Journal of Autoimmunity xxx (2016) 1–9

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Human adipose mesenchymal stem cells as potent anti-fibrosis therapy for systemic sclerosis

Alexandre T.J. Maria [a, b, c], Karine Toupet [a, b], Marie Maumus [a, b], Guillaume Fonteneau [a, b], Alain Le Quellec [b, c], Christian Jorgensen [a, b, d], Philippe Guilpain [a, b, c, 1], Danièle Noël [a, b, d, *, 1]

[a] Inserm, U 1183, Saint-Eloi Hospital, 80 ave Augustin Fliche, Montpellier, F-34295, France
[b] Montpellier University, Medical School, 2 rue de l'Ecole de Médecine, Montpellier, F-34967, France
[c] Department of Internal Medicine, Multiorganic Diseases, Saint-Eloi Hospital, 80 ave Augustin Fliche, Montpellier, F-34295, France
[d] Clinical Unit for Osteoarticular Diseases and Department for Biotherapy, Lapeyronie Hospital, ave du Doyen Giraud, Montpellier, F-34295, France

ARTICLE INFO

Article history:
Received 11 February 2016
Received in revised form
21 March 2016
Accepted 23 March 2016
Available online xxx

Keywords:
Systemic sclerosis
Fibrosis
Mesenchymal stem cells
Adipose stem cells

ABSTRACT

Objectives: Displaying immunosuppressive and trophic properties, mesenchymal stem/stromal cells (MSC) are being evaluated as promising therapeutic options in a variety of autoimmune and degenerative diseases. Although benefits may be expected in systemic sclerosis (SSc), a rare autoimmune disease with fibrosis-related mortality, MSC have yet to be evaluated in this specific condition. While autologous approaches could be inappropriate because of functional alterations in MSC from patients, the objective of the present study was to evaluate allogeneic and xenogeneic MSC in the HOCl-induced model of diffuse SSc. We also questioned the source of human MSC and compared bone marrow- (hBM-MSC) and adipose-derived MSC (hASC).

Methods: HOCl-challenged BALB/c mice received intravenous injection of BM-MSC from syngeneic BALB/c or allogeneic C57BL/6 mice, and xenogeneic hBM-MSC or hASC (3 donors each). Skin thickness was measured during the experiment. At euthanasia, histology, immunostaining, collagen determination and RT-qPCR were performed in skin and lungs.

Results: Xenogeneic hBM-MSC were as effective as allogeneic or syngeneic BM-MSC in decreasing skin thickness, expression of *Col1*, *Col3*, *α-Sma* transcripts, and collagen content in skin and lungs. This anti-fibrotic effect was not associated with MSC migration to injured skin or with long-term MSC survival. Interestingly, compared with hBM-MSC, hASC were significantly more efficient in reducing skin fibrosis, which was related to a stronger reduction of *TNFα*, *IL1β*, and enhanced ratio of *Mmp1/Timp1* in skin and lung tissues.

Conclusions: Using primary cells isolated from 3 murine and 6 human individuals, this preclinical study demonstrated similar therapeutic effects using allogeneic or xenogeneic BM-MSC while ASC exerted potent anti-inflammatory and remodeling properties. This sets the proof-of-concept prompting to evaluate the therapeutic efficacy of allogeneic ASC in SSc patients.

1. Introduction

Systemic sclerosis (SSc) is an orphan disease characterized by tissue fibrosis, microangiopathy and autoimmunity, still exhibiting poor prognosis in many patients [1]. One of the most current promising therapeutic approaches is cell therapy, including hematopoietic stem cell [2] and mesenchymal stem/stromal cell (MSC) transplantation [3,4]. MSC are multipotent stromal progenitor cells that can be isolated from numerous tissues including bone marrow (BM), adipose tissue, synovium, dental pulp, umbilical cord, etc. They display immunomodulatory and trophic properties, among which their anti-fibrotic capacity is well described [5]. MSC have proven efficacy in several animal models of fibrosis [6–8] and we recently demonstrated in a murine model of HOCl-induced SSc,

* Corresponding author. Inserm U 1183, Institute for Regenerative Medicine and Biotherapy (IRMB), Hôpital Saint-Eloi, 80 avenue Augustin Fliche, 34295, Montpellier cedex 5, France.
E-mail address: daniele.noel@inserm.fr (D. Noël).
[1] Equally contributing authors.

http://dx.doi.org/10.1016/j.jaut.2016.03.013
0896-8411/

that an infusion of murine syngeneic MSC could alleviate skin and lung fibrosis through the modulation of inflammation, oxidative status and extracellular matrix remodeling [9].

While MSC-based clinical trials enrolling patients in phase I/II studies are ongoing, the finding of SSc-related alterations of MSC in their niche is of importance [10–14]. The question of using an allogeneic rather than autologous approach is therefore under debate. Another important issue regarding MSC-based therapy concerns the tissue source from which the cells are to be isolated. The most commonly used source of MSC is BM but an increasing number of studies investigate the potential of MSC isolated from subcutaneous fat, for obvious easier accessibility and higher recovery yield [15]. BM-derived MSC (BM-MSC) and adipose-derived MSC (ASC) share a common phenotype, differentiation potential and trophic function but exhibit disparities in the range of their functional and therapeutic activity [16–19]. Moreover, the different MSC sources have scarcely been compared in preclinical or clinical studies [20–23] and never investigated in the specific conditions of SSc.

We therefore evaluated the therapeutic potential of BM-MSC according to antigen compatibility and compared the efficacy of allogeneic and xenogeneic BM-MSC versus autologous/syngeneic BM-MSC in the murine preclinical model of HOCl-induced diffuse SSc. In this model, we also investigated the therapeutic effect of human ASC, obtained from several donors, by comparison with human BM-MSC.

2. Materials and methods

2.1. Experimental design and animals

SSc was induced by daily intradermal injections of hypochlorite (HOCl) as previously described [9,24] and according to the Laboratory Animal Care guidelines with approval from the Regional Ethics Committee on Animal Experimentation (CEEA-LR-11054). A healthy control group was injected with phosphate buffered saline (PBS). All experiments were performed in BALB/c mice, except for the biodistribution study performed in C57BL/6 mice. At day 21, homogeneous HOCl-challenged groups of mice were formed according to skin thickness and 2.5×10^5 MSC were injected in the tail vein. Upon injection, mice were mixed to avoid cage effect bias and allow a blinded evaluation of skin thickness. Skin, lung and blood samples were taken at euthanasia and fixed in 3.7% formaldehyde for 48 h for histology or stored at −80 °C for molecular analyses.

2.2. Isolation and culture of MSC

BM-MSC from BALB/c and C57BL/6 mice were isolated by flushing the BM of mouse femurs, characterized and used before passage 15 as previously described [25]. Human samples were obtained from informed patients whose written consent was collected as approved by the French Ministry of Higher Education and Research (DC-2010-1185). Human BM-MSC were isolated from patients undergoing hip replacement surgery and ASC from healthy donors undergoing plastic surgery as already described [26,27]. BM-MSC and ASC were used before passage 4 and 2, respectively.

2.3. Histopathology

Paraffin-embedded samples (5 μm thick) were stained with Masson trichrome or immunostained with DAPI (Sigma) or antibodies for α-sma (Abcam, 1/500), CD3-epsilon (Santa Cruz Biotechnology, 1/250) and F4/80 (Invitrogen, 1/50). Histological slides were scanned using Nanozoomer (Hamamatsu) and immunofluorescence acquisition was made using a confocal laser microscope (Leica, SP5) and LAS AF Lite software.

2.4. RT-qPCR analysis

RNA was extracted from crushed samples using the RNeasy mini kit (Qiagen). Total RNA (1 μg) was reverse-transcribed (M-MLV RT, Invitrogen). qPCR was performed on 20 ng cDNA using specific primers (Supplemental data, Tables 1 and 2) and SYBRGreen I Master-mix by real-time PCR (LightCycler 480, Roche Applied Science). Samples were normalized to mRNA expression of TATA binding protein *(Tbp)* for tissue samples or *GAPDH* for cell extracts. Results were provided either as relative expression to these housekeeping genes using the formula $2^{-\Delta Ct}$ or as fold change using the formula $2^{-\Delta\Delta Ct}$.

2.5. qPCR analysis for Alu expression

DNA was extracted using DNeasy blood and tissue kit (Qiagen). qPCR was performed with 10 ng DNA on real-time PCR instrument Viia7 (Applied Biosystems) using SYBRGreen Master-mix and Alu primers (Supplemental data, Table 2). Results were compared with 3 standard curves of serial dilutions of hBM-MSC, and extrapolated to the whole organ for quantification, as previously described [27].

2.6. Collagen content in tissues

Collagen content assay was based on the quantitative dye-binding Sircol method using acid-pepsin extraction (Biocolor). Results were expressed as the collagen content in μg/mm^2 of skin or μg/mg of lung.

2.7. Statistical analyses

All quantitative data were expressed as mean ± SEM. Data were compared using Mann-Whitney's test for nonparametric values, Student's *t*-test for parametric values and one-way ANOVA for more than two groups in case of parametric values (Kruskall-Wallis if nonparametric). All statistical analyses were performed using Prism 6 GraphPad software (California). A P value < 0.05 was considered significant.

3. Results

3.1. Isolation and characterization of MSC

Murine BM-MSC (mBM-MSC) were isolated from BALB/c and C57BL/6 mice as previously described and further characterized [9]. mBM-MSC were made of a homogeneous population of cells that expressed the conventional markers for stromal progenitors Sca-1, CD29, CD44 and did not express the hematopoietic markers CD11b, CD45 or F4/80 (Fig. 1A). Human MSC isolated from BM (hBM-MSC) or adipose tissue (hASC) highly expressed the stromal progenitor markers CD73, CD90, CD13, and CD105 (Fig. 1A). Both cell types did not express the hematopoietic markers CD11b, CD14, CD34 and CD45.

Under specific inductive conditions, all these cells showed a trilineage differentiation potential, as demonstrated by the up-regulation or expression of adipogenic markers (*Fabp4, Lpl* and *Pparγ*), osteogenic markers (*Oc, Ap* and *Runx2*), and chondrogenic markers (*Acan*, *Col2B* and *Sox9*) as compared to non-induced cells (Fig. 1B).

Please cite this article in press as: A.T.J. Maria, et al., Human adipose mesenchymal stem cells as potent anti-fibrosis therapy for systemic sclerosis, Journal of Autoimmunity (2016), http://dx.doi.org/10.1016/j.jaut.2016.03.013