Human monocytes educated with exosomes from TLR4 primed mesenchymal stem cells treat acute radiation syndrome by promoting hematopoietic recovery

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Abstract

Total body irradiation is often used as a conditioning regimen for bone marrow transplants but can cause life threatening damage to host tissues especially the bone marrow. Developing a cellular therapy that can protect the bone marrow from acute radiation syndrome (ARS) and stimulate hematopoiesis is a priority for patients exposed to therapeutic or even accidental radiation injury. In this study, exosomes derived from mesenchymal stem cells (MSCs) stimulated with the TLR4 agonist lipopolysaccharide (LPS) were used to alternatively activate human monocytes, termed LPS EEMos, as a potential novel radioprotective cellular therapy. LPS EEMos expressed higher levels of PD-L1 ($p<0.0001$), and lower levels of CD16 ($p<0.01$), CD86 ($p<0.01$), and CD206 ($p<0.0001$) by flow cytometry compared to monocytes educated with exosomes from unstimulated MSCs (EEMos). Using qPCR, increased gene expression in LPS EEMos of IL-10 ($p<0.05$), IDO ($p<0.001$), FGF2 ($p<0.05$), IL-15 ($p<0.05$), and IL-6 ($p<0.0001$) were detected compared to EEMos. Using a xenogeneic radiation injury model, infusion of human LPS EEMos 4 hours after lethal radiation led to reduced clinical scores and an increased survival at 40 days postinfusion, as compared to infusions of PBS, EEMos, and monocytes alone, all of which led to worse clinical scores and 0% survival with uniform death by 20 days ($p<0.05$). Complete blood cell counts in LPS EEMo recipients showed leukocyte, erythrocyte and platelet counts equivalent to non-irradiated mice, demonstrating complete restoration of hematopoiesis. Infusion of LPS EEMos may be a useful strategy to protect the bone marrow from acute radiation syndrome by expression of anti-inflammatory molecules and cytokines that promote hematopoiesis/engraftment.

Background

- Mesenchymal stem cells (MSCs) are a supportive cell subset that have been developed for acute radiation syndrome (ARS) but have shown inconsistent efficacy in animal models.
- We have previously demonstrated *in vitro* that co-culturing MSCs with macrophages leads to development of a regenerative, anti-inflammatory macrophage subset called an MSC educated macrophage (MEM) enhances survival from lethal ARS using a xenogeneic mouse model, as compared to infusions of MSCs or macrophages alone.
- One of the limitations of translating MEMs to the clinic is the time needed to generate them – 10 days. A patient could easily succumb to a toxicity of ARS during this critical time period, and cells that can be generated more quickly will be more practical for clinical application.
- Exosomes from MSCs can be used to educate monocytes (EEMos) reduce production time from 10 days to just 24 hours, allowing for a cell therapy that can be deployed quickly and effectively after the radiation insult.

Hypothesis

We hypothesize that LPS EEMos are a useful cell subset to protect the bone marrow against ARS by expression of anti-inflammatory molecules and augmentation of host hematopoiesis.

Acknowledgments

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Conflicts of interest: P.H. and C.C. have a patent related to using MSCs and exosomes to educate monocytes/macrophages for radiation injury (US Patent 10,166,254 B2. Issued Jan 1, 2019).

Methods

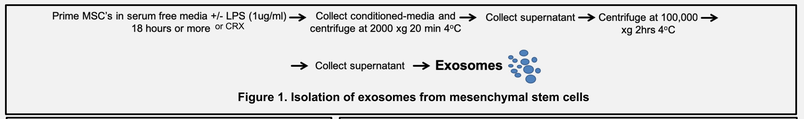


Figure 1. Isolation of exosomes from mesenchymal stem cells

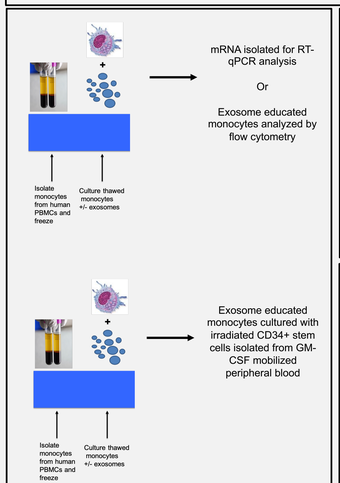


Figure 2. Methods for *in vitro* characterization of EEMos

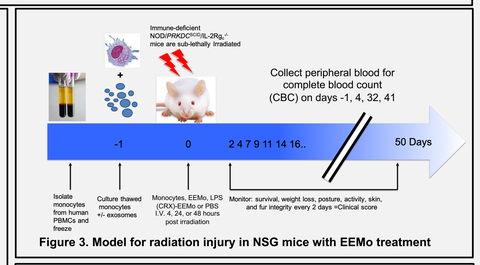


Figure 3. Model for radiation injury in NSG mice with EEMo treatment

Figure 2. Methods for *in vitro* characterization of EEMos

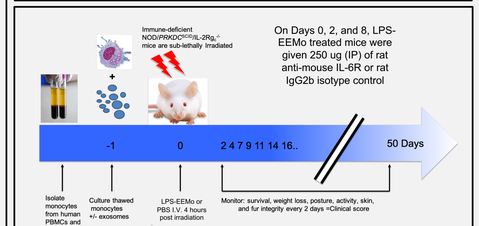
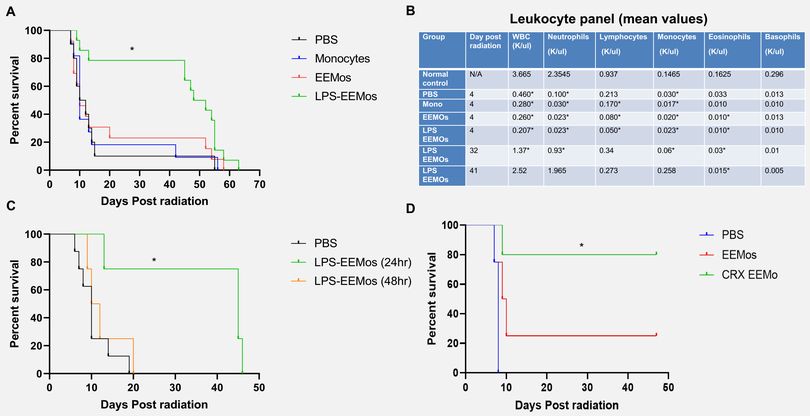


Figure 4. Investigating IL-6 for mechanism of LPS-EEMo radioprotective effect

Results



Group	Day post radiation	WBC (K/ul)	Neutrophils (K/ul)	Lymphocytes (K/ul)	Monocytes (K/ul)	Eosinophils (K/ul)	Basophils (K/ul)
Normal control	N/A	3.665	2.3545	0.937	0.1465	0.1625	0.296
PBS	4	0.460*	0.100*	0.213	0.030*	0.033	0.013
Mono	4	0.280*	0.030*	0.170*	0.017*	0.010	0.010
EEMos	4	0.260*	0.023*	0.080*	0.020*	0.010*	0.013
LPS EEMOs	4	0.207*	0.023*	0.050*	0.023*	0.010*	0.010
LPS EEMOs	32	1.37*	0.93*	0.34	0.06*	0.03*	0.01
LPS EEMOs	41	2.52	1.965	0.273	0.258	0.015*	0.005

Figure 5. Results from in vivo NSG radiation model with PBS, monocyte, EEMo, or LPS (CRX) EEMo treatment. (A) Survival of NSG mice over time (treatment 4 hours post irradiation). Results pooled from three separate experiments, with 10 to 14 mice/group. **(B)** Mean values of CBC leukocyte panel (taken from 1 experiment). **(C)** Survival of NSG mice over time (treatment 24 or 48 hours post irradiation). Results pooled from two separate experiments, with 4 to 8 mice/group **(D)** Survival of NSG mice treated with CRX-EEMos (4 hours post irradiation) One experiment with 4 mice/group. * $P < 0.05$

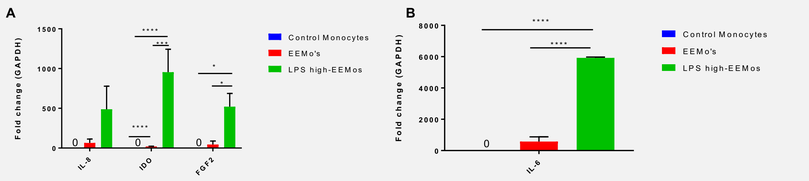


Figure 6. RT-qPCR analysis of control monocytes, EEMos, and LPS-EEMos. (A) Fold change in mRNA expression of IL-8, IDO (indoleamine 2,3-dioxygenase), and FGF2 (fibroblast growth factor 2) compared to housekeeping gene GAPDH. **(B) Fold** change in mRNA expression of IL-6 compared to housekeeping gene GAPDH. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Results

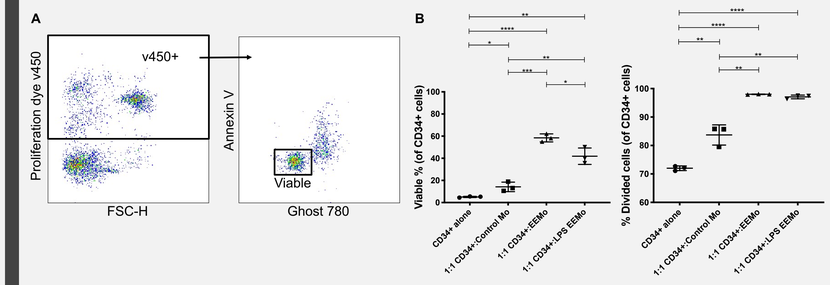


Figure 7. Co-culture of irradiated, v450 labelled CD34+ stem cells with control monocytes, EEMos, or LPS EEMos. (A) Representative flow cytometry plots of v450 labelled CD34+ stem cells (from GM-CSF mobilized peripheral blood) irradiated with 4 GY and then co-cultured with LPS EEMos. Analysis was performed 3 days after culture was initiated. **(B)** Compiled data from co-culture in (A). 3 wells of a 96-well plate were allocated for each condition, in 1:1 CD34:EEMo ratios. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

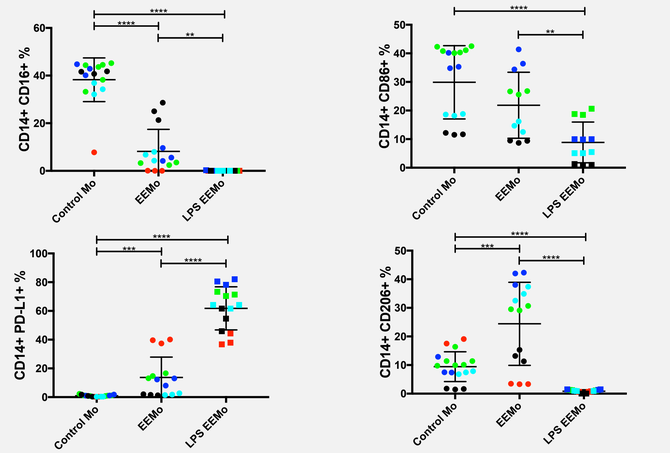


Figure 8. Flow cytometric characterization of control monocytes, EEMos, and LPS EEMos. Viable CD14+ cells were analyzed for the expression of surface molecules CD16, CD86, PD-L1, and CD206. Each color represents a separate CD14+ isolate that was uneducated (control Mo), educated with MSC exosomes (EEMos), or educated with LPS stimulated MSC exosomes (LPS EEMos). . * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

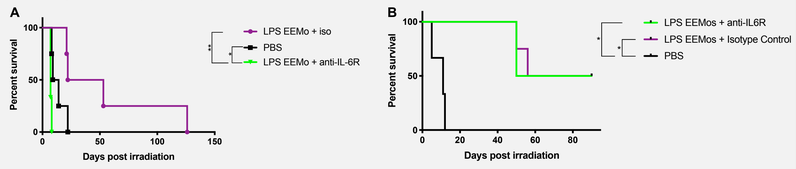


Figure 9. Results of IL-6R inhibition on the radioprotective effect of LPS-EEMos. (A) Female NSG mice were irradiated (4 mice/group) and given the indicated treatment 4 hours later. **(B)** Same as (A), except with Male NSG mice. * $P < 0.05$, ** $P < 0.01$

Conclusions

- Human LPS high EEMos attenuate weight loss, reduce clinical scores and improve overall survival in a xenogeneic ARS model.
- Irradiated NSG mice treated with LPS EEMos show leukocyte levels equivalent to non-irradiated mice.
- LPS EEMos express high gene expression of IL-6, IDO, and FGF2.
- LPS EEMos are a $CD14^+$ cell subset that consist of a high percentage of $PD\text{-}L1^+$ cells and low percentage of $CD16^+$, $CD86^+$ and $CD206^+$ cells.
- EEMos and LPS EEMos are able to enhance the survival, proliferation, and differentiation of irradiated human CD34+ stem cells, making them a promising cellular therapy for acquired bone marrow failure from lethal ARS.