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Histocompatibility testing of cultivated human bone marrow stromal cells – A promising step towards pre-clinical screening for allogeneic stem cell therapy

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ABSTRACT

Mesenchymal stem cells (MSCs) lack major histocompatibility complex (MHC)-II and only show minimal MHC-I expression. Despite MSCs demonstrating T-cell anergy, there are no established methods to evaluate their suitability. It is crucial to evaluate the complete mismatch of MHC compatibility in view of the hypo-immunogenic nature and immunomodulatory properties of MSCs with respect to their proliferation potential (PP) and utility in terms of passage number. With bone marrow (BM) being the major source of MSCs, the use of these cells becomes even more complicated, due to many other receptors coming to fore and triggering alternative pathways. This prospective study included five BM aspirates for MSC cultures and five allogeneic peripheral blood mono nuclear cells (PBMNCs) from healthy volunteers. MHC compatibility was assessed by polymerase chain reaction-sequence specific primer (PCR-SSP). The PP and a T-cell response to MSCs was addressed in mixed cultures and evaluated on the basis of their stimulation index (SI). Allogeneic circulatory antibodies against the donor MSCs was performed by cytotoxicity assay. The PP of MSCs during interactions with PBMNCs (T-cells) demonstrated T-cell anergy and the response to circulatory antibodies was minimal, in consonance with other published reports. Although, the results are encouraging for potential clinical application of MSC transplantation, autologous is always preferable to allogeneic, at least until the long-term safety of these cells is established in clinical trials.

1. Introduction

MSCs are pluripotent stromal cells that can differentiate into various lineages [1,2] and are known to be hypo-immunogenic in nature. These unique properties have attracted the attention of cell biologists, immunologists as well as clinicians leading to initiation of pilot clinical trials for various indications. However, MSC transplantation has its own benefits and risks. The presence of MHC-I and up-regulation of MHC-II expression in the presence of host interferon-gamma are important determinants of their use as immunomodulators [3–6]. Safety profiling in terms of cytogenetic instability, support of tumor growth, ectopic differentiation, fetal calf serum response, effect of inflammatory molecules released from MSCs, and cell product purification must be carefully considered prior to incorporating into clinical practice, specially for allogeneic MSC transplantation [7,8].

Mesenchymal stem cells have a large number of receptors that can make them intolerant of T-cells and activate the MHC-II up-regulation [9–11]. Autologous/allogeneic MSC transplantation often follows a cascade of immune function modulation events resulting in complex cell–cell interactions and varying responses to different cell types. On most occasions autologous cells may not pose as serious a threat of rejection, graft versus host disease (GvHD) or immune deviation as does allogeneic MSC transplantation [12–15]. The contrasting reports of the MSC immune modulation and subversion of the same on the host milieu or under other inflammatory conditions triggering the reversal of the same functions indicate that these cells must be thoroughly evaluated for the cell–cell compatible factors [16,17]. Further, MSC passage, cell infusion number, frequency, duration, concurrent immunosuppression and pre-injection manipulation of MSCs are needed to derive optimal therapeutic benefit from MSC transplantation. With the gaps in existing literature, we felt the need to explore the feasibility of developing a pre-clinical screening for histocompatibility testing of donor mesenchymal cells, similar to the protocols used for screening potential donors for bone marrow transplantation.

2. Material and methods

The prospective study design includes five bone marrow aspirates and HLA mismatched PBMNCs from five healthy individuals.

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2.1. Human MSC culture and phenotypic characterization

Bone marrow mononuclear cells (BMMNCs) were separated using Ficoll-hypaque (Sigma) gradient at 2500*g* for 30′. Mononuclear cells were then plated at a density of 10×10^4 cells/cm^2 in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal calf serum (Sigma, USA). When cultures reached near confluence, cells were detached with 0.05% trypsin (Sigma, USA) and 0.53 mM ethylenediamine tetra acetic acid (EDTA) (Sigma, USA), and were passaged at a density of 1000 cells/cm^2. The adherent bone marrow stromal cells (BMSCs) were harvested between passage 2, where a homogenous population of cells was microscopically observed, and passage 5.

Phenotypic characterization was performed by immunocytochemistry and immunofluorescence. For immunofluorescence analysis, cells were fixed with acetone:methanol (1:1), washed with phosphate buffered saline (PBS) and incubated with the primary mAb against CD29, CD90, CD11a, CD11c, CD25, CD45, CD34, and Vimentin at 1:50 dilution for 30 min at room temperature. Washed slides were then incubated in the dark with a secondary fluorescein isothiocyanate-conjugated goat anti-mouse antibody (FITC), (Dako, USA) for 30 min and counterstained with Propidium Iodide. Fluorescence was visualized using a Carl Zeiss confocal microscope.

2.2. MSCs differentiation

MSCs of the third passage were differentiated into osteocytes in DMEM + 1× insulin transferrin selenium (ITS) containing 1 μM/ml hydrocortisone (Sigma, USA), 120 μM/ml indomethacin (Sigma, USA) for 20 days and then switched to B27 serum supplemented media for 10 days. Chondrogenic differentiation was achieved by inducing soft aggregate of MSCs (by centrifugation) in DMEM + 1× ITS with 10 ng/ml transforming growth factor-β (TGF-β) (Sigma, USA) for 14 days. The differentiated adipocytes and chondrocytes were characterized by Oil red 'O' staining for lipid globules and alizarin red staining for calcium mineral deposits, respectively.

2.3. MHC compatibility

HLA typing of MSCs and PBMNCs was done by PCR-SSP for the HLA-ABDR combination using One Lambda SSP trays. Histocompatibility was done for the 6 Ag match to assess the level of matching.

2.4. Antibody responses to MSCs

MSCs were incubated for 45 min at a 1:50 dilution of Stro-1, HLA-DR, ALS (anti-lymphocytic serum), and test serum for MSC crossmatching by flowcytometry. AB positive serum was treated as negative serum, and anti-lymphocytic serum (ALS) was used to rule out the haematopoeitic lineage induced cytotoxic antibodies, while Stro-1 was used as positive control against IgG isotype to demonstrate the positivity of stromal cells. The cells were washed twice with PBS containing 2% bovine serum albumin and 0.1% NaN_3 (washing buffer). The cells were further treated with secondary fluorescein isothiocyanate-conjugated goat anti-mouse antibody (FITC), (Dako, USA) for 45 min. After the cells were washed twice with washing buffer, 10,000 viable cells were analyzed by flow cytometry using Cell Quest Pro (FACS Calibur). All the staining procedures were performed at 4 °C.

2.5. MHC and MSCs: Co-culture and mitogenic stimulation

Co-culture experiments were set-up for five pairs of allogeneic PBMNCs and MSCs. Proliferation potential was evaluated by MTT assay [18] and the results calibrated using stimulation indices. These cultures were followed for 5 days prior to termination of the assay. Similarly, the test was set-up until passage 5 to take note of any differential responses that could be detrimental for the allograft or up-regulation of MHC-II expression. The MTT assay was chosen over the radio-isotope method owing to its simplicity of use and the quick results. Phytohemagglutinin (PHA) was used as positive control for mitogenic stimulation.

3. Results

3.1. MSCs culture and phenotypic characterization

MSCs were successfully culture-expanded from all donors (Fig. 1a) and formed colony forming units when seeded at low density in culture flasks (Fig. 1b). The cultured mesenchymal cells, isolated from the second to third passage, were seen to comprise a single phenotypic population by immunohistochemical, immunofluorescence analysis of surface-expressed antigens. MSCs were uniformly positive for CD90, CD29 and Vimentin and negative for CD34 (lipopolysaccharide receptor), CD45 (leukocyte common antigen), CD11a, CD11c, CD25, and HLA-DR, suggesting that there was no-contamination with cells of hematopoietic lineage (Fig. 1e–l).

3.2. MSCs differentiation

Differentiated MSCs into adipocyte and chondrocyte lineages stained positive with Oil red 'O' for lipid laden cells (Fig. 1c) and alizarin red for calcium mineral deposits (Fig. 1d).

3.3. Antibody responses to MSCs

Histocompatibility testing showed a complete mismatch profile between PBMNCS volunteers and MSC donors. MSC crossmatching was done on five normal healthy sera to determine cytotoxic antibodies. The results demonstrate lack of circulatory antibodies to donor MSCs (Fig. 2).

3.4. Co-culture and mitogenic stimulation

To determine whether MSCs could induce a proliferative response by allogeneic lymphocytes, PBLs were co-cultured with MSCs (2-way MLR) and mitomycin-treated MSCs (1-way MLR) to evaluate the T-cell responses to MSCs [18]. The mean stimulation index with increasing passages demonstrates that the PP of MSCs decreases considerably, supporting the T-cell anergy. One-way MLR had little influence on the outcome of the allogeneic response. MSC inhibition of lymphocyte response was most marked when MSCs were co-cultured simultaneously. The MSCs inhibited mitogenic responses from the third passage onwards to the fifth passage (Fig. 3). PHA stimulation on co-cultures of MSC–MHC demonstrated T-cell suppression.

4. Discussion

Adult MSC therapy is fast emerging as a treatment of choice in most end-stage immune-mediated diseases due to its immunomodulatory properties and tissue regeneration potential. It has been used to reverse GvHD [19–21]. However, reports on clinical application of MSCs are ambivalent; while on the one hand it supports T-cell anergy, on the other it demonstrates a propensity to affect cytokine production in the inflammatory milieu [4–6,12,22]. Results from clinical studies are promising, particularly in terms of treating patients with cancer [24], reducing the incidence of