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

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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,

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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).

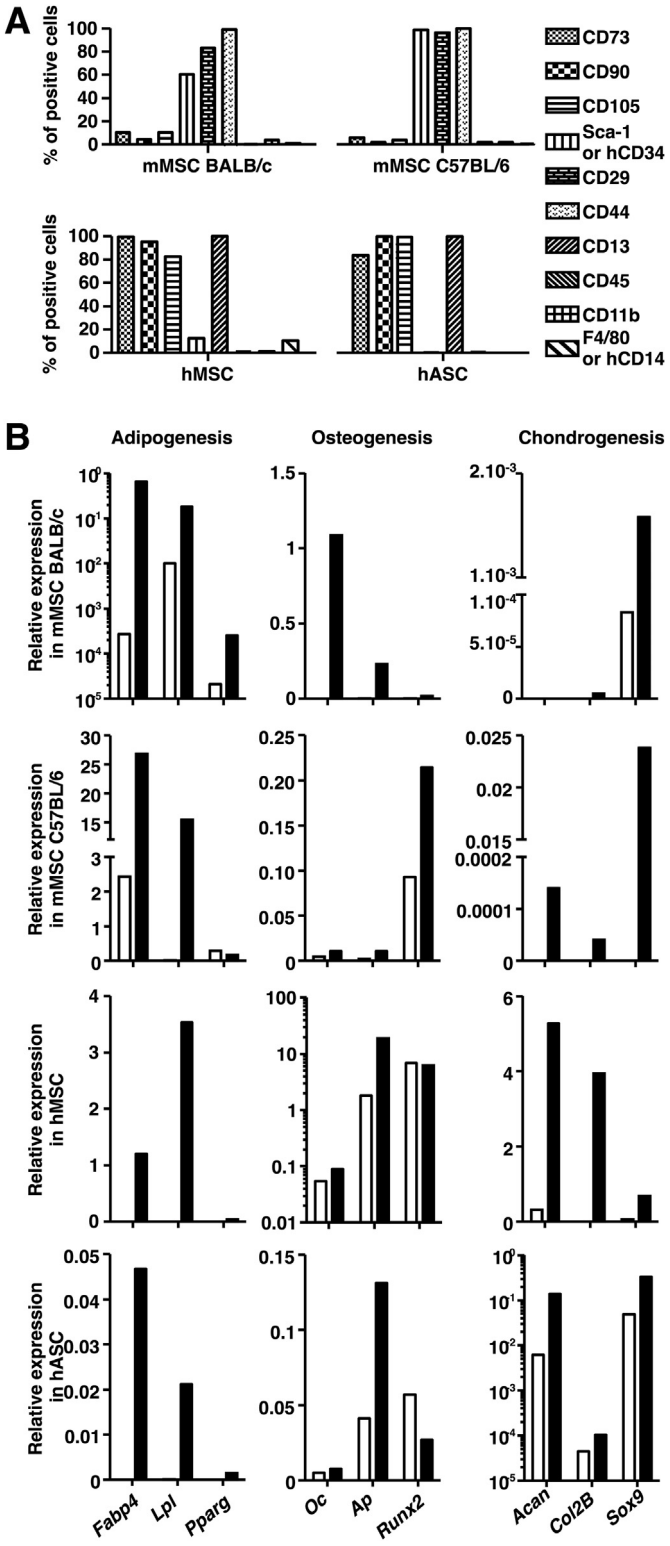

Fig. 1. Characterization of murine and human MSC. Murine bone-marrow (BM) derived MSC from C57BL/6 or BALB/c mice (mMSC) and human MSC from BM (hMSC) or adipose tissue (hASC) were used. (A) Percentage of BALB/c, C57BL/6 mMSC, hMSC and hASC, positive for the markers listed on the right by flow-cytometry analysis. (B) mRNA expression of murine or human genes in the abovementioned MSC at day 0 (in white histograms) or 21 days after adipogenesis, osteogenesis or chondrogenesis induction (in black histograms); mRNA expression normalized to *Gapdh* or *Rps9* expression for murine or human genes respectively.

3.2. Human and murine BM-MSC exert a similar therapeutic effect in the HOCl-induced SSc murine model

Given that the properties of hBM-MSC from SSc patients may be altered [10–14], we wondered whether the use of allogeneic hBM-MSC could be of interest for SSc treatment. In a first series of experiments, we evaluated the anti-fibrotic effect of BM-MSC with matched/unmatched antigen compatibility in the murine HOCl-induced SSc model. HOCl-challenged mice were infused at day 21 with 2.5×10^5 BM-MSC, isolated from syngeneic BALB/c, or allogeneic C57BL/6 mice, or from a human donor (xenogeneic approach). A significant inflexion in the progression of skin thickness was observed in all MSC-treated mice compared with control HOCl-mice (Fig. 2A). In the syngeneic approach, the increase in skin thickness was significantly lower as soon as 1 week after cell injection, but no significant difference between the three treated conditions was noted after 3 weeks. Decrease of skin thickness was associated with significant decrease in total collagen deposition in skin and lungs of treated mice, whatever the origin of BM-MSC (Fig. 2B). Accordingly, gene expression of the fibrotic markers *Col1, Col3* and *α-Sma* was decreased in the three treated groups, both in skin and lungs (Fig. 2C). In lungs, the impact of BM-MSC infusion was high since the expression of *Col3* and *α-Sma* was similar to that of normal tissues (normalized at 1 in PBS-injected mice) and even lower using hBM-MSC (Fig. 2C). At the histological level, we previously reported that low skin thickness increase observed after syngeneic BM-MSC injection was related with low collagen fiber deposition [9]. Such observation was again observed in the present study, both with syngeneic and allogeneic mBM-MSC (data not shown) and, hBM-MSC. The reduction in collagen deposition in hBM-MSC-treated mice, compared with control HOCl-mice, was illustrated by Masson Trichrome staining of skin and lung sections (Fig. 2D). Altogether, the xenogeneic approach using hBM-MSC was as efficient as syngeneic or allogeneic mBM-MSC for reducing fibrosis in the HOCl-induced SSc model. This allowed to further investigate the effect of human MSC in this model.

3.3. Human BM-MSC are rapidly cleared and do not migrate to the injured skin in the HOCl-induced SSc model

We then wanted to determine whether the therapeutic effect was related to migration of BM-MSC to the injured skin tissues. We therefore injected hBM-MSC in the tail vein of HOCl mice and looked for the presence of human specific *Alu* sequences in skin at different time points following infusion, by qPCR. Using this technique, no *Alu* sequence could be detected in skin of mice infused with hBM-MSC at any time point. In contrast, about half of the injected hBM-MSC was found in the lungs of all treated mice during the first 48 h post-infusion, but not after 7 days (Fig. 3). Indeed, the therapeutic effect of hBM-MSC was not related to migration to injured skin or to long-term survival.

3.4. Human ASC are more potent than human BM-MSC to reduce skin fibrosis in the murine HOCl-induced SSc model

In order to have access to another MSC source that could be of interest for clinical application, we wondered whether hASC could be substituted to hBM-MSC as a more efficient cell source in SSc patients. We therefore aimed at comparing the efficacy of hASC versus hBM-MSC in the HOCl-induced SSc model. We used hBM-MSC from 3 different donors and hASC from 3 other donors, each injected in groups of 6–8 HOCl-challenged mice at d21. As shown in Fig. 4A, a significant reduction of skin thickness was obtained in treated mice as soon as 2 weeks after infusion, with a significantly greater impact at d42 in hASC-treated mice compared with hBM-

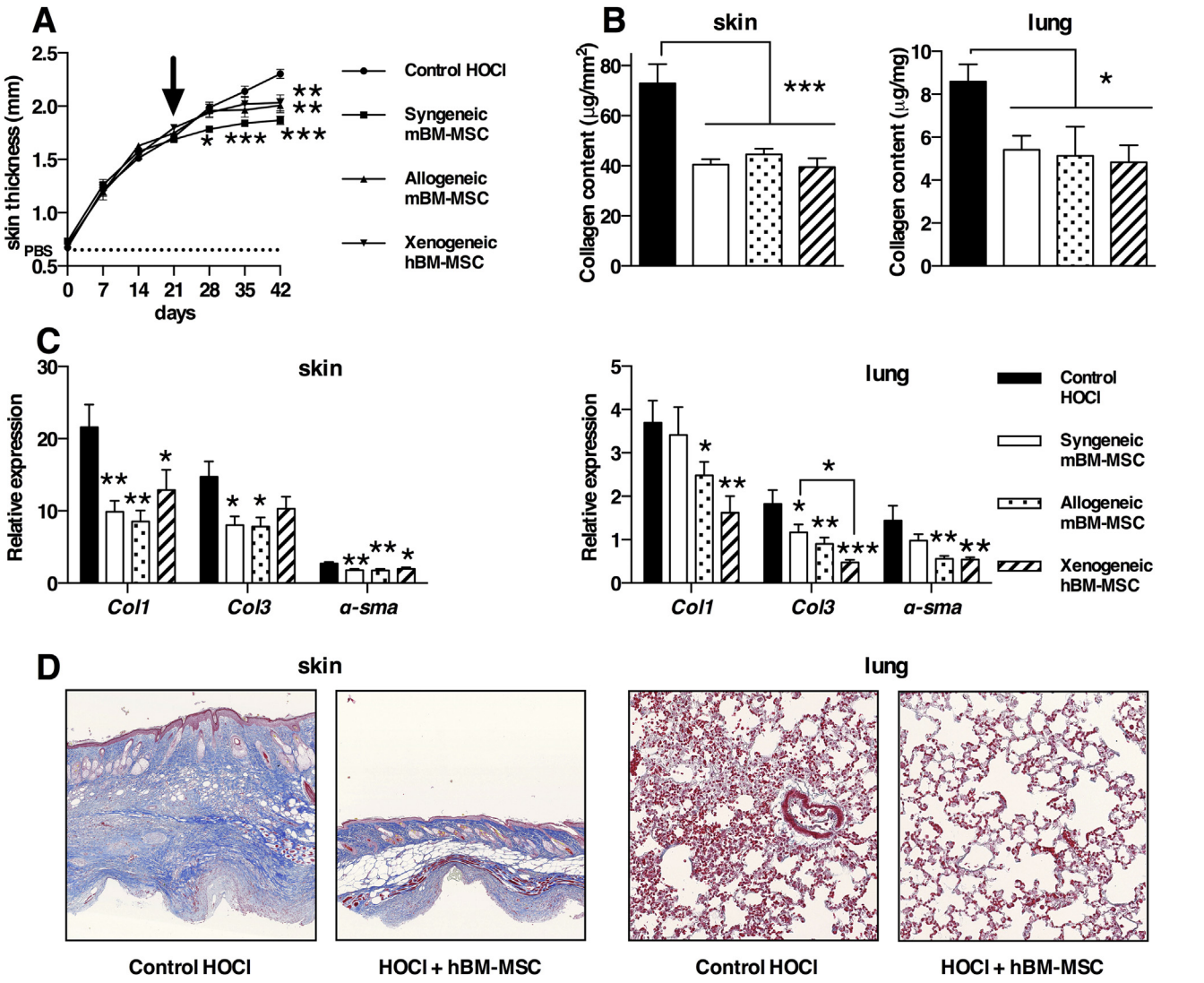

Fig. 2. Effect of xenogeneic hBM-MSC compared with syngeneic or allogeneic mBM-MSC in the HOCl-induced SSc murine model. (A) Skin thickness from control HOCl-mice, and HOCl-mice treated at d21 (arrow) with an infusion of 2.5×10^5 syngeneic BALB/c, allogeneic C57BL/6 or xenogeneic human BM-MSC. (B) Collagen content at euthanasia (d42) in skin and lungs from the groups described in (A). (C) mRNA expression of *Col1*, *Col3*, and *α-Sma* normalized to *Tbp* expression in skin and lungs, expressed as fold change *vs* PBS-mice. (D) Representative skin (on the left, magnification 10×) and lungs (on the right, magnification 20×) sections of hBM-MSC-treated mice vs control HOCl-mice at d42 stained with Masson trichrome. Data are presented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

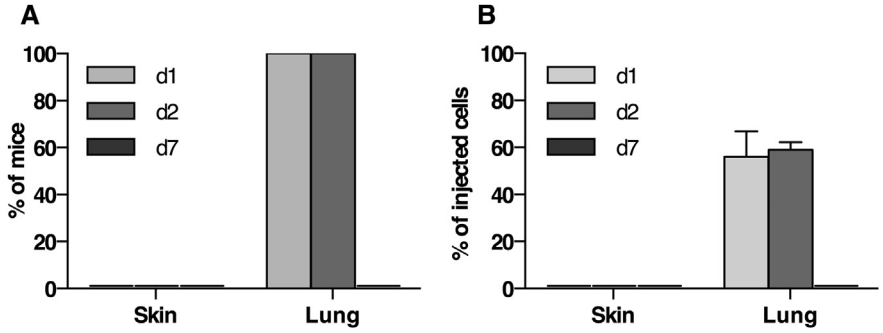

Fig. 3. Biodistribution of hBM-MSC in the HOCl-induced SSc model. (A) Percentage of mice with *Alu* sequences detected in skin or lung tissues by qPCR analysis at day 1, 2 or 7 after infusion. (B) Percentage of hBM-MSC detected in skin or lung tissue at day 1, 2 or 7 after infusion, based on *Alu* sequences expression by qPCR analysis (n = 4 per group). Data are presented as mean ± SEM.

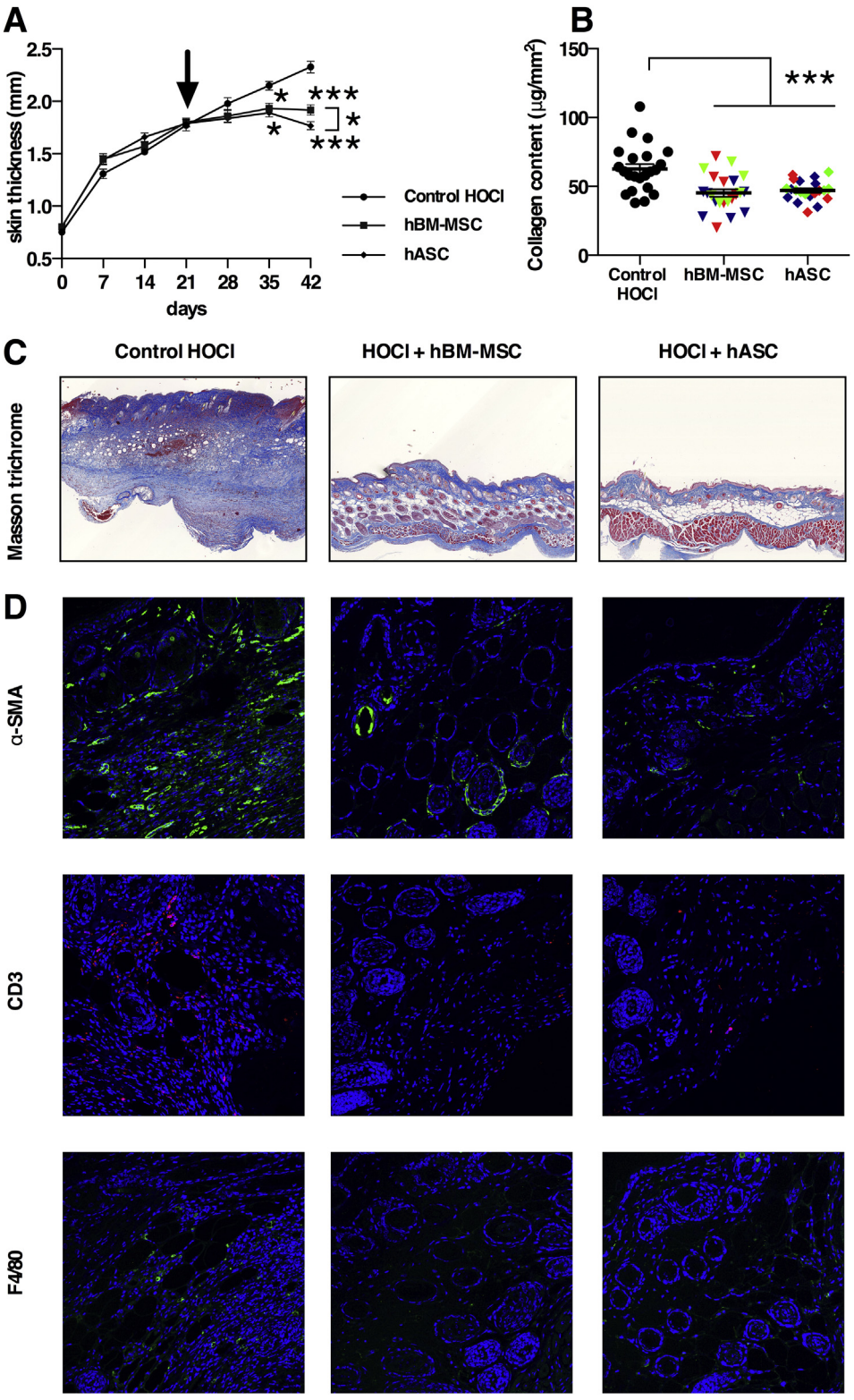

Fig. 4. Effect of hBM-MSC or hASC injection in the HOCl-induced SSc model. (A) Skin thickness from HOCl-mice treated with an infusion of 2.5×10^5 hBM-MSC or hASC at d21 (arrow) compared with control HOCl-mice. 3 different samples of hBM-MSC and 3 of hASC were evaluated (each sample infused to 6–8 mice). (B) Collagen content at d42 in skin from control HOCl-mice, hBM-MSC- or hASC-treated mice. Each hBM-MSC or hASC sample is represented by a different colour. (C) Representative skin sections of mice at d42 stained with Masson trichrome, magnification 10×. (D) Immunostaining with DAPI (in blue) and antibodies for α-SMA (upper panels in green), CD3 (middle panels in red) or F4/80 (lower panels in green), in skin from control HOCl-mice, hBM-MSC- or hASC-treated mice, magnification 20×. Data are presented as mean ± SEM. $^*P < 0.05$, $^{***}P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MSC-treated mice. Whatever the sample used, a similar reduction in total skin collagen content was obtained (Fig. 4B), and histological analysis with Masson Trichrome staining revealed less extracellular matrix deposition (Fig. 4C). We also noticed lower cellularity as shown by DAPI staining (Fig. 4D) along with less α-SMA staining in hBM-MSC- and hASC-treated mice compared with control HOCl-mice (Fig. 4D). Finally, skin from hBM-MSC- and hASC-treated mice exhibited less infiltrates of $CD3^+$ T lymphocytes and $F4/80^+$ macrophages compared with HOCl-challenged mice (Fig. 4D).

Concurrently, a significantly lower expression of the fibrotic markers *Col1* and *α-SMA* was detected following hBM-MSC and hASC treatment, whatever the donor (Fig. 5). Interestingly treatment with hASC led to a higher increase of *Mmp1/Timp1* ratio, suggesting higher matrix remodeling activity and a stronger decrease of *TNFα*, *IL1β* and *IL10* in skin, compared with hBM-MSC treatment. Altogether, these results indicated that beyond donor variability, a higher remodeling and anti-inflammatory activity of hASC contributed to a stronger benefit on skin thickness.

3.5. Human ASC concurrently reduce lung fibrosis in murine HOCl-induced SSc model

Using the model of HOCl-induced diffuse SSc, we were able to evaluate the impact of hBM-MSC and hASC injection in lung tissue at d42. First, histological analysis revealed an improvement of pulmonary fibrosis as shown by a normal architecture of lung parenchyma in hBM-MSC- and hASC-treated mice contrasting with high extracellular matrix depositions and cell infiltrates in control HOCl-mice (Fig. 6A). Second, we noted a similar decrease of the expression of *Col1* and *α-Sma* transcripts after hBM-MSC or hASC injection (Fig. 6B). Reduction of the fibrotic markers was associated with an increase in *Mmp1/Timp1* ratio in tissue, which was even more significant with hASC. Of note, a donor-dependent effect of both hBM-MSC and hASC was observed. The inflammatory cytokines *TNFα* and *IL1β* were both decreased after treatment, but a higher reduction of *IL1*β expression was noted using hASC while the level of *IL10* was not affected by hBM-MSC or hASC treatment. Except for *IL10*, all the fibrotic and inflammatory markers were modulated in the lung following hBM-MSC and hASC treatment, confirming a local and systemic effect of cell therapy in this model of diffuse SSc.

4. Discussion

In the last decades, MSC have been shown to exert potent immunosuppressive properties, affecting both the innate and adaptive immune responses, through the inhibition of immune cell proliferation and differentiation, and the promotion of immune tolerance by the generation of regulatory cells [28]. Hence, there has been a rising interest for MSC-based therapy in the field of autoimmune diseases, with promising results in various animal models and phase I/II clinical trials in multiple sclerosis, rheumatoid arthritis or systemic lupus erythematosus [29]. Among autoimmune disorders, SSc appears as a peculiar multifaceted disease in which aberrant immune system activation coexists with fibroblast and endothelial cell dysfunction, leading to multi-organ fibrosis and vasculopathy. Therefore, maybe more in SSc than in any other autoimmune diseases, the therapeutic potential of MSC has to be evaluated (for review, see Maria et al., 2016). In a first preclinical study in the HOCl-induced murine model of SSc, we demonstrated antifibrotic and immunosuppressive effects of syngeneic BM-MSC [9]. However, the alterations observed in MSC from SSc patients might lead to turn towards allogeneic transplantation, with a possible loss of efficacy in case of immune rejection. This has prompted us to investigate the importance of antigen compatibility and tissue source of MSC in the HOCl-induced SSc murine model. Herein, we showed that allo-/xenogeneic BM-MSC transplantation was as efficient as syngeneic transplantation to reduce fibrotic lesions in immunocompetent mice. These results are in line with

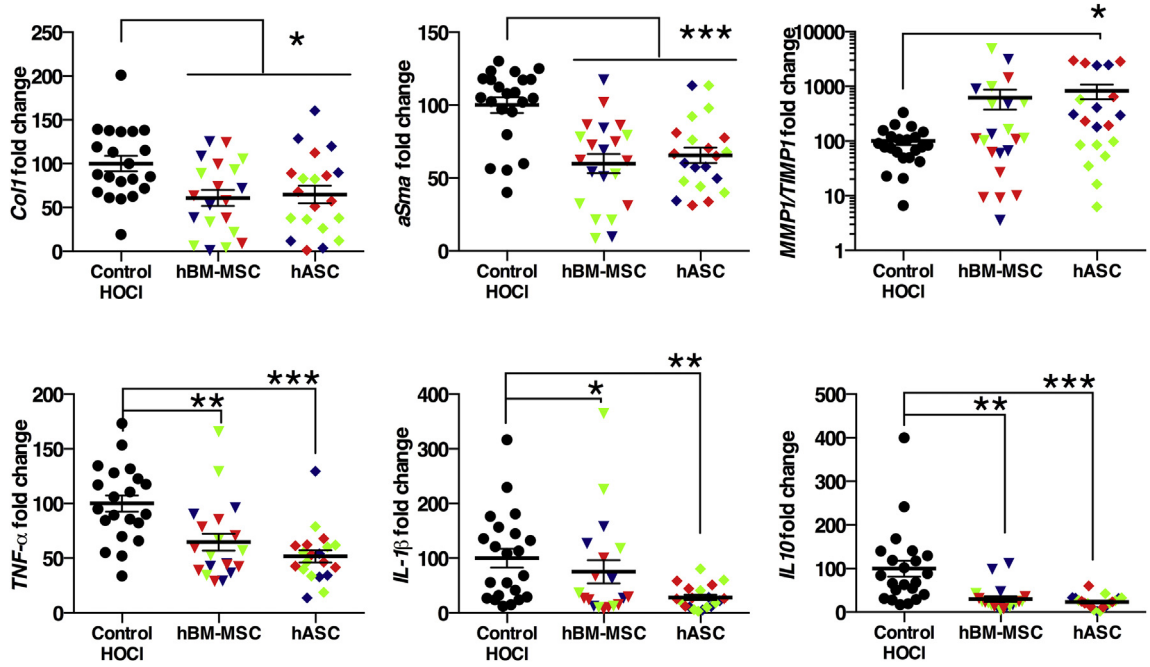

Fig. 5. Effect of hBM-MSC or hASC injection in skin in the HOCl-induced SSc model. mRNA expression of fibrotic, remodeling and inflammatory marker genes, normalized to *Tbp* expression, in skin samples from hBM-MSC- or ASC-treated HOCl-mice, expressed as fold change *vs* control HOCl-mice. Each hBM-MSC or hASC sample is represented by a different colour. Data are presented as mean ± SEM. n = 20–22 per group, $^*P < 0.05$, $^{**}P < 0.01$.

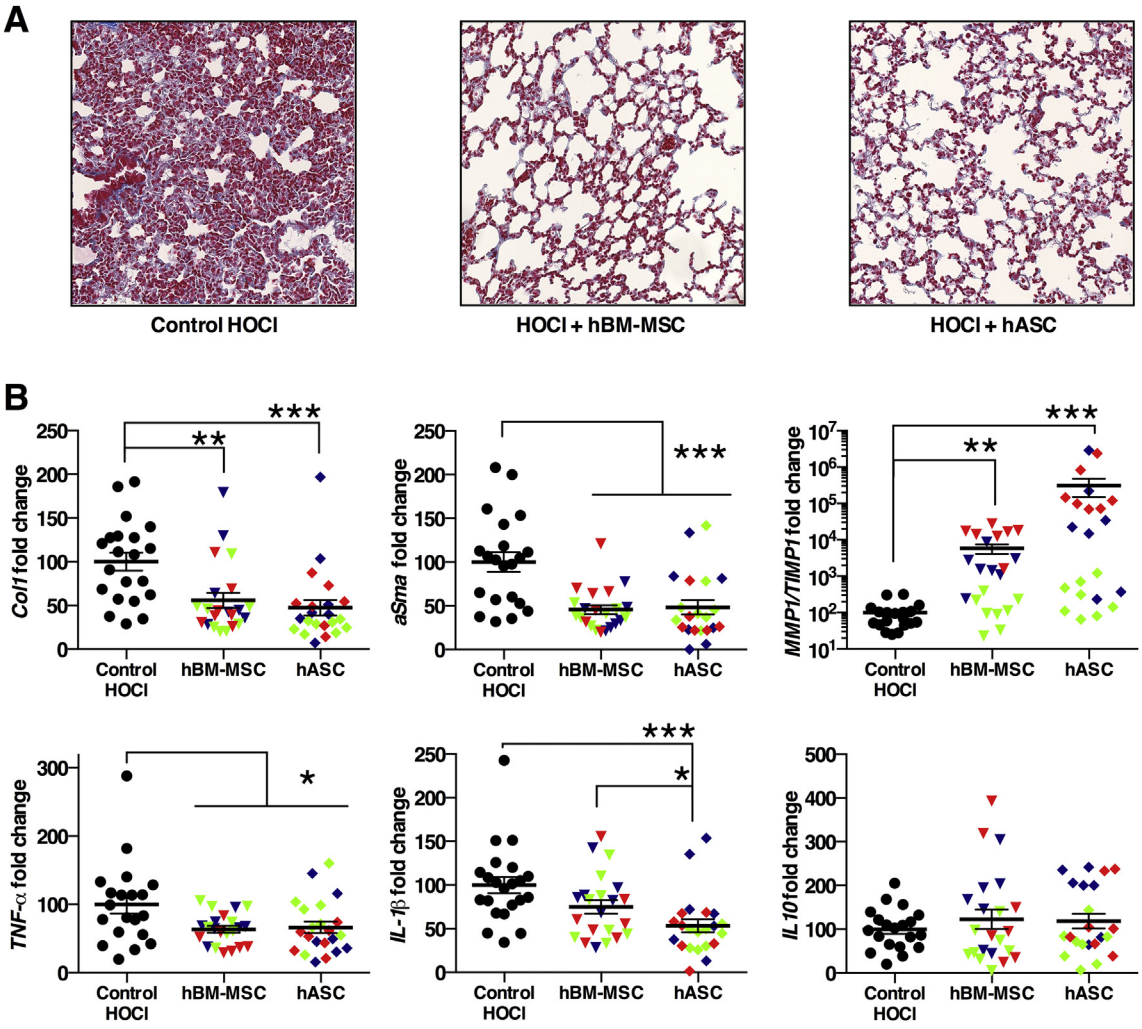

Fig. 6. Effect of human BM-MSC and ASC in lungs in the HOCl-induced SSc model. (A) Representative lung sections stained with Masson Trichrome, from hBM-MSC- or hASC-treated HOCl-mice compared with control HOCl-mice (magnification 20×). (B) mRNA expression of fibrotic, remodeling and inflammatory marker genes, normalized to *Tbp* expression, in lung samples from hBM-MSC- or hASC-treated mice, expressed as fold change *vs* control HOCl-mice. Data are presented as mean ± SEM. Each hBM-MSC or hASC sample is represented by a different colour. n = 20–22 per group, $^{*}P < 0.05$, $^{**}P < 0.01$.

some studies on the efficacy of allo/xeno implantation in other preclinical models of various diseases [30–32]. Indeed, BM-MSC have long been considered as immune privileged since they do not induce potent alloreactivity when infused into another organism [33]. Nonetheless, they have been shown to elicit cellular and humoral responses in vivo [33,34] and MHC mismatch could even be responsible for a lack of effect [35]. While the host immune reaction could lead to rapid clearance of transplanted cells, BM-MSC and ASC are proposed to act principally through a "hit and run mechanism", which does not preclude their therapeutic efficacy at least on the short- or middle-term [33,36]. Indeed, most of MSC functions do not require cell-to-cell contact, but rather paracrine mechanisms through the release of cytokines, growth factors and/or extracellular microvesicles in the surrounding environment [37]. This may explain that in case of allo- and xenogeneic transplantation, the benefit of BM-MSC and ASC can be observed long after their clearance [34]. BM-MSC and ASC produced many anti-inflammatory mediators, notably Indoleamine 2,3-Dioxygenase activity, IL-6, IL1RA, TSG6, PGE2, as well as anti-fibrotic factors (HGF, bFGF, CTGF, TSG-6), which could account for the therapeutic effect observed in the present study but still require further investigation [37]. Here, we showed that the clearance of hBM-MSC occurred during the first week following MSC infusion, consistently with literature and our previous results [9,33,36,38]. As a whole, MHC-matching of transplanted BM-MSC does not seem essential for the therapeutic benefit in the present model, at least on the short term. This result was very encouraging in the context of SSc, where autologous treatment may be considered as unsuitable, with regard to the alterations of endogenous mesenchymal progenitors in the disease [10–14].

We also addressed the question of the interest of using adipose tissue as a convenient source of MSC in the murine model of SSc. This point is of particular importance in the perspective of clinical applications in humans. Indeed, the relative accessibility of subcutaneous adipose tissue, and the higher yield of progenitor cells at isolation are two major reasons why ASC could supplant BM-MSC in clinical trials [16]. Whatever the tissue they originate from, all MSC meet the criteria defined by the international society for cell therapy (ISCT) and thus share common biological features in terms of plastic adherence in culture, surface marker expression or tri-lineage differentiation potential [39]. However, tissue specificity has been suggested and mainly concerns functional properties of MSC (expression profile and/or secretome) [40,41] [26], (for review, see Maria et al., 2016). These observations make the

concept of a unique MSC controversial [42], but support the preferential use of one source of cell according to specific therapeutic applications. Hence, beyond similar phenotype and differentiation potential, BM-MSC and ASC are different cell populations with preferential commitments [18], making the comparison of their functional potentialities crucial. To date, this question has not been addressed in SSc or pulmonary fibrosis models and most of the published studies focused on *in vitro* properties. Thus, few comparisons between BM-MSC and ASC have been made in preclinical models [16–18,21–23,43,44]. Interestingly, when compared to MSC from other sources, ASC were shown to display the strongest immunosuppressive and angiogenic capacities [16–18,45–47]. Here, we demonstrated that hASC were at least as effective as hBM-MSC at reducing fibrosis in murine HOCl-induced SSc. Even though it has been suggested that endogenous adipose progenitors could contribute to fibrosis in SSc [48], the trophic potential of adipose-derived progenitors has been reported in two recent studies. First, Scuderi et al. reported a beneficial effect of autologous ASC, administered locally in affected skin areas (face or limbs) of six SSc patients in a non-controlled study [3]. Second, a recent study by Granel et al. evaluated the feasibility and safety of local injections of autologous stromal vascular fraction (SVF) in the fingers of 12 SSc patients, with promising results [49]. The limitation of SVF for broader applications such as systemic infusions is likely the heterogeneity of preparations, with variable numbers of immune and endothelial cells, and difficulty of standardization for GMP applications. On the whole, these data and our findings argue for the interest of evaluating the therapeutic effect of ASC in human SSc.

Interestingly, we observed functional differences between ASC and BM-MSC. Indeed, ASC exhibited a stronger anti-inflammatory effect in tissues as reported in literature [45,46], but also an enhanced capacity to induce extracellular matrix remodeling by increasing the balance between metalloproteases and inhibitor of metalloproteases. However, in contradiction to existing literature [50], ASC tended to have a lower ability to induce antioxidant defenses, as suggested by lower total antioxidant capacity of the host's serum (data not shown). In addition, beyond differences in anti-inflammatory or remodeling capacities among BM-MSC and ASC, our study also pointed out a heterogeneity between human individual donors that could impact potency of the cells, as suggested by distinct responses in MMP1/TIMP1 ratios in tissues. The availability of potency assays and biomarkers would be useful to predict specific patterns of MSC functionalities [33,51,52]. In that sense, the goal would be to offer a personalized therapy by selecting the most appropriate donors for each disease profile (for example, more immune-modulatory-prone MSC for inflammatory signatures, more pro-angiogenic MSC for ischemic presentations), thus improving the outcomes of MSC therapy.

This point seems particularly relevant when treating SSc, a multifaceted disease in which clinical presentation, disease course, prognosis and outcome are notably heterogeneous [53]. In other terms, the optimization of MSC treatment in SSc could rely on the accurate selection of MSC donor, whose characteristics best match those of that patient. Therefore, efforts made to improve classification, for instance establishing new ACR/EULAR criteria [54] or searching for prognostic factors [55,56], are very useful to predict disease outcome and define the best therapeutic strategies. The search for new biomarkers or the use of transcriptomic analyses in SSc might also help in dismembering subsets of patients in relation with disease phenotype and predictable response to therapy [53,57]. On the whole, MSC-based therapeutic approaches could benefit from a better definition of disease status, allowing an optimal matching between functional properties of selected donor cells and disease characteristics.

5. Conclusion

To conclude, this preclinical study demonstrated that, beyond major histocompatibility antigen mismatch, MSC-based therapy still remained efficient in reducing skin and lung fibrosis in murine SSc, which is promising concerning allogeneic approaches in the human refractory disease. The potent effect obtained with human ASC underlined the interest of using subcutaneous adipose tissue rather than BM as a source of MSC in future clinical trials. However, the wide clinical heterogeneity of the disease, as well as that of MSC itself opens a new field of investigation in order to offer efficient individualized MSC-based therapy in SSc.

Competing interests

ALQ declares speaking fees from Actelion Pharmaceuticals. None of the other authors has any potential conflict of interest related to this manuscript.

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Appendix A. Supplementary data

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