Therapeutic Effect of Exogenous Treg Cells on Collagen-Induced Arthritis and Rheumatoid Arthritis

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Research article

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Abstract

Background Regulatory T (Treg) cells have anti-inflammatory and anti-autoimmune functions. The proportion and functions of Treg cells are perturbed in rheumatoid arthritis (RA) patients. **Methods** Human Treg cells were induced to amplify in vitro and cocultured with RA synovial fibroblast cells (RASFs). The proliferation and apoptosis of RASFs were determined by the cell counting kit-8 (CCK-8) assay and flow cytometry, respectively. Human Treg cells were also injected to collagen-induced arthritis (CIA) rats via the tail vein. Changes in lymphocyte subtypes and cytokines in the peripheral blood and spleen were observed by flow cytometry. **Results** After coculture with the Treg cells, the proliferation of RA synovial fibroblast cells decreased ($p < 0.01$), and the rate of apoptosis increased ($p = 0.037$). The human Treg cells were injected into the tail veins of collagen-induced arthritis (CIA) rats. The severity of the CIA was reduced ($p < 0.01$) following the injection, the percentages of rat endogenous Treg cells in the peripheral blood and spleen increased significantly ($p = 0.007$ and $p < 0.01$, respectively), and the proportion of B cells decreased ($p = 0.031$). The levels of interleukin IL-5 and IL-6 and the Th1/Th2 ratio in the peripheral blood were significantly decreased ($p = 0.013$, 0.009 and 0.012 , respectively). The number of NK cells and the levels of IL-4, IL-13, TNF- α , IFN- γ and GM-CSF in the peripheral blood and spleen did not change significantly. **Conclusion** These results suggest that exogenous Treg cells play a therapeutic role in RA and CIA. Treg cell treatment could serve as a therapy for RA.

Background

Rheumatoid arthritis (RA) is a common autoimmune disease. The main manifestation of RA is chronic, systemic joint inflammation. Abnormal proliferation of a large number of RA synovial fibroblast (RASf)-like cells, infiltration of inflammatory cells, abnormal secretion of inflammatory factors, thickening of the synovial lining layer and the destruction of articular cartilage and bone are observed in diseased joints [1]. Currently, the pathogenesis of RA is not entirely clear. Dysregulation of the naïve T cell (T_n) subset is an important step in RA pathogenesis [2]. Due to the influence of cytokines, T_n cells differentiate into a variety of cell subsets with different functions. Interleukin (IL)-2 and a high concentration of tumor growth factor beta (TGF- β) induce T_n cells to differentiate into CD4⁺ CD25⁺ regulatory T (Treg) cells [3], which secrete the anti-inflammatory cytokines IL-10 and TGF- β to inhibit inflammatory responses [4]. Treg cells also interfere with the overactivation of T cells through direct contact with effector T cells [5]. Moreover, Treg cells inhibit effector T cell proliferation by competing with them for IL-2 binding, and together with IL-2, TGF- β enhances Treg cell polarization [6]. A decrease in the proportion of Treg cells results in a decrease in these functions and can lead to unrestrained autoimmune responses. The number of Treg cells in the peripheral blood of RA patients is significantly decreased, their immunosuppressive function is lower than normal [7], and both of these features are especially important during the active stage of RA disease. After anti-rheumatism treatment of RA patients, the number of Treg cells in the peripheral blood increases significantly [8].

The proportion of Treg cells is also robustly decreased in collagen-induced arthritis (CIA) rats [9]. The inflammation associated with CIA is aggravated in CD4⁺CD25⁺ Treg cell-deficient rats [10]. The injection of bone marrow-derived inhibitory cells increases the in vivo expression of Foxp3 and restores the Treg cell population to normal levels in CIA rats, resulting in a reduction in the severity of the arthritis [11]. In this study, human Treg cells and RASFs were cocultured to observe the effects of Treg cells on RASFs. Human Treg cells were also injected into CIA rats via the tail vein to assess the therapeutic effects of Treg cells on these rats. The purpose of this study was to explore the therapeutic effects of Treg cells on RA through in vitro cytological and animal experiments.

Methods

Tissue collection

Human synovial tissue samples were collected from RA patients (n=7, 4 females, 30-68 years old, mean age of 54) during knee joint arthroscopic synovectomy procedures. The diagnosis conformed to the revised criteria of the American College of Rheumatology. The RA patients were medicated with nonsteroidal anti-inflammatory drugs to help reduce the pain and swelling of the joints and to decrease stiffness. Patients were enrolled between June 2017 and October 2018 at Shandong Provincial Qianfoshan Hospital. All of the patients signed written informed consent statements for participation in the study. The study protocol was approved by the Medical Ethical Committee of Shandong Provincial Qianfoshan Hospital at Jinan (Approve number 20170306), China.

Induced amplification of Treg cells

Healthy human (n=7) peripheral blood was aseptically collected. The volunteer signed written informed consent for participation in the study. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation and were then plated in a culture flask coated with 5 µg/ml anti-human-CD3 antibody (Sungene, China). Treg cell differentiation and amplification were induced in Dulbecco's Modified Eagle's Medium (DMEM, HyClone, USA) containing TGF-β (PeproTech, USA), recombinant human IL-2

(T&L, China), anti-human CD28 antibody (Sungene, China) and 10% fetal bovine serum (FBS, Gibco, USA). Fresh growth medium was added every 2 days, and the first round of amplification was completed after 6 days. The cells were transferred to a culture flask without the antibody and were further cultured for 2 days in DMEM containing recombinant human IL-2 and 10% FBS. After 2 days, the cells were transferred to a culture flask coated with anti-human CD3 antibody and were amplified following the same method used for the first amplification cycle. The entire process required 14 days.

Assessment of Treg cell population expansion by flow cytometry

One-hundred microliter samples of the unamplified PBMCs and the Treg cell suspension following 14 days of amplification were collected, and anti-human CD25-fluorescein isothiocyanate (FITC, BioLegend, USA) and anti-human CD4-PerCP (BioLegend, USA) antibodies were added. The samples were then incubated in the dark for 30 min at 4°C. The cells were washed twice with phosphate-buffered saline (PBS) and then resuspended in PBS to form single-cell suspensions. Flow cytometry was used for detection (ACEA, USA) and, after phenotypic characterization, 5 Treg cell samples were pooled in equal proportions for the subsequent experiments.

Primary culture of RASFs

RA synovial tissue samples were aseptically disrupted, and DMEM containing 4% type II collagenase (Solarbio, China) was added, and the samples were then incubated in a 5% CO₂ at 37°C for 4 h until the tissue pieces were dispersed into a cell suspension. The cell suspension was filtered through a 70-µm cell strainer and then centrifuged at 1200 rpm for 5 min. The cells were resuspended in DMEM containing 10% FBS and incubated at 37°C in a 5% CO₂ incubator to obtain primary RASFs. Cells passaged for 3-8 generations were used in the subsequent experiments.

Assessment of RASF amplification via the cell counting kit-8 (CCK-8) assay

A single-cell suspension of RASFs was seeded into 96-well plates. Induced mature Treg cells were washed with PBS and seeded into the 96-well plates at a 1:1 ratio of RASFs to Treg cells. The cells were cocultured for 24-72 h. At the end of the coculturing, the suspended Treg cells were washed away with PBS, and CCK-8 (Dojindo, Japan) solution was added to the plates, which were then incubated for 3 h. The OD₄₅₀ values were measured using a microplate reader. The data were statistically analyzed using an independent samples t-test (BioTek, USA).

Assessment of RASF apoptosis using annexin V-propidium iodide (PI)/FITC

A single-cell suspension of RASFs was seeded into 6-well plates. Induced mature Treg cells were seeded into the 6-well plates at a 1:1 ratio of Treg cells to RASFs, and the cells were cocultured for 48 h. At the end of the coculturing, the suspended Treg cells were washed away with PBS, and a single-cell suspension of RASFs was collected and resuspended in binding buffer. FITC-conjugated antibody and PI-conjugated antibody (BioLegend, USA) were added, and the samples were then mixed well and incubated at room temperature for 15 min in the dark. Apoptosis was assessed by flow cytometry. The data were statistically analyzed using an independent samples t-test.

Assessment of the effects of coculture on cytokine levels via flow cytometry

A single-cell suspension of RASFs was seeded into 6-well plates. Induced mature Treg cells were seeded into the 6-well plates at a 1:1 ratio of Treg cells, and the cells were cocultured for 48 h. After the coculturing, the supernatant was collected, centrifuged at 1500 rpm for 10 min, and assayed for changes in cytokine levels using a human Th1/Th2 cytokine assay kit (Cell-Genebio, China). The data were statistically analyzed using one-way analysis of variance (ANOVA).

Treg cell treatment of CIA rats

A total of 30 rats (six week old) were purchased from Shandong Laboratory Animal Center (Jinan, China) and were randomly divided into a normal control (NC) group, a CIA group and a Treg treatment group (n=10 rats per group). The CIA rat model was established in all of the groups except for the NC group. Bovine type II collagen (Chondrex, USA) was mixed with complete Freund's adjuvant (Sigma, USA) in equal amounts and the mixture was fully emulsified. The initial immunization with 0.2 ml of emulsion per rat was performed by intracutaneous injection into the tail root. One week later, bovine type II collagen was mixed with incomplete Freund's adjuvant (Sigma, USA) in equal amounts and the mixture was fully emulsified. A booster immunization was performed via intracutaneous injection of 0.2 ml of emulsion per rat into the tail root. The rats in the Treg treatment group were injected with Treg cells (10^7 Treg cells/kg) via the tail vein simultaneously with the booster immunization, and the injection was repeated again one week later. The NC and CIA groups were injected with the same dose of PBS at the same time points. All mice were humanely euthanized by a lethal dose of ketamine and xylazine.

The breeding and operation of the experimental animals were carried out in accordance with the Helsinki Convention on Animal Protection and the Regulations of the People's Republic of China on the Administration of Experimental Animals. The study protocol was approved by the Medical Ethical Committee of Shandong Provincial Qianfoshan Hospital at Jinan (Approve number 20170306), China.

Evaluation of the general CIA conditions

After the start of treatment, the degree of ankle joint swelling was measured with a Vernier caliper, and the joints were photographed every 3 days. At the end of the experiment, an inflammation curve was plotted based on the degree of joint swelling over time. The data were statistically analyzed by repeated measurements ANOVA.

Histopathological examinations

The experiment was terminated on the 20th day after the booster immunization, following the start of treatment. Tissue samples located 1 cm above and below the right knee joint were collected, fixed in 4% paraformaldehyde (Solarbio, China) for 48 h, decalcified with EDTA and embedded in paraffin. Pathological changes in the joint tissue samples were observed under light microscopy after hematoxylin-eosin (HE) staining.

Assessment of the lymphocyte subsets in the peripheral blood and spleen of rats

The experiment was terminated on the 20th day after treatment. The animals were anesthetized via intraperitoneal injection of 30 mg/kg 3% sodium pentobarbital. For sample collection, each rat was fixed on its back, the abdominal cavity opened, and a blood sample was collected from the inferior vena cava. The spleen was removed and placed in 2 ml PBS where it was then cut into pieces with ophthalmic scissors and filtered through a strainer to obtain a single-cell suspension. Samples of both the peripheral blood and the spleen single-cell suspensions were collected after red blood cell lysis using red blood cell lysis buffer. Next, anti-rat CD4-FITC (BioLegend, USA) and anti-rat CD25-phycoerythrin (PE, BioLegend, USA) were added for the detection of Treg cells, anti-rat CD3-allophycocyanin (APC, BioLegend, USA) and anti-rat CD161-FITC (BioLegend, USA) were added for the detection of NK cells, and anti-rat CD3-APC (BioLegend, USA) and anti-rat CD45RA-PE (BioLegend, USA) were added for the detection of B cells. The samples were incubated at 4°C for 30 min in the dark, washed twice with 500 µl PBS, and examined after resuspending in PBS. The data were statistically analyzed using one-way ANOVA.

Assessment of cytokine concentrations in rats

The obtained blood samples were coagulated at room temperature for at least 30 min and then centrifuged at 3000 rpm for 20 min at 4°C to obtain serum. Changes in cytokine expression levels in the peripheral blood were detected by flow cytometry using a rat Th1/Th2 cytokine assay kit (BioLegend, USA). Th1 cells are characterized by interferon gamma (IFN-γ) secretion, and Th2 cells mainly secrete IL-4. The Th1/Th2 ratio was

calculated based on the average fluorescence intensities detected for IFN- γ and IL-4. The data were statistically analyzed using one-way ANOVA.

Statistical analysis

Normal and variance homogeneity tests were performed using SPSS 17.0 software, and data that met the test criteria are represented as $\bar{x} \pm s$. Independent and paired sample t-tests were used to determine significant differences between two groups. Repeated measurements and one-way ANOVA were used to determine significant differences between multiple groups. The least significant difference (LSD) method or the Tamhane method was used for pairwise comparisons. $P < 0.05$ was considered statistically significant.

Results

Amplification of human Treg cells

Fourteen days after induction of PBMC differentiation with anti-human CD3 and anti-human CD28 antibodies, IL-2 and TGF- β , the Treg cell phenotypes were examined by flow cytometry. The percentage of CD4⁺CD25⁺Treg cells in the cultures increased from 6.85% (unamplified) to 74.33% after amplification, indicating successful Treg cell amplification (**Fig. 1**).

Effects of Treg cells on RASFs

Treg cells were cocultured with RASFs for 24 , 48 , and 72 h and then washed with PBS to remove the suspended Treg cells. The proliferation of the adherent RASFs was measured via CCK-8 assays. Compared with the results obtained with for the NC group, the RASF proliferation was significantly reduced after 24 and 48 h of coculturing (1.17 ± 0.15 vs. 0.83 ± 0.14 , $P < 0.01$; and 1.30 ± 0.47 vs. 0.10 ± 0.12 , $P < 0.01$, respectively), suggesting that Treg cells inhibit RASF amplification (**Fig. 2**).

Treg cells were cocultured with RASFs for 48 h, and the apoptosis of the RASFs was measured by flow cytometry. Compared with the rate of apoptosis in the NC group (in which the RASFs were cultured alone), the apoptosis rate of the RASFs cocultured with Treg cells was significantly higher (1.73 ± 1.21 vs 10.31 ± 4.69 , $P=0.035$), suggesting that coculture with Treg cells promotes RASF apoptosis (**Fig. 3**).

Effects of Treg cell treatment on CIA

Rats were treated with Treg cells simultaneously with the second booster immunization. One week later, joint swelling was obvious in the rats in the CIA group compared with those in the NC group, which were not treated with collagen II, while the joint swelling observed in the rats in the Treg cell treatment group was mild (**Fig. 4A**). HE staining showed that the joint structure of the rats in the NC group was intact, the joint cavity was clean, the articular cartilage on both sides was even and smooth, and the synovial membrane showed no obvious hyperplasia. The CIA rats showed detached necrotic tissue masses in their joint cavities. Furthermore, local calcification basophilic enhancement was accompanied by infiltration of many inflammatory cells, consisting mainly of neutrophils and a small number of pus cells. The articular cartilage and bone tissue were absent locally, and a single layer of inflammatory cells was attached to the surface of the cartilage. There was clear synovial hyperplasia, along with an increase in the number of capillaries, which was accompanied by obvious vasospasm. In the rats in the Treg cell treatment group, the joint structure was intact, the joint cavity was clean, the articular cartilage on both sides was even and smooth, and the synovial membrane showed no obvious hyperplasia (**Fig. 4B**). The inflammation curve showed that the degree of joint swelling in the rats in the Treg cell treatment group was significantly lower than that of the rats in the CIA group ($F=5.023$, $P<0.01$, **Fig. 4C**).

The peripheral blood and spleen lymphocyte subsets following Treg cell treatment were examined by flow cytometry. The proportions of rat Treg cells in the peripheral blood and spleen of the animals in the CIA group were significantly lower than those of the animals in the NC group ($P=0.020$; $P<0.01$). After treatment with human Treg cells, the proportion of

rat Treg cells increased significantly compared with the number detected in the animals in the CIA group ($P=0.007$; $P<0.01$). The proportions of NK cells in the peripheral blood and spleen of the animals in the CIA group were higher than those of the animals in the NC group ($P=0.012$; $P=0.017$), and the proportions of NK cells in the animals in the Treg cell treatment group were even higher ($P=0.003$; $P=0.001$). However, there was no significant difference between the animals in the CIA group and those in the Treg treatment group. The proportion of peripheral blood B cells in the animals in the CIA group was significantly higher than that in the animals in the NC group ($P=0.004$). Furthermore, the proportion of B cells in the animals in the Treg treatment group was lower than that of the animals in the CIA group; however, the difference was not statistically significant. The proportion of B cells in the spleen was the highest in the animals in the CIA group, and the difference was statistically significant compared with the B cell proportions in the NC and Treg treatment groups ($P=0.036$; $P=0.031$, **Fig. 5**).

Flow cytometry was used to measure cytokine levels in the peripheral blood of rats treated with Treg cells. The levels of TNF- α , IFN- γ , granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, IL-5, IL-6, IL-10 and IL-13 were higher in the peripheral blood of the rats in the CIA group compared with the levels detected in the animals in the NC group ($P=0.007$, 0.019, 0.016, 0.043, 0.002, 0.002, 0.002 and $P=0.030$, respectively). The levels of IL-5, IL-6 and IL-10 in the peripheral blood of animals in the Treg treatment group were lower compared with the levels detected in the animals in the CIA group ($P=0.013$, 0.009 and 0.006, respectively); furthermore, there were no statistically significant differences between the levels of these cytokines in the animals in the Treg treatment group and those in the NC group, suggesting that IL-5, IL-6 and IL-10 had recovered to normal levels (**Fig. 6A**). We also analyzed the Th1/Th2 ratio based on the IFN- γ and IL-4 levels. The Th1/Th2 ratio in the animals in the CIA group was higher than that in the animals in the NC group. After Treg cell treatment, the Th1/Th2 ratio in the treated animals was significantly lower compared with that of the animals in the CIA group ($P=0.012$), and there was no significant difference compared with the NC group (**Fig. 6B**).

Discussion

Abnormal RASF proliferation and apoptosis are important pathological features of RA [12]. In this study, we cocultured RASFs with human Treg cells derived from in vitro induction and differentiation, and we found that Treg cells inhibited RASF proliferation and promoted their apoptosis, suggesting that Treg cells play an inhibitory role in the excessive activation of RASFs in the pathogenesis of RA. The pathogenesis of RA is closely related to immune system disorders. Previous studies showed that the number of Treg cells in RA patients is negatively correlated with the Disease Activity Score 28 (DAS28), the C-reactive protein (CRP) level and the erythrocyte sedimentation rate (ESR) [13]. Furthermore, RA patients have fewer Treg cells, and the immune suppressive function of these cells is defective [14]. Therefore, Treg cells play a key role in RA pathogenesis. The results of the animal experiments described herein revealed that the proportions of rat Treg cells in the peripheral blood and spleen of CIA rats were lower than those of normal rats. The inflammatory curve and histochemical results of CIA rats treated with human Treg cells indicated an alleviation of their arthritis. The proportion of rat Treg cells was increased in the animals treated with human Treg cells. Moreover, the IL-6 level in the peripheral blood of CIA rats was higher than that in the animals in the NC group, while the IL-6 level was significantly lower after Treg cell treatment. These results indicate that human Treg cells have a significant therapeutic effect on CIA. Previous studies showed that Treg cells obtained by in vitro induction had more robust therapeutic effects on colitis in mice compared with thymus-derived natural Treg cells and that they can reduce the IL-6 expression in mesenteric lymph nodes [15], observations that complement the results of this study. Moreover, in vitro induction can yield a large number of induced Treg cells, which are more stable than natural Treg cells [16]. Treg cells can induce transformation of Tn cells into Treg cells when TGF- β on the surface of the Treg cells comes into direct contact with Tn cells, a process known as infectious immune tolerance [17]. In this study, human Treg cells had a therapeutic effect on rat CIA, while no rat immunological rejection responses were observed. Furthermore, the number of rat Treg cells in the animals in the Treg cell treatment group was higher than that in the animals in the CIA group; therefore, we speculate that human Treg cells may stimulate the rats to produce more Treg cells. In addition to antibody production, B cells also promote the activation and proliferation of T cells, stimulate cytokine production, participate in cell-cell interactions, and promote RA pathogenesis [18]. Our results showed that the number of B lymphocytes in the spleens of animals in the Treg cell-treated group was lower than that in the spleens of animals in the CIA group, suggesting that Treg cell therapy may also play a role by reducing B cell levels. Previous studies showed that coculture of Treg cells with B cells inhibits B cell activation and proliferation [19], consistent with our results. The numbers of NK cells in the peripheral blood and spleen and the IL-4, IL-13, TNF- α , IFN- γ and GM-CSF levels in the peripheral blood of the animals in the CIA group were significantly higher than those of the animals in the healthy control group, although these features were not significantly different from those of the animals in the Treg cell treatment group. These results indicate that the therapeutic effects of Treg cells on CIA are not exerted via regulation of the number of NK cells or the levels of IL-4, IL-13, TNF- α , IFN- γ or GM-CSF. RA pathogenesis is closely related to immune system disorders. Disruption of the Th1/Th2 cell ratio can play an important role in the development of RA [20]. Th1 cells secrete cytokines such as IFN- γ , IL-2, TNF- α , and GM-CSF to

mediate cellular immunity and are mainly characterized by IFN- γ secretion. Th2 cells secrete IL-4, IL-5, IL-6, IL-10, IL-13 and other cytokines to mediate fluid immunity and are mainly characterized by IL-4 secretion [21]. It is traditionally thought that the numbers of both Th1 and Th2 cells, although mainly Th1 cells, are high in RA [22]. Both clinical studies and animal experiments support the idea that a decrease in the Th1/Th2 ratio can favorably contribute to RA treatment [23]. The results of the current study showed that on the 20th day after Treg cell treatment, the levels of Th1- and Th2-related cytokines in the peripheral blood of animals in the CIA group were elevated and that the Th1/Th2 ratio was higher compared with that of the animals in the healthy control group, indicating a relatively low abundance of Th2 cells. After treatment with Treg cells, the levels of several cytokines, including IL-5, IL-6 and IL-10, were significantly lower in the treated animals compared with their levels in the animals in the CIA group; furthermore, the Th1/Th2 ratio also decreased and was near that of the animals in the healthy control group. Taken together, these results indicate that there are relatively more Th2 cells under these conditions and suggest that Treg cell therapy can exert a therapeutic effect on CIA by restoring a normal Th1/Th2 balance. The results of present study also showed that the levels of IL-5, IL-6 and IL-10 were higher in the peripheral blood of CIA rats and were significantly lower in the animals in the Treg cell treatment group. A clinical dynamic monitoring study revealed a significant increase in the serum IL-5 level in patients during the progression from joint pain to RA that was absent in patients who did not develop RA [24]. Injection of anti-IL-5 antibody into heart-transplanted mice can reduce collagen deposition and eosinophil infiltration in the graft and prevent a rejection response [25]. IL-6 is an important inflammatory factor in RA that is significantly elevated in the serum of RA patients, and it can damage inflamed joints [26]. The experiments in the present study showed that the expression levels of IL-6 and IL-5 were significantly lower following Treg cell therapy, supporting the therapeutic effects of Treg cells on CIA and their inhibitory effects on immune rejection. Although the IL-10 level was lower after Treg cell therapy, the Th1/Th2 ratio was also lower under these conditions; therefore, the CIA disease symptoms were still robustly alleviated.

Conclusion

Summarily, the results of the current study showed that Treg cells can inhibit RASF proliferation, while promoting their apoptosis. The introduction of human Treg cells into CIA model rats effectively inhibited the CIA symptoms, increased the number of rat Treg cells, and reduced the number of B cells, the Th1/Th2 ratio, and the secretion levels of IL-5 and IL-6. The results of this study clarify the cellular immunological mechanisms underlying the effects of Treg cells in the treatment of RA and suggest that Treg cell therapy is a potential treatment for RA.

Abbreviations

Cell counting kit-8 (CCK-8); collagen-induced arthritis (CIA); Dulbecco's Modified Eagle's Medium (DMEM); fetal bovine serum (FBS); fluorescein isothiocyanate (FITC); granulocyte-macrophage colony-stimulating factor (GM-CSF); interleukin (IL) interferon gamma (IFN- γ); nature killer (NK); naïve T cell (Tn); peripheral blood mononuclear cells (PBMCs); propidium iodide (PI); regulatory T (Treg)–rheumatoid arthritis (RA); rheumatoid arthritis synovial fibroblasts (RASFs); tumor growth factor beta (TGF- β); tumor necrosis factor alpha (TNF- α).

Declarations

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. The raw data can be requested from the corresponding author.

Authors' contributions

SL, HXW and HW performed the experiments and analyzed the data. GZ was the main contributor in sample acquisition. XC contributed to the study design, data analysis and interpretation and was a main contributor to the writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Medical Ethical Committee of Shandong Provincial Qianfoshan Hospital at Jinan (Approve number 20170306), China. All of the patients signed written informed consent statements for participation in the study.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Phenotypic analysis of Treg cells before and after 14 days of amplification.

Figure 2

Detection of RASF proliferation by the CCK-8 method. NC indicates RASF culture alone and RASF+Treg indicates coculture. *** represents a P value less than 0.001.

Figure 3

Detection of RASF apoptosis by annexin V-PI/FITC double staining. (A) Apoptosis of RASFs in the NC and coculture groups. (B) Statistical analysis of the proportion of RASF apoptosis. * represents a P value less than 0.05.

Figure 4

Therapeutic effects of human Treg cells on CIA rats. (A) Rat joint swelling degree. (B) HE staining of rat joints. (C) Inflammation curve. *** represents a P value less than 0.001.

A

Figure 5

Effects of Treg cell therapy on peripheral blood and spleen lymphocyte subsets in CIA rats. (A) Changes in peripheral blood lymphocyte subsets. (B) Changes in spleen lymphocyte subsets. * represents a P value less than 0.05, ** less than 0.01, and *** less than 0.001.

Figure 6

Effects of Treg cells on cytokine levels in the peripheral blood. (A) Changes in cytokine levels in the peripheral blood of rats after Treg cell therapy. B. Changes in the Th1/Th2 ratio in the peripheral blood of rats. * represents a P value less than 0.05, ** less than 0.01, and *** less than 0.001.

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