

Original Article

IL-1RA gene-transfected bone marrow-derived mesenchymal stem cells in APA microcapsules could alleviate rheumatoid arthritis

Jianhua Hu, Hongjian Li, Guanhao Chi, Zhao Yang, Yi Zhao, Wei Liu, Chao Zhang

Department of Orthopedics, First People's Hospital of Yunnan Province, Kunming 650032, Yunnan Province, China

Received November 19, 2014; Accepted January 9, 2015; Epub January 15, 2015; Published January 30, 2015

Abstract: Objectives: In order to investigate the encapsulation of interleukin 1 receptor antagonist (IL-1RA) gene-modified mesenchymal stem cells (MSCs) in alginate-poly-L-lysine (APA) microcapsules for the persistent delivery of interleukin 1 receptor antagonist (IL-1RA) to treat Rheumatoid arthritis (RA). Methods: We transfect mesenchymal stem cells with IL-1RA gene, and quantify the IL-1RA proteins released from the encapsulated cells followed by micro-encapsulation of recombinant mesenchymal stem cells, and thus observe the permeability of APA microcapsules and evaluate clinical effects after induction and treatment of collagen-induced arthritis (CIA). The concentration of IL-1RA in the supernatant was determined by IL-1RA ELISA kit by run in technical triplicates using samples from three separate mice. Results: Encapsulated IL-1RA gene-transfected cells were capable of constitutive delivery of IL-1RA proteins for at least 30 days. Moreover, the APA microcapsules could inhibit the permeation of fluorescein isothiocyanate-conjugated immunoglobulin G. Also, it has been found that the APA microcapsules can significantly attenuate collagen induced arthritis after delivering of APA microcapsules to rats. Conclusions: Our results demonstrated that the nonautologous IL-1RA gene-transfected stem cells are of potential utility for RA therapy.

Keywords: Rheumatoid arthritis, IL-1RA gene-modified mesenchymal stem cells, encapsulation, alginate-poly-L-lysine microcapsules, persistent delivery

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by pain and inflammation, progressive joint destruction, significant disability, systemic manifestations and premature mortality [1]. The pathogenesis of RA is complex and involves the activation of several synovial cell populations and the production of many proinflammatory and destructive mediators including cytokines, prostaglandins and proteases. Many investigators have suggested that tumor necrosis factor (TNF) and interleukin 1 (IL-1) play a critical part in the pathogenesis of RA [2]. The IL-1 family includes IL-1, IL-1 and IL-1 receptor antagonist (IL-1Ra) [3]. IL-1Ra has been given therapeutically in several experimental models of arthritis with dramatic effects [4]. Importantly, these treatments have demonstrated efficacy in 60% of RA patients. Despite the many benefits of these systemic agents, their side effects, short dura-

tion of effect, need for long-term treatment and inability to cure the disease, cause them to fall short of optimal RA therapy. For these reasons, the development of targeted gene therapy is an increasingly attractive option for long-term RA disease control. In order to make gene therapy a viable therapeutic option for RA the following need to be optimized: gene-delivery, vectors, candidate molecules and targets and methods to regulate transgene expression [5].

To overcome existing problems associated with drug delivery to articular tissues, we have explored the application of gene therapy to the treatment of RA and other joint pathologies [6]. The development of effective gene delivery systems capable of secreting proteins with anti-arthritic or therapeutic properties into the tissues of the joint space may provide an effective method for treating RA. Viral vectors currently provide the most efficient system for high-level transgene expression in vivo. Retrovirus (RV)

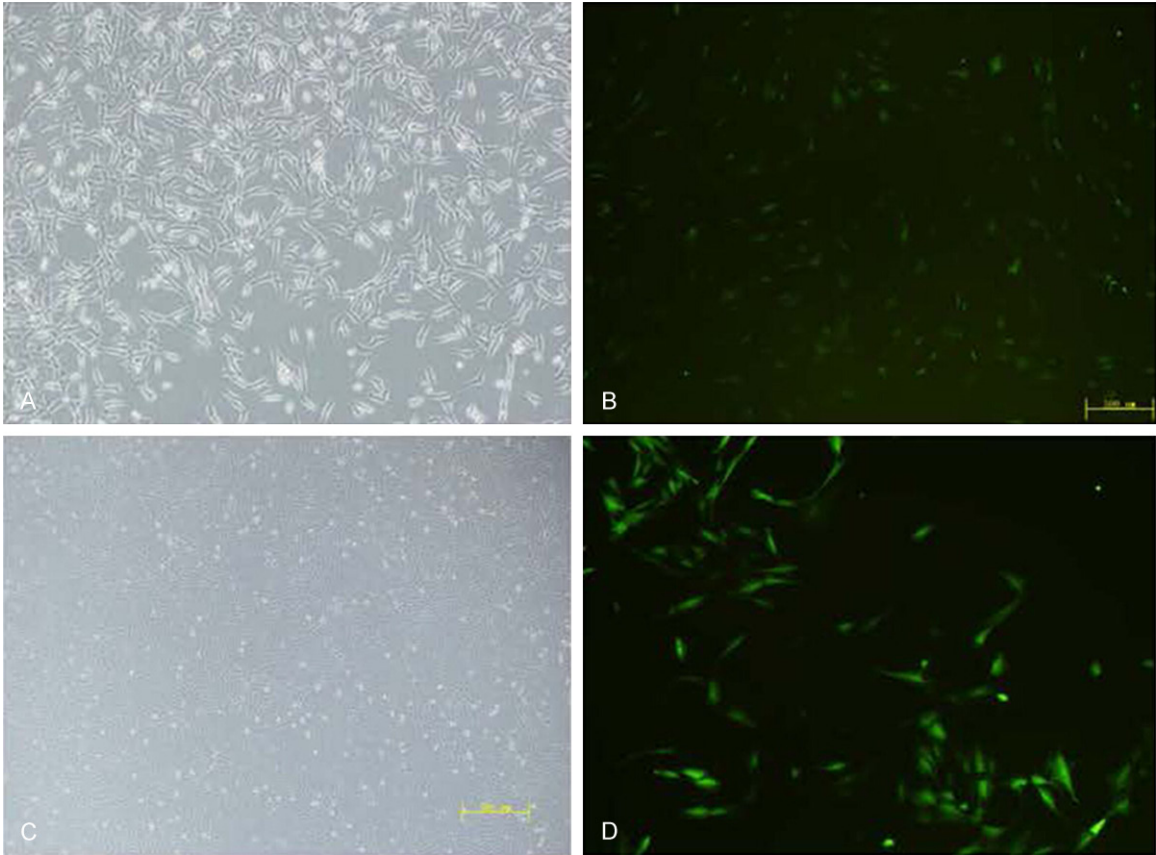


Figure 1. Preparation of PLXRN-IL-1Ra transfected MSCs. A: White field of Pt67 cells; B: PLXRN-IL-1Ra lentivirus packed in Pt67 cells; C: White field of BMSCs; D: BMSCs transfected with PLXRN-IL-1Ra lentivirus.

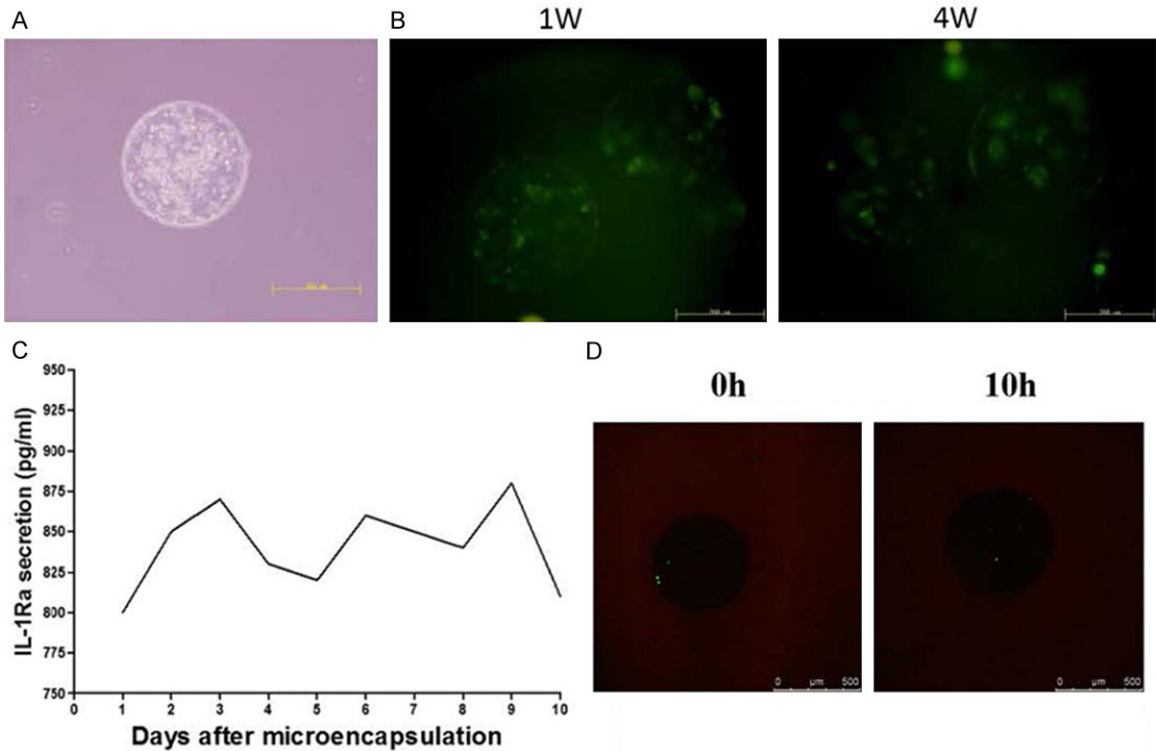


Figure 2. IL-1RA secretion and immunoisolation characteristics of the microcapsules. A: White field microcapsules, which were spherical, smooth, and of uniform size; B: Fluorescence of microcapsules at 1 week and 4 weeks; C: IL-1RA secretion from microcapsules; D: The intact APA microcapsules prevented the entry of the fluorescein isothiocyanate-immunoglobulin G.

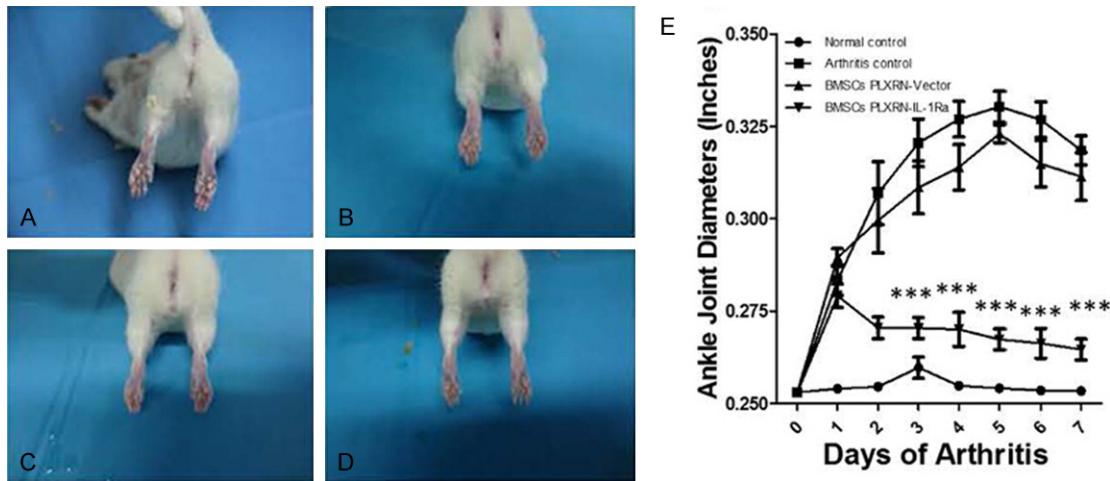


Figure 3. Microcapsules improved histological changes and arthritis scores in SD rats. Representative photographs of tarsotibial joint swelling of the hind paws in mice treated with normal control (A), arthritis control (B), arthritis PLXRN-vector (C) or arthritis PLXRN-IL-1Ra (D); (E) Arthritis scores during the progression of CIA. Error bars represented SEM, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ indicated significant differences from the control group.

and lentivirus (LV) vectors are commonly used vehicles for ex vivo gene transfer owing to their ability to integrate transgenes directly into the host genome, providing the advantage of stable expression. Several studies have shown effective transduction and protein expression within human synovial cells using RV/LV vectors [7, 8]. Drawbacks to the use of RV vectors include the poor transduction of nondividing cells and poor in vivo transduction, although there has been some success with direct in vivo transfer at very high retroviral titers [9]. Lentivirus vectors, derived from RVs, have the benefit of infecting quiescent cells by penetration through the nuclear membrane. Another concern with RV/LV vectors is the risk for mutagenesis as a result of random genome insertion [10].

Microencapsulated universal cell lines were introduced to overcome the drawbacks of the RV therapy. The microcapsule membrane is fabricated so that its permeability permits the exit of the recombinant product and allows glucose and other nutrients to diffuse freely through the membrane but excludes the larger immune mediators such as complement, lymphocytes, and macrophages responsible for the destruction of allogeneic cells [11]. This immunoisolation ensures that the same recombinant encapsulated cell line can be implanted into different

affected individuals to deliver the desired product, potentially reducing the cost and increasing the safety of gene therapy. In this article, we prepared microencapsulated Mesenchymal stem cells (MSCs) which were transfected with IL-1RA lentivirus to treat RA. The microencapsulated MSCs showed fine immunoisolation effect and stable IL-1RA secretion, therefore, can significantly attenuate collagen-induced arthritis. The findings above provided a safety and effective therapy on RA treatment.

Materials and methods

Ethics statement

This study was carried out in strict accordance with the recommendations of the First People's Hospital of Yunnan Province. The in vivo study protocol was approved by the Committee on the Ethics of Animal Experiments of the First People's Hospital of Yunnan Province.

Cell culture and transfection of mesenchymal stem cells with IL-1RA gene

Isolation of bone marrow mesenchymal stem cells (BMSCs) from Sprague-Dawley (SD) rats has been illustrated before [3]. In brief, bilateral femurs of SD rats were recovered by dissec-

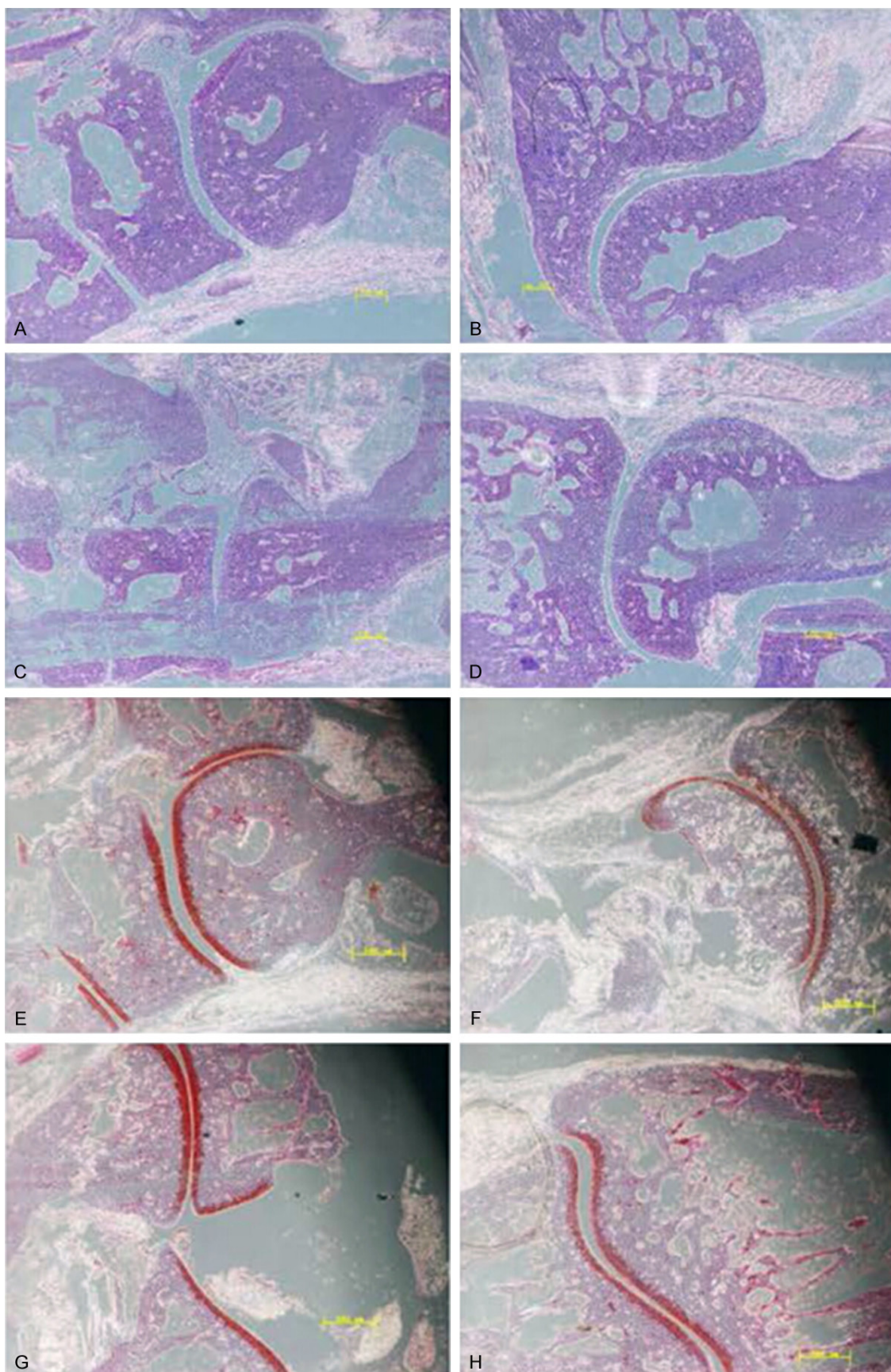


Figure 4. Microcapsules improved cartilage destruction in CIA model. Representative histopathologies of the ankle joints stained with H&E in CIA treated with normal control (A), arthritis control (B), arthritis PLXRN-vector (C) or arthritis PLXRN-IL-1Ra (D). And representative histopathologies of the ankle joints stained with Oil red O in CIA treated with normal control (E), arthritis control (F), arthritis PLXRN-vector (G) or arthritis PLXRN-IL-1Ra (H).

tion, and the soft tissues were detached aseptically. Metaphysis from both ends were resected, and bone marrow was collected by flushing the diaphysis with 10 mL of DMEM (Life Technology, Gaithersburg, MD, USA) containing 10% heat-inactivated FBS (Hyclone, Logan, UT, USA) and antibiotics (penicillin, 100 U/mL; Streptomycin, 100 g/mL). Single-cell suspensions were obtained by drawing bone marrow into a syringe through a 22-gauge needle. Confluent BMSCs were released by 5-min exposure to a solution of 0.05% trypsin and passaged at a 1:3 split. Passage 2 BMSCs were incubated with the lysate of the recombinant lentivirus carrying the human IL-1RA gene (PLXRN-IL-1Ra) or the recombinant lentivirus carrying the firefly luciferase gene only (PLXRN-vector). The PLXRN-IL-1Ra and PLXRN-vector were constructed by in vivo homologous recombination in Pt67 cells Clontech company's protocol. Infected cells were continuously cultured in complete DMEM with FBS after removal of transfection medium.

Microencapsulation of recombinant mesenchymal stem cells

APA microcapsules were produced by a special high-voltage electrostatic microcapsule generator, as previously described, with minor modifications [3]. Briefly, a suspension of cells at a concentration of 1×10^6 /mL was mixed with 1.5 g/mL alginate in a syringe and extruded as droplets through a 27-gauge needle with a syringe pump. The gelled droplets were collected in a 1.1% CaCl_2 solution. The outer alginate layer was cross-linked with PLL for 6 min and then coated with alginate. The core of unpolymerized alginate was dissolved with sodium citrate for 5 min to yield microcapsules with the cells suspended. All the above washing procedures were performed at 4–10°C. The collected microcapsules were cultured in the dish at 37°C for further determination.

Quantification of the IL-1RA proteins released from the encapsulated cells

Five aliquots of encapsulated IL-1RA gene-transfer BMSCs were cultured in 15 mL DMEM with

2.5×10^6 cells in each aliquot for 30 days. The same amounts of encapsulated non-gene transfer BMSCs served as the control. Each day, the supernatant was collected and kept the medium unchanged. The concentration of IL-1RA in the supernatant was determined by IL-1RA ELISA kit (R&D Systems, Minneapolis, MN, USA). The assay was performed according to the protocol recommended by the manufacturer.

Observation of the permeability of APA microcapsules

The APA microcapsules were added into 200 L of 0.05% weight per volume FITC-Ig G solution. The microcapsules were then shaken in solution at room temperature for 1 h and 10 h, and then examined by confocal laser scanning microscopy.

Induction and treatment of collagen-induced arthritis (CIA) and evaluation of clinical effects

CIA induction and clinical evaluation were described as previously with minor revise [12]. In brief, female rats (8 per group) were given intradermal/subcutaneous (SC) injections of bovine type II collagen (2 mg/mL in Freund's incomplete adjuvant) at a single site at the base of the tail and over the back at 2 sites (250 µL in divided doses) on day 0 and day 7. Arthritis onset occurred on days 12, 13, and 14; as rats developed disease, they were randomized divided into four groups: rats with no treatment (normal control), rats were induced with collagen but did not treat with microcapsules (arthritis control), rats induced with collagen and treated with microcapsules containing PLXRN-vector transfected BMSCs (arthritis PLXRN-vector), rats induced with collagen and treated with microcapsules containing PLXRN-IL-1Ra transfected BMSCs (arthritis PLXRN-IL-1Ra). Treatment was initiated on the first day that clinical signs of arthritis were clearly visible, as evidenced by ankle joint swelling. Treatment with microcapsules containing PLXRN-IL-1Ra or PLXRN-vector transfected BMSCs was administered SC beginning on day 1 of arthritis and continuing through day 6. Caliper

measurements of ankle joint diameter were made prior to the onset of arthritis, on the day of randomization (day 1 of arthritis), and on each subsequent study day until termination of the study on day 7 of arthritis. At termination, hind paws and knee joints were then collected into formalin for Histopathological evaluation.

Histopathology

Histopathological changes were described as before [12]. Ankle joints were collected into 10% neutral buffered formalin and maintained for at least 24 hours prior to placement in SurgiPath Decalcifier I solution (Grayslake) for 1 week. When decalcification was complete, the digits were trimmed, and the ankle joint was transected in the longitudinal plane to give 2 approximately equal portions. These were processed for paraffin embedding, sectioned, and stained with hematoxylin and eosin for general evaluation and with Oil red O for specific evaluation of cartilage changes. Multiple sections were prepared to ensure that the distal tibia was present with both cortices and that abundant distal tibial medullary space was available for evaluation.

Statistical analysis

Two-way analysis of variance was used to compare means across groups. A level of $P < 0.05$ was used to indicate statistical significance.

Results

Generation of IL-1RA transfected BMSCs

Retroviral vector PLXRN carrying IL-1Ra (PLXRN-IL-1Ra) gene was packed and amplified in the PT67 cells. Viral titer was determined by infecting NIH/3T3 cells with serially diluted viral supernatants (**Figure 1A, 1B**). BMSCs from SD rats showed characteristic morphology of a small cell body with a few long and thin cell processes (**Figure 1C**). BMSCs were transfected with PLXRN-IL-1Ra lentivirus with proper viral titer, and showed high IL-1RA expression (**Figure 1D**).

IL-1RA secretion and immunoisolation characteristics of the microcapsules

The microcapsules were generally spherical, smooth, and of a uniform size, with a diameter of about 250 m (**Figure 2A**). The fluorescence

of the encapsulated cells was still positive 28 days after encapsulation (**Figure 2B**). This indicated that the cells were viable and could express the gene products in the microcapsules. Then, the IL-1RA proteins in the supernatants from the encapsulated IL-1RA gene-transfected MSCs were collected and tested. And found that the IL-1RA secretion was high and continuous (**Figure 2C**). Little IL-1RA protein was detected in the supernatant from the control cells. A photograph taken by confocal laser scanning microscopy showed that the intact APA microcapsules prevented entry of the FITC-Ig G as long as 10 hours (**Figure 2D**), which indicates that the APA microcapsules in vivo could play immune-isolation effect.

IL-1RA transfected MSCs can attenuate collagen induced arthritis

It has reported that 100 mg/kg body weight IL-1RA could attenuate 50% of the ankle diameter [13]. According IL-1RA doses and BMSCs IL-1RA secretion, about 1×10^6 BMSCs were delivered to rats paws subcutaneously. The arthritic signs characterized by erythematous edema appearing in limbs and joint swelling were markedly improved. Representative photographs of the tarsotibial joint swelling of the hind paws from SD rats were shown in **Figure 3A-D**. IL-1RA transfected BMSCs significantly decrease the ankle joint diameter in CIA mice (**Figure 3E**). H&E staining of the ankle joints indicated that administration of IL-1RA resulted in a significant suppression of these Histopathological changes compared to control group (**Figure 4A-D**). The degradation and destruction of articular cartilage tissues was assessed by Oil red O staining, a method to detect the matrix proteoglycan depletion. Proteoglycan loss in the ankle joints of groups treated with IL-1RA was significantly decreased than that of control groups (**Figure 4E, 4F**), stating that the cartilage profiles in RA have been significantly improved after the administration of S IL-1RA transfected BMSCs as illustrated by the increased deep Oil red O staining in cartilage tissues (**Figure 4F**).

Discussion

In this study, we constructed IL-1RA lentivirus, and transfected them into BMSCs successfully. The APA encapsulated PLXRN-IL-1Ra transfected BMSCs could secret IL-1RA stably at a high

concentration, furthermore, could isolate the antibody outside to avoid elimination by innate immunity. In CIA model, encapsulated PLXN-IL-1Ra transfected BMSCs could attenuate collagen induced arthritis.

To obtain a sufficient therapeutic level of IL-1RA secretion, we tried to encapsulate the cells at as high a concentration as possible. But capsules prepared with the highest concentration of cells exhibited some irregular shapes and broken membranes, 1×10^6 cells/mL of alginate were proper that showed uniformly-sized intact spheres with a diameter of about 200-300 μ m [14].

Cell survival and long-term expression of transgenes is important for the success of somatic gene therapy with cell encapsulation. We found that, 4 weeks after encapsulation, the BMSCs were still alive by fluorescence detection. ELISA array results indicated that the IL-1RA proteins could be secreted stably from the microcapsules for 1 month. Further CIA animal experiment showed that delivery of IL-1RA proteins by encapsulation is enough to ameliorate collagen induced arthritis.

The main advantage of cell microcapsules is their protective from innate immunity, which could permit the transplantation extra tissue cells. BMSCs have been reported to suppress the activity of a broad range of immune cells, but recently data indicated that BMSCs interact with natural killer cells and act as antigen presenting cells with interferon-stimulation [15-17]. Lenti-virus vectors, which have been used in most gene therapy strategies, are reported to induce immune response [9, 10]. Our data have showed that intact APA microcapsules can inhibit permeation of FITC-Ig G. Because the rejection of xenograft is mediated by the humoral immune system and because this response is initiated through the binding of Ig M and Ig G antibodies, these results indicate that APA microcapsules could be used to encapsulate xenogeneic cells. APA microcapsules could prevent antibody conjugation, thereby facilitating immunologic acceptance of xenografts.

Our results demonstrate that gene-modified stem cells can survive in APA microcapsules and prevent immune response. Encapsulated IL-1RA gene-transfected cells are capable of constitutive synthesis and delivery of biologi-

cally active IL-1RA proteins for at least 30 days. In collagen induced arthritis, encapsulated IL-1RA gene-transfected cells could ameliorate ankle joint diameter and degradation and destruction of articular cartilage tissues. Our results provide a new strategy to cure RA.

Acknowledgements

This research was supported by a grant from Yunnan Science Fund (Grant No. 2009CD200).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chao Zhang, Department of Orthopedics, First People's Hospital of Yunnan Province, Kunming 650032, Yunnan Province, China. Tel: +86-87163638549; Fax: +86-87163638549; E-mail: chaozhang286@fexmail.cn

References

- [1] Pincus T. Long-term outcomes in rheumatoid arthritis. *Br J Rheumatol* 1995; 34 Suppl 2: 59-73.
- [2] Arend WP, Dayer JM. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor alpha in rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 151-60.
- [3] Ding HF, Liu R, Li BG, Lou JR, Dai KR, Tang TT. Biologic effect and immunisolating behavior of BMP-2 gene-transfected bone marrow-derived mesenchymal stem cells in APA microcapsules. *Biochem Biophys Res Commun* 2007; 362: 923-927.
- [4] Van den Berg WB. Lessons for joint destruction from animal models. *Curr Opin Rheumatol* 1997; 9: 221-228.
- [5] Kay J, Calabrese L. The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. *Rheumatology (Oxford)* 2004; 43 Suppl 3: iii2-9.
- [6] Evans CH, Robbins PD. Gene therapy of arthritis. *Intern Med* 1999; 38: 233-239.
- [7] Byers S, Rothe M, Lalic J, Koldej R, Anson DS. Lentiviral-mediated correction of MPS VI cells and gene transfer to joint tissues. *Mol Genet Metab* 2009; 97: 102-108.
- [8] Gouze E, Pawliuk R, Pilapil C, Gouze JN, Fleet C, Palmer GD, Evans CH, Leboulch P, Ghivizzani SC. In vivo gene delivery to synovium by lentiviral vectors. *Mol Ther* 2002; 5: 397-404.
- [9] Thomas CE, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. *Nat Rev Genet* 2003; 4: 346-58.
- [10] Themis M. Monitoring for potential adverse effects of prenatal gene therapy: genotoxicity

- analysis in vitro and on small animal models ex vivo and in vivo. *Methods Mol Biol* 2012; 891: 341-370.
- [11] Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980; 210: 908-910.
 - [12] Cornelissen F, Asmawidjaja PS, Mus AM, Corneth O, Kikly K, Lubberts E. IL-23 dependent and independent stages of experimental arthritis: no clinical effect of therapeutic IL-23p19 inhibition in collagen-induced arthritis. *PLoS One* 2013; 8: e57553.
 - [13] Bao J, Yue T, Liu W, Zhang Q, Zhou L, Xu HJ, Dai SM. Secondary failure to treatment with recombinant human IL-1 receptor antagonist in Chinese patients with rheumatoid arthritis. *Clin Rheumatol* 2011; 30: 697-701.
 - [14] Liu R, Ma GH, Wan YH, Su ZG. Influence of process parameters on the size distribution of PLA microcapsules prepared by combining membrane emulsification technique and double emulsion-solvent evaporation method. *Colloids Surf B Biointerfaces* 2005; 45: 144-153.
 - [15] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005; 105: 1815-1822.
 - [16] Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, Muroi K, Ozawa K. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood* 2007; 109: 228-234.
 - [17] Chan JL, Tang KC, Patel AP, Bonilla LM, Pierobon N, Ponzio NM, Rameshwar P. Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon-gamma. *Blood* 2006; 107: 4817-4824.