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# Management of Airway Remodeling in a Mouse Model of Allergic Airways Inflammation Using Extracellular Vesicles from Human Bone Marrow-Derived Mesenchymal Stromal Cells

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**HIGHLIGHTS**

- Extracellular vesicles (EV) from human bone marrow were able to reduce airway inflammation in a mouse model.
- Treatment applied by Intratracheal injection was more effective than systemic administration by caudal vein injection.
- IT administration of EVs appears to act therapeutically by restoring the basal BAL cell counts and attenuating early lung remodeling.

**Abstract:** Asthma is a chronic respiratory disease affecting 300 million people worldwide. It results in several structural changes in the airways, which are minimally accessible in clinical practice. Cell therapy using mesenchymal stromal cells (MSCs) is a promising strategy for treating asthma due to the paracrine activity of MSCs, which influences tissue regeneration and modulates the immune response. Studies using extracellular vesicles (EV) released by MSCs have demonstrated their regenerative properties in animal models. The aim of this study was to evaluate the potential of EVs isolated from human bone marrow MSCs (hBM-MSCs) to control lung tissue remodeling in ovalbumin-induced allergic asthma in Balb/c mice. We isolated hBM-MSCs from a single donor, expanded and characterized them, and then isolated EVs. Asthma was induced in 43 male Balb/c mice, divided into four groups: control, asthmatic (AS), asthmatic plus systemic

EVs (EV-S), and asthmatic plus intratracheal EVs (EV-IT). Upon completion of asthma induction, animals were treated with EVs either locally (EV-IT) or intravenously (EV-S). Seven days after, we performed bronchoalveolar lavage (BAL) and the total nuclear cells were counted. The animals were euthanized, and the lungs were collected for histopathological analysis of the airways. The EV-S group showed improvement in only the total BAL cell count compared with the AS group, while the EV-IT group showed significant improvement in almost all evaluated criteria. Therefore, we demonstrate that the local application of EVs derived from hBM-MSCs may be a potential treatment in controlling asthma.

**Keywords:** Airway remodeling; asthma; Balb/C mice; extracellular vesicles; stromal cells; pulmonary inflammation.

## INTRODUCTION

Asthma is a chronic respiratory disease affecting approximately 300 million people worldwide, including both children and adults [1]. Pathological structural changes in the airways resulting from asthma include goblet cell hyperplasia, hyper-secretion of mucus, and thickening of the epithelial and smooth muscle layers [2].

Cell therapy using mesenchymal stromal cells (MSCs) is a promising strategy for the treatment of several respiratory diseases due to their paracrine activity, which influences tissue regeneration and modulates the immune response [3]. MSCs release soluble factors and extracellular vesicles (EVs) into the extracellular environment, targeting different cells and promoting biological responses to repair damaged tissues [4]. Studies conducted in animal models of different diseases have demonstrated the regenerative potential of EVs from MSCs and other stem cell types, indicating their vast therapeutic potential [5]. Asthma doesn't have a cure, the current treatments only alleviate the symptoms and controls episodes of asthma exacerbation making it an expensive disease to handle, then as if MSCs represents an alternative treatment for some pulmonary diseases, and the role of extracellular vesicles on airways inflammatory processes was still undisclosed a cell-free treatment for asthma was needed. Hence, the hypothesis of this study is that EVs from human bone marrow MSCs could modulate the inflammatory process in an animal model of asthma. The aim of this study was to evaluate the potential of EVs isolated from hBM-MSCs in the control of lung tissue remodeling in a previously validated ovalbumin-induced allergic airway inflammation in Balb/c mice.

## MATERIAL AND METHODS

**Ethics** - This study was analyzed and approved by the Pontifícia Universidade Católica do Paraná ethical committee in research (registration number: 34309914.0.0000.0020). A single bone marrow sample was collected from a patient from Santa Casa de Misericordia de Curitiba, Brazil, after the donor provided written informed consent. The ethical committee in animal use from Pontifícia Universidade Católica do Paraná analyzed and approved this study (approval number: 904).

**MSC isolation** - A sample of hBM was used for MSC isolation by centrifugation with Histopaque (Sigma-Aldrich, MO, USA) (d=1.077 g/mL) as described [6] and adapted by our group [7]. MSCs were cultivated in Iscove's modified Dulbecco's medium (Gibco Invitrogen, NY, USA) supplemented with 15% fetal bovine serum (FBS) (Gibco Invitrogen, NY, USA), in a humidified incubator at 37°C with 5% CO2. Cells were used up to the third passage.

**MSC characterization** - MSCs were characterized at the time of vesicle collection (passage 3). Cells (1×106) were incubated with monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PE-Cy5), and allophycocyanin (APC) from BD Pharmingen4, against the following human antigens: CD45 (FITC; cat #555482), CD14 (FITC; cat #555397), CD19 (FITC; cat #555412), CD90 (PE; cat #555596), CD73 (PE; cat #550257), CD166 (PE; cat #559263), HLA-DR (PE-Cy5; cat #551375), CD105 (APC; cat #562408), CD34 (APC; cat #555824), and CD29 (APC; cat #559883), (BD Pharmingen, San Jose, CA, USA). All incubations were performed at room temperature for 30 min. Isotype-identical antibodies for FITC (cat #5555748), PE (cat #555787), PE-Cy5 (cat #555750), and APC (cat #555751) (BD Pharmingen, San Jose, CA, USA) were used as controls. After incubation, cells were washed with phosphate buffered saline (PBS) and fixed with PBS containing 1% paraformaldehyde. At the same time, viability and apoptosis tests were performed using annexin V (cat #556422) and 7-

aminoactinomycin D (7-AAD, cat #559925). All flow cytometry data was acquired on a FACS Calibur (BD Biosciences San Jose, CA, USA), and analyzed using FlowJo software (Flowjo Ashland, OR, USA).

**EV isolation and characterization** - Extracellular vesicles were obtained from supernatants of MSCs cultured under hypoxia (1% O2). After the cell culture reached confluence the cells were washed twice with phosphate buffered saline (PBS) and grown in DMEM (Gibco Invitrogen, NY, USA) with 2% FBS depleted of vesicles to avoid cross-contamination. After 24 h of incubation, the conditioned medium was centrifuged at 700 g for 5 min to remove cellular debris and then centrifuged at 4,000 g for 20 min to remove apoptotic bodies. For EV collection, the supernatant was ultracentrifuged at 100,000 g for 1 h and 20 min at 4ºC, discarded, and the vesicles were recovered from the bottom of the tubes with an equal volume and stored at 4ºC. This procedure was performed for three consecutive days, and at the end of the third day, the vesicles were diluted with 30 mL of PBS and submitted to a second ultracentrifugation at 100,000 g for 2 h at 4ºC for cleaning. The purified EVs were resuspended in small volumes of PBS after which the protein concentration of each sample was determined using the Qubit® 2.0 (Life TechnologiesTM, Invitrogen, NY, USA) assay. All samples were stored at -80ºC until used.

**Allergic Airways Inflammation Induction** - Forty-three male Balb/c mice were divided in 4 groups: control (C, n=11), asthmatic (AS, n=11), asthmatic treated intratracheally (IT) with EVs (EV-IT, n=10) and asthmatic treated intravenously with EVs (EV-S, n=11). A protocol validated in our laboratory (8) was used for allergic airways inflammation induction (Figure 1a). In brief, six intraperitoneal (IP) injections of 100 µL of sterile saline (for the control group), or 10 µg of ovalbumin (Sigma Aldrich, MO, USA) diluted in 100 µL of sterile saline, were administered to the animals on days 0, 2, 4, 7, 9, and 10. On days 15, 18, and 21, IT injections were administered as an allergenic challenge. The control group received 20 µL of sterile saline, other groups received 20 µg of ovalbumin in 20 µL of sterile saline. During IT injections, all animals received ketamine hydrochloride (Vetnil, São Paulo, SP, Brazil) (Quetamina injetável, 100 mg/kg) and xylazine hydrochloride (Vetnil, São Paulo, SP, Brazil) (Sedanew 2%, 10 mg/kg) via IP injection for anesthesia.

**Treatment** - On day 22, animals in groups C and AS received 20 µL of sterile saline by IT injection. Animals in the EV-IT group received 15 µg/animal EVs by IT injection, and the EV-S group received 15 µg/animal EVs through the tail vein, the dosage applied was based on another paper from our group [9]. During these procedures, all animals received ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) anesthetic via IP injection.

**Bronchoalveolar lavage (BAL), euthanasia, and histopathology** – On day 29, the animals were anesthetized with ketamine and xylazine as described above, and BAL was performed by exposing the trachea and injecting and retrieving 1 mL of sterile saline three times. All BAL fluid samples were kept on ice. The animals were then euthanized by anesthetic overdose (400 mg/kg ketamine, 50 mg/kg xylazine). The lungs were identified, washed with sterile saline, and stored in 2 labeled tubes per animal (left and right lungs) with 25 mL of 10% paraformaldehyde, which was changed weekly. After euthanasia, the BAL fluid was centrifuged at 300 x g for 8 min [8], and the supernatant was stored. The pellet was re-suspended in 500 µL of Phosphate-buffered saline to count the total nucleated cells in the BAL fluid with a Neubauer's chamber and an Olympus CK41 optical microscope (Olympus Scientific Solutions Americas Inc., MA, USA). The lungs were gravity-fixed with 8 centimeters of water in a 10% formalin solution and paraffinized in histological cassettes. To produce histological slides, the pieces were sliced with a microtome to 5 µm thickness. Before staining, all slides were deparaffinized by submerging them three times in histological grade xylol (Labmaster, Pinhais, PR Brazil) for 5 min, three times in absolute ethanol, 95% ethanol, and 70% ethanol (Labmaster, Pinhais, PR Brazil) for 1 min each, and then in distilled water for 5 min. Eighty-six slides (from 43 animals, left and right lung) were generated for each stain.

**Lung Staining** – All slides were stained with Hematoxylin and Eosin (HE), Periodic Acid-Schiff (PAS), and Masson's trichrome (MT) following standard protocols for each one. HE staining was used to evaluate pulmonary morphology, the presence of inflammatory infiltrate on airways, thickening of the epithelial basement membrane, obstructions of the lumen of bronchioles, hyperplasia, and hypertrophy of smooth muscle cells (8). PAS staining was used to evaluate the mucus production by goblet cells in the airways. MT staining (Easypath, São Paulo, SP, Brazil) allows to evaluate increases in collagen production in the airways.

**Semi-quantitative analyses** - After all slides were stained, 15 bronchiole images per lung were acquired at 400× magnification on an Nikon DS-Ri1 camera attached to an Nikon Eclipse Ni microscope, using NIS-Elements D 4.20.01 software (all from Nikon Corp., Tokyo, Japan). For the analysis's numbers were given randomly to each bronchiole image. Images were analyzed for all parameters, which were scored from 0–3, using a scoring system based on the evaluation methods used in [8]. A score of 0 represented bronchioles with no detectable inflammation, unmodified bronchial architecture, and basal levels of mucus and collagen production, while a score of 3 represented bronchioles with intense inflammation, airway remodeling with significant structural changes, a high number of inflammatory cells, intense thickening of epithelial and smooth muscle cell layers, frequent desquamation of epithelial cells, and increased production of mucus by goblet cells on airways. Quantitative (BAL) and semi-quantitative (histology, score 0–3) analyses were performed by the same blinded operator.

**Statistical Analysis** - BAL cell counts were analyzed by analysis of variance, then by Tukey's test for multiple comparisons. The scores obtained from other variables were analyzed using a non-parametric Kruskall-Wallis test, and an extension of the test was used for multiple comparisons between groups. $P<0.05$ indicated statistical significance. Data were analyzed with IBM SPSS Statistics software v.20 (IBM Corporation, NY, USA).

## RESULTS

Flow cytometry analysis was conducted to confirm the identity of passage 3 hBM-MSCs. The immunophenotypic profiles indicated that the stromal cells isolated from hBM stained positively for CD105 (97.5%), CD73 (93.8%), CD90 (98%), CD29 (93.5%), and CD166 (92.7%), and were negative for CD14 (0.21%), CD34 (0.15%), CD45 (0.091%), CD19 (0.099%), and HLA-DR (0.082%), indicating canonical MSC characteristics. Since the culture medium used to obtain EVs was DMEM with a low concentration of EV-depleted FBS (2%), the percentages of apoptotic and necrotic cells were estimated at the time of EV isolation. Low percentages of apoptotic and necrotic cells (0.81% and 3.96% respectively) were observed, similar to the percentages of apoptotic/necrotic cells growing in standard culture conditions as previously described by our group [10].

To evaluate the size distribution and concentration of the vesicles, an EV sample was examined using NTA. EVs derived from hBM-MSCs displayed a heterogeneous population, with particles ranging from 77.8–283.6 nm and a mean size of 167.8 nm (Figures. 1a and b). In total, 8.42×1010 EVs were isolated from 3.0×107 hBM-MSCs. TEM of the purified EVs revealed spherical morphology and the presence of double-layered membrane vesicles, with particle diameters comparable to those determined by NTA. In addition, TEM with immunogold staining displayed a CD105+ and CD63+ MSC-EV population (Figures. 1c and d). The size distribution, determined by NTA and confirmed by TEM, indicated the presence of microvesicles and exosomes in the purified EV population. For treatment, 4.2×109 EVs, or the equivalent of 15 µg of protein, were administered to each animal.

After allergic airways inflammation induction and histological analyses, the AS group displayed all the characteristics of diseased lungs (Figure 2) and had histology scores of 2–3. Histological modification included intense inflammation with a high number of inflammatory cells, and airway remodeling with significant structural changes, such as intense thickening of epithelial and smooth muscle cell layers, frequent desquamation of epithelial cells, and increased mucus production by goblet cells in the airways. An atypically high number of cells were present in the BAL fluids of the AS group. Non-asthmatic control animals (C group) had scores of 0–1 for all the histological parameters evaluated (Figure 2), and these values were always significantly lower than the AS group. Significant differences were also consistently observed between the C group and the EV-treated groups, but in several cases, the symptoms of EV-treated animals were significantly ameliorated when compared to the positive control group (AS group). No animals in the EV-treated groups achieved complete recovery. Animals in the EV-S group showed little or no overall improvement compared to the AS group, except for a statistically significant decrease in the number of total cells in the BAL fluid (EV-S= 15.42±0.70, AS= 23.32±0.62, $p < 0.001$; Figure 3a). Interestingly, the EV-IT group showed significant improvement compared to the AS group in almost all of the parameters evaluated. The BAL fluid total cell count decreased by half (EV-IT= 11.50±0.62, AS= 23.32±0.62, $p < 0.001$; Figure 3a) and the presence and intensity of inflammatory infiltrate was reduced more than 40% (EV-IT= 0.93±0.09, AS = 1.61±0.14, $p < 0.001$; Figure 3b).

Histopathological characteristics such as smooth muscle cell layer thickening (EV-IT = 0.63±0.06, AS = 1.32±0.14, p = 0.001; Figure 3c) and mucus production (EV-IT = 0.63±0.14, AS = 1.21±0.12, p = 0.001; Fig. 3d) were reduced over 50%. Thickening of the epithelial cell layer also significantly decreased (1.59±0.15 vs. 2.11±0.09, p = 0.003; Figure 4a) as did the production of collagen surrounding the airways (1.48±0.06 vs. 1.66±0.07, p = 0.049; Figure 4b). Although a reduction of 27% in epithelial cell desquamation, which can result in airway obstruction, was observed between the AS and EV-IT groups, it was the only parameter evaluated that did not show statistical significance (0.33±0.06 vs. 0.45±0.06, p = 0.094; Figure 4c). Descriptive statistics and p values resulting from comparisons of all parameters analyzed in this study are shown in the supplementary material (Tables S1-14).

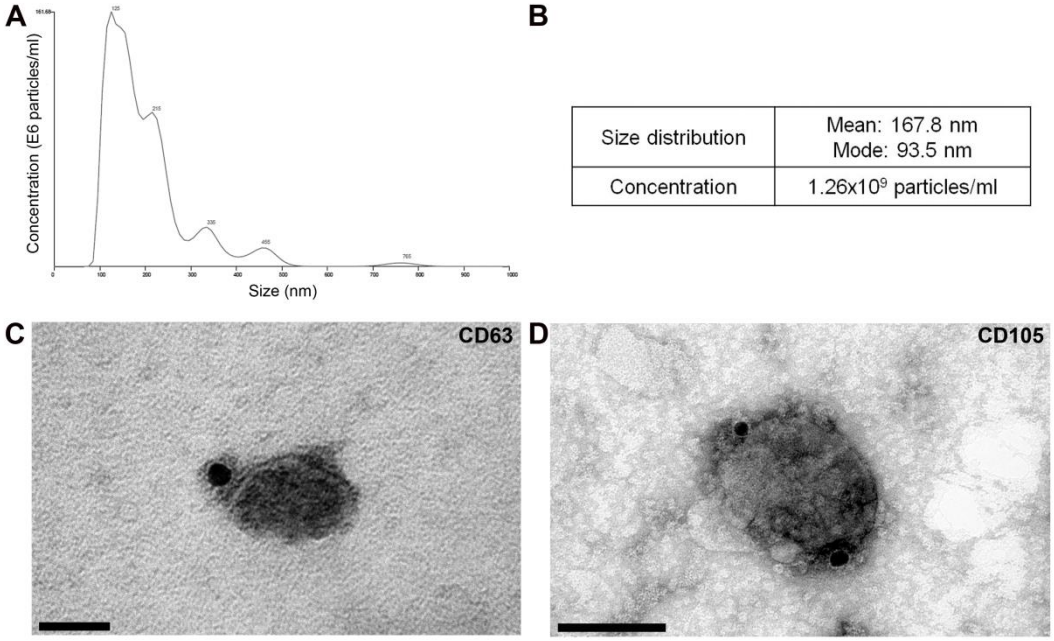



**Figure 1.** Characterization of extracellular vesicles (EVs) derived from human bone marrow mesenchymal stem cells (hBM-MSCs) (A) Representative graphic of the size distribution from NTA. (B) Table summarizing the NTA parameters. (C) and (D) Representative transmission electron microscopy images of EVs derived from hBM-MSCs. Extracellular vesicles were labeled with antibodies against CD63 (C) and CD105 (D), conjugated to colloidal gold. Scale bar in C = 50 nm; Scale bar in D = 100 nm.

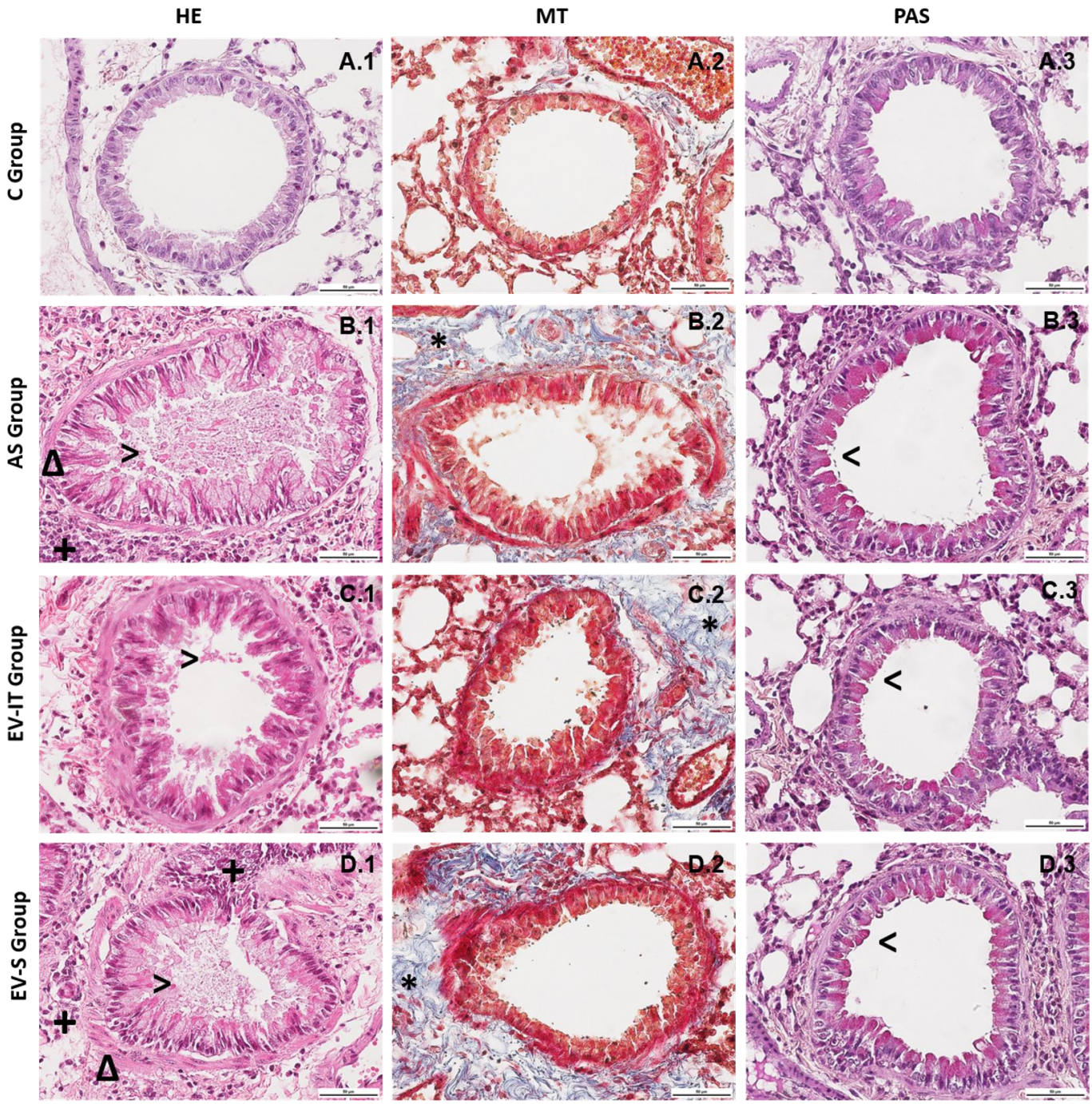
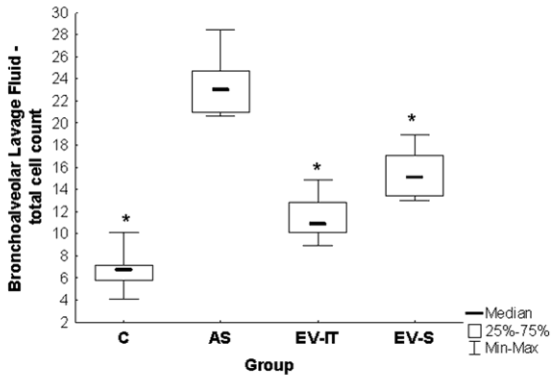



**Figure 2.** Photomicrographs of bronchial sections at 400× HE: hematoxylin & eosin; MT: Masson's trichrome; PAS: Periodic acid-Schiff. *: collagen deposition (B.2, C.2 and D.2); +: inflammatory infiltrate (B.1 and D.1), >: airway obstruction (B.1, C.1 and D.1), <: increased mucus production (B.3, C.3 and D.3), and Δ: smooth muscle thickening (B.1).

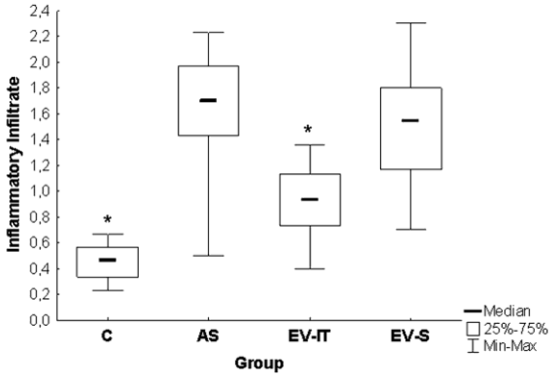
**A**




*p* values resulting from the comparisons between all groups

| Compared Groups | *p* value |
|---|---|
| C x EV-IT | <0.001 |
| C x EV-S | <0.001 |
| C x AS | <0.001 |
| EV-IT x EV-S | <0.001 |
| EV-IT x AS | <0.001 |
| EV-S x AS | <0.001 |

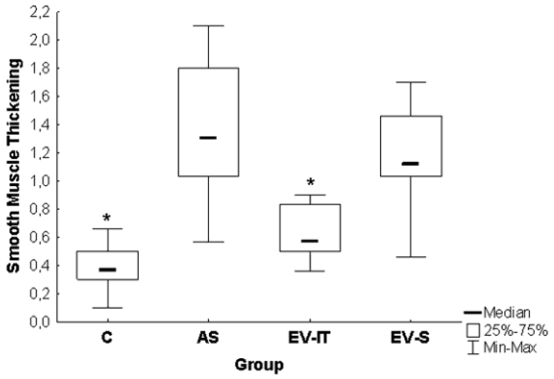
**B**




*p* values resulting from the comparisons between all groups

| Compared Groups | *p* value |
|---|---|
| C x EV-IT | 0.003 |
| C x EV-S | <0.001 |
| C x AS | <0.001 |
| EV-IT x EV-S | 0.003 |
| EV-IT x AS | <0.001 |
| EV-S x AS | 0.478 |

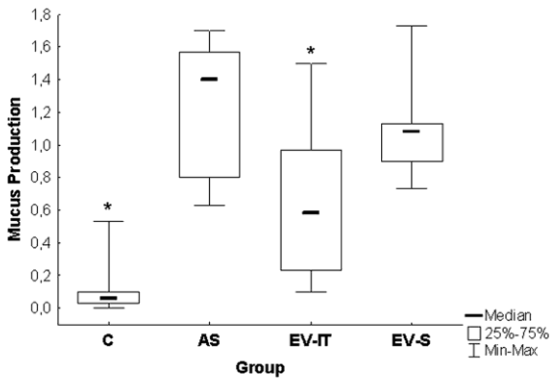
**C**




*p* values resulting from the comparisons between all groups

| Compared Groups | *p* value |
|---|---|
| C x EV-IT | 0.038 |
| C x EV-S | <0.001 |
| C x AS | <0.001 |
| EV-IT x EV-S | 0.001 |
| EV-IT x AS | <0.001 |
| EV-S x AS | 0.400 |

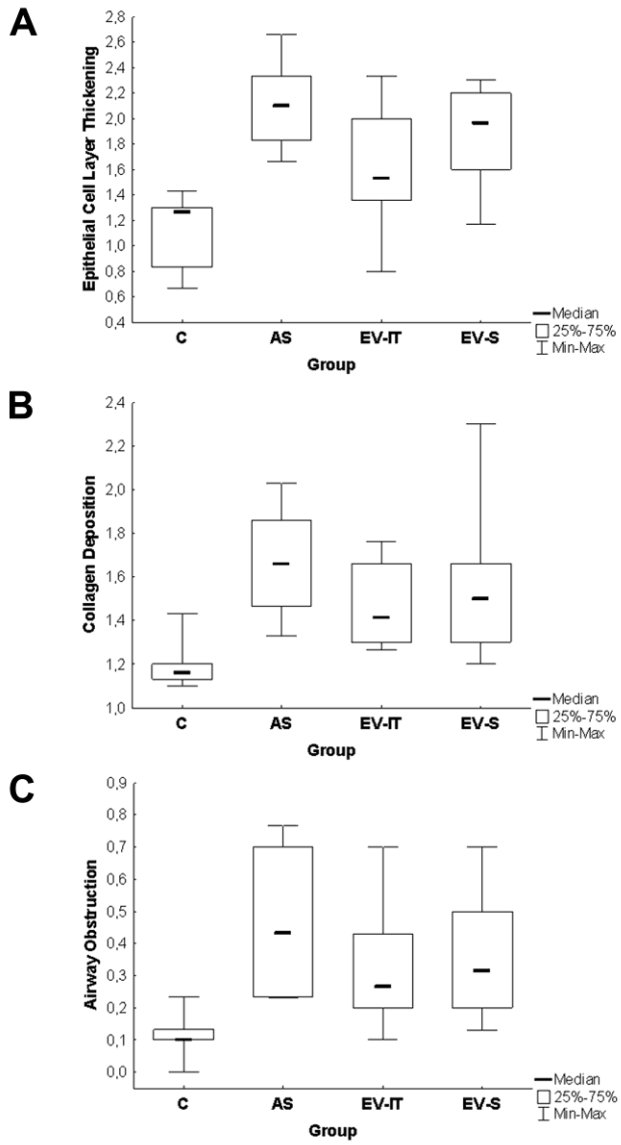
**D**




*p* values resulting from the comparisons between all groups

| Compared Groups | *p* value |
|---|---|
| C x EV-IT | 0.003 |
| C x EV-S | <0.001 |
| C x AS | <0.001 |
| EV-IT x EV-S | 0.003 |
| EV-IT x AS | 0.001 |
| EV-S x AS | 0.769 |

**Figure 3.** Effect of MSC-EV administration on inflammation, smooth muscle thickness and mucus production of murine lungs following ovalbumin induction of allergic asthma Statistical analysis of the total cell count in bronchoalveolar lavage fluid (A), inflammatory infiltrate (B), smooth muscle thickening (C) and mucus production (D). Lung sections were stained with hematoxylin and eosin (B) and (C) or periodic acid-Schiff (D). Abbreviations: C: control (n = 9); EV-IT: asthmatic animals treated with EVs by intratracheal pathway (n = 10); EV-S: asthmatic animals treated with EVs by systemic pathway (n = 10); AS: asthmatic animals without treatment (n = 11).




*p* values resulting from the comparisons between all groups

| Compared Groups | *p* value |
|---|---|
| C x EV-IT | 0.011 |
| C x EV-S | <0.001 |
| C x AS | <0.001 |
| EV-IT x EV-S | 0.096 |
| EV-IT x AS | 0.003 |
| EV-S x AS | 0.150 |

*p* values resulting from the comparisons between all groups

| Compared Groups | *p* value |
|---|---|
| C x EV-IT | 0.001 |
| C x EV-S | <0.001 |
| C x AS | <0.001 |
| EV-IT x EV-S | 0.887 |
| EV-IT x AS | 0.049 |
| EV-S x AS | 0.067 |

*p* values resulting from the comparisons between all groups

| Compared Groups | *p* value |
|---|---|
| C x EV-IT | 0.002 |
| C x EV-S | 0.001 |
| C x AS | <0.001 |
| EV-IT x EV-S | 0.743 |
| EV-IT x AS | 0.094 |
| EV-S x AS | 0.176 |

**Figure 4.** Effect of MSC-EV administration on epithelial cells, collagen deposition and airway obstruction of murine lungs following ovalbumin induction of allergic asthma Statistical analysis of epithelial cell layer thickening (A), collagen deposition (B), and airway obstruction (C). Lung sections were stained with hematoxylin and eosin (A) and (C), or Masson's trichrome (B). Abbreviations: C: control (n = 9); EV-IT: asthmatic animals treated with EVs by intratracheal pathway (n = 10); EV-S: asthmatic animals treated with EVs by systemic pathway (n = 10); AS: asthmatic animals without treatment (n = 11).

## DISCUSSION

Significant effort has gone into the development of effective and low-cost treatments for respiratory diseases. Recently, EVs have become the subject of intense investigation, and specifically, MSC-derived EVs have been indicated as a promising cell-free therapy enabling tissue repair. In this study, we examined the potential effects of treatment with EVs isolated from human BM-MSCs to control ovalbumin-induced allergic asthma in Balb/c mice. To our knowledge, this is the first study to show lung tissue amelioration by IT administration of MSC-EVs in the allergic asthma model. Our model was based in a xenogeneic transplant without the use of immunosuppressive drugs or humanized mice, as previously demonstrated in studies by our group [8].

MSCs are hypoimmunogenic, often lacking MHC-II and costimulatory molecule expression, and are a highly regulated self-renewing population of cells with potent mechanisms to avoid rejection [11]. EVs released by MSCs reportedly have the same behavior allowing their use in xenogeneic transplants. Analysis of MSC-derived EVs in animal models of inflammation suggests that the stem cell-derived vesicles are also immunosuppressive [12].

Over 1010 EVs were harvested and purified from hypoxic hBM-MSC cultures. Our data is consistent with a mix of microvesicles and exosomes [13] in the EV population. Hypoxic cultures were used because low

oxygen concentrations increase EV release and modifies their content, potentiating their reparative effects [14]. In an in vitro study, it has been demonstrated that MSCs could respond to several stimuli to change the content of the EVs increasing the concentration of anti-inflammatory cytokines [15]. Here, we used hypoxia and serum starvation during EV collection. These EVs were able to reduce significantly lung inflammation and remodeling.

We demonstrated that the asthmatic induction protocol showed many common features of allergic asthma in humans, as previously described by our group [8]. The asthmatic animal group exhibited a significant increase of inflammatory cell infiltration and higher BAL cell counts. In addition, our in-depth histological analyses revealed pathological lung changes in the AS group compared to the controls. One of the most interesting conclusions that could be drawn from our study is that the MSC-EVs seem to mimic, at least for the parameters analyzed here, what it has been shown for asthma treatment using MSCs themselves. Xenogeneic or syngeneic transplants of MSCs from different origins to treat lung injuries that result in inflammation and tissue remodeling are known to exert potent immunosuppression anti-inflammatory effects and lung remodeling attenuation/prevention [4,16,17]. In most cases, a parallel might be established between MSC and MSC-EV treatments. Recently, other research groups studying asthma with a similar mouse model [17] or in a model of allergic-airway inflammation [18], but with differences in treatment and evaluated parameters, confirmed the parallel effects observed between stem cells and their vesicles injected systemically. [19] used 37 µg of EVs derived from human adipose mesenchymal stromal cells (hATMSCs) administered in the jugular vein and compared it with the administration of hATMSCs themselves. The effects were largely similar, and few differences were observed during outcome assessment of lung mechanics and inflammation. In accordance with these findings, we observed that the cell count in the BAL fluid was significantly diminished when hBM-MSC-EV were injected systemically. In an endotoxin-induced model of acute lung injury, intratracheal and systemic administration of hMSC-EVs similarly decreased the influx of inflammatory cells in BAL fluid and reduced lung remodeling [16]. Conversely, in our study we observed that IT injection of EVs (single application of 15 µg) was more effective than systemic EV treatment, leading to a significant improvement of all parameters investigated. The partial discrepancies among works might be due to the differences intrinsic to the animal/disease model and concentration of vesicles, among others. However, previous studies have shown that after systemic administration, EVs tend to accumulate preferentially in the liver, followed by the spleen, gastrointestinal tract, and lungs [20,21]. In addition, it was reported differences in EV localization when they were administered intravenously, intraperitoneally, or subcutaneously, demonstrating that different routes of systemic administration influenced EV tissue distribution [21]. We hypothesize that vesicles administered by the systemic route may have been retained in other organs, whereas the IT-administered vesicles are more likely to be retained in the injured area. Moreover, the beneficial effects observed in this study were obtained with only 15 µg of EVs. We speculate that achieving the same effects by a systemic route might require a higher concentration of vesicles [17] or multiple applications.

Regarding the comparison between IT application of MSC vs MSC-EV, in an ovalbumin-induced murine model of asthma IT applications of both human [22] and murine MSC [23,24] reduced airway inflammation and suppressed histopathological changes in a similar way to what we have observed using hMSC-EVs. Thus, reinforcing the idea that MSC-EVs can reproduce the cells effects on a model of allergic asthma. More preclinical studies are necessary to improve the protocols by adjusting vesicle sources, vesicle concentration and number of doses.

## PATENTS

In conclusion, we have demonstrated that the local application of hBM-MSC-derived EVs might be as effective as MSCs in controlling allergic airways inflammation. IT administration of EVs appears to act therapeutically by restoring the basal BAL cell counts and attenuating early lung remodeling.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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