Remission of collagen-induced arthritis by adoptive transfer of peritoneal cells

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Abstract

Objective

The collagen-induced arthritis (CIA) model shares both immunological and pathological features with human rheumatoid arthritis (RA), thus it has been used extensively as a model to study the pathogenesis of RA and for testing therapeutics. It is well-known that the T helper cell 17 (Th17) responses are involved in the pathogenesis of RA, while the regulatory T cells (Tregs) are considered to limit the progress of disease. Previously, we found that peritoneal cells (PCs) possess immunosuppressive characteristics and it is conceivable that PCs potentially have the therapeutic benefits for RA. In this study, we investigated whether PCs are capable of Treg induction and therefore suppress Th17-mediated CIA.

Methods

Naïve PCs were intravenously transferred into CIA mice at the early clinical signs of arthritis. The treatment commenced on day 0 and then every other day until day 14. Clinical symptoms of arthritis, histological changes, cytokine expressions and cell population profiles were investigated.

Results

Intravenously administrating PCs ameliorated the severity of CIA. Further investigations unveiled that the reduction of Th17 cells and the induction of Tregs is ascribed to the remission of the disease. Specifically, when splenic PBMC were cultured with PCs, the expression of FOXP3 and IFN-γ was markedly induced. It is suggested that IFN-γ secreted by PCs plays an important role in the conversion of $CD4^+$T cells to Tregs.

Conclusion

The adoptive transfer of PCs is effective in the treatment of CIA by regulating the T cell differentiations. Our findings might provide a new strategy for RA therapy.

Key words
peritoneal cells, collagen-induced arthritis, Treg, Th17, IFN-γ

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Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune disease characterised by chronic inflammation, swelling and destruction of multiple joints (1-3). The aetiology and pathogenesis of RA have been associated with multi-factor, including the interaction of environmental, genetic, microbe and immune factors. To date, there is still a lack of effective therapy method to prevent the long-term progression of joint damage in RA patients. Clinically, it is hardly to cure the RA patients completely, and a cure of RA requires both anti-inflammation and re-establishment of immunological tolerance.

During the last decades, many new strategies have been developed to treat RA. Infliximab, a monoclonal antibody to TNF-α, is now broadly used in the treatment of RA. Although such treatments are effective in many cases of RA, a large patient population suffers tuberculosis and chronic infection thereafter (4, 5). Notably, cell therapy has been demonstrated to be of improved safety and certain efficacy for curing of RA by growing investigations. Regulatory T cells (Tregs) are well-known suppression cells, which characterised by highly expression of Foxp3. It is reported that Tregs can possess many immunosuppressive characteristics and the disturbances of the Tregs repertoire have been linked to the pathogenesis of autoimmune diseases (6). Other investigations found that the adoptive transfer of Tregs can effectively cure the antibody-mediated autoimmune diseases, such as experimental colitis and collagen-induced arthritis (7-9). However, the major limitation of this subset is that it is difficult to acquired sufficient number of Tregs from peripheral blood and tissues under non-elicited condition. Clinically, 127.1±9.0 CD4+CD25^{high} T cells can be separated from 1 μl health human blood, which means the CD4^{+}CD25^{high} T cell subset characterised in human peripheral blood normally represents 3% of lymphocytes (10). If we take the expression of FOXP3 into account, the number of Tregs could be less than 3% in lymphocytes. In addition, in order to get more Tregs, the CD4^{+} T cells would be stimulated with anti-CD3/CD28 and IL-2 *in vitro* (11). Obviously the expansion of immune cells is potentially associated with serious adverse effects such as uncontrolled cell proliferation, pan immunosuppression and consequent tumour development (12-14). Yet, it is still difficult to generate sufficient Tregs for adoptive therapy. Therefore, further investigations of novel therapeutics are warranted.

Peritoneal cells (PCs) in peritoneal cavity are mainly composed of 50–60% B cells, ~30% macrophages and 5–10% T cells (15). Clinically, PCs are easy to be acquired and the process to obtain the PCs does less harm to donors. Previous studies have proven that PCs possess immunosuppressive capacities under stimuli or infections (16-18). It is reported that in acute inflammation, PCs were identified to show an anti-inflammation ability through the secretion of inhibitory cytokines, such as IL-10 (19). Especially, the peritoneal macrophages induced by various agents significantly suppress the immune response *in vivo* and *in vitro* (16). In our recent research, we found that the adoptive transfer of PCs can regulate the inflammation and immune tolerance in dextran sulfate sodium (DSS)- induced colitis model through the regulation of inhibitory macrophages and inhibitory B cells (20). Furthermore, we found that the adoptive transfer of PCs can reduce the titres of OVA-specific antibody in the serum of OVA-immunised mice (20). These results suggested the comprehensive inhibition of immune response by PCs transfer. Therefore, it is conceivable that PCs potentially have the therapeutic benefits for other autoimmune diseases, such as RA. To date, PCs have not been engaged in treating RA.

Collagen-induced arthritis (CIA) model shares both immunological and pathological features with human RA, thus it has been used extensively as a model to study the pathogenesis of RA and for testing therapeutics (21). Most recently, both the pro-inflammatory T helper cell 17 (Th17) and Tregs have been suggested to play an important role in the induction and maintenance of CIA (22, 23). Thus, in order to investigate the potential therapeutic effect of PCs in RA

and also its role in T cell differentiation, we employed CIA model in this study. Here, the aim of this study was to explore the therapeutic effect of PCs in RA with CIA animal model. We also investigated the role of PCs transfer in T cell differentiation. In addition, the underlying mechanisms by which PCs function to treat CIA were examined. Thus, our findings might provide a novel strategy for RA therapy.

Materials and methods

Mice

All studies were approved and supervised by the State Key Laboratory of Biotherapy Animal Care and Use Committee (no. 2018-25, Sichuan University, Chengdu, Sichuan, China). DBA/1 mice were provided by State Key Laboratory of Biotherapy and Collaborative Innovation Center for Biotherapy, Sichuan University (Chengdu, China). All mice were housed and fed in a dedicated pathogen-free facility and maintained at 22°C under 12-hour day and night cycles throughout the experiment.

Peritoneal cells

Under sterile conditions, each DBA/1 mice's peritoneal cavity was injected with 5-10 ml cold PBS. After abdominal kneading (24), peritoneal cells were harvested with syringe and centrifuged at 1500 rpm for 3 min. Then, cells were re-suspended in RPMI-1640 or normal saline at a concentration of 1×10^6 cells/100ul.

Collagen-induced arthritis (CIA)

Male DBA/1 mice were immunised with 100 μl of 2 mg/ml bovine type II collagen (CII) emulsified with 5 mg/ml complete Freund's adjuvant (CFA) intradermally at several sites at the base of the tail, boosting immunised with CII and incomplete Freund's adjuvant (IFA) after 3 weeks (Chondrex, Redmond, WA, USA). The onset of the arthritis was approximately 1 week after the final immunisation. Arthritis clinical scores were assessed as follows: 0, normal; 1, slight swelling and/or erythema; 2, pronounced edematous swelling; and 3, ankyloses (25, 26). Each limb was graded, resulting in a maximum score of 12.

Treatment protocols

The mice were randomly divided into two groups, CIA and CIA+PC. CIA group were treated with saline, and used as disease control. CIA+PC group were treated with PCs, and used to check the potential therapeutic effect of PCs. The treatment commenced at the early clinical signs of arthritis and intravenous administrated every other day until day 14. The PCs concentrations used were 1×10^6/100 μl, and the clinical disease severity was monitored daily. At the end of experiment, we measured the thickness of paw and ankle, and also collected the spleen and limbs for further studies.

Histologic analysis

For haematoxylin and eosin (H&E), the hind limbs were washed with saline, and immediately fixed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Then the limbs were decalcified in EDTA, embedded in paraffin, sectioned, stained and examined at ×200 magnifications by light microscopy (Leica DFC425C, LAS v. 3.8 software). The histological evaluation of severity of arthritis was graded on a scale of 0–3 using a previously validated scoring system (27-29): a) cell infiltration: none=0; slight=1; moderate=2; severe=3; b) synovial hyperplasa: none=0; slight=1; moderate=2; severe=3; c) bone erosion: none=0; slight=1; moderate=2; severe=3. The final scores are the averages of five individual slides, and the total inflammation scores are the sum of the three parts.

Flow cytometry

Fresh spleen cells were collected at the end of experiment. After erythrocyte lysis, cells were washed with PBS and stained with anti-CD3-APC, anti-CD4-PerCP, anti-CD25-PE and anti-Foxp3-FITC following the guide of mouse Treg staining kit (eBioscience, USA). We also stained the cells with anti-CD4-PerCP and anti-IL-17-FITC following the guide of BD Cytofix/Cytoperm. To test the characteristic of PCs, we checked the surface-markers expression with anti-F4/80-PE, anti-B220-FITC, anti-CD11b-PerCP-Cy5.5, anti-CD3-DAPI and anti-CD5-APC. All antibodies were purchased from BD Bioscience, eBioscience and BioLegend.

Cytokines

The hind limbs were collected and stored in liquid nitrogen. After retrieval, tissues were ground into a powder, weighed and re-suspended with medium strong RIPA buffer containing 1% protease inhibitor cocktail (Sigma-Aldrich, USA) for 2 hours, then homogenate and centrifuged at 17,000×g for 20 minutes at 4°C. Tissue supernatants were collected for luminex (Merck Millipore, USA) and ELISA (eBioscience, USA) testing.

Cell culture

Peritoneal cells were isolated as described previously. Breifly, 2×10^6 peritoneal cells were cultured in 2 ml complete RPMI-1640 medium for 4h and 8h at 37°C, 5% CO2, and stimulated with or without 1 μg/ml LPS. The supernatants were collected with syringe and checked the expression of IFN-γ by ELISA testing (eBioscience, USA). To get the PMBC, we centrifuged the fresh splenocytes with percoll solutions, and then washed with RPMI-1640 medium. Briefly, 2×10^6 PBMC were cultured with 2×10^6 PCs in 2ml complete RPMI-1640 medium in 6-well plates. After 6h and 12h we collected the cells and checked the level of Foxp3, INF-γ and β-actin.

Western blot

Total cell protein extracts were prepared in RIPA buffer and subjected to western blot assays as described before. Anti-FOXP3 was purchased from Santa Cruz. Anti-IFN-γ was purchased from BD Biosciences. Mouse monoclonal anti-β-actin was purchased from Sigma-Aldrich.

Statistical analysis

The SPSS statistical software was used to analysis. Results were presented as means ± SEM unless otherwise stated. Statistical significance was determined by the Student's *t*-test and two-way analysis of variance (ANOVA). Values of $p<0.05$ were considered statistically significant.

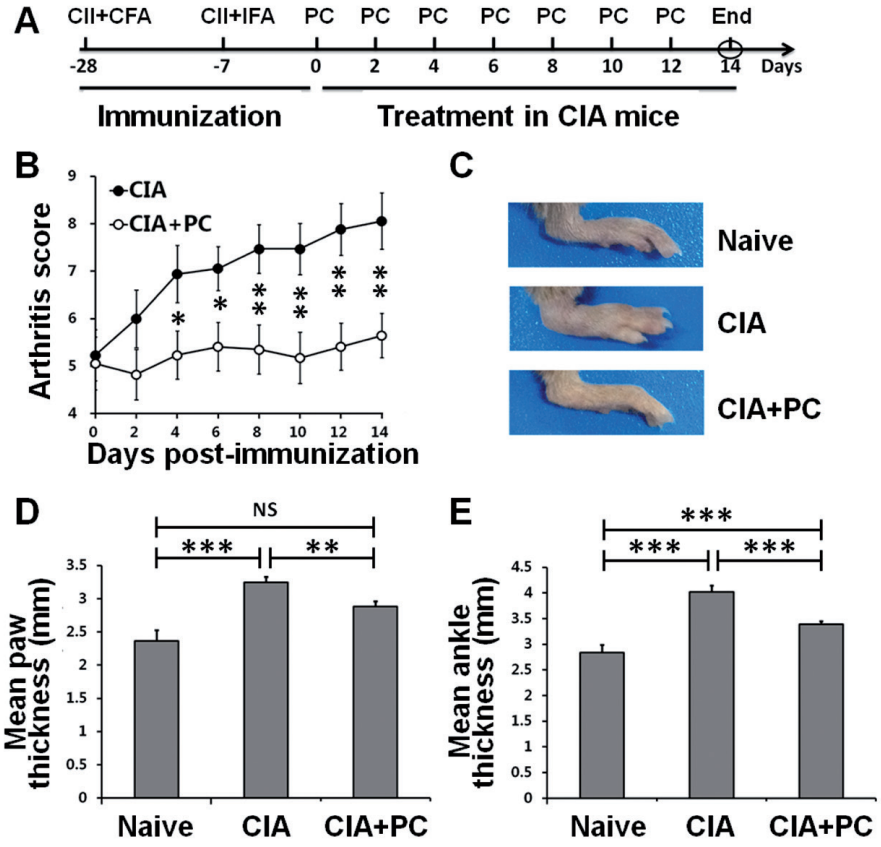

Fig. 1. Attenuation of collagen-induced arthritis by adoptive transfer PCs.
A: Arthritis was induced by immunisation with type II collagen for twice. CIA and CIA+PC group were intravenous injected with 100ul saline and 1x10^6 PCs every other day, respectively. B: The time course of arthritis score was monitored every other day. On day 14, the thickness of (**C**) paw and (**D**) ankle were analysed.
E: Photographs of representative mice ankle. These observations clearly demonstrate the therapeutic potential of peritoneal cells to reduce the development and progression of collagen induced arthritis. Data presented are the mean ± SEM of seventeen mice. Similar results were observed in three independent experiments (*p<0.05, **p<0.01 and ***p<0.001, CIA+PC *vs*. CIA. NS stands for no significant difference).

Results

Attenuation of collagen-induced arthritis by adoptive transfer PCs

We previously demonstrated that the adoptive transfer of PCs can regulate the inflammation and immune tolerance in DSS-induced colitis model (20). Thus, in order to investigate the potential effect of PCs in RA, we employed CIA model. CIA is a classical model with stable occurrence of disease and maximum likelihood compared with the human RA occurred clinically. We induced the arthritis by immunised the mice with CII for twice and the mice with early symptoms were treated every other day with 100μl 1x10^6 freshly isolated PCs (CIA+PC; n=17). Mice treated with saline were set as disease control (CIA; n=17) (Fig. 1A). The arthritis score is clinically used to describe the severity of joint inflammation. The mean arthritis score of CIA+PC mice was significantly lower than that of CIA mice at the indicated time points (Fig. 1B). The thickness of paw and ankle are decreased in CIA+PC group, which indicated that the effective relief of joint inflammation (Fig. 1C-E). As shown in Figure 1C and 1D, in the CIA group, the thickness of paw and ankle are 3.25±0.08 mm and 4.02±0.06 mm at day 14 respectively. Mice in the CIA+PC group have 2.88±0.16 mm and 3.39±0.14 mm, respectively. As shown in Figure 1E, CIA group mice gradually displayed changes in joint swelling and erythema. In contrast, CIA+PC group mice displayed less severe and delayed symptoms. These observations clearly demonstrate the ability of PCs to reduce the development and progression of type II collagen induced arthritis.

PCs treatment reduced the joint inflammation and bone destruction

Further histological analysis supports the detections in macroscopic assessments. The CIA mice exhibited extensively distributed inflammation with destruction of joint, while CIA+PC mice had mild or moderate inflammation (Fig. 2A). The histological scores for cell infiltration, synovial hyperplasia and bone erosion in the CIA+PC mice were significantly lower compared to that of the CIA mice (p<0.05, Fig. 2B). As shown in Figure 2A and 2B, both cartilage and bone destruction as well as joint inflammation were inhibited by the treatment of PCs, These findings confirmed that the adoptive of PCs can reduce the development and progression of CIA. In addition, we also investigated whether PCs treatment could alter the cytokine profile in the joint of arthritis. As shown in Figure 2C, the protein levels of cytokines in the joint of CIA and CIA+PC mice were measured by Luminex and ELISA. We found that the pro-inflammatory cytokines, such as IL-17, IL-1β, TNF-α, IL-6 and IL-12 were down-regulated, while the anti-inflammatory cytokines, such as IL-4, IL-10 and TGF-β1 were up-regulated. This cytokines profile is similar with the profile that previously we determined in DSS-induced colitis model with PCs treatment (20). However, it is worth mentioning that the expression of IFN-γ is significantly increased in CIA+PC mice, which indicated that the IFN-γ may play an important role in CIA model with PCs treatment. These findings suggest the pivotal effects of cytokine alteration led by PCs transfer in amelioration of CIA.

PCs treatment reduced Th17 cells and induced Tregs in CIA mice

To investigate whether the adoptive transferred PCs could specifically regulate the T cell differentiation, we firstly studied the characteristic of PCs. Fresh PCs were obtained from un-manipulated 6-7 weeks old naive mice, and the surface-markers expressions on PCs were measured by FACS (Fig. 3A). We

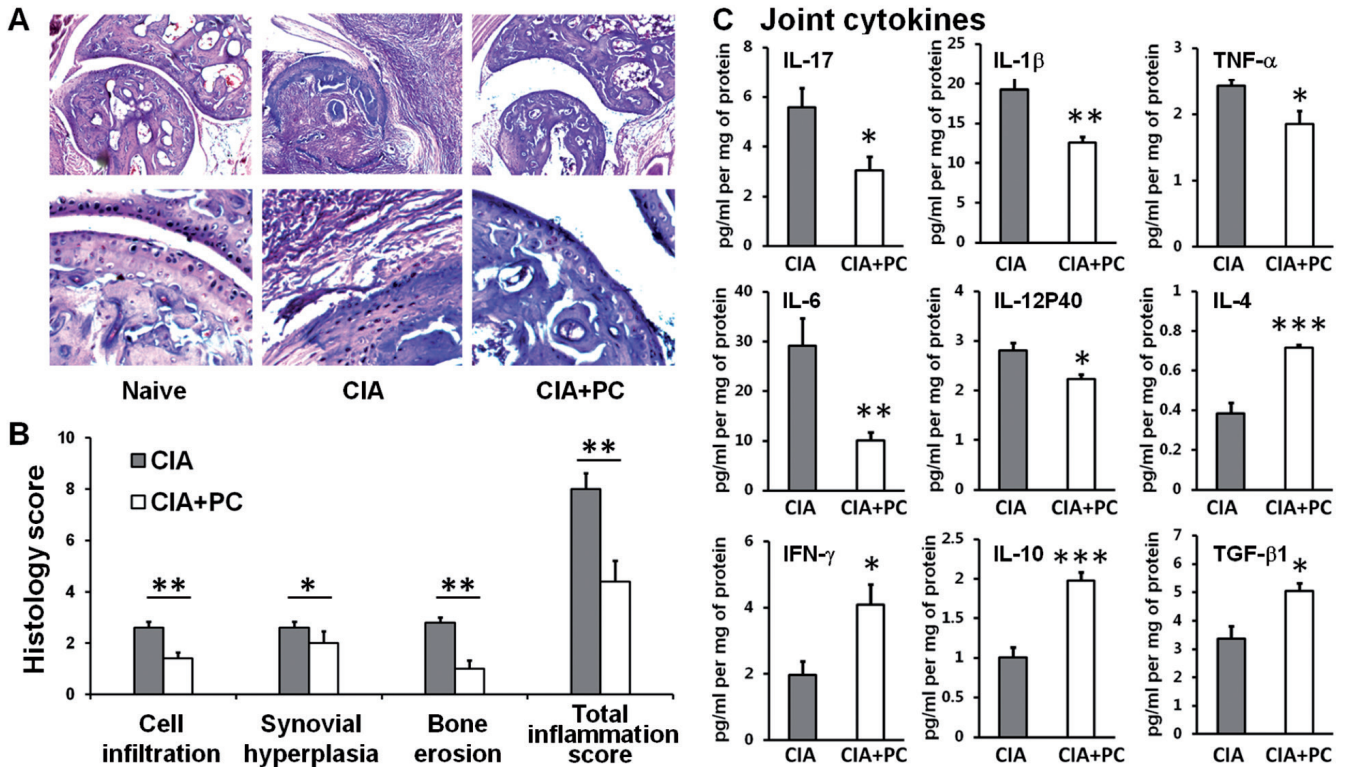

Fig. 2. PCs treatment reduces the joint inflammation and bone destruction. At the end of the experiment, the histology of the joints was evaluated.
A: The representative histological sections of H&E staining joints were displayed.
B: According to the histology score, the CIA group mice were characterised by severe cell infiltration, synovial hyperplasia and bone erosion. The total inflammation score in CIA group is significantly higher than that in CIA+PC group.
C: Joints were harvested and analysed by Luminex and ELISA for the protein level of indicated cytokines. The down-regulation of pro-inflammatory cytokines and the up-regulation of anti-inflammatory cytokines are ascribed to the remission of the disease. These observations confirmed that the adoptive of PCs can reduce the joint inflammation and bone destruction in CIA mice. Data presented are the mean ± SEM (*p<0.05, **p<0.01 and ***p<0.001, CIA+PC *vs*. CIA).

found that 3.1±0.22 million live peritoneal cells can be obtained from one operation, and among these cells 50±3% are B cells, 41±5% are macrophages and 4±1% are T cells (Fig. 3B). More than half of the peritoneal B cells are characterised by a high level of CD5 expression. Furthermore in animal models, the fresh spleen cells were separated from mice to trace the percentage of Th17 and Tregs in vivo by FACS. CIA mice that received the treatment of PCs showed less $CD4^+IL\text{-}17^+$ T cells (CIA *vs*. CIA+PC, 3.13% *vs*. 0.787%) (Fig. 3C) and more $CD3^+CD4^+CD25^+Foxp3^+$ Tregs (CIA *vs*. CIA+PC, 3.46% *vs*. 7.24%) (Fig. 3D). These finding indicated that the adoptive transfer of PCs inhibits the Th17 mediated response and also plays an important role in the conversion of $CD4^+$ T cells to Tregs.

IFN-γ is a key mediator by which PCs induce the Tregs

Transferring of PCs intravenously altered the expression of cytokines closely related to inflammation, with the pro-inflammatory cytokines decreased and the anti-inflammatory cytokines increased (Fig. 2C). Among these cytokines, interferon γ (IFN-γ) a cytokine pivotal for immunostimulation and immunoregulation, were up-regulated. However, in previously studies, the IFN-γ was identified as an important activator of innate and adaptive immunity against viral. It is also reported that the deficiency of IFN-γ increased Th17 cell number and up-regulate the expression of IL-17 and TNF-α in the joints of mice with CIA (30). Thus, we inferred that IFN-γ play key roles in PCs mediated immunosuppression. To demonstrate our hypothesis, we detect the expression of IFN-γ after culturing PCs for 4 and 8 hours. As shown in Figure 4A, the expression of IFN-γ was significantly increased in PCs stimulated with or without LPS. These data suggest that the PCs are able to secret the IFN-γ, especially upon the inflammatory stimulations. In this study, we found that PCs can autocrine a small amount of IFN-γ when culture *in vitro*, but this amount is limited. LPS was added in the medium to simulate inflammatory stimulation. In this case, inflammation-activated PCs, including activated peritoneal macrophages and peritoneal T cells, can further secrete a large amount of IFN-γ. Then, we cultured the splenic PBMC together with PCs for 6 and 12 hours, and check the conversion of T cells with western blot and flow cytomery. We found that the level of FOXP3 and IFN-γ are up-regulated in PCs treated group (Fig. 4B). In addition, the percentage of $CD3^+CD4^+IL\text{-}17$ T cells decreased with time, while the $CD3^+CD4^+CD25^+FOXP3^+$ T cells increased with time (Fig. 4C). These data suggest that upon the inflammatory stimulation, PCs can secret a large amount of IFN-γ and consequently up-regulate the expression

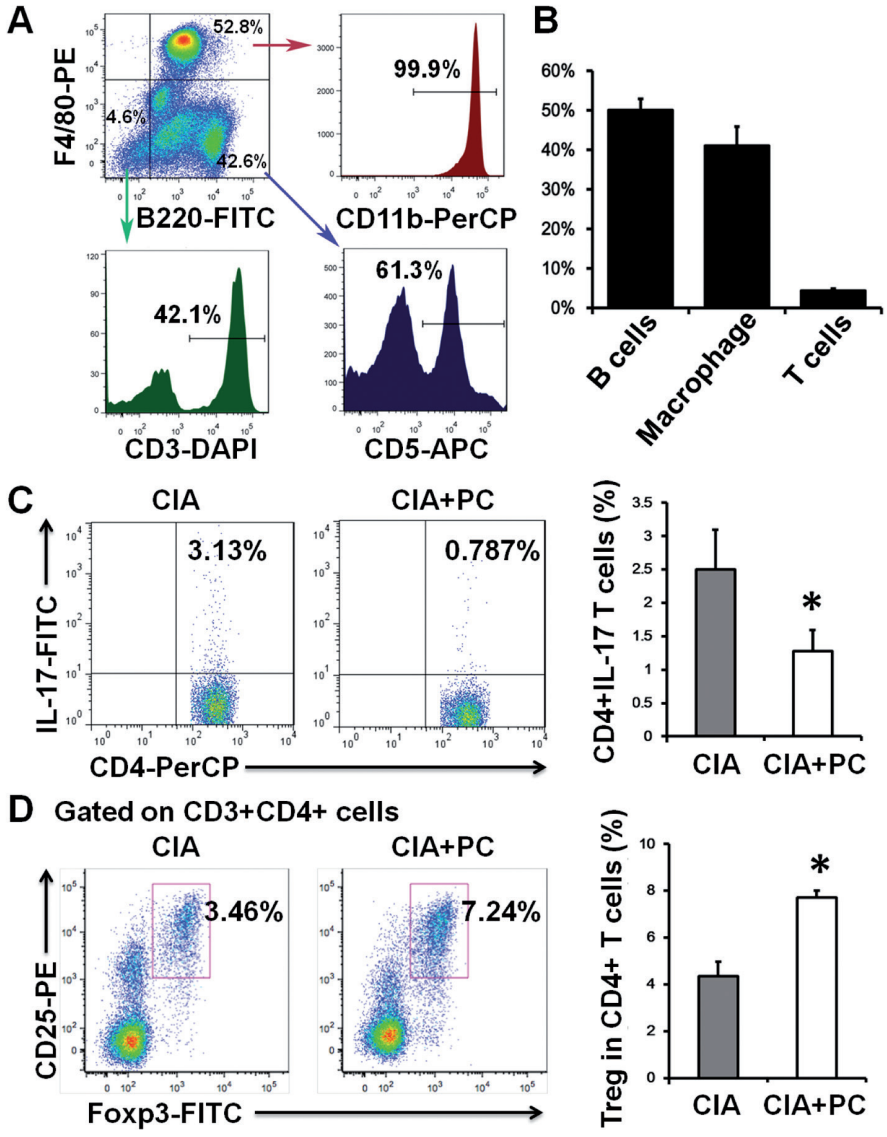

Fig. 3. PCs treatment regulates the T cell differentiation in arthritis mice.
A: The representative FACS analysis of surface-markers expression on PCs obtained from 6-7 weeks old naive mice.
B: PCs are mainly composed of 50±3% B cells, 41±5% macrophages and 4±1%% T cells. At the end of the experiment, mice spleen were harvested and analysed by FACS for the T cell differentiation. The percentage of (**C**) $CD4^+IL\text{-}17^+$ Th17 cells and (**D**) $CD3^+CD4^+CD25^+Foxp3^+$ regulatory T cells were analysed, and the left are the representative flow cytometry. These observations clearly demonstrate the PCs are competent at collagen-induced arthritis therapy, ameliorate disease by reducing the Th17 cells and increasing Tregs. Data presented are the mean ± SEM (n=4-5/group). Similar results were observed in three independent experiments (*p<0.05, **p<0.01 and ***p<0.001, CIA+PC *vs.* CIA).

of FOXP3, therefore functioned in the conversion of $CD4^+$ T cells to Tregs.

Discussion

Several observations have been made in this study concerning the therapeutic potential of PCs on CIA animal model and its underlying mechanisms. To our knowledge, prior to the presented study here, no study has reported the therapeutic efficacy initiated by PCs in RA. Firstly, we checked the therapeutic effect of PCs in CIA model. We found that the vaccine based on adoptive transfer of PCs as cell therapy could be effective to suppress the inflammation and immune response in arthritis mice. For example, the swelling, bone destruction and also the physiological function of joints are mitigated by PCs treatment, which indicated the effective relief of joint inflammation by PCs transfer. Further experiments were placed to check the expression of cytokines on protein level in arthritis joints. We found that the treatment of PCs have the potential to suppress the inflammatory cytokines and up-regulate the anti-inflammatory cytokines. Thus, the therapeutic effects of PCs are determined.

Secondly, we checked the composition and also the phenotype of PCs, we found that 3.1±0.22 million live peritoneal cells can be obtained from one mouse, which means nearly three doses of PCs can be collected from one operation. Among these cells, peritoneal macrophages and peritoneal B lymphocytes are the major cell types of PCs (15, 31). In our study, the peritoneal macrophages and peritoneal B lymphocytes are account for 41±5% and 50±3%, respectively. As well-recognised before, both peritoneal macrophages and peritoneal B lymphocytes are capable to play defense effects upon infections by microbes and superantigens (32). And these two cell types have long been regard as defense of peritoneum by secreting many kinds of cytokines and chemokines (33). In our study, more than half of the peritoneal B cells are characterised by a high level of CD5 expression. It is reported that the B lymphocytes can be divided into B1 and B2 subsets based on the CD5 expression, and the CD5 positive B1 cells are represent a potent regulatory B cells, which capable in control of T cell dependent inflammatory responses (34, 35). What is more, the peritoneal macrophages are found to highly express CD206 mRNA previously and the CD206 positive peritoneal macrophages are represent a potent regulatory macrophages, which capable in moderate the inflammation and overactive immune response (36-38). Therefore, the PCs could possess immunosuppressive characteristics and these literatures support our concept that PCs can effectively prevent the initiate and development of collagen induced arthritis.

Additionally, a number of studies have shown that the differentiation of Th17 cells is implicated in the pathogenesis of RA, mainly through the secretion of IL-17 and other Th17 cytokines such as IL-22 and IL-21 (39-41). And it is reported that the quantity of Th17 cell

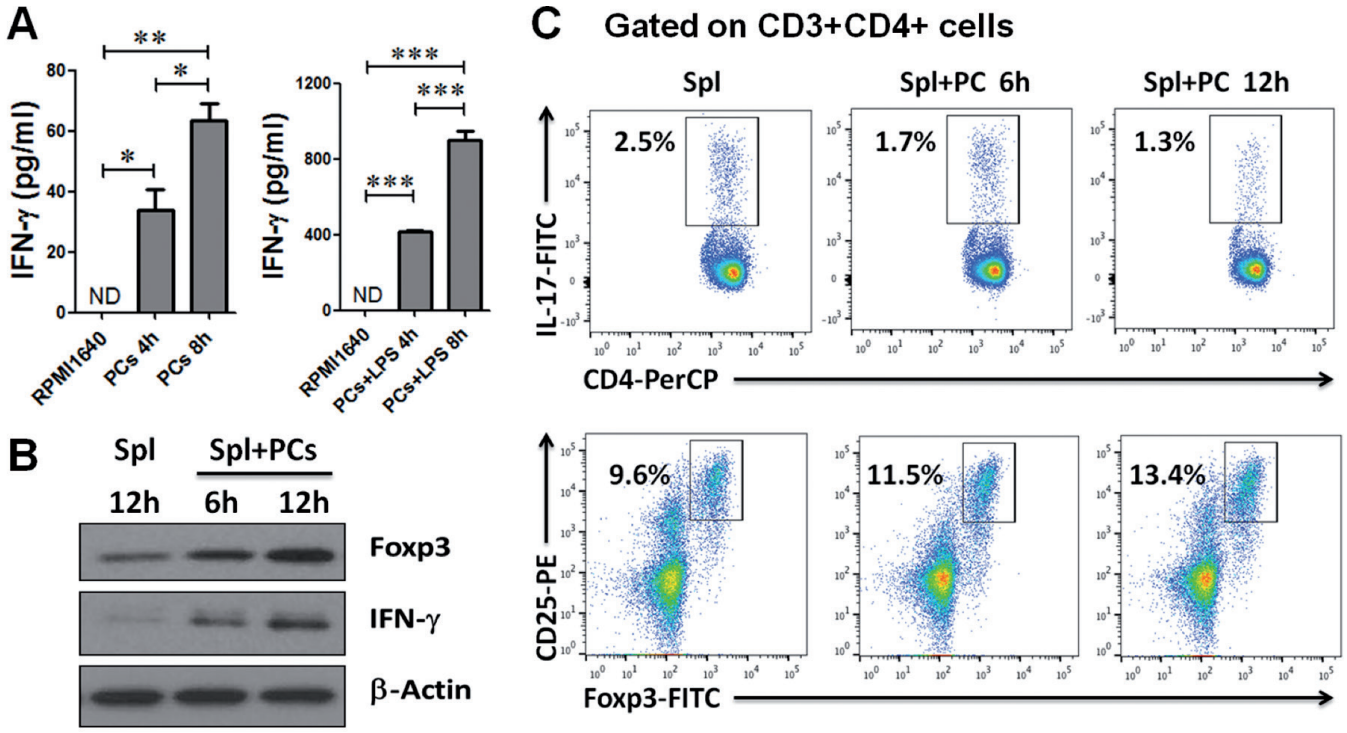

Fig. 4. PCs induce the Tregs through the secretion of IFN-γ.
A: 2×10^6 PCs were cultured in 2 ml complete RPMI-1640 medium, and stimulated with or without 1 μg/ml LPS. After 4 and 8 hours, the supernatants were collected and then detected the expression of IFN-γ by ELISA.
B: 2×10^6 splenic PBMC were cultured with 2×10^6 PCs for 6 and 12 hours, and the expression of FOXP3 and IFN-γ were measured by Western blot.
C: $CD3^+CD4^+IL\text{-}17^+$ Th17 cells and $CD3^+CD4^+CD25^+Foxp3^+$ regulatory T cells were analysed. These observations clearly demonstrate the IFN-γ secreted by PCs is responsible for the induction of FOXP3 in T cells.
Data presented are the mean ± SEM. Similar results were observed in three independent experiments (*p<0.05, **p<0.01 and ***p<0.001, PCs 4h, PCs 8h *v*.RPMI1640. ND stands for not detected).

and IL-17 expression are correlated with disease activity and severity (42). Notably, the balance of Th17 and Tregs is responsible for the joint inflammation, and consequently play a major role in the development of arthritis (6, 43, 44). In our study, we further checked the Th17 and Tregs population in fresh spleen cells from CIA and CIA+PC mice. And we found that the proportion of IL-17 secreting $CD4^+$ T cells are decreased in PCs treated group, while the proportion of Foxp3-positive Tregs are significantly increased. These finding definitely support the previous findings and also suggest that the treatment of PCs can effectively influence the differentiation of T cells, and therefore contribute to the therapeutic effects of PCs in rheumatoid arthritis.

Thirdly, although we already proved that the treatment of PCs can regulate the balance of Th17/Treg proportions and result in inflammation remission in CIA model. We still want to figure out the underlie mechanism that how PCs regulate the T cell differentiation. It's worth mentioning that among the cytokines that the PCs regulated in joints, IFN-γ, a cytokine pivotal for immune regulation and stimulation, is dramatically increased. Previously, a number of studies have demonstrated that the IFN-γ is an important Th1 pro-inflammatory cytokine, and commonly engaged in innate and adaptive immunity against viral. However, a recent study found that the deficiency of IFN-γ increased Th17 cell number, and up-regulate the expression of IL-17 in the joints of mice with CIA (30). Especially, the deficiency of either IFN-γ or IFN-γ receptor in CIA mice will lead to an exacerbation of joint inflammation and cartilage damage (30, 45). These literatures in turn support our concept that IFN-γ might participates in the progress of Th17 cell differentiation initiated by PCs. Furthermore, a number of studies reveal that the IFN-γ is functioned in inducing FOXP3 expression in T cells, and thus promote the conversion of $CD4^+CD25$- T cells to Tregs (46-48). In our study, the Th17 response was inhibited by adoptive transfer of PCs *in vivo*. What's more *in vitro* test, the IFN-γ expressed in PCs supernatant was found efficiently increasing the expression of FOXP3 in splenic PBMC. Therefore, high amounts of IFN-γ induced and self-secreted by PCs are likely to accumulate locally in inflammatory joints, which effectively functioned in triggering the expression of FOXP3 in T cells during the inflammation of arthritis.

Finally, PCs have many advantages over other cell types when they are adoptively transferred for treating rheumatoid arthritis. I) the bio-availability of PCs is easier than Tregs and other stem cells. Autologous PCs can be easily collected from peritoneal cavity and there are sufficient autologous PCs for cell therapy, since we demonstrated that nearly three doses of PCs can be acquired from one mouse in one operation. II) Same with the function of Tregs in CIA, PCs ameliorate arthritis

through down-regulation of several pro-inflammatory cytokines, including IL-17, IL-1β, TNF-α, IL-6 and IL-12, and up-regulation of several anti-inflammatory cytokines, including IL-10, TGF-β1 and IL-4 (9, 49, 50). III) Upon inflammatory stimulation during the arthritis, PCs can produce a large amount of IFN-γ, which contribute to the regulation of T cell differentiation. In our study, the IFN-γ is likely to suppress Th17 response and trigger the production of Tregs *in vivo*. IV) Freshly collected PCs were immediately administrated in CIA mice without any additional ex vivo culture. Thus the safety and physiological function of PCs are enhanced.

In conclusion, the adoptive transfer of PCs we presented here provides a new efficacious regimen for rheumatoid arthritis therapy. The treatment with PCs successfully suppressed the pro-inflammatory cytokines and induced the anti-inflammatory cytokines *in vivo*. Particularly, the induction and self-secretion of IFN-γ after adoptive transfer of PCs are essential for the anti-inflammation properties. During the joint inflammation, the IFN-γ induced by PCs is critically required for the conversion of CD4+CD25- T cells to Tregs. Therefore, our findings might provide a new strategy for rheumatoid arthritis therapy. However, the findings still require confirmation in the clinical settting.

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