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An engineered biomarker system to monitor and modulate immune clearance of cell therapies

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Abstract

Background aims. Cell transplants offer a new opportunity to deliver therapies with novel and complex mechanisms of action. Understanding the pharmacology of cell transplants is important to deliver this new therapy effectively. Currently, however, there are limited techniques to easily track cells after intravenous administration due to the dispersion of the graft throughout the entire body. *Methods.* We herein developed an engineered cell system that secretes a luciferase enzyme to sensitively detect cell transplants independent of their locale by a simple blood test. We specifically studied a unique feature of cell transplant pharmacology—namely, immune clearance—using mesenchymal stromal cells (MSCs) as a proof-of-concept cell therapy. MSCs are a clinically relevant cell therapy that has been explored in several disease indications due to their innate properties of altering an immune response. *Results.* Using this engineered reporter, we observed specific sensitivity of cell therapy exposure to the preparation of cells, cytolysis of MSCs in an allogeneic setting and a NK cell-mediated destruction of MSCs in an autologous setting. *Conclusions.* Our cellular tracking method has broader implications at large for assessing *in vivo* kinetics of various other cell therapies.

Key Words: *engineered MSCs, immune clearance, immunogenicity, luciferase, MSC, NK cell*

Introduction

Cell therapies are a burgeoning class of medicines that can broaden the spectrum of drug action compared with conventional molecular therapeutics. Mesenchymal stromal cells (MSCs) are at the forefront of clinical testing, and currently there are 493 active clinical trials that administer MSCs as a therapeutic intervention [1]. MSCs have many useful properties that make them a clinically attractive therapy. They are easily isolated and expanded from bone marrow or clinically discarded tissue (e.g., umbilical cord blood, adipose tissue). MSCs can also be engineered *ex vivo* using both viral and nonviral methods to act as a drug delivery platform [2,3]. A number of preclinical studies observed the potential of MSCs to enhance wound-healing responses by secreting molecules that stimulate angiogenesis, endogenous stem cell populations, and a pro-resolving inflammatory response. An intravenous route of administration is the most prevalent use of MSCs for the systemic modulation [4–7] of a dysfunctional immune system. However, despite these great strides in recent years, there lies a significant bottleneck in passing phase III clinical trials for U.S. Food and Drug Administration approval [8]. One hypothesis for the ineffectiveness of MSCs in clinical studies is a short cell half-life *in vivo* based on estimates in mouse models [3].

A critical question in the field of cell therapy is the consideration of autologous or allogeneic cells for use. Autologous cells, in principle, have the advantage of minimizing immune rejection of a cell transplant. Yet managing supply chain logistics of autologous cell therapies is a challenge that can limit the scalability of this approach. The use of allogeneic MSCs overcomes scale-up challenges of cell therapies to meet the clinical and ultimately commercial needs of >10 000 patients per year for highly prevalent indications. Unfortunately, typical allogeneic cells suffer from immunogenicity. MSCs, however, have been considered minimally immunogenic through a lack of MHC Class II molecules and have been safely evaluated in mismatched transplant settings without severe immunogenic reactions

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[9,10]. Whether as an autologous or allogeneic cell therapy, the immune clearance of MSCs has been underexplored with respect to their relatively short *in vivo* life span. Current literature supports that active immunologic processes may be responsible for their short *in vivo* persistence. Allogeneic MSCs elicited a memory T-cell response and rapid clearance of subsequent intravenous doses by the immune system [11]. Activated natural killer (NK) cells, known to eliminate foreign cells, have also been shown to facilitate MSC lysis [12,13]. Furthermore, immunocompromised mice demonstrated higher levels to MSC-derived secreted factors after a cell transplant compared with wild-type mice [14]. Collectively, these studies suggest that recognition and immune clearance of MSCs could severely limit the bioavailability thereby greatly reducing therapeutic efficacy.

Studying immune clearance of a cell therapy such as MSCs is particularly challenging with existing tools. Whole body imaging requires specialized equipment and is only of high utility when cells are densely concentrated; the tracking of a diffusely spread cell transplant is hindered by signal to noise limits of conventional imaging techniques. Other approaches using flow cytometry or tracer-labeled cells are invasive and require sacrificing animals over time to detect cell levels, which becomes tedious and can be time- and resource-intensive [15–17]. Furthermore, these methods are equally challenged when searching for rare engrafted cells in a large tissue bed.

Because MSCs broadly distribute and encounter the immune system throughout the entire body, we engineered a reporter MSC as a cell-based biosensor that secretes a blood-based readout for minimally invasive, continuous measurements. Mouse and human MSCs were genetically engineered with constitutive expression of a secreted Gaussia luciferase (gLuc) reporter. gLuc activity can be easily quantified in a small aliquot of blood (5 μL) *ex vivo* by adding its substrate coelenterazine and measuring emitted photons using a luminometer. gLuc has a half-life of 5–20 min in mice circulation and has been used as a highly sensitive reporter (detection of ~1000 cells) for quantitative assessment of stem cells *in vivo* by measuring its level in the blood [18–20]. This reporter system was put to the test to track MSCs in clinically relevant situations of autologous and allogeneic treatments and was found to reveal new insights into formulation of MSCs, potential cytolysis of allogenic MSCs and NK-cell–mediated clearance of autologous MSCs.

Methods

Culture and expansion of human and mouse MSCs

Human MSCs were isolated from whole bone marrow (Lonza) following previously established protocols [21,22]. Frozen vials of a C57BL/6 murine MSC line were purchased from Gibco. Cells were cultured in MSC media comprising α-minimum essential medium (Gibco) with 10% fetal bovine serum, 1% penicillin-streptomycin (Gibco), 1% antibiotic-anti-mycotic (Gibco) and 2.5 μg/L human fibroblast growth factor (R&D Systems). Cells were cultured at 37°C, 5% carbon dioxide. Cells were expanded by plating 1000 cells/cm^2 in a polystyrene cell culture treated flask (Corning) and allowed to reach 90% confluence rate before sub-culturing. MSCs from passages 3–5 were used for *in vitro* experiments. For *in vivo* experiments, MSCs harvested at passage 3 were used. Brefeldin A (BA)-treated MSCs were incubated for 24 h in 5 μg/mL BA [14] before use.

Genetic engineering of MSCs

MSCs were harvested at passage 2 for lentiviral infection. Lentivirus was obtained from the Massachusetts General Hospital Vector Core. The lentivirus was engineered to contain a G-luciferase gene along with a green fluorescent protein (GFP) reporter gene. Following protocols previously established as a guide, MSC media containing 100 μg/mL protamine sulfate was used for transduction [2]. Multiplicity of infection (MOI) of 1 to 10 was used for producing cell bank. Transduced GFP positive cells were sorted using a BD FACS Aria III (BD Biosciences) cell sorter (Harvard Stem Cell Institute Flow Cytometry Core at Massachusetts General Hospital, Boston). GFP positive cells were then cultured, expanded and used for subsequent studies.

Animals

All mice, female, C57BL/6J and BALB/cJ, were purchased from Jackson Laboratories (Jackson Laboratories) at 6–8 weeks old. Animals were housed at Massachusetts General Hospital Animal Facility according to Institutional Animal Care and Use Committee–approved protocols.

Injection of cell suspensions and depletion of NK cells

Tail vein injections of human and mouse MSCs were done at a concentration of 1×10^6 cells/200 μL in serum-free MSC media. Depletion of NK cells was achieved by injecting mice with 100 μg per dose of Mouse Anti-Mouse NK-1.1 Clone PK136 (BD Biosciences) [21,23,24]. Sham mice were treated with 100 μg per dose of Rat Anti-Mouse CD16/CD32 Clone 2.4G2 (BD Biosciences). Intraperitoneal injections were performed every 3 days for a week before injection with MSC cell suspension. Spleens were collected from mice post mortem, and cells were collected using single cell suspension methods. Depletion was