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Bone marrow stem cells modified with human interleukin 10 attenuate acute rejection in rat lung allotransplantation†

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Abstract

OBJECTIVES: The aim of this study was to investigate new therapeutic options to attenuate acute rejection in a rat lung allograft model. Cell-based gene therapies have recently been reported as a novel curative option in acute and chronic diseases for which conventional treatments are not available. We studied the effect of human interleukin 10 (hIL-10) on expressing bone marrow-derived mesenchymal stem cells (BMSCs) in combination with cyclosporine A (CsA) on acute rejection of lung allografts in the rat.

METHODS: Lung allotransplantation was performed from male Brown Norway donor to male Fisher (F344) rats. Rat BMSCs were transfected with hIL-10 *in vitro* and introduced in the graft prior to implantation. Group A ($n = 5$) received CsA intraperitoneally (2.5 mg/kg body weight) for 5 days post-transplant; Group B ($n = 5$) received BMSC and CsA and Group C ($n = 5$) received hIL-10-BMSC before implantation and CsA. Graft function was assessed by blood gas levels only from the graft on day 5; tissue was sampled for histological grading of rejection and measurement of the wet-to-dry ratio.

RESULTS: All Group A control animals showed severe signs of rejection. On Day 5, all grafts in Group C showed improved gas exchange (mean arterial partial pressure of oxygen 222.2 ± 40.38 mmHg vs 92.36 ± 20.92 mmHg in Group B and 42.72 ± 18.07 mmHg in Group A). Histological examination revealed moderate-to-severe rejection in all animals in Group A [International Society for Heart and Lung Transplantation Level III B (ISHLT)] in contrast to low-to-moderate rejection in Group B (II–IIIA) and much improved histological grade in Group C (I–IIA). Moreover, the wet-to-dry ratio was also reduced in Group C (4.8 ± 1.19 compared with 4.78 ± 0.62 in Group B and 9.36 ± 0.90 in Group A).

CONCLUSIONS: The hIL-10 BMSC represent a promising novel method for localized cell-based gene therapy for acute rejection in a rat lung allograft model.

Keywords: Lung transplantation • Stem cells • Acute rejection • IL-10

INTRODUCTION

Lung transplantation is the only treatment option for a variety of end-stage lung diseases such as chronic obstructive pulmonary disease, interstitial lung disease and cystic fibrosis [1]. However, survival after a lung transplant is still not particularly promising compared with that after other solid organ transplants [2, 3], with only 53% survival reported 5 years after the transplant [1]. Approximately 33% of the patients develop acute rejection in the first year after the transplant, which is related to the development of chronic rejection and reduced survival. Various attempts have been made to attenuate acute rejection; however, the results are not promising [2]. Induction therapy, which involves intensive immunosuppressant therapy given perioperatively to reduce acute rejection and to delay the initiation of maintenance immunosuppression, was recently introduced [2]. The most commonly applied induction agent is the interleukin 2 receptor antagonist [4]. Despite these advances, the survival rate is low. Therefore, novel approaches that could attenuate acute rejection and improve survival are required.

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In previous studies, we successfully applied the electroporation-mediated gene transfer of human interleukin 10 (hIL-10) to the recipient prior to implantation to attenuate acute rejection in a lung transplant model [5]. However, the dose of hIL-10 was high and the route of administration was systemic. Therefore, we reduced the dose of hIL-10 and provided a more localized application using IL-10 modified bone marrow-derived stem cells.

Bone marrow-derived mesenchymal stem cells (BMSCs) are known to have immunogenic and anti-inflammatory properties and have been tested in various disease models [6], including a rat heart transplant model [7]. BMSC cell therapy has been reported in acute and chronic lung injury models [8, 9]. Stem cells can also be used as a vehicle to carry the gene [10–12]. Moreover, cell-based gene therapy is a promising approach for localized and targeted applications and has been applied in a chronic lung injury model [13]. The BMSCs are transfected with a gene of interest *in vitro* and administered *in vivo*. We hypothesized that this approach has an additive effect locally and can be of benefit in attenuating acute rejection in a lung transplant model. In the current study, we tested whether BMSCs that overexpress IL-10 when administered in the graft before implantation can attenuate acute rejection in a rat lung allograft model.

MATERIALS AND METHODS

Animal ethics approval

All animals received humane care in compliance with the 'Principles of Laboratory Animal Care', formulated by the National Society for Medical Research, and the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Research. Experiments were performed in accordance with the standards of the European Convention of Animal Care. The animals were kept in air- and temperature-controlled cages. At no time were the animals deprived of food and water. The protocol was approved by the University of Bern animal study committee. ARRIVE guidelines were followed to report the data (see Supplementary material, File S1).

Isolation and culture of rat bone marrow-derived mesenchymal stem cells

BMSCs were isolated from recipients (Fisher F344 rats). The animals were anaesthetized by inhalation of 4% isoflurane in a glass chamber, followed by intraperitoneal administration of thiopental (50 mg/kg body weight). The femur and tibia from the hindlimbs were dissected, and the skin and muscles were removed. The isolated femur and tibia were placed in 70% ethanol for 30 s and then transferred in fresh phosphate-buffered saline (PBS; 1× PBS). The bones were cut at the ends with a bone cutter and flushed with 1 ml of 1× PBS using a 22-G needle. The flow-through liquid was collected in a sterile tube; each bone was flushed 2–3 times with a volume of 1 ml each time. After centrifugation, the cells were resuspended in Iscove's modified Dulbecco's medium supplemented with 10% PBS and 1% penicillin/streptomycin medium and plated in 75-cm flasks and placed at 37°C in a 5% carbon dioxide incubator. After 3 days, the medium was changed; subsequently, the medium was changed every day. For the experiments, the cells were used at Passages 2–4. Ten F344 animals were used to isolate the BMSCs. The cells were counted using the Countess Cell Counter (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

Transfection of rat bone marrow-derived mesenchymal stem cells

Rat BMSCs were transfected with the plasmid pCikhIL-10, hIL-10 driven by the human cytomegalovirus early promoter enhancer as described [5] with the Amaxa nucleofection (Lonza, Allendale, NJ, USA) using U-23 settings according to the manufacturer's protocol; 5×10^5 cells were transfected in 1 cuvette with 1 μg of hIL-10 plasmid. After transfection, the cells were kept in warm media and placed at 37°C in a 5% carbon dioxide incubator overnight. Next morning, a total of 2×10^6 cells were introduced in the explanted graft approximately 2 h before implantation in the recipient. The Amaxa (Lonza AG, Switzerland) nucleofection works on the concept of *in vitro* electroporation. Electroporation is a physical method of gene transfer that involves the application of a high voltage to the cells for a very short period. Electroporation makes the cell membrane permeable, thus enabling the transfer of the DNA inside the cell for a successful transfection. For experimental purposes, all of the cells were used, and no prior sorting of the transfected cells was performed.

Flow cytometry

The immunophenotype of the cell surface was analysed by staining the BMSCs with fluorescein isothiocyanate-labelled monoclonal antibodies against antihuman IL-10 antibody. After trypsinization, the cells were resuspended in PBS to obtain a single-cell suspension, washed and incubated for 45 min at 4°C in the dark with the staining antibody. Unbound antibody was removed by washing the cells 3 times in staining buffer (1% bovine serum albumin and 0.1% sodium azide in PBS) at 4°C. The cells were then resuspended in staining buffer and analysed with the FACScan (Becton Dickinson, Franklin Lakes, NJ, USA). The stem cells were divided in 2 tubes, with 1 tube containing cells that were not transfected. These are indicated by the red graph in Fig. 1 and represent the negative population. The stem cells in the second tube were transfected with hIL-10 as explained previously and were gated based on the threshold of the autofluorescence obtained from the non-transfected cells. We then measured the intensity of the fluorescence in the transfected stem cells (the blue graph in Fig. 1). After transfection, BMSC were not sorted for positive cells; rather we used the whole population of BMSC for the experiments.

Experimental groups

Left lung allotransplantation was performed in rats with major full mismatch and 1 minor immunological mismatch (donor: Brown Norway; recipient: Fischer F344) [5]. Recipients were randomly divided into 3 groups ($n = 5$): Group A animals received CsA 2.5 mg/kg/day intraperitoneally starting on the day of the transplant. Group B ($n = 5$) received CsA daily in combination with BMSC introduced into the graft 2 h before the implantation. Group C ($n = 5$), the implanted graft, was given BMSC-hIL10, i.e. BMSC transfected with 1 μg of hIL-10 plasmid DNA (pCik hIL-10) 24 h before the operation. All animals in Group C also received

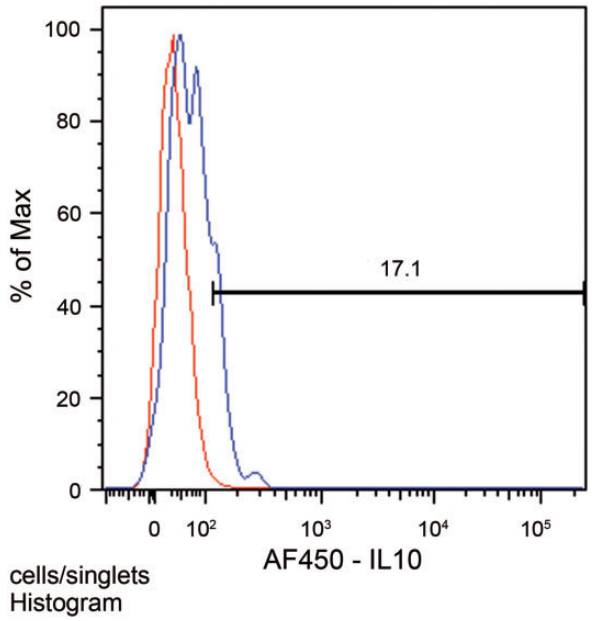

Figure 1: Flow cytometer analysis to determine the number of IL-10-transfected BMSCs before administration in the animals. The histogram gives the percentage of cells in relation to the largest fraction of living cells on the *Y* axis. The *X* axis represents the intensity of the fluorescence. The red curve indicates unstained cells (negative population), and the blue curve indicates the transfected cells. Stem cells transfected with hIL-10 were gated based on the threshold of the autofluorescence obtained from the non-transfected cells (red graph). The transfected stem cells were then compared for the intensity of the fluorescence, which is the measure of the transfection, indicated by the blue line. The 2 blue curves are too close; the difference between them is less than 5%, which represents a small population of cells with slightly different transfection efficiency. IL-10: interleukin 10.

CsA. During the course of the experiments, 2 recipients were sacrificed prematurely because of technical issues and were excluded; no animals died after the procedure. We used 34 animals for this study.

OPERATIVE PROCEDURE

Donors

The donor animals were anaesthetized in a glass chamber by inhaling 4% isoflurane. Thiopental (Pentotal®, Abbot AG, Baar, Switzerland) at a dosage of 50 mg/kg body weight was administered intraperitoneally. Heparin (Liquemin®, Roche Pharma, Rheinach, Switzerland) was administered by injection into the penile vein (500 IU/kg body weight). A tracheostomy was performed, and the animals were ventilated with a 14 GA catheter (Insyte®, Becton Dickinson, Sandy UT, USA) with fraction of inspired oxygen = 1.0, a breathing frequency of 100 breaths/min and a tidal volume of 8 ml/kg body weight by a rodent ventilator (Model 683, Harvard Apparatus, South Natick, MA, USA). After we cut the inferior vena cava and the left appendix of the heart, we inserted a small silicon tube into the main pulmonary artery (PA) via an incision in the right ventricle. Both lungs were flushed with 20 ml of a low-potassium dextran solution (Perfadex®, Vitrolife Pharmaceutics, Uppsala, Sweden) at 20 cmH_2O pressure. The trachea was then tied with the lungs in the end respiratory position. The heart–lung block was removed, and the left lung was separated *ex vivo*. Small plastic cuffs were placed around the PA and the left pulmonary vein (PV), the vessels were everted, tied on the cuffs and fastened with 8-0 monofilament thread (Surgipro®, USSC, Norwalk, CT, USA). The grafts were stored in low-potassium dextran solution.

Introduction of bone marrow-derived mesenchymal stem cells in the graft

The hIL-10-BMSC (2×10^6 cells) in a volume of 250 µl was introduced into the isolated graft, which was immediately stored at 10°C until implantation in the recipient. The implantation procedure was usually performed within 2 h.

Recipients

The recipients were anaesthetized by breathing 4% isoflurane in the glass chamber. Intubation was carried out using a 14-GA catheter placed into the trachea. Anaesthesia was maintained with 2.5% isoflurane. The recipient was ventilated with 1 cm water positive end-expiratory pressure, a breathing frequency of 100 breaths/min and a tidal volume of 8 ml/kg body weight by a rodent ventilator. A left thoracotomy was performed. The left hilum was dissected, and microclips were placed on the left PA and the left PV. The left main bronchus was ligated with 6-0 polyfilament thread (Sofsilk®, USSC, Norwalk, CT, USA) and cut. An incision was made in both the PV and the PA. The vessels were flushed with saline solution. The cuffs of the donor lung were inserted into the recipients' vessels, and 6-0 polyfilament ligatures (Sofsilk) were placed around the cuffs and tied. The native PA and PV were cut-off beyond the anastomoses, and the native lung was removed. A 9-0 monofilament running over-and-over continuous suture (Monosof®, Tyco Healthcare, Wollerau Switzerland) was used for the bronchial anastomosis. Ventilation was started. The microclips were removed to allow retrograde and subsequently anterograde perfusion of the graft. A small chest drain was inserted into the left pleural space; the thoracotomy was closed with 3 layers of continuous sutures. The chest drain was removed after spontaneous breathing was restored; extubation followed.

Cyclosporin CsA (Sandimmune®) (Novartis Pharma AG, Basel, Switzerland) was diluted in castor oil in a ratio of 1:9 and injected intraperitoneally every day. One ampoule of cyclosporine 50 mg in 1 ml was added to 9 ml of castor oil to prepare 10 ml of the solution. The volume of the daily administered cyclosporine solution depended on the body weight of the recipient.

ASSESSMENT

Graft function

Five days after the transplant, the recipient was preanaesthetized in a glass chamber by inhaling 4% isoflurane and was given thiopental (50 mg/kg body weight) intraperitoneally. The animal was ventilated via a tracheostomy with the Harvard Apparatus Rodent Ventilator with fraction of inspired oxygen = 1.0, a frequency of 100 breaths/min and a tidal volume of 8 ml/kg. A thoraco-laparotomy in the anterior midline was done. The right hilum was dissected. Microvascular clips were placed on the right main bronchus and the right PA to ventilate and perfuse only the isolated left lung graft. Five minutes after the occlusion, 1 ml of

blood was aspirated from the aortic arch to a syringe (Radiometer Pico 50, Copenhagen, Denmark) for blood gas assessment (Gastat-navi, Techno Medica Co. Ltd, Japan) using special chips. We collected 1 ml of blood from the inferior vena cava to measure the serum hIL-10 levels. Preocclusion blood was not collected due to technical issues; it is not representative of the graft function, and drawing blood twice from the same animal at a short interval can be fatal. Subsequently, the inferior vena cava and left appendix of the heart were incised, and a small silicon tube was inserted into the main PA via an incision in the right ventricle. The lungs were then flushed with 20 ml of a 0.9% saline solution under pressure of 20 cmH_2O. The heart–lung block was explanted, and tissue samples were collected for histological analysis, wet-to-dry (W/D) ratio and protein extraction.

Histology

After explantation, the graft was isolated. The lung was cut into 3 pieces. The upper part was taken for histological analysis and fixed in 4% paraformaldehyde. The middle section was frozen for further analysis, and the lower portion was used to determine the dry/wet ratio. After the graft was fixed in paraffin, sections were cut and stained with haematoxylin and eosin. The histological assessment was done by a trained lung pathologist in blinded fashion according to the Working Formulation for the Classification of Pulmonary Allograft Rejection of the International Society for Heart and Lung Transplantation (ISHLT) [1].

Enzyme-linked immunosorbent assay for human interleukin 10 expression

Levels of hIL-10 circulating in the serum and in the lung graft were measured using an hIL-10 immunoassay kit (R&D Systems, Abingdon, UK) according to the manufacturer's protocol. The antibody is specific, so no cross-reaction with rat IL-10 occurs. A total of 1 ml of blood was drawn from the inferior vena cava on Day 5 at sacrifice, centrifuged and stored at -20°C until we were ready to make the measurements. The graft tissue was snap frozen, and the total protein was extracted using the T-PER™ Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA) using 50 mg of tissue. The protein was extracted according to the manufacturer's protocol. After centrifugation, the sample was stored at -20°C until we were ready to make the measurements.

Wet-to-dry lung weight ratio

The lung W/D weight ratio was measured as an index of the accumulation of lung water in the graft. To measure the total amount of lung water, the lung weight was measured immediately after its excision (wet weight). The lung tissue was then dried in an oven at 60°C for 48 h and reweighed as dry weight. The W/D weight ratio was calculated by dividing the wet by the dry weight.

Statistics

Analysis of variance was followed by a planned comparison with the contrast vector. The level of rejection was graded using the non-parametric Kruskal–Wallis analysis of variance and a *post hoc* analysis with Dunnett's test using the control group. A *P*-value <0.05 was considered significant. All the analyses were performed using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA).

RESULTS

Rat bone marrow-derived mesenchymal stem cells were successfully transfected and produced a significant amount of human interleukin 10

Rat BMSCs were transfected by *in vitro* electroporation; 24 h after the transfection, 17.1% of the cells were positive for hIL-10 (Fig. 1). The level of hIL-10 level found in the rBMSC 24 h after transfection was 1184 ± 547.6 pg/ml.

High levels of human interleukin 10 were detected in the sera and the grafts of the recipients after pretreatment with human interleukin 10 bone marrow-derived mesenchymal stem cells

Levels of hIL-10 were measured in the sera and the lung grafts of the recipients in the treated group. Significantly higher levels of hIL-10 were found in the sera and in the grafts (97.71 ± 13.57 pg/ml and 76.86 ± 35.57 pg/ml, respectively) in Group C; no hIL-10 was noted in either control group (Groups A and B) at Day 5 using enzyme-linked immunosorbent assay.

Arterial blood gas levels indicated significantly improved graft function after administration of human interleukin 10 bone marrow-derived mesenchymal stem cells

Arterial partial pressure of oxygen levels in arterial blood measured 5 min after clamping the right hilum were significantly improved in the hIL-10 BMSC group (Group C) (222.2 ± 40.38 mmHg) compared with those in the BMSC group (Group B) (92.36 ± 20.92 mmHg) and in the CsA group (Group A) (42.72 ± 18.00 mmHg) (Fig. 2A).

Graft oedema was lower in the recipients treated with human interleukin 10 bone marrow-derived mesenchymal stem cells

The W/D ratio in the grafts of the hIL-10 BMSC group was significantly reduced in Group C (4.8 ± 1.19) compared with those in Group A (CsA group) (9.36 ± 0.90). The ratio in the BMSC-only group (Group B) was also reduced to 4.78 ± 0.62 with no significant difference between the hIL-10 BMSC group (Group C) and the BMSC-only group (Group B) (Fig. 2B).

The rejection score was significantly improved in recipients treated with human interleukin 10 bone marrow-derived mesenchymal stem cells

The histopathological assessment demonstrated improvement in lung architecture in the hIL-10 BMSC group compared with the

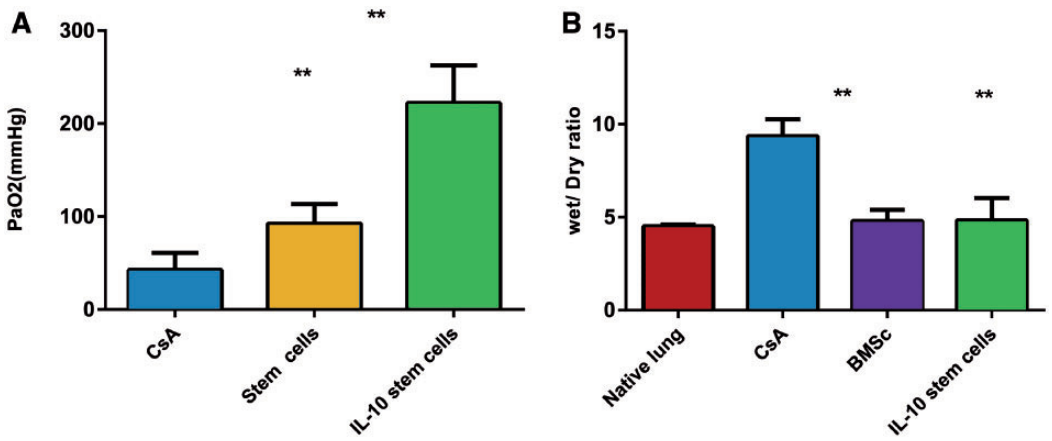

Figure 2: Function of the graft after 5 days, determined by measuring the arterial blood gas levels (**A**) and lung oedema, measured by determining the wet/dry ratio (**B**) ($n = 5$ in each group). *$P < 0.05$; **$P < 0.001$. BMSC: bone marrow-derived mesenchymal stem cell; CsA: cyclosporine A; hIL-10: human interleukin 10.

Table 1: Histological grading using the classification system of the International Society for Heart and Lung Transplantation (ISHLT)

	Vascular	Airway
CsA	A3 (A2–A4)	B1 (B1–B0)
BMSC	A1 (A1–A2)	B0 (B1–B0) (1 B1)
hIL-10 BMSC	A1 (A1–A0)	B1 (B1–B0) (1 B1)

A: acute rejection; A0: no rejection; A1: minimal rejection; A2: mild rejection; A3: moderate rejection; A4: severe rejection; B: airway inflammation; B0: no inflammation; B1: low-grade inflammation; B2: high-grade inflammation; BMSC: bone marrow-derived mesenchymal stem cell; CsA: cyclosporine A; hIL-10: human interleukin 10.

BMSC-only and CsA groups, as indicated by the rejection scores of I–II A in the hIL-10 BMSC group (Group C) compared with II–IIIA in the BMSC-only group (Group B) and III B in the CsA group (Group A) (Table 1, Fig. 3).

DISCUSSION

This study demonstrates the beneficial effect of hIL-10 BMSC in the graft of the recipients as a novel method to attenuate acute graft rejection in a rat model of lung allotransplantation. This method leads to better graft function, reduced graft oedema and improved histological characteristics.

Immunomodulation to attain improved graft function and reduce acute rejection has been tried and tested in the past using various methods. We also reported the use of gene therapy to overexpress hIL-10 in the recipient to attenuate acute rejection in a rat lung allotransplantation model [5]. IL-10 is a potent suppressor of interferon γ, tumour necrosis factor α, interleukin 1β and interleukin 6 and facilitates the induction of tolerance after allogenic transplantation [14]. It inhibits monocyte-/macrophage-dependent synthesis of cytokines by TH1 cells [15]. IL-10 also inhibits monocyte-/macrophage-dependent T-cell activation and antigen presentation as well as alloreactivity *in vivo* [16]. Because it has these properties, IL-10 has been tested in various transplantation models using different gene therapy approaches [17, 18].

Despite recent advances, gene therapy is still not readily accepted in clinical applications. Therefore, novel techniques appropriate for clinical translation need to be evaluated and developed. In this study, our goal was to identify a potent partner for IL-10 that could exert a synergetic, beneficial effect and attenuate acute lung rejection by more effective anti-inflammatory and immunosuppressive properties.

The immunomodulatory effect of mesenchymal stem cells was initially reported to be the ability to suppress T-cell proliferation [19]. It was further established that mesenchymal stem cells could inhibit proliferation of B cells, natural killer cells and dendritic cells. They also inhibit dendritic cells maturation, activation and antigen presentation; cytokine secretion and cytotoxicity of T and natural killer cells; and B-cell maturation and antibody secretion [20, 21]. The use of stem cells as carriers for genes appears promising [13, 22, 23]. In this study, we transfected BMSC with interleukin-10 (hIL-10 BMSC) to evaluate its effect on acute lung rejection in an allotransplant model. The synergetic effect of this approach was evident in the improved lung function and histological analysis.

However, a few *in vivo* studies using mesenchymal cells for immunomodulation have reported controversial results [24–26]. Most of these studies were done using mice models in which the cells were administered after transplantation. We administered BMSC or hIL-10 BMSC prior to transplantation and achieved attenuation of graft rejection using the hIL-10 BMSC in line with the islet transplantation study in a rat model [27]. These results indicate that hIL-10 BMSC exerts a protective effect. However, we did not test BMSC or hIL-10 BMSC in our model after transplantation to evaluate the effect post-transplant due to the pleotropic nature of IL-10 [28]. Moreover, it has been shown in some studies that BMSC collect at the site of injury [13]. Contrary to these finding, we could not find BMSC or hIL-10 BMSC in the lung graft (data not shown); however, we did find an increased level of hIL-10 in the serum. We speculate that this increase is due to the effect of the acute inflammatory process that leads to cell death. However, this process needs further study using advance imaging systems. Finally, Kim *et al.* [27] reported the production of IL-10 by the BMSC as an important immune

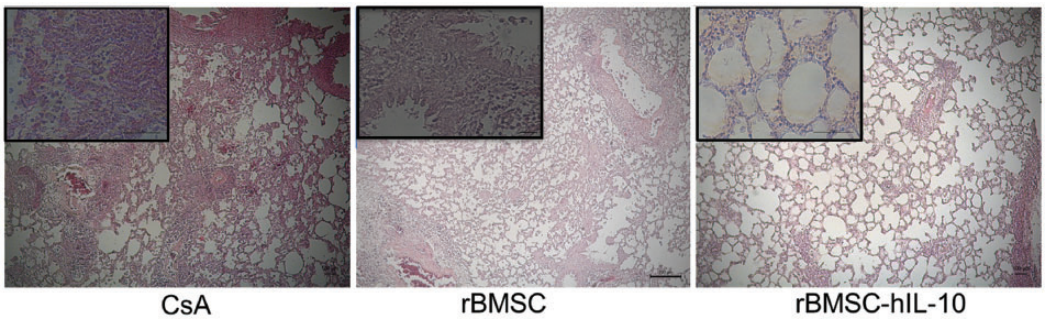

Figure 3: Histological representation of the graft on Day 5 for the different groups. In the control group with CsA only (International Society for Heart and Lung Transplantation (ISHLT; IIIB), a dense peribronchiolar, perivascular and interstitial leucocytic infiltration, slight alveolar oedema and moderate macrophages are seen in the alveoli; also, the inflammatory infiltrate involves the epithelium, causing epithelial damage. The inset, which is at a higher magnification, shows increased numbers of cellular infiltrates in the epithelium. In the rBMSC-only group (ISHLT Level II–IIIA), areas of expansion of the perivascular interstitium by mononuclear cells and the extension of the same cells into the adjacent perivascular alveolar septa are visible. Moreover, relatively clear alveoli are visible with comparatively less cellular infiltrate and rescued epithelium. In the hIL-10B MSC group (ISHLT Level I–IIA), perivascular mononuclear infiltrate is visible, with minimal peribronchiolar mononuclear cell infiltrate, sparing the respiratory epithelium. Moreover, the alveoli are much clearer, with minimal cellular infiltrate and clear alveoli; the alveolar epithelium looks unchanged and intact (n = 5 in each group). BMSC: bone marrow-derived mesenchymal stem cell; CsA: cyclosporine A; hIL-10: human interleukin 10; MSC: mesenchymal stem cell.

modulator in their islet transplant study. We engineered the BMSC to produce more IL-10, thereby enhancing the efficiency of the BMSC and exerting a synergistic effect. We only tested local delivery to the graft, because IL-10 acts as a proinflammatory cytokine when it is overexpressed after the onset of an inflammatory process [29]. Multiple doses of BMSC alone after transplantation could also be an interesting approach to further enhance the beneficial effect; however, we did not test it.

Limitations

Although we have preliminary data suggesting that hIL10 BMSC is a promising approach for treating acute graft rejection, we do not yet have detailed mechanistic proof. Moreover, another limitation is the short-term follow-up period. It would be interesting to see the effect after a longer period. Also, our model does not allow us to introduce more volume in the graft after implantation; therefore, we were unable to test the effect of administering more BMSC after implantation. Although the current study offers a good model, further preclinical study is required using a larger animal model to prove the concept before clinical translation can occur.

CONCLUSION

In conclusion, hIL-10 BMSC is a promising immunomodulatory agent for attenuating acute lung rejection. In addition, BMSCs are easily isolated in culture and can be transfected *in vitro* using non-viral vectors. Thus, modified BMSC therapy has the potential to reduce the severe toxicity of immunosuppressant drugs, to suppress an immune rejection and to induce immune tolerance following clinical transplantation. However, further investigations are required to understand the detailed mechanisms. Moreover, researchers have recently agreed to standardize the methods used to characterize the immunomodulatory effect of mesenchymal cells for regulatory issue, thus facilitating advanced clinical studies and their eventual acceptance for clinical applications [30].

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Conflict of interest: none declared.

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