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 xenogeneic 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<0.01), and lower levels of CD163 (p<0.01), CD86 (p<0.01), and CD206 (p<0.001) 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<0.001) were detected compared to EEMos. Using a xenographic 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 post irradiation, 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 to treat acute radiation syndrome (ARS) but have shown inconsistent efficiency 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) that enhances survival from lethal ARS using a xenogenic 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 cell therapy to 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.

Methods

Figure 1. Isolation of exosomes from mesenchymal stem cells

Figure 2. Methods for in-vitro characterization of EEMos

Figure 3. Result from in vivo ARS model with PBS, monocytes, EEMos, or LPS EEMos treatment. (A) Survival of NSG mice post irradiation (treatment 4 hours post irradiation). Results pooled from three separate experiments, with 10-12 mice per group. (B) Survival of NSG mice post irradiation (treatment 4 hours post irradiation). Results pooled from two separate experiments, with 4-8 mice per group. (C) Survival of NSG mice treated with CD34 EEMos (9 hours post irradiation) One experiment with 2 mice per group. P < 0.05

Figure 4. Investigating IL-6 for mechanisms of LPS-EEMs radioprotective effect

Figure 5. Model for radiation injury in NSG mice with EEMos treatment

Figure 6. RT-qPCR analysis of control monocytes, EEMos, and LPS-EEMos. (A) Fold change in mRNA expression of IL-6, EAS (proinflammatory 3.3 downregulation), and FGF2 (fibroblast growth factor 3) 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.

Figure 7. Co-culture of irradiated, iV040 labeled CD34+ stem cells with control monocytes, EEMos, or LPS EEMos. (A) Representative flow cytometry plots of iV040 labeled CD34+ stem cells from iV040 labeled peripheral blood irradiated with 4 Gy and then co-cultured with LPS EEMos. Analysis was performed 3 days after culture was initiated. (B) Flow cytometry data from co-culture at 48 hours. Flow cytometry plots were allocated for each condition, in 1:1 EEMos:cells ratio. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Figure 8. Flow cytometric characterization of control monocytes, EEMos, and LPS EEMos. Viability CD34+ cells were assessed prior to the expansion of surface molecules CD16, CD68, FOXP3, and CD86. Each color represents a separate CD14+ subset that was unduced (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.