise to myeloid and erythroid cells that transiently engraft and functionally protect mice from death due to invasive fungal infections in MHC disparate recipient mouse strains (12, 16). Here we demonstrate that administration of culture-derived, cryopreserved mMPC as late as 7 days after irradiation improves survival in unmatched, allogeneic recipient mice against high doses of ionizing radiation (\(\gamma\) and X rays). Unlike other radiation countermeasures, mMPC do not stimulate sustained cytokine induction. This is the first report of a radiation countermeasure for ARS demonstrating efficacy across a broad range of high radiation doses when administered as late as 7 days after irradiation. These results make mMPC a very promising radiation countermeasure for ARS among all candidate therapeutics currently under development regarding efficacy, timing and practicality of administration.

**MATERIALS AND METHODS**

**Mice**

Male 6–8 weeks old specific pathogen free (SPF) CD2F1 mice were purchased (Harlan, Indianapolis, IN) and housed (8 per cage) in an air conditioned facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International at AFRRI. Upon arrival, mice were held in quarantine for 1 week. A microbiological examination of representative samples ensured the absence of *Pseudomonas aeruginosa*. Mice were provided certified rodent rations (Harlan Teklad Rodent Diet, Harlan Teklad, WI), acidified water (HCl, pH = 2.5–2.8) ad libitum, sterilized bedding and housed in rooms with a 12-h light/dark cycle. The mouse holding room was maintained at 21 ± 2°C with 10–15 h cycles of fresh air and a relative humidity of 50 ± 10%. Research was conducted according to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council, U.S. National Academy of Sciences. At the time of the experiment, mice were 8–9 weeks old. Experimental mice were observed daily in the morning and any mice found sick/morbid, but not moribund, were allowed to continue in the experiment. Mice were considered moribund when they showed an inability to remain upright, were cold, unresponsive or displayed decreased or labored respiration (19, 20). Mice found moribund were euthanized by CO\(_2\) overdose followed by cervical dislocation. Once mice were considered moribund, we monitored them at least twice daily, early morning and late afternoon. In addition to principal investigator research staff, mice were also observed twice a day independently by veterinary science department staff.

Bab/lc and AKR/J mice were procured from Jackson Laboratories, Chicago, IL. C57BL6/Ka mice were either bred at Cellerant Therapeutics (San Carlos, CA) or procured from Jackson Laboratories, Bar Harbor, ME. FVB mice were procured from Charles River Laboratories, Hollister, CA. All mice used at Cellerant Therapeutics were SPF males and were received at 6–8 weeks of age, quarantined for at least 1 week and subjected to experimental procedures at 8–12 weeks of age. Mice were housed in individually ventilated cages of up to 5 mice per cage on a 12 h light/dark cycle at 21 ± 2°C with 10–15 h cycles of fresh air and given sterilized bedding and acidified water. Antibiotics (10\(^{6}\) U/L polymixin B sulfate and 1.1 g/L neomycin sulfate) were added to drinking water of irradiated mice. Mice were monitored twice daily during survival studies. Mice found moribund (as described above) were euthanized.

All survival studies at AFRRI and Cellerant were carried out using CD2F1 and Bab/lc mice, respectively. A KR/J, FVB and C57BL6/Ka or B6.Pi-Thy1.1 mice were used for preparing myeloid progenitors. All animal procedures were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the respective institutes.

**Derivation of Mouse Myeloid Progenitor Cells (mMPC)**

mMPC were generated separately from three MHC disparate mouse strains: AKR/J, FVB, and either C57BL6/Ka or B6.Pi-Thy1.1 mice.
HSC for mMPC derivation were isolated from the bone marrow of donor mice as described previously (21). Monoclonal antibodies used for immunofluorescence staining for HSC isolation included PE-Cy7-conjugated CD117 (2B8), APC-conjugated Sca-1 (Ly6A/E), FITC-conjugated Thy1.1 (HIS51), and a lineage cocktail of PE-conjugated B220 (RA3-6B2), CD3 (145-2C11), CD4 (GK1.5), CD5 (53-7.3), CD8 (53-6.7), CD11b (M1/70), Gr-1 (RB6-8C5), and Ter119 (TER-119) (eBioscience, San Diego, CA). HSC were double sorted for purity as CD117-Thy1.1lowSca-1linlow (22) using a 3-laser FACSAria (BD Biosciences, San Jose, CA). Sorted HSC from C57BL/6Ka, B6.PI-Thy1.1, AKR, or FVB mice were plated in X-VIVO 15 media (Lonza, Walkersville, MD) supplemented with recombinant mouse stem cell factor (rm SCF, Invitrogen, Carlsbad, CA), rm thrombopoietin (rm TPO) (Invitrogen, San Diego, CA), rm Fms-like tyrosine kinase 3 ligand (Flt3L) (R&D Systems, Minneapolis, MN), Primocin (Invivogen), 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), and Glutamax (Invitrogen). On day 9 of culture, C57BL/6/Ka or B6.PI-Thy1.1 mMPC were harvested and cryopreserved. AKR and FVB mMPC were harvested and cryopreserved on day 10. mMPC from each strain were cryopreserved separately in X-VIVO 15 media containing 10% dimethyl sulfoxide (ProMune Pharmaceuticals, Lake Zurich, IL) and 25% FCS at 20 million cells/ml.

**Irradiation**

Mice were placed in ventilated Plexiglas boxes compartmentalized to accommodate 8 mice per box and exposed to bilateral irradiation in the AFRRI cobalt-60 facility between the hours of 8 and 11 a.m. at a dose rate of 0.6 Gy/min to total midline doses indicated in experiments as recently described (23). After irradiation, mice were returned to their cages and monitored. Sham-irradiated mice were treated in the same manner as irradiated animals except that the 0.5 C rods were not raised from the pool of shielding water. Radiation dosimetry was based on the alanine/EPR (electron paramagnetic resonance) system (24, 25), currently accepted as one of the most accurate methods and used for intercomparison between national metrology institutions. The calibration curves used in dose measurements at Armed Forces Radiobiology Research Institute (spectrometer e-Scan, Burker Biospin, Inc., Madison, WI) are based on standard alanine calibration sets purchased from the United States National Institute of Standards and Technology, Gaithersburg, MD.

Mice irradiated at Cellerant Therapeutics were placed in a compartmentalized pie-shaped Plexiglas irradiation chamber able to accommodate up to 12 mice. Irradiation was performed between 10 and 12 a.m. using a Faxitron CP-160 X-ray system, at 0.72 Gy/min (160 kV, 6.3 mA, filtration 0.8 mm Al). Single radiation doses were delivered to mice using a calibrated rotating turntable. Previous dose validation studies at Cellerant Therapeutics established 9 Gy as the dose that results in an LD100/30 in male Balb/c mice.

**Administration of mMPC to Mice**

Mice were anesthetized in a ComPac® anesthesia system (VetEquip Inc., Pleasanton, CA) with isoflurane (Abbott Laboratories, Chicago, IL) aerosol used as the anesthetic agent. Anesthetized mice were transfused intravenously (retro-orbital sinus) with a 0.5 ml insulin syringe and 28 G needle. Each mouse received 100 or 150 µl of cell suspension containing the desired number of mMPC or vehicle. Mice were continuously monitored until regaining consciousness before transfer to cages.

**Blood Collection and Serum Separation**

Mice were anesthetized with isoflurane and blood samples were collected from the caudal vena cava, transferred to CapiJect serum separator tubes (3T-MG; Terumo Medical Corp., Elkton, MD), allowed to clot for 30 min and centrifuged at 1000g for 10 min. Serum was collected and stored at −70°C until used for cytokine analysis by multiplex Luminex.

**Analysis of Cytokines by Multiplex Luminex in Serum Samples**

The Luminex protocol is a sandwich immunoassay system that allows for the simultaneous detection of different cytokines in the Luminex-200 (Luminex Corp., Austin, TX) dual-laser flow analyzer. Mouse serum samples were analyzed for interleukin-1β (IL-1β), IL-6, IL-10, IL-12(p70), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), keratinocyte-derived chemokine (KC), and tumor necrosis factor-α (TNF-α), as described earlier (26). Cytokine analysis kits were custom ordered (M200003JZX, Bio-Rad Inc., Hercules, CA) and included all necessary reagents for analysis. In brief, cytokine antibody-conjugated beads were added to each well of a flat-bottom 96-well plate (Bio-Rad Inc.). Serum samples were diluted 1:4 and samples were analyzed per manufacturer’s instructions as described earlier (27).

**Statistical Analysis**

Means with standard error or percentage were reported, if applicable. Analysis of variance (ANOVA) was used to detect whether there was a significant difference among groups. If the difference was significant, a pairwise comparison by Tukey-Kramer was used to identify which groups were different from the others. For survival data, a log-rank test was used to compare survival curves. Fisher’s exact test was used to compare survival rates at the end of 30 days, with Bonferroni correction used to control type-I error if multiple comparisons were used. A significance level was set at 5% for each test. All statistical tests were two-sided. Statistical software PC SAS was used for statistical analyses.

**RESULTS**

**mMPC Administration to Balb/c Mice Mitigates Death from a Potentially Lethal Dose of X-Ray irradiation**

To investigate whether mMPC can be used to mitigate radiation injury, we evaluated the effects of mMPC on survival of Balb/c mice exposed to potentially lethal doses of radiation. Cryopreserved mMPC from three MHC-disparate mouse strains, AKR (H-2k), C57Bl/6 (H-2b) and FVB (H-2v) were thawed, washed, and pooled in equal parts and multiple doses of mMPC (1.5, 3 and 6 million/mouse) were administered to unmatched Balb/c mice (H-2d) irradiated with 9 Gy (LD90/30). mMPC were administered 4 h after X-ray exposure and observed for 30 days after irradiation. All mice in the vehicle control group died by day 14 after irradiation (Fig. 1). Mice treated with 1.5, 3, and 6 million mMPC demonstrated a cell-dose response relationship for mitigation of animal deaths exposed to 9 Gy X-ray irradiation (Fig. 1).

**mMPC Administration to Balb/c can be Delayed Up to Five Days and Still Convey Survival Benefit at a Potentially Lethal Dose of X-Ray Irradiation**

To evaluate the potential of using mMPC as a radio-mitigator, we assessed the effect of delayed mMPC administration on survival. Five million mMPC were administered to five groups of irradiated Balb/c mice (9
mMPC Mitigates Death from a Potentially Lethal Dose of $^{60}$Co $\gamma$ Radiation in CD2F1 Mice

To determine whether mMPC mitigate death from radiation independent of mouse strain and radiation source, CD2F1 mice (H-2d) were irradiated using $^{60}$Co $\gamma$ radiation (LD$_{90/30}$ dose, 9.2 Gy) and transfused with either 5 or 3 million mMPC pooled from AKR, C57Bl/6, and FVB mice 2 h, 2 or 4 days after radiation exposure in 3 different experiments. For each administration time a third group of control mice was irradiated with $^{60}$Co $\gamma$ radiation and received vehicle. The irradiated mice were monitored for survival over 30 days and Kaplan Meier analysis was used for plotting survival curves. As shown in Fig. 3A, all vehicle control treated mice died by day 20 after irradiation, while 100% ($P < 0.0001$) and 94% ($P < 0.0001$) of mice survived in groups receiving 5 and 3 million mMPC, respectively, when administered 2 h after irradiation. When mMPC were administered 2 days after irradiation, 100% and 94% of mice survived in groups receiving 5 and 3 million mMPC, respectively, while 32% of vehicle-treated mice survived (Fig. 3B). When mMPC were transfused 4 days after irradiation, 88% and 69% of mice survived with administration of 5 and 3 million mMPC, respectively (Fig. 3C).

To investigate how long mMPC administration can be delayed after irradiation and whether increased numbers of mMPC provide increased mitigation, administration was further delayed using higher mMPC doses. When administered 5 or 6 days after $^{60}$Co $\gamma$ radiation (LD$_{90/30}$ dose, 9.2 Gy), 88% of mice treated with 4 million mMPC survived compared to 13% in the vehicle control treatment groups (Fig. 4A). Administration of 6 million mMPC 7 days after $^{60}$Co $\gamma$ radiation significantly mitigated death in CD2F1 mice compared to vehicle control group (Fig. 4B). When 12 million mMPC were administered, there was no additional benefit (data not shown).
KC, which were induced at a later time. Twelve hours after administration of mMPC to irradiated mice an additional increase was noted for all cytokines tested except G-CSF. There were no differences in cytokine levels 24 h after cell administration between vehicle control and mMPC-treated and irradiated mice suggesting that this minor increase in cytokines was transient. Administration of 5 million mMPC to unirradiated mice had no effect on cytokine levels.

Because mMPC derived cells are present in recipients for more than two weeks (unpublished data), we were interested in determining cytokine levels at later time points in irradiated mice receiving mMPC. Two groups of mice were irradiated at 9.2 Gy ($^{60}$Co γ radiation) and 5 million mMPC were administered to one group 2 h after irradiation. A second group of irradiated mice received vehicle only. A third and fourth group of mice received mMPC or vehicle only without exposure to radiation. Blood samples from these mice were collected 6 and 12 days after irradiation, and serum was separated for cytokine analysis by multiplex Luminex. Results presented in Fig. 6 demonstrate that there was no difference in the levels of various cytokines in the four treatment groups 6 days after irradiation, except IL-10, which was lower in the irradiated vehicle control group compared with irradiated mice that received mMPC. Additionally, the irradiated vehicle control group had higher levels of G-CSF compared to unirradiated vehicle controls. On day 12 after irradiation, the irradiated vehicle control group had significantly higher levels of all cytokines (except G-CSF), compared with the unirradiated control group. Interestingly, irradiated mice receiving mMPC had significantly lower levels of all cytokines compared with irradiated mice that received vehicle. This observation suggests that mMPC administration results in a significant decrease in levels of cytokines tested in the serum of treated mice by day 12 after irradiation.

**mMPC Mitigate Death from Supralethal Doses of Radiation**

Since we observed 100% survival at lethal levels of irradiation primarily causing death due to hematopoietic failure, we tested the efficacy of mMPC against higher doses of $^{60}$Co γ irradiation. Mice were irradiated with different doses of $^{60}$Co γ radiation (9, 9.5, 10, 11, 12, 13, 14, 15, 15.5, 16 and 16.5 Gy) and 5 million mMPC were administered 24 h after irradiation to determine the range of irradiation for which mMPC provide survival benefit. Survival analysis, shown in Fig. 7A, demonstrated that mMPC mitigated 100% of death from radiation doses as high as 15 Gy. All mice in the vehicle group receiving 10 Gy radiation exposure died by day 17. All mice treated with mMPC receiving 15.5 Gy or higher doses of radiation exposure died. A probit analysis was carried out to determine the dose reduction factor (DRF). The slopes for the probit lines for the mMPC and vehicle were not different, but significantly shifted to the right for mMPC-treated mice compared with the vehicle-treated mice. The LD$_{50}$/30 radiation doses were 15.17 Gy and 8.79 Gy for the mMPC and vehicle-treated groups, respectively. The DRF for mMPC was calculated to be 1.73. The study did not include doses with intermediate responses, due to the steep slope of the dose response curve. As a result, confidence limits could not be calculated using the probit method for the LD$_{50}$/30 and resulting DRF.
Based on these findings, we tested the efficacy of mMPC against supralethal doses of $^{60}$Co $\gamma$-irradiation (11, 13 and 14 Gy) when mMPC administration was delayed. We transfused mice with 6 million mMPC 4 or 5 days after irradiation in two separate experiments (Fig. 8A). When mMPC were transfused 4 days after irradiation, mMPC significantly increased survival of mice at 11, 13 and 14 Gy radiation doses compared to vehicle control. When mMPC were transfused 5 days after irradiation, mitigation of death at all radiation doses was significantly higher in mMPC-treated groups compared with the respective vehicle control group (Fig. 8B). We also infused 12 million mMPC/mouse to investigate whether administration of higher cell numbers increased the radiomitigation in mice subjected to supralethal doses of radiation. No additional survival benefit was noted (data not shown).

**DISCUSSION**

Despite 50 years of research and the existence of several promising drugs currently under investigation, no agent has been approved as a radiation countermeasure for ARS (7, 28–31). There is an ever-increasing risk of military personnel, civilians, and first responders being exposed to potentially lethal doses of ionizing radiation due to nuclear proliferation and terrorist activity. In any given nuclear or radiological mass casualty event, there will be a mix of victims receiving low, moderate, or high levels of ionizing radiation (32). While some victims would suffer radiation injury too severe for effective medical intervention, the majority of victims would have received enough radiation to injure, but not completely ablate, the blood-forming capacity of the bone marrow. These victims can recover from their injuries, but must receive substantial supportive care so that sufficient levels of blood granulocytes and platelets are maintained during the critical 30–60 day period when they are at significant risk of life-threatening infections, as well as uncontrolled bleeding and anemia. Countermeasures to bridge the acute cytopenic period and counteract overlapping gastrointestinal damage have not been developed to date.

Here we demonstrate that infusion of cryopreserved, culture-derived mMPC pooled from several MHC-disparate donors is effective in preventing death across a wide range
CD2F1 mice were injected IV with 5 million mMPC or vehicle 96 h after 60Co γ irradiation (11 Gy) or no radiation exposure. Blood samples were collected at different times after mMPC administration (n = 8). Serum samples were analyzed by multiplex Luminex for cytokines. Significantly different levels (P < 0.05) of cytokines were observed at indicated times. *Irradiated mice compared with unirradiated control; †irradiated mice treated with vehicle compared to irradiated mice injected with mMPC. (▲) Vehicle; (○) Rad 11 Gy + 5 million mMPC; (●) Rad 11 Gy + vehicle; (△) 5 million mMPC.
of high-radiation doses in unmatched recipient mice. Even when administration of mMPC was delayed up to 5 and 7 days in lethally irradiated Balb/c and CD2F1 mice, respectively, a significant increase in 30-day survival was observed. We have previously used tocopherol succinate to mobilize progenitors through G-CSF induction, and used such mobilized progenitors as a radiomitigator to protect $^{60}$Co-irradiated mice as late as 24 h after irradiation (33). The ability to delay the administration of any radiation countermeasure is critical to allow for dosing of radiation victims in an emergency situation. Additionally, the ability to cryopreserve mMPC prior to use and administer it without matching donor and recipient eliminates time-consuming procedures and logistics needed for bone

FIG. 6. Effects of mMPC administration on cytokine induction in irradiated CD2F1 mice 6 and 12 days after cell administration. Mice were injected IV with 5 million mMPC or vehicle 2 h after $^{60}$Co γ irradiation (9.2 Gy). Blood samples were collected 6 and 12 days after mMPC administration ($n=8$). Serum samples were analyzed by multiplex Luminex for cytokines. *Significant difference in cytokine levels between two different groups is indicated. The symbol key for all cytokines is the same as shown in the panel for IL-6.
marrow transplant. More importantly, it allows for the development of a therapeutic that can be stockpiled and that would readily be available on demand in case of an emergency. Currently there is no report of any radiation countermeasure under development that can be administered as late as this mMPC product to a wide range of people and still confer significant mitigation of ARS (7).

We have previously reported production of various cytokines for 24–48 h in response to administration of several radiation countermeasures currently under development, including genistein, 5-androstenediol, CBLB502 (truncated flagellin agonist of Toll-like receptor 5), CBLB612 and CBLB613 (synthetic lipopeptide of mycoplasma origin, agonist of Toll-like receptor 2/6) (18, 34–36). Our data demonstrate that administration of mMPC do not induce cytokine production in unirradiated mice and cause a minor transient elevation of cytokines 12 h after administration in mice exposed to 11 Gy (60Co γ irradiation) (Fig. 5). Overall, the levels of cytokines were low and may not be relevant biologically (37, 38). Recently, we demonstrated that the radioprotective efficacy of tocopherol succinate against acute hematopoietic and GI-related injury is mediated through G-CSF (26, 39). Our current study suggests that mMPC mitigation from radiation lethality is not mediated through an immediate effect on G-CSF levels. We did, however, observe a peak in cytokines tested 12 days after irradiation (9.2 Gy, 60Co γ irradiation) in vehicle control mice irradiated mMPC treated mice (Fig. 6). These cytokines play an important role in the inflammatory response (40–43) and their inhibition by mMPC-treatment indicates that mMPC may have anti-inflammatory activity. Whether the reduction of cytokines 12 days after irradiation results from direct inhibition of cytokines by mMPC or from an indirect effect on hematopoietic injury in general, remains to be determined.

MMPC are mouse myeloid progenitor cells derived in culture from bone marrow hematopoietic stem cells with varying degrees of maturity and lack lymphoid surface markers. MMPC can be cryopreserved, stored and thawed without compromise in function. All transplantations described in this report were performed with cryopreserved mMPC pooled after thaw from 3 MHC-disparate mouse strains (AKR1b-2a, FVB1b-2a and C57BI/6J-2a) and transplanted into unmatched recipient mice (CD2F1b-2a or Balb/c1b-2a).
No obvious signs of GVHD, e.g., diarrhea and skin irritations, were observed in any mMPC treated mice monitored for up to 90 days. Our results suggest that mMPC do not mitigate radiation injury by means of early cytokine induction. Instead, mMPC provide functional cells to the hematopoietic system, which was compromised by irradiation, thereby preventing death from hematopoietic failure. In a related model, Balb/c mice were transplanted with mMPC 1 day after irradiation (9 Gy) and challenged with a lethal dose of fungus on day 7 after irradiation, demonstrating the presence of functional mMPC progeny in these mice. These mice showed autologous recovery of the hematopoietic system by day 30 after irradiation (unpublished data).

Even at high radiation doses (11, 13 and 14 Gy, \(^{60}\text{Co} \gamma\) irradiation) 6 million mMPC provided significant mitigation when administered as late as 5 days after irradiation. At these supralethal radiation doses the primary cause of death is GI damage as indicated by the early death of the control animals. This observation points toward an effect of mMPC outside the hematopoietic system (44). We will perform follow-on studies to further evaluate the effect of mMPC administration on injury of GI and other nonhematopoietic tissues in mice irradiated with supralethal radiation doses. In addition, we will perform long-term survival studies to see whether (1) there is a benefit to chronically-injured tissues (e.g., lung) and (2) whether there is long-term engraftment from the small subset of HSC/MPP within the MPC product.

Our results showing that administration of mMPC enhances survival after high-dose radiation exposure suggest that a human MPC (hMPC) product is a promising candidate as a countermeasure to radiation exposure. Cellerant Therapeutics has developed a culture system using serum-free medium and a defined cytokine cocktail free of any animal products to expand CD34\(^+\) enriched

**FIG. 8.** Efficacy of mMPC against supralethal doses of \(^{60}\text{Co} \gamma\) radiation. CD2F1 mice were transfused with 6 million mMPC 4 or 5 days after \(^{60}\text{Co} \gamma\) irradiation (11, 13 and 14 Gy) and observed for 30 days. Death by radiation was significantly mitigated in mice treated with mMPC 4 (panel A) or 5 (panel B) days after irradiation compared with vehicle control (\(n = 16\)). *Denotes statistically significant difference from vehicle control.
human HSC in vitro and to direct their differentiation into human myeloid progenitor cells. These hMPC (CLT-008) are significantly depleted of long term engrafting HSC and lymphoid cells. The active component of CLT-008 consists mainly of hematopoietic progenitor cells at various developmental stages that are committed to the myeloid lineages. CLT-008 is currently in two phase 1 clinical trials in patients undergoing cord blood transplants and in patients receiving high-dose chemotherapy, in both cases for the treatment of hematological malignancies.

Three major advantages make myeloid progenitors ideal for the treatment of a large number of ARS victims: (1) myeloid progenitor cells can be cryopreserved and stored without compromise in function, (2) treatment can be delayed up to 7 days and still provide survival benefit, and (3) myeloid progenitors provide radiomitigation in unmatched irradiated recipients without signs of rejection or GVHD. Together these characteristics make myeloid progenitors a prime candidate for an off-the-shelf bridging therapy for acute radiation victims that can be administered in the field with minimal infrastructure requirements, and can be delayed until disaster response teams are in place.

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REFERENCES

27. Singh VK, Ducey EJ, Fatami OO, Singh PK, Brown DS, Purmal A, et al. CBLB613: A TLR 2/6 Agonist, Natural Lipopeptide of


42. Pappu R, Ramirez-Carrozzi V, Sambandam A. The interleukin-17 cytokine family: critical players in host defence and inflammatory diseases. Immunology 2011; 134:8–16.