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Contribution of human adipose tissue–derived stem cells and the secretome to the skin allograft survival in mice

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ABSTRACT

Background: Despite considerable evidence showing the immunosuppressive properties of mesenchymal stem cells (MSCs) *in vitro*, such properties have not been fully demonstrated *in vivo*. The aim of this study was to evaluate the effect of MSCs and/or MSC secretome in inducing tolerance in a mouse skin transplantation model.

Methods: After receiving full-thickness skin allotransplantation on the back of the mouse, the recipient mice were infused with phosphate-buffered saline, adipose tissue–derived stem cells (ASCs), conditioned media (CM), and control media. Specifically, ASCs (1.0×10^6/0.1 mL) were transplanted to ASC-infused mice and 25-fold concentrated CM, which had been obtained from ASC culture were infused to CM-infused mice. Graft survival rates and the parameters reflecting immunologic consequences were assessed.

Results: The serum level of proinflammatory cytokine interleukin 6 decreased in mice treated with ASCs or CM compared with the control groups after infusion ($P < 0.05$). Interferon gamma, interleukin 10, and tumor necrosis factor alpha messenger RNA levels in the skin graft seemed to be decreased in the ASC-infused mice and CM-infused mice. Hyporesponsiveness was identified in mixed lymphocyte reaction assay at 30-d posttransplantation in ASC- or CM-infused mice. And, administering ASCs and CM markedly increased skin allograft survival compared with control animals ($P < 0.001$).

Conclusions: These findings suggest that ASCs and their secretome have the potential to induce immunologic tolerance. Moreover, our results demonstrate that the immunosuppressive properties of ASCs are mediated by the ASC secretome. Our approach could provide insights into a promising strategy to avoid toxicities of chemical immunosuppressive regimen in solid organ transplantation.

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1. Introduction

Immunologic tolerance in the clinical setting can be defined as long-term allograft survival in the absence of immunosuppressive treatment [1]. Immunologic tolerance has been considered an ultimate goal in human solid organ transplantation. Currently, the immunologic barriers after organ transplantation have been somewhat successfully overcome by immunosuppressive regimens, which can be toxic and have many side effects, possibly resulting in allograft loss. In such circumstances, recent sporadic reports have indicated overcoming immunologic barriers by using the immunosuppressive properties of stem cells [2–5].

Mesenchymal stem cells (MSCs) are derived from fetal and adult organs and have the capacity to self-renew and differentiate into various tissues including muscle, fat, stroma, tendon, cartilage, and bone [6]. Numerous *in vitro* experiments have revealed that MSCs have potent immunosuppressive properties [7,8]. MSCs suppress the T cell response to mitogenic stimuli and directly inhibit T cell proliferation [9,10]. MSCs also inhibit B cell proliferation and differentiation into antibody-secreting cells [11]. Furthermore, MSCs affect immunologic functions of antigen-presenting cells and inhibit monocyte differentiation into mature dendritic cells [12].

Although *in vitro* results support the immunosuppressive properties of MSCs, their effects *in vivo* have been poorly investigated. Recently, infusing donor bone marrow with epidermal cells was shown to improve skin allograft survival [13] and bone marrow–derived MSCs were reported to improve skin graft survival [14]. However, there is also a report to show that MSC infusion did not improve heart allograft survival [15].

Based on the above-mentioned findings, the purpose of this study was to evaluate the role of MSCs in inducing skin allograft tolerance in a mouse skin transplantation model. In particular, we compared MSCs' immunosuppressive properties with the properties of the conditioned media (CM), most of which include MSC secretome. We think that our approach can provide not only an understanding of how MSCs work but also provide new insight into a novel therapy based on the MSC's mechanism.

2. Materials and methods

2.1. Animals and study design

This study was carried out in compliance with the guidelines of the Institute for Laboratory Animal Research, Korea. Eight-week-old male C57BL/6 mice (Damool Science, Daejeon, Korea) were used as skin graft donors, and 8-week-old male BALB/c mice (Damool Science) were used as skin graft recipients. Obtaining specimens, such as serum samples or tissue, usually kills mice or influences the graft survival, limiting validation of survival analysis. Therefore, we designed two experimental sets: a set for graft survival analysis ($n = 48$) and a set for specimen attainment ($n = 64$). The set for graft survival was also used for mixed lymphocyte reaction (MLR) at posttransplantation 30 d. According to the materials injected via tail vein after skin allograft transplantation, each set was divided into four groups: phosphate-buffered saline (PBS) group, adipose tissue–derived stem cells (ASC) group, CM group, and M group. PBS group was infused with PBS; ASC group, ASCs; CM group, CM; and M group, control media. Specifically, ASCs at a concentration of 1.0×10^6/0.1 mL were given to ASC-infused mice, 25-fold concentrated CM which had been obtained from ASC culture to CM-infused mice, and control media (MesenPro RS medium; Gibco. Grand Island, NY) to control media-infused mice, respectively.

2.2. Preparation of ASCs and CM

2.2.1. Preparation of ASCs

Passage-three human MSCs were kindly donated by Hurim Biocell Co. (Seoul, Korea). The MSCs were obtained from human subcutaneous adipose tissue and thus are also called human ASCs. After thawing, the cells were cultured in MesenPro RS medium (Gibco) with growth supplement and antibiotics (amphotericin B, streptomycin, and penicillin) at 37°C in 5% CO_2. On reaching 80% confluence, the cells were digested with 0.25% trypsin/ethylenediaminetetraacetic acid at 37°C, centrifuged, and resuspended in the medium. The cell suspensions were plated in new flasks and cultured. Cells were used after three passages in our laboratory.

2.2.2. Identification of ASCs

Expression of cell-surface antigens in the cultured cells was analyzed by flow cytometry. The passage 3 cells were incubated with fluorescein isothiocyanate- or phycoerythrin-conjugated anti-CD44, anti-CD45, anti-CD29, or mouse IgG isotype control antibodies (BD Biosciences, Heidelberg, Germany) for 30 min at 4°C in PBS. Cells were washed and analyzed with a FACSCalibur kit (BD Biosciences, Pharmingen, CA).

2.2.3. 4, 6-Diamidino-2-phenylindole labeling

To allow identification of ASCs in transplant specimens, ASC nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO) before infusion to mice. DAPI was added to the medium at a final concentration of 50 mg/mL. The cells were incubated for 30 min at 37°C with 5% CO_2 and washed six times in PBS. Cells were detached by digestion with 0.25% trypsin/ethylenediaminetetraacetic acid at 37°C, centrifuged, and resuspended in PBS at 2×10^8 cells/mL.

2.2.4. Preparation of CM

ASCs that reached 70%–80% confluence were refed with serum-free MesenPro RS basal medium without growth supplement. CM was prepared by collecting the serum-free medium after culturing for 48 h. The medium was then concentrated approximately 25-fold using ultrafiltration units (Amicon Ultra-PL 3; Millipore, Bedford, MA) with a 3-kDa molecular weight cutoff. The CM was stored at 4°C or −80°C until use.

2.3. Full-thickness skin graft model and transplantation

2.3.1. Skin grafting

After mice were sedated with tiletamine-zolazepam (Zoletil 20) (30 mg/kg intraperitoneally), 3.0 × 3.0 cm full-thickness