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Stem cells and necrotizing enterocolitis: A direct comparison of the efficacy of multiple types of stem cells

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ABSTRACT

Purpose: Necrotizing enterocolitis (NEC) is a leading cause of gastrointestinal morbidity and mortality in premature infants. While studies have shown potential for stem cell (SC) therapy in experimental NEC, no study has compared different SC side-by-side. Our purpose was to determine whether one type of SC may more effectively treat NEC than others.

Methods: Four SC were compared: (1) amniotic fluid-derived mesenchymal SC (AF-MSC); (2) amniotic fluid-derived neural SC (AF-NSC); (3) bone marrow-derived mesenchymal SC (BM-MSC); and (4) neonatal enteric neural SC (E-NSC). Using an established rat model of NEC, pups delivered prematurely received an intraperitoneal injection of SC. Control pups were injected with PBS. Additional controls were breast-fed by surrogates and not subjected to experimental NEC. Intestinal tissue was graded histologically.

Results: NEC incidence was: PBS, 61.3%; breast-fed unstressed, 0%; AF-MSC, 19.1%; BM-MSC, 22.9%; AF-NSC, 18.9%; E-NSC 22.2%. All groups demonstrated statistical significance ($p<0.05$) compared to controls, and there was no difference between SC groups.

Conclusion: All four SC groups reduced the incidence and severity of experimental NEC equivalently. AF-MSC may be preferable because of availability of AF at delivery and ease of expansion, increasing potential for clinical translation.

Level of Evidence: V (Animal study).

Necrotizing enterocolitis (NEC) is a leading cause of gastrointestinal morbidity and mortality in premature infants [1]. Stem cell therapy has been shown in in vivo animal models to protect the intestines from several different types of injury including NEC [2–9]. While previous studies have shown promise with administration of stem cells, no study has directly compared the efficacy of different types of stem cells side-by-side. Our purpose was to determine whether stem cells from four different sources have varying efficacies, enabling the identification of stem cell(s) that may more effectively treat NEC.

1. Methods

1.1. Cell culture

All cell cultures were derived from timed-pregnant Lewis rats (Rat Resource and Research Center, University of Missouri, Columbia, MO), sacrificed at E14.5 of gestation (estimated day 14.5 of an average 22 day gestation).

AF-MSC cells were obtained using modifications of previously described procedures [10,11]. AF was harvested via 25ga needle aspiration of amniotic sacs. Cells were cultured in Minimum Essential Medium Alpha with GlutaMAX™ (MEM- α, ThermoFisher, Waltham, MA), supplemented with 10% Embryonic Stem Cell Qualified Fetal Bovine Serum (ES-FBS, ThermoFisher), 18% Chang B and 2% Chang C (Irvine Scientific, Santa Ana, CA), and 1% penicillin/streptomycin/amphotericin B (PSA, ThermoFisher). Media was changed every three days. Cells were routinely passaged using 0.25% Trypsin–EDTA (Trypsin, ThermoFisher) at 80–90% of confluence. All cells used for studies were from passages 4–9.

AF-NSC primary cell cultures were established from AF, with NSC induction performed using modifications of previously described protocols [12,13]. Cells were cultured in NSC growth medium consisting of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12, ThermoFisher) supplemented with 4% chicken embryo extract (Gemini Bio-Products, West Sacramento, CA), 2% PSA, 1X N-2 supplement (ThermoFisher), 20 ng/ml recombinant rat fibroblast growth factor basic (FGF, R&D Systems, Minneapolis, MN), and 20 ng/ml recombinant rat epidermal growth factor (EGF, R&D Systems). NSCs grew in this medium as cellular aggregates known as neurospheres. Media was changed every four days.

BM-MSC primary cell cultures were established from marrow harvested from femurs and tibias as previously described [14–16]. Bones

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were dissected of surrounding tissue and marrow flushed with culture media through a 25ga needle. Culture media was composed of MEM-α with 10% MSC-Qualified Fetal Bovine Serum (MSC-FBS, ThermoFisher), and 1% PSA. The first media change occurred after five days, and subsequently every three days. Cells were passaged using Trypsin at 80–90% confluence. All cells used for studies were from passages 4–9.

E-NSC primary cell cultures were established from neonatal rat pups 4–7 days after birth using a previously described method [6,17–19]. Intestine from duodenum to ileum was harvested, opened longitudinally, and the mucosa and submucosa stripped away. The muscular layer was digested in DMEM/F12 containing 1 mg/mL Collagenase and 1 mg/mL Dispase (Worthington Biochemical Corporation, Lakewood, NJ) for 45–60 min. Cells were passed through a 70 μm filter, centrifuged,

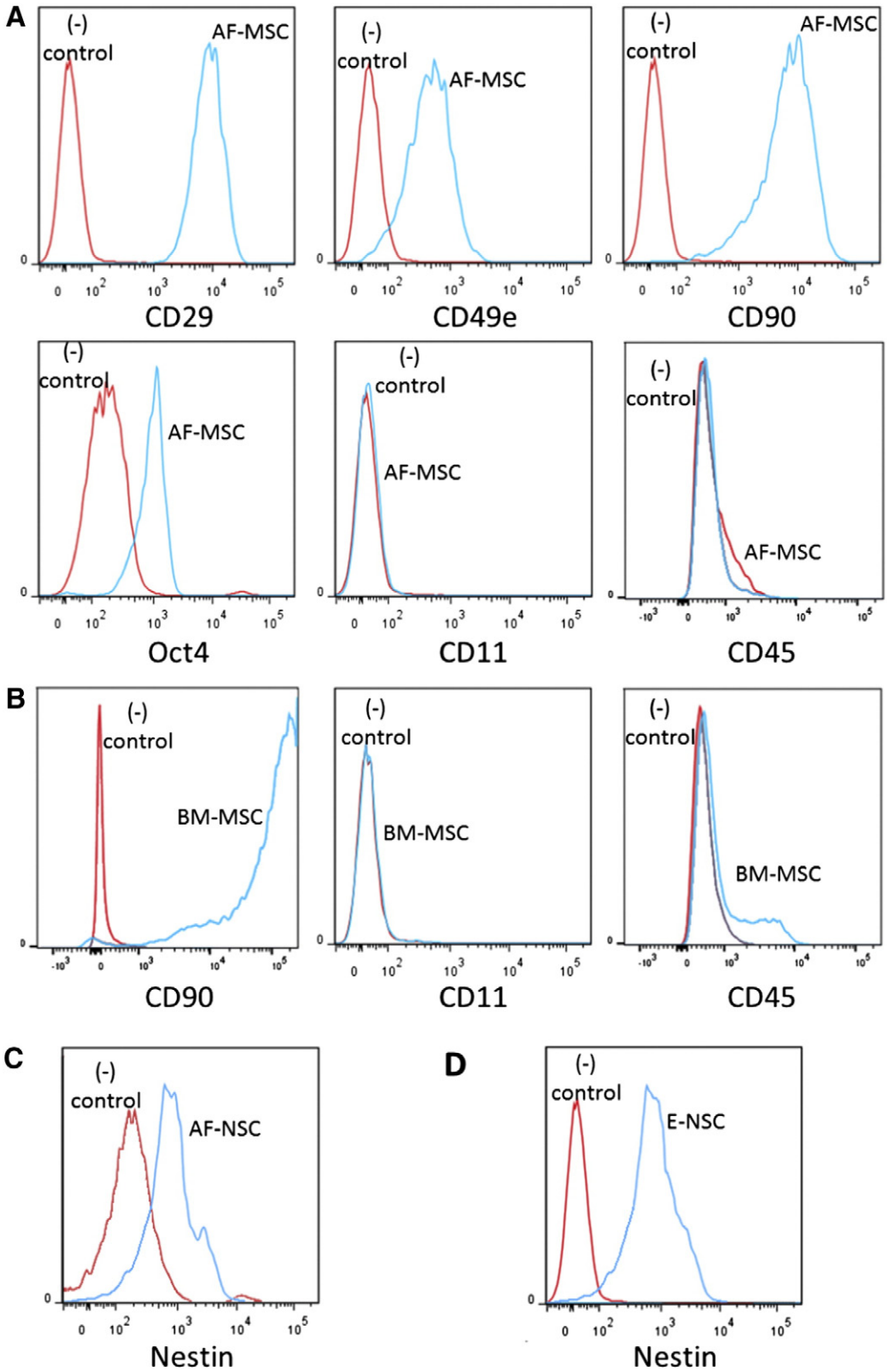

Fig. 1. Flow cytometric analysis of stem cells. Red lines represent negative controls and blue lines represent populations of interest. (A) AF-MSC, positive for CD29, CD49e, CD90; partially positive for Oct4; negative for CD11 and CD45; (B) BM-MSC, positive for CD90 and negative for CD11 and CD45; (C) AF-NSC, positive for Nestin; (D) E-NSC, positive for Nestin.