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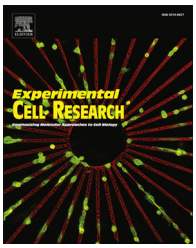

Research Article

Bone-derived mesenchymal stromal cells from HIV transgenic mice exhibit altered proliferation, differentiation capacity and paracrine functions along with impaired therapeutic potential in kidney injury

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ABSTRACT

Mesenchymal stem cells (MSCs) secrete paracrine factors that could be cytoprotective and serve roles in immunoregulation during tissue injury. Although MSCs express HIV receptors, and co-receptors, and are susceptible to HIV infection, whether HIV-1 may affect biological properties of MSCs needs more study. We evaluated cellular proliferation, differentiation and paracrine functions of MSCs isolated from compact bones of healthy control mice and Tg26 HIV-1 transgenic mice. The ability of MSCs to protect against cisplatin toxicity was studied in cultured renal tubular cells as well as in intact mice. We successfully isolated MSCs from healthy mice and Tg26 HIV-1 transgenic mice and found the latter expressed viral Nef, Vpu, NL4-3 and Vif genes. The proliferation and differentiation of Tg26 HIV-1 MSCs was inferior to MSCs from healthy mice. Moreover, transplantation of Tg26 HIV-1 MSCs less effectively improved outcomes compared with healthy MSCs in mice with acute kidney injury. Also, Tg26 HIV-1 MSCs secreted multiple cytokines, but at significantly lower levels than healthy MSCs, which resulted in failure of conditioned medium from these MSCs to protect cultured renal tubular cells from cisplatin toxicity. Therefore, HIV-1 had adverse biological effects on MSCs extending to their proliferation, differentiation, function, and therapeutic potential. These findings will help in advancing mechanistical insight in renal injury and repair in the setting of HIV-1 infection.

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Introduction

Bone marrow- or bone-derived mesenchymal stem cells (MSCs) are multipotent with the ability to differentiate along various lineages and to serve roles in tissue homeostasis, repair, and immunoregulation [1]. Such properties of MSCs are relevant for HIV-1 and related pathophysiological mechanisms, since MSCs express HIV receptors, CD4, and also coreceptors, CCR5 and CXCR4, and are thus

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susceptible to HIV infection or its persistence [2–5]. Previously, HIV was found to suppress clonogenicity and differentiation potential of MSCs [6], which was in agreement with reduced bone density in HIV-infected patients [7,8], because osteoblasts are needed for new bone formation may originate, in part, from bone marrow MSCs [9,10]. At the molecular level, HIV-1 proteins, such as p55, gag and REV, may perturb osteogenic signaling in differentiating MSCs [11]. However, whether HIV-1 could affect paracrine signals emanating from MSCs has not yet been defined. This will be important since MSCs may influence other cells via paracrine signals.

MSCs secrete multiple substances, including growth factors, cytokines, and chemokines [12,13], and respond to chemoattractants while migrating to sites of tissue inflammation or injury [14,15]. These properties of MSCs generated much interest for cell transplantation, including for immunomodulation and tissue engineering [16]. For instance, cell therapy with transplantation of MSCs has been studied in the model of cisplatin-induced acute kidney injury (AKI) [13,15,17]. Recently, we found transplanted MSCs improved outcomes in cisplatin-induced AKI by paracrine signals and not by replacing lost cells [18]. These MSCs secreted several soluble factors, including GCSF, VEGF, HGF, IL-10, EGF, IGF, etc. [18], that are well-known to possess antiapoptotic, anti-inflammatory, mitogenic, angiogenic [13], and renoprotective properties [19–25].

As acute kidney injury is a common problem in patients with HIV-1, we hypothesized that expression of HIV-1 genes in MSCs might compromise their biological properties, including lower efficacy of cell therapy with such MSCs for recovery of injured renal tubular cells, especially via paracrine signaling from transplanted MSCs. In the present study, we isolated MSCs from long bones of Tg26 HIV-1 transgenic mice characterized by the proviral transgene, pNL4-3:d1443, which encodes all HIV-1 genes, except for *gag* and *pol* [26], and express viral Nef, Vpu, NL4-3 and Vif mRNAs. Studies of these Tg26 HIV-1 MSCs in comparison with MSCs from healthy mice showed that HIV-1 deleteriously affected proliferation, differentiation, and paracrine functions of MSCs.

Materials and methods

Animals

The Ethics Review Committee for Animal Experimentation of Feinstein Institute for Medical Research-North Shore LIJ Health System and the Animal Care and Use Committee of Albert Einstein College of Medicine approved study protocols. C57BL/6J, FVB/N and C57BL/6-TgCAG-EGFP/1Osb/J mice (GFP mice) with ubiquitous expression of green fluorescent protein (GFP) were from Jackson Labs (Bar Harbor, ME). Tg26 HIV-1 transgenic mice in FVB/N background were from Dr. P.E. Klotman (Mount Sinai Medical Center, New York, NY). Tg26 mice typically develop proteinuria and glomerulosclerosis by 4 weeks with worsening of renal functions by 8–10 weeks of age [26]. We used 6–8 weeks old mice. All animals were housed under standard lighting conditions with unlimited access to pelleted food and water.

Isolation and characterization of MSCs

MSCs were isolated from long bones of C57BL/6J, C57BL/6-GFP, FVB/N and Tg26 HIV-1 transgenic mice, essentially as described previously [27]. Briefly, fragments of compact bones were suspended in Mesencult basal medium (StemCell Technologies Inc; Vancouver, CA) containing 10% FBS and 1 mg/ml of collagenase II (Sigma-Aldrich, USA), followed by digestion for 1–2 h at 37 °C. Cells and bone fragments were incubated in Mesencult basal medium with supplements (StemCell Technologies Inc, CA) in humidified 37 °C incubator with 4% O_2 and 5% CO_2 for 3 days. Non-adherent cells and tissue debris were removed by changing medium after 3 days and twice weekly. MSCs were used for studies after three to five passages in culture. To characterize cellular phenotype, MSCs were stained with PE-conjugated monoclonal antibodies specific for CD11b, CD14, CD34, CD86, CD90, and CD105 (BD Pharmingen) and analyzed by flow cytometry (FACSAriaII, Biosciences).

Proliferation and osteogenic differentiation of HIV-1 MSCs

To examine proliferation ability, MSCs were continuously subpassaged 1:3 with trypsin-EDTA. Population doublings were measured by manual counting of cell numbers. To induce osteogenic differentiation, MSCs were cultured in Mesencult medium containing 10% FBS, 100 nM dexamethasone, 50 μM ascorbic acid-2-phosphate and 10 mM β-glycerophosphate (Sigma) for 3 weeks with fresh medium every 3–4 days. To examine osteogenic differentiation, cells were fixed in 10% formalin for 1 h, washed with PBS, and incubated with 40 mM Alizarin Red (Sigma) for 10 min.

Viral gene expression in Tg26 HIV-1 MSCs

Total RNA was extracted from MSCs at 90% confluence by Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA). After incubation with Amplification Grade DNase I (Invitrogen), RNAs were reverse transcribed with Omniscript RT system (Qiagen Inc., Valencia, CA). Polymerase chain reactions (PCR) for HIV-1 genes were performed with following primers: Nef (forward: 5′-GGTGGGTTTTCCAGT CACAC-3′, reverse: 5′-GGGAGTGAATTAGCCCTTCC-3′); NL4-3 (forward: 5′-GGCGTTACTCGACAGAGGAG-3′, reverse: 5′-TGCTTTCATAGA-GAAGCTTGATG-3′); Vif (forward: 5′-ACCTGGCAGACCAACTAATT CACCT-3′, reverse: 5′-GGCCCTTGGTCTTCTGGGGCTT-3′); and Vpu (forward: 5′-AGCACCTTGGAACGATACCTGGGA-3′, reverse: 5′-TGGTCCT TTGATGGGAGGGGCA-3′). Mouse β-actin (forward: 5′-GAGCTATGAG CTGCCTGAC-3′, reverse: 5′-CTGATCCACATCTGCTGGAA-3′) was used as control for integrity of input RNA. PCR used equal amounts of templates in Platinum PCR Supermix (Life Sciences, Carlsbad, CA) with following conditions: denaturation at 94 °C × 5 min, 35 cycles at 94 °C × 30 s, annealing at 55 °C × 30 s, and extension at 72 °C × 1 min. PCR products were resolved in 1.5% agarose gels containing ethidium bromide.

Protective effect of transplanted MSCs in cisplatin-induced AKI

HIV-1 MSCs and FVB/N MSCs were trypsinized and passed through 70-μM nylon mesh. Male mice ($n=5$ each) were transplanted with either 5×10^5 HIV-1 MSCs or FVB/N-MSCs through tail vein injection 24 h before cisplatin (Sigma Chemical Co., St. Louis, MO), which was freshly dissolved in saline to 1.2 mg/ml. Mice were injected subcutaneously with 12 mg/kg cisplatin. The vehicle-treated controls received saline alone. To demonstrate differences in renal histology and renal tests, kidney and blood samples were collected on day 3 after cisplatin or vehicle alone.

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