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Conditioned medium from amniotic membrane-derived cells prevents lung fibrosis and preserves blood gas exchanges in bleomycin-injured mice—specificity of the effects and insights into possible mechanisms

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Abstract

Background and aims. We recently demonstrated that injection of conditioned medium (CM) generated from cells of the mesenchymal region of human amniotic membrane (AMTCs) reduces bleomycin-induced lung fibrosis in mice, suggesting a crucial role of paracrine factor(s) secreted by AMTCs in these beneficial effects. We further investigated this hypothesis, the mechanisms involved, the effects on some lung functional parameters and whether AMTC-secreted effector(s) are specific to these cells and not produced by other cell types, extending the time of analysis up to 28 days after treatment. *Methods.* Bleomycin-challenged mice were either treated with AMTC-CM or CM generated from human skin fibroblasts, human peripheral blood mononuclear cells or Jurkat cells, or were left untreated. Mouse lungs were analyzed for content of pro-inflammatory and pro-fibrotic molecules, presence of lymphocytes and macrophages and for fibrosis level (through histological semi-quantitative evaluation and quantitative measurement of collagen content). Arterial blood gas analysis was also performed. *Results.* Up to 28 days after delivery, AMTC-CM-treated mice developed reduced lung fibrosis with respect to mice treated with other CM types. AMTC-CM-treated mice had comparatively better preservation of blood gas parameters and showed lower lung content of interleukin-6, tumor necrosis factor-α, macrophage inflammatory protein-1α, monocyte chemoattractant protein-1 and transforming growth factor-β associated with reduced lung macrophage levels. *Conclusions.* AMTC-CM prevents lung fibrosis in bleomycin-challenged mice, improving survival and preserving lung functional parameters such as blood gas exchanges. The specificity of AMTC-CM action was indicated by the absence of fibrosis reduction when other CM types were used. Finally, we provide some insights into the possible mechanisms underlying AMTC-CM-mediated control of fibrosis.

Key Words: *amniotic membrane-derived cells, amniotic mesenchymal tissue cells, conditioned medium, human term placenta, lung fibrosis, mesenchymal stromal cell*

Introduction

We and others have previously described *in vitro* studies that show the modulatory ability of cells derived from the amniotic membrane both on T lymphocytes (1–3) and on antigen-presenting cells (4).

In vivo studies have also demonstrated anti-inflammatory and anti-fibrotic properties of placenta-derived cells (5–7). In particular, we have shown that human placental fetal membrane-derived cells—specifically, a mixture composed of cells from the mesenchymal layer of the human amniotic and chorionic membranes and cells from the amniotic epithelial layer—were able to reduce the progression of lung fibrosis when transplanted into bleomycin-challenged mice (8). Furthermore, in a similar animal model, Moodley *et al.* (9) reported that treatment with human amniotic epithelial cells (hAECs) reduced lung inflammation and fibrosis and that these cells, when engrafted in host lungs, differentiated into cells with an alveolar epithelial phenotype. More recently, Murphy *et al.* (10) confirmed the anti-inflammatory and anti-fibrotic effects of hAECs, although these

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authors did not detect significant cell engraftment in the lungs of transplanted mice; meanwhile, Vosdoganes *et al.* (11) found that hAECs that had been injected into a sheep fetus affected by intra-amniotic lipopolysaccharide-induced pulmonary inflammation were able to cause a reduction in levels of lung inflammatory cytokines.

Despite these evident beneficial effects of placental cells on lung fibrosis, there have been no clear reports to date on the mechanism(s) through which these cells act. The fact that cell treatment efficacy has also been observed in the absence of significant levels of placenta-derived cell engraftment (8,10) suggests that these cells could work through a paracrine mechanism by releasing soluble factors that are able to exert trophic actions on host lung cells and modulatory actions on inflammatory cells recruited to the injury site. Indeed, our group has reported strong evidence in support of such a mechanism by administering conditioned medium derived from the culture of cells isolated from the mesenchymal region of human amniotic membrane (amniotic mesenchymal tissue cells, AMTCs) to bleomycin-challenged mice, rather than administering the cells themselves (12). Even in the absence of cells, mice treated with AMTC-derived conditioned medium (AMTC-CM) developed less pronounced lung fibrosis in terms of fibrosis distribution, fibroblast proliferation, collagen deposition and alveolar obliteration when compared with untreated mice.

However, several questions remain open in regard to the beneficial effects exerted by AMTC-CM on pulmonary fibrosis, such as their ultimate outcome on pulmonary function, the mechanism(s) through which these effects are exerted and whether these positive effects are specific to AMTC-CM, or if they are instead also exerted by CM obtained from other cell types of different nature.

In the current study, with the use of the same murine model of bleomycin-induced lung fibrosis described previously (8,12), we addressed some of these open questions, thus going beyond our previous results (8,12), as well as further exploring the properties of AMTC-CM. In particular, besides confirming the ability of AMTC-CM to reduce the severity and progression of lung fibrosis, we explored these effects for up to 28 days after delivery and examined the potential of AMTC-CM in protecting pulmonary function from impairment induced by fibrotic lesions. In addition, in attempting to elucidate the mechanism(s) that may underlie the beneficial effects of AMTC-CM, we also quantified the inflammatory and pro-fibrotic cytokine levels in the lungs of bleomycin-challenged mice that had been treated with AMTC-CM and evaluated T-lymphocyte and macrophage infiltration in the mouse lungs. Finally, for all of these experiments, we investigated whether the paracrine effector(s) secreted by AMTCs are specific to these cells by comparing the effects of AMTC-CM with those exerted by conditioned media generated from other cell types sharing a similar mesodermal origin with AMTCs and that were representative of both non-adherent hematopoietic and adherent non-hematopoietic (stromal) cell lineages, namely, human peripheral blood mononuclear cells (PBMCs), human T-leukemia cells (Jurkat cells) and human skin fibroblasts.

Methods

Ethics statements

Human term placentas and skin biopsies were collected after obtaining written informed consent according to the guidelines of the Ethical Committee of the Catholic Hospital (CEIOC) and of the Ethical Committee of the hospital Fondazione Poliambulanza-Istituto Ospedaliero (Brescia, Italy), respectively.

Animal experiments were carried out in accordance with the guidelines established by the European Community (No. 2007/526/CE) and by the Italian law 116/92 on the accommodation and care of animals used for experimental purposes. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Milano.

Cells used to prepare CMs

CMs were generated from the culture of the following four cell types:

(i) Amniotic mesenchymal tissue cells are cells isolated from the mesenchymal region of human amniotic membrane, following a well-established protocol of our laboratory (13,14). As previously described (14,15), at passage 0, their phenotype (by fluorescence-activated cell sorting analyses) is CD90 (82% ± 3%), CD73 (66% ± 6%), CD13 (89% ± 2%), CD44 (57% ± 10%), CD105 (6% ± 4%), CD166 (14% ± 4%), CD45 (6% ± 3%), HLA-DR (6% ± 3%) and CD14 (6% ± 3%) and negative for CD34. AMTCs used in this study were obtained from six different placentas.

(ii) Human fibroblasts were taken from a dermal human fibroblast cell line, from skin biopsies, established in our laboratory (15).

(iii) Human peripheral blood mononuclear cells were obtained from heparinized blood samples

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