ORIGINAL ARTICLE

PJ34, a PARP1 inhibitor, attenuates acute allograft rejection after murine heart transplantation via regulating the CD4$^+$ T lymphocyte response

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SUMMARY

Acute allografts rejection is the most important factor causing allograft disability for many patients undergoing organ transplantation. PJ34, which is a specific inhibitor of poly(ADP-ribose) polymerase 1, is involved in immune regulation, may be effective in preventing acute cardiac rejection. We performed the models of abdominal heterotopic heart transplantation. PJ34 was injected intraperitoneally daily (20 mg/kg/day) starting the day after surgery. The severity of rejection was determined by histology. The mRNA expression levels of cytokines and transcription factors in the grafts were measured by quantitative polymerase chain reaction (qPCR). The proportion and number of T-cell subpopulations in the spleens were analyzed by flow cytometry. *In vitro*, the effect of PJ34 on allogeneic responses was investigated. We found treatment with PJ34 prolonged allograft survival compared with normal saline treatment. Compared with the control group, PJ34 treatment reduced the proportion of CD4$^+$IFN-γ$^+$ and CD4$^+$IL-17A$^+$ cells and increased the percent of CD4$^+$IL-4$^+$ and CD4$^+$Foxp3$^+$ cells in the spleens. *In vitro*, PJ34 treatment significantly inhibited the mRNA levels of IFN-γ and IL-17A and promoted the mRNA levels of TGF-β and FOXP-3 in activated CD4$^+$ T cells. Modulating the CD4$^+$ T lymphocyte response with PJ34 could attenuate acute allografts rejection after murine heart transplantation. These findings indicate that PARP1 may be a promising therapeutic target to attenuate acute cardiac allograft rejection.

Transplant International 2021; 34: 561–571

Key words
acute allograft rejection, CD4$^+$ T lymphocyte, heart transplantation, PARP1 inhibitor, PJ34

Received: 30 August 2020; Revision requested: 16 October 2020; Accepted: 21 December 2020

Introduction

Heart transplantation (HT) is the most effective therapy for end-stage heart failure. And acute rejection causing allograft disability is a terrible challenge for patients undergoing heart transplantation. At present, cyclosporin A (CsA) and tacrolimus (FK506) targeting the phosphatase calcineurin (CN) are the main immunesuppressive medicines to prevent and treat acute cardiac allograft rejection. However, sustained applications of CsA and FK506 can lead to numerous side effects, like nephrotoxicity, neurotoxicity, cancerogenesis, and hypertension. Due to these deleterious negative effects, many researches have been undertaken to find novel

doi:10.1111/tri.13809

treatments. However, there are no ideal approaches existing to prevent or inhibit allograft rejection, partly because the mechanisms of allograft rejection are heterogeneous and complex. $CD4^+$ but not $CD8^+$ T cells, components of the adaptive immune system, are essential for allo-rejection [1]. In solid organ transplantation, $CD4^+$ T lymphocytes are principal mediators for acute allograft rejection. $CD4^+$ T cells are divided further into effector T helper (Th) cells (Th1, Th2, and Th17), regulatory T cells (Tregs) and T follicular helper (Tfh) cells [2]. Among these subsets, Th1 promotes allograft rejection through promoting cytotoxic T-cell activity, inducing delayed type hypersensitivity and Fas/Fas ligand-mediate cytotoxicity [3]. Th2 promotes transplantation rejection majorly through the recruitment of eosinophils depend on Th2 cytokines, such as interleukin (IL)-4 and IL-5 [4]. Th17 can also cause allograft damage by increasing early allograft inflammation and vasculopathy [5,6]. Likewise, Tfh makes contributions to allograft rejection by assisting B-cell differentiation into antibody-producing plasma cells [7,8]. By contrast, an extensive number of previous researches have indicated that Tregs might protect the transplanted organs by suppressing allo-reactive Th1 and Th2 cells and inducing transplantation tolerance [9,10].

Poly(ADP-ribose) polymerase 1 (PARP1), which is a dominant member of the PARP family in eukaryotes, makes contributions to approximately 90% of cellular PARP activity. Upon activation, PARP1 can catalyze extensive ADP-ribose polymer chains from its substrate nicotinamide adenine dinucleotide (NAD^+) to target proteins, a process called poly(ADP-ribosyl)ation, and conduce to the process of DNA repair [11,12]. Beyond DNA repair, increasing evidence demonstrated that PARP1 is also involved in a variety of cellular and biological processes, such as immune response and inflammation, stress signaling, cell differentiation, and death [12–15]. Excessive hyperactivation of PARP causing the depletion of intercellular NAD^+ and ATP levels maybe lead to cellular metabolic disorder and death. Moreover, insufficiency in PARP1 function is related to multiple diseases, including autoimmunity, chronic inflammation, cardiovascular diseases, and cancers [15–17]. However, the role of PARP1 in allograft rejection particularly cardiac allograft rejection is poorly understood.

PJ34 is a PARP1 inhibitor attenuating poly(ADP-ribosyl)ation of ADP-ribose polymer. It was reported that PARP inhibition could protect against alcoholic and non-alcoholic steatohepatitis [18]. After stroke and thrombolysis in mice, PJ34, which attenuated vascular inflammation and brain edema, was able to improve reperfusion and vasculoprotection [19]. In experimental allergic encephalomyelitis, PJ34 making pro-inflammatory Th1 shift to Th2 was associated with immunomodulation [20]. In addition, PJ34 also reduced the spinal cord number of autoreactive Th17 cells and prevented central nervous system migration of dendritic cells during encephalomyelitis module [20]. However, few studies have been performed to explore the effects of PJ34 on solid organ allograft rejection. We investigated the role of PARP1 in the acute allograft rejection of heart transplantation. We constructed the acute rejection model of abdominal heterotopic cardiac transplantation in mice and detected the expression of the PARP1 in the grafts. Then, we investigated whether PJ34 could attenuate acute rejection and lengthen allograft survival times via modulating the $CD4^+$ T lymphocyte response *in vivo*. Furthermore, we treated naïve T cell from mice spleen with anti-CD3, anti-CD28, and cytokines, and confirmed effects of PJ34 *in vitro*. Our results provide a key role for PJ34 in acute cardiac allograft rejection and new approaches for potential therapy.

Materials and methods

Animals

C57BL/6 (B6, H-2b) and BALB/c (H-2d) mice were used as recipients and donors, respectively (aged 6–-10 weeks; weighed 18–25 g; Beijing HFK Bioscience Co. Ltd, Beijing, China). All animal experiments were conducted strictly in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology.

Heterotopic heart transplantation and post-transplantation treatments

Heterotopic cardiac transplantation was performed as described previously [21]. Briefly, heart grafts harvested from donor mice (BALB/c or C57BL/6) were transplanted into the abdominal cavity of recipient mice (C57BL/6) via end-to-side anastomosing that was the aorta and pulmonary artery of the heart graft was anastomosed to the aorta and vena cava of the recipient mice, respectively. To observe the survival time of the heart grafts, all mice were divided into three groups: the isograft group (C57BL/6 to C57BL/6, $n = 8$), the

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control group (BALB/c to C57BL/6, normal saline treatment, $n = 8$), and the PJ34 group (BALB/c to C57BL/6, PI34 treatment, $n = 8$). Cardiac allograft pulsation was monitored daily by direct abdominal palpation performed in a double-blinded manner. The endpoint was complete cessation of cardiac contractility. PJ34 (20 mg/kg/day; Selleck, Houston, TX, USA) or normal saline was intraperitoneally injected daily starting the day before surgery. The doses refer to those used in previous studies. Cardiac graft survival was reported as the MST ± SD.

Isolation of naïve CD4$^+$ cells

The spleens of 4–6 weeks old mice ($n = 6$) were used to prepare single-cell suspensions. After erythrocyte lysis, pooled splenocytes were depleted of CD11b$^+$, CD8$^+$ and CD19$^+$ cells using biotinylated primary antibodies (BioLegend, San Diego, CA, USA) and streptavidin-coated secondary magnetic particles (Stem Cell Technologies, Vancouver, Canada). The enriched cells were sorted on the FACS Aria for CD4$^+$CD25$^-$CD44$^-$CD62L$^+$. Purity was >98%.

T helper cells differentiation

Naïve CD4 T cells (0.4×10^6) in 24 well plates (Costar) pre-coated with anti-CD3 (154-2C11, 5 μg/ml, BioXcell) and anti-CD28 (37.51, 2 μg/ml, BioXcell) were cultured in RPMI containing 10%FCS and polarizing cytokines. The cytokines were anti-mIL-12(10 ng/ml) and mIL-4 (2 μg/ml) for Th1; mIL-2 (20 ng/ml), anti-mIL-4, hTGF-β1 (3 ng/ml) and anti-mIFN-γ (2 μg/ml) for Treg; mIL-6 (10 ng/ml), anti-mIL-4, hTGF-β1 (2 ng/ml) and anti-mIFN-γ for Th17. The conditions of stimulated cells called "neutral" (anti-mIL-4 plus anti-mIFN-γ but without added cytokines) were considered Th0 cells. Testing compounds were added at the start of culture. After 3 days, PMA (50 ng/ml) and ionomycin (750 ng/ml) stimulated differentiated cells for 4 h.

Flow cytometry

Single splenic cell suspensions were analyzed by flow cytometry. According to the manufacturer's protocols, these cells were stained with FITC-anti-CD4 (BioLegend) and PE-anti-CD25 (eBioscience, San Diego, CA, USA), after which they were stained with PerCP-Cy5.5-anti-IFN-γ (eBioscience), PE-anti-IL-4 (eBioscience), PE-anti-IL-17A (eBioscience), and APC-anti-FoxP3 (eBioscience). The FACS Calibur Flow Cytometer (BD Biosciences, San Diego, CA, USA) was used to assess marker expression. All data were analyzed with FLOWJO V10 software.

RNA isolation and real-time PCR

Total RNA was extracted with Trizol reagent (Takara Bio, Tokyo, Japan) according to the protocols of manufacturers. RNA PCR Kit (Takara Bio) was used to reverse-transcribe RNA. Quantitative polymerase chain reaction (qPCR) amplification was performed via SYBR Green PCR Master Mix (Takara Bio) and ABI PRISM 7900 Sequence Detector system (Applied Biosystem, Foster City, CA, USA). The method of $2^{-\Delta\Delta C_t}$ was used to calculate relative gene expression. The 18s was used as the endogenous control and the real-time PCR (RT-PCR) primer sequences are shown in the following.

Gene	Forward primer	Reverse primer
IFN-γ	CGGCACAGTCATT GAAAGCCTA	GTTGCTGGCCTGA TTGTC
IL-17A	ACTACCTCAACC GTTCCACG	TTCCCTCCGCATTG ACACAG
IL-4	CCATATCCACGGA TGCGACA	CTGTGGTGTTCTT CGTTGCTG
TGF-β	AGGGCTACCATGC CAACTTC	CCACGTAGTAGACG ATGGGC
FOXP-3	GGCAGAGAGGTATT GAGGGTG	CCACAGCATGGG TCTGTCTTC
IL-10	GGTTGCCAAGCCTT ATCGGA	GAGAAATCGATG ACAGCGCC
GATA-3	AAGG AGAGCAGG GACATCCT	TTGTAGTACAGCCC ACAGGC
18s	TTGACGGAAGGG CACCACCAG	GCACCACCACCCACG GAATCG

Western blot

With a 4% proteinase inhibitor (Sigma-Aldrich, St. Louis, MO, USA), cells or tissue was harvested in an ice-cold suspension buffer. Protein was quantified with BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Equal proteins were separated by SDS polyacrylamide mini-gels and subsequently transferred onto the PVDF membrane. This PVDF membrane was followed by washing with Tris-buffered saline (TBS) and blocking with 5% nonfat dry milk for, and then incubated overnight with primary antibodies: phosphor-NF-κB p65 (diluted at 1:1000, 3039S, CST), NF-κB p65 (diluted at 1:1000, 8242T, CST), phospho-IκBα (diluted at 1:500, 5209S, CST), IκBα (diluted at 1:500, 4812S, CST) and GAPDH (diluted at 1:1000, 5174S, CST).

Membranes were then washed with TBST and incubated with appropriate peroxidase-conjugated secondary antibody for 1 h at room temperature. Target protein bands using the ECL kit were detected with a Bio-Rad (Hercules, CA, USA) imaging system.

Histologic analyses

On 5 and 7 days after transplantation, the cardiac grafts were harvested from the recipients of the isograft group, the control group, and the PJ34 group (each group $n = 3$–5) and then were fixed in formalin. The paraffin-embedded heart sections were processed for immune-histochemical and hematoxylin–eosin staining and the degree of PR was graded as described previously [22] using the following scale: 0R = no rejection, 1R = focal mononuclear cell infiltrates without necrosis, 2R = focal mononuclear cell infiltrates with necrosis, 3R = multifocal infiltrates with necrosis and 4R = widespread infiltrates with hemorrhage and vasculitis.

Statistical analysis

Graft survival was assessed using the Kaplan–Meier graphs and log-rank test. Data are presented as mean ± SEM. Unpaired, two-tailed Student's *t*-test was used to analyze data from two groups. One-way analysis of variance (ANOVA) was used to analyze multiple comparisons, followed with Tukey's post-test. We considered differences with $P < 0.05$ statistically significant. And all experiments were repeated for at least three replicates per condition.

Results

PARP activity was increased in cardiac allografts

To investigate the expression of PARP1 in allograft, we successfully performed the acute rejection model of murine heterotopic cardiac transplantation. Histological analysis of heart grafts revealed the allografts on days 5

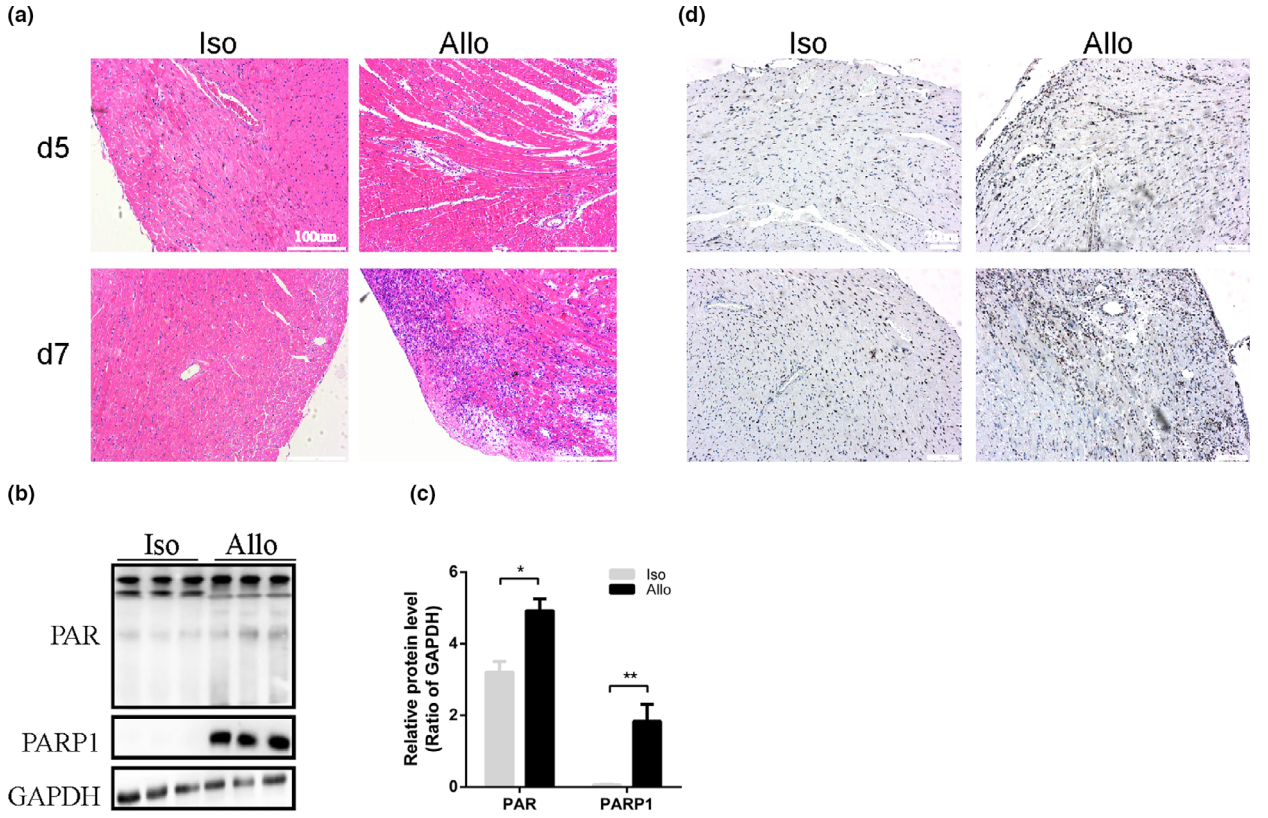

Figure 1 PARP activity was increased in cardiac allografts. (a) The tissue sections of cardiac grafts harvested on days 5 and 7 after transplantation were stained with hematoxylin–eosin (HE). Inflammatory cells infiltrating in the myocardium and the perivascular regions were markedly increased in the allograft group. Scale bars = 100 μm. (b and c) Poly(ADP-ribose) polymerase 1 (PARP1) and total poly(ADP-ribosyl)ation levels were significantly decreased in the allograft group compared with the isograft group by Western blot analysis. *$P < 0.05$, **$P < 0.01$. (d) Immunohistochemical staining of PARP1 in the tissue sections from the isograft group and allograft group. Scale bars = 20 μm.

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and 7 after surgery displayed severe pathological feature of acute rejection compared with the syngeneic transplantation. The allografts showed a lot of inflammatory cells infiltration and rigorous vasculopathy (Fig. 1a). Furthermore, compared to syngeneic grafts, increased PARP1 expression and total poly(ADP-ribosyl)ation levels were observed in the rejection group by Western blot (Fig. 1b,c, $P < 0.05$ or $P < 0.01$). Meanwhile, results of immunohistochemical analysis also indicated the level of PARP1 was conspicuously elevated in cardiac allografts on days 5 and 7 post-transplantation. (Fig. 1d). These results demonstrated clear acute rejection and the relevance of activated PARP in cardiac allograft.

PJ34 attenuated acute rejection and prolonged murine cardiac allograft survival

Because PARP1 is activated in allograft, we hypothesized that inhibition of PARP1 activity might have beneficial effects on the onset and development of acute rejection. To test the hypothesis, we established BALB/C-to-C57BL/6 murine heart transplantation. PJ34 (the PJ34 group, 20 mg/kg/day) or normal saline (the control group) was intraperitoneally injected daily from the day before the operation until the grafts were rejected. We found that treatment ~~treated~~ with PJ34 prolonged allograft survival compared with normal saline treatment [mean survival time (MST), 15.1 ± 1.9 vs. 7.05 ± 0.59 days, $P < 0.001$; Fig. 2a]. Hematoxylin–eosin staining demonstrated that cardiac allografts of the control group were infiltrated with a large number of cells particularly in perivascular distribution on days 5 or 7 after heart transplantation. Treatment with PJ34 significantly ameliorated myocardial damage and vasculopathy of the cardiac allografts (Fig. 2b). Moreover, we quantified the signs of parenchymal rejection (PR) in cardiac grafts tissue sections. In line with the decreased inflammatory cells infiltration, the PR scores of the allografts in the PJ34

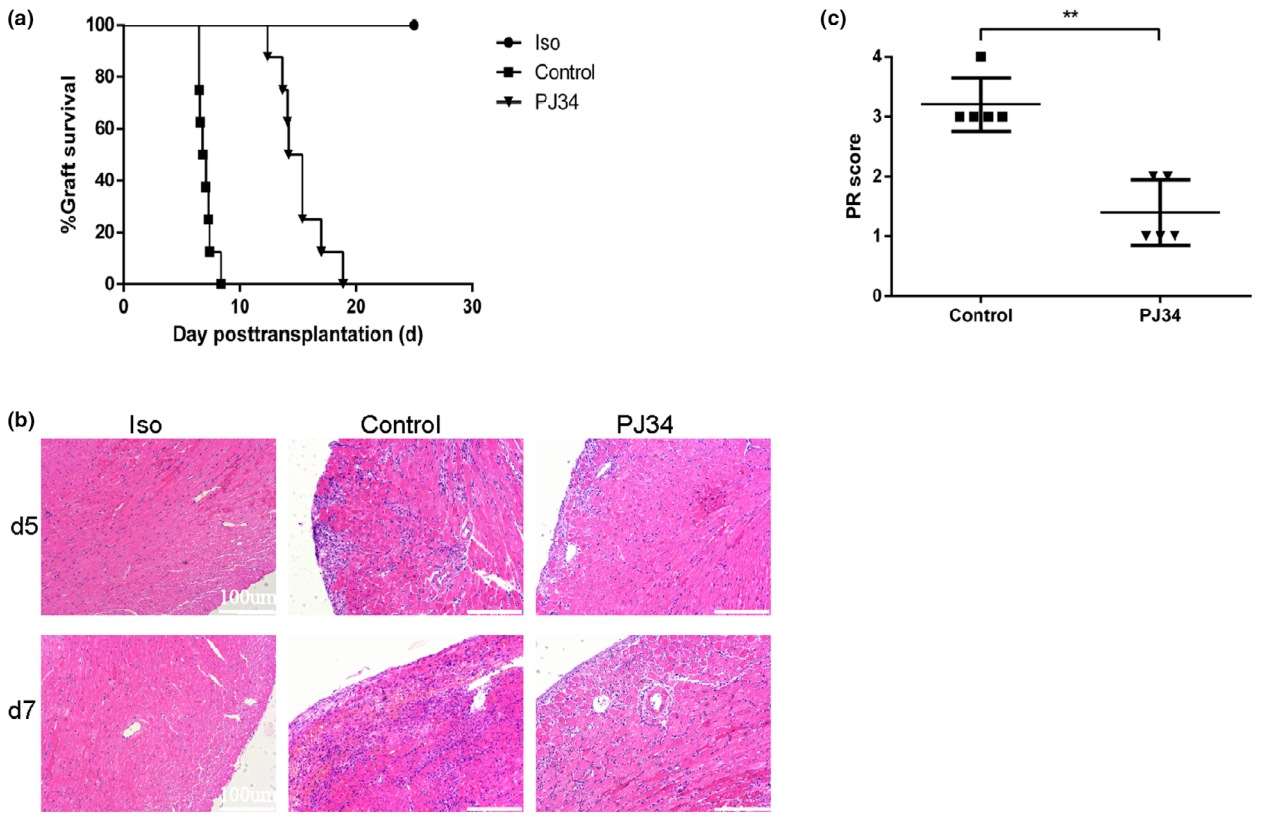

Figure 2 PJ34 attenuated acute rejection and prolonged murine cardiac allograft survival. (a) Survival of the cardiac grafts in each group. PJ34 prolong murine cardiac allograft survival (MST, the PJ34 group, 15.1 ± 1.9 days, $n = 8$; the control group, 7.05 ± 0.59 days, $n = 8$). (b) Hematoxylin–eosin staining of the tissue sections in cardiac grafts. PJ34 can significantly ameliorate inflammatory cells infiltration and vasculopathy of the cardiac allografts. Scale bars = 100 μm. (c) The PR scores of the tissue sections in cardiac allografts were evaluated on day 7 after murine heart transplantation. $N = 5$. $^{**}P < 0.01$.

group were conspicuous lower than the control group on the 7th day post-transplantation (Fig. 2c, $P < 0.01$). Therefore, it was likely that the PJ34, a PARP1 inhibitor, could not only prolong murine cardiac allograft survival, but also attenuate acute rejection.

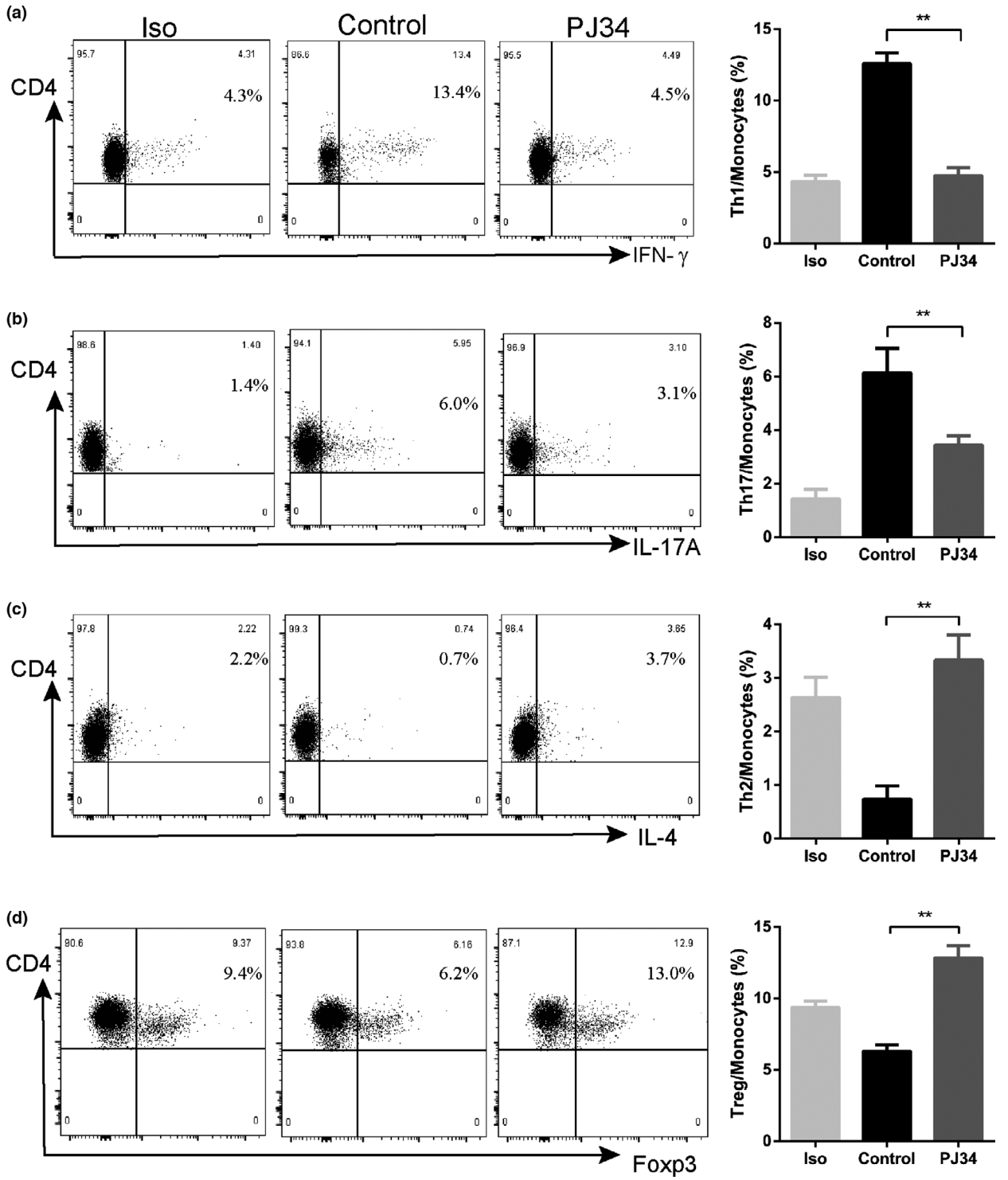

Figure 3 PJ34 modulated the percentages of $CD4^+$ T cells subsets in recipient spleen. Splenocytes isolated from allograft recipients in each group on day 5 after heart transplantation were activated and stained with different antibodies. Representative graphs of $CD4^+IFN\text{-}\gamma^+$ Th1 cells (a), $CD4^+IL\text{-}17A^+$ Th17 cells (b), $CD4^+IL\text{-}4^+$ cells Th2 cells (c), and $CD4^+Foxp3^+$ Treg cells (d) in the spleen by flow cytometric (FCM) analysis ($n = 3$–5). $^{**}P < 0.01$.

PJ34 modulated the percentages of $CD4^+$ T cells subsets in recipient spleen

The spleen acting as guardians is closely involved in allograft rejection. To assess the role and mechanism underlying PJ34-mediated allograft protection, we used the flow cytometry to analyze the $CD4^+$ T cells subpopulation/percentage. Th1 cells ($CD4^+IFN\text{-}\gamma^+$ cells) comprised a large percentage in the control group; and it was notably reduced after PJ34 treatment (Fig. 3a, $P < 0.01$). In parallel with the results of Th1 cells, we found that the proportion of Th17 cells ($CD4^+IL\text{-}17A^+$ cells) was also decline in the PJ34 group compared with the control group (Fig. 3b, $P < 0.01$). Th2 can also affect transplantation rejection; however, the percentage of Th2 cells ($CD4^+IL\text{-}4^+$ cells) was drastically increased with PJ34 treatment after heart transplantation (Fig. 3c, $P < 0.01$). Subsequently, we assessed the percentage of splenic Tregs ($CD4^+Foxp3^+$ cells). We found that PJ34 treatment gave a rise to a remarkable increase in the Tregs percentage compared with the control group (Fig. 3d, $P < 0.01$).

PJ34 reduced the inflammatory cells infiltrating into cardiac allografts

By detecting expression of related inflammatory cells involved in acute rejection, we could assess the effects of PJ34 on cardiac allografts. Immunohistochemical analysis showed that the infiltration of inflammatory cells was noted in the epicardium and myocardium particularly in perivascular distribution. A significant infiltration of $CD3^+$ cells, $CD4^+$ cells and $CD8^+$ cells was observed in the allogeneic grafts, whereas all $CD3^+$ cells, $CD4^+$ cells and $CD8^+$ cells were reduced in the PJ34-treated group compared with the control group (Fig. 4a–c). Neutrophil infiltration is a well-recognized feature of AMR and is thought to cause injury through the production of reactive oxygen species and release of preformed enzymes [23]. Subsequently, we analyzed neutrophils in the cardiac grafts, but there was no significant difference between the PJ34 group and the control group (Fig. 4d).

PJ34 regulated the expression of inflammatory cytokines

After verifying that PJ34 was conducive to prolong survival of cardiac allografts, we examined whether it regulated the expression of certain inflammatory cytokines related to acute transplant rejection. The grafted hearts harvested from mice sacrificed on 5th day after heart transplantation were subjected to qPCR analysis. The results revealed that the mRNA levels of IFN-γ and IL-17A were significantly reduced in the PJ34 group compared to those in the control group (Fig. 5a,b, $P < 0.05$). In contrast, the mRNA levels of TGF-β, FOXP-3, and IL-10, known as anti-inflammatory cytokines, were markedly increased with PJ34 treatment (Fig. 5d–f, $P < 0.05$). However, the expression of IL-4

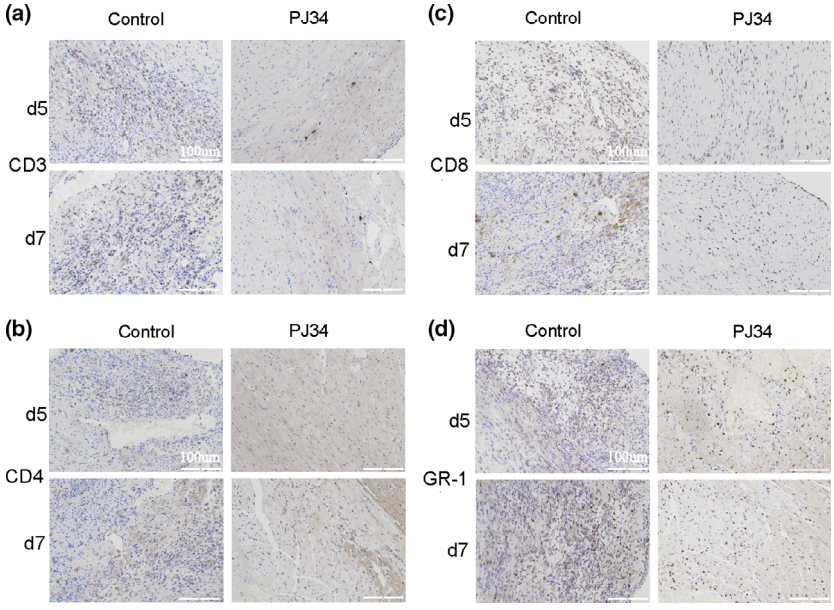

Figure 4 PJ34 reduced the inflammatory cells infiltrating into cardiac allografts. Immunohistochemical staining of CD3 (a), CD4 (b), CD8 (c), and Gr-1 (d) in the tissue sections from the control group and the PJ34 group. Scale bars = 100 μm.

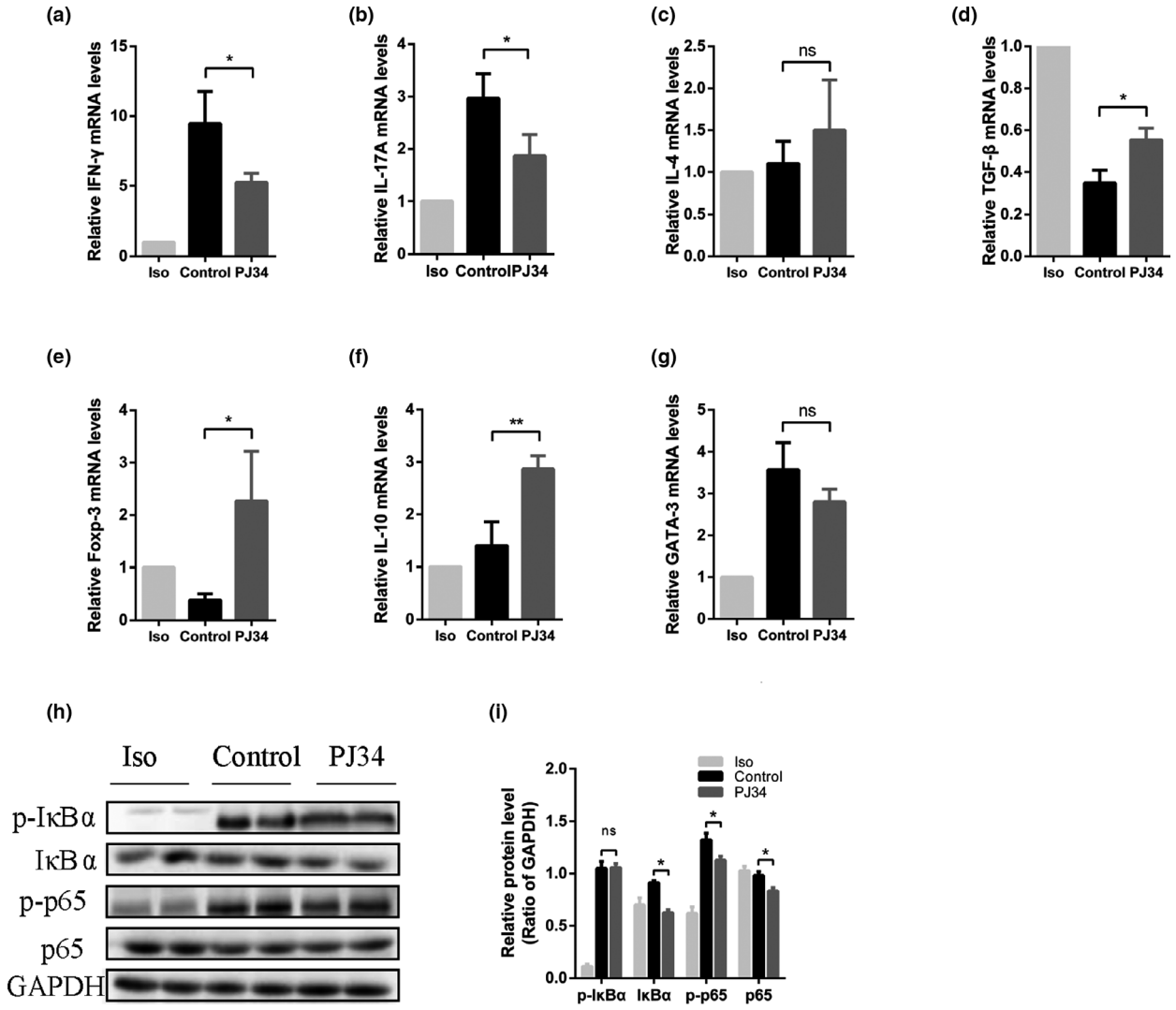

Figure 5 PJ34 regulated the expression of inflammatory cytokines. (a–g) The expression level of IFN-γ, IL-17A, IL-4, TGF-β, FOXP-3, IL-10, and GATA-3 was quantitative by real-time polymerase chain reaction (RT-PCR). *$P < 0.05$, **$P < 0.01$. Ns indicates no significance. (h and i) IκBα, p-p65, and p65 were significantly decreased in the PJ34 group compared with the control group by Western blot analysis. *$P < 0.05$. Ns indicates no significance.

and GATA-3 was not significantly different from the control group (Fig. 5c,g). Furthermore, we explored the effects of PJ34 on NF-κB in grafted hearts with Western blotting. Treatment with PJ34 can inhibit the expression of IκB and p65 but except for p-IκBα, we found no significance between the control group and the PJ34 treatment group (Fig. 5h,i, $P < 0.05$).

PJ34 inhibits Th1/Th17 cell differentiation and promotes Treg differentiation *in vitro*

To further investigate the role of PJ34 in allogeneic responses, we performed studies using naïve $CD4^+$ T cells from murine spleens *in vitro*. After stimulation with different cytokines, we found that PJ34 treatment significantly inhibited the mRNA levels of IFN-γ and IL-17A and promoted the mRNA levels of TGF-β and Foxp-3 (Fig. 6a, $P < 0.05$). In the supernatant, PJ34 also reduces the expression of IFN-γ and IL-17A and increase the levels of IL-10 (Fig. 6b, $P < 0.05$). By the use of Western blotting, PJ34 treatment, which exhibited the similar effects *in vivo*, was shown to inhibit the expression and activation of IκBα and p65 (Fig. 6c,d, $P < 0.05$).

Discussion

In this work, we found that increased PARP1 expression and total poly(ADP-ribosyl)ation levels of the allogeneic

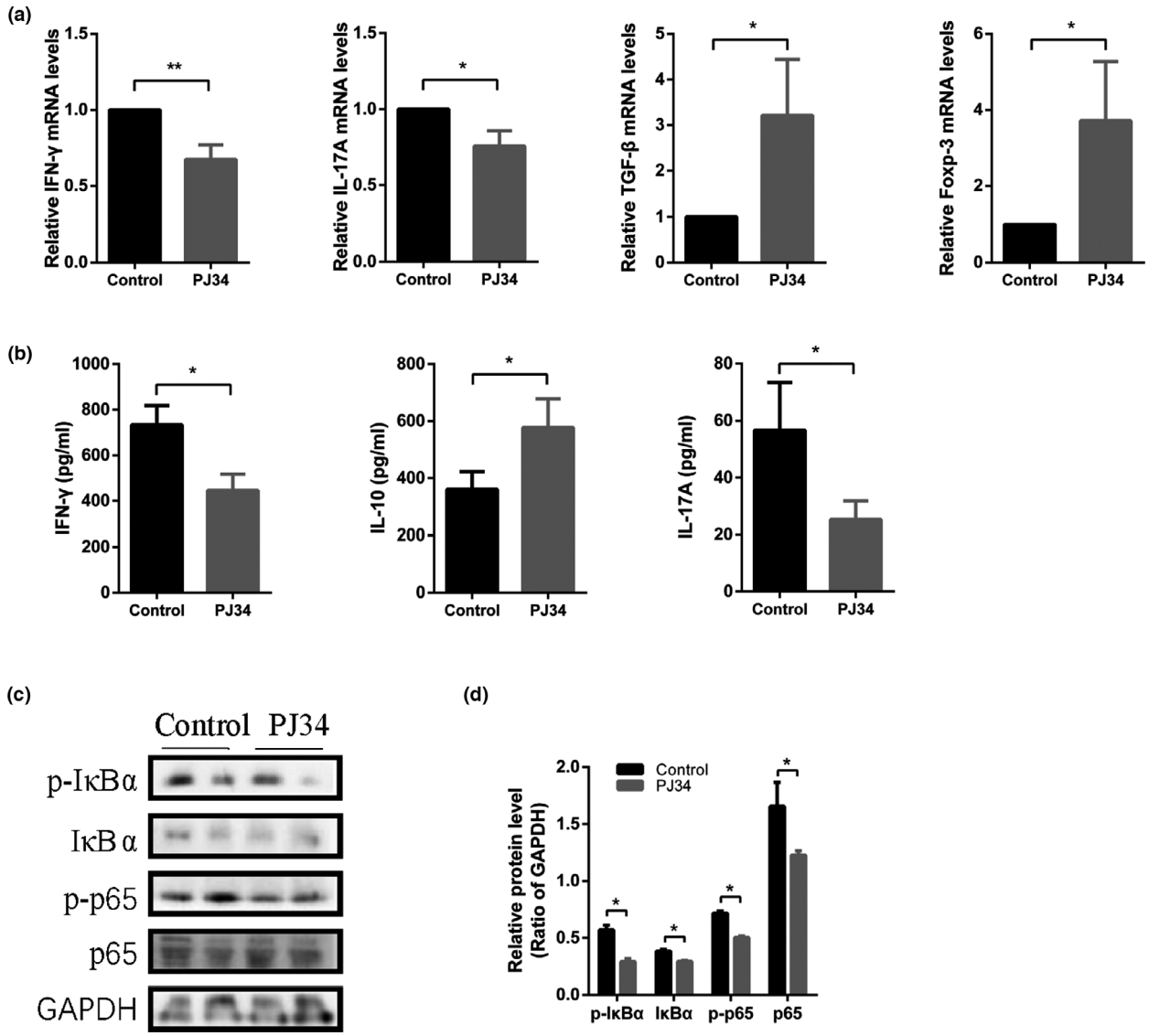

Figure 6 PJ34 inhibits Th1/Th17 cell differentiation and promotes Treg differentiation *in vitro*. (a) The expression level of IFN-γ, IL-17A, TGF-β, and Foxp-3 was quantified by real-time polymerase chain reaction (qRT-PCR). $^{*}P < 0.05$, $^{**}P < 0.01$. (b) The concentration of IFN-γ, IL-17A, and IL-10 in the supernatant. $^{*}P < 0.05$. (c and d) PJ34 can decrease p-IκBα, IκBα, p-p65, and p65 in differentiated $CD4^{+}$ cells by Western blot analysis. $^{*}P < 0.05$.

grafts were observed. The treatment with PJ34, which was an inhibitor of PARP1, could attenuate acute rejection and prolong murine cardiac allograft survival. PJ34 modulated the percentages of $CD4^{+}$ T cells subsets with inhibiting Th1/Th17 cell differentiation and promoting Treg differentiation.

Allograft rejection is the primary obstacle to long-term graft survival in clinical heart transplantation. T-cell-mediated alloimmune responses is affected via the antigen-presenting cells (APCs) presenting the allo-antigens to $CD4^{+}$ and $CD8^{+}$ T cells [24]. Furthermore, allo-reactive $CD4^{+}$ T cells especially Th1 and Th17 mediate prominent allograft rejection. During acute rejection, allo-reactive Th1 produces IFN-γ and relevant transcript factor T-bet and FasL are upregulated [25]. Th17 might contribute to allograft rejection through producing cytokines IL-17 and IL-21. In this study, we confirmed that PJ34 can reduce the percentage of Th1 and Th17 in spleen; meanwhile, the levels of IFN-γ and IL-17A had a significant decline with PJ34 treatment in allogeneic hearts. In contrast to Th cells, Tregs are considered potent immunosuppressive cells are crucial for keeping host immune tolerance after allogeneic transplantation [26]. We also found that promoting the differentiation of Tregs was associated with prolonging the allogeneic grafts survival. Therefore, regulating the

$CD4^+$ T cells subsets or modulating the balance of Th1/17 and Treg may have some therapeutic effects.

$CD4^+$ T-cell-mediated allograft rejection depends to a large degree upon the relevant inflammatory cytokines. Subsequently, we measured mRNA levels of IFN-γ, IL-17A, TGF-β, FOXP-3, IL-10, IL-4, IL-12 and GATA-3 in cardiac grafts. Consistent with previous studies, our studies demonstrates that inhibiting the activation of PARP1 can reduce the levels of IFN-γ and IL-17A and increase the levels of TGF-β and IL-10 [27–29]. IFN-γ is produced by the Th1, which is known to contribute to graft rejection. Previous studies also suggest that most acute allograft rejection exhibit high levels of IFN-γ and low levels of TGF-β [30]. With anti-inflammatory and suppressive effects on alloimmune responses, Th2 cell-produced IL-10 and Tregs-produced TGF-β may also shift the immune response from the Th1 pathway to Th2 or Tregs responses, favoring Ig production [31]. This result could imply that TGF-β and IL-10 can attenuate the acute rejection process and protect allografts.

PJ34 modulated the percentages of $CD4^+$ T cells subsets involves a series of molecular mechanisms. NF-κB is a crucial signal transduction molecule, participating in regulation of cytokines, chemokines, and adhesion molecules [32]. Previous research indicated that NF-κB was activated during acute rejection after renal or liver transplantation [33,34]. Cooper *et al.* [35] found a time-dependent increase in myocardial NF-κB activity in untreated allografts compared with isografts using PhosphorImage analysis and demonstrated that the role of NF-κB mainly in the allo-dependent pathway in the rejection process. Treatment with PDTC, a specific inhibitor of NF-κB, suppressed NF-κB activity in rejection of cardiac allograft in rat via inhibiting of the P65 subunit of NF-κB, or inhibiting of IκB degradation, thereby reducing NF-κB subunit P65/P50 in the nucleus [36]. Consistent with these results, in this study, we found treatment with PJ34 attenuated acute cardiac allograft rejection, as well as reducing the expression of IκB and p65 *in vivo* and *in vitro*.

PARP1 induces the process of DNA repair and function which is at the center of the stress responses. A variety of stimulation can activate PARP1 that subsequently determine cellular fate [37]. A growing body of evidence suggests that PARP1 is closely related to immune response and chronic inflammation, including autoimmunity [38,39]. However, no researches have linked PARP1 to cardiac allograft rejection. Here, our experiments provide evidence that PARP1 was highly activated in the allogeneic grafts after heart transplantation. Our data showed the allograft rejection accompany with PARP1 activation and various cellular mechanisms might be triggered in the process. PARP1 likely contributes to DNA repair protecting cell from damage when immune responses is mild or moderate, but it aggravates myocardial death and vasculopathy when immune responses is excessive. Persistent DNA damage and cell death might also be involved in PARP1 activation after cardiac transplantation and these require further research.

Conclusions

In summary, PJ34, a specific PARP1 inhibitor, could attenuate acute allografts rejection and prolong murine cardiac allograft survival by modulating the $CD4^+$ T lymphocyte response. This study indicates that PARP1 may be a promising therapeutic target to attenuate acute cardiac allograft rejection.

Authorship

XC: involved in methodology, investigation, and writing the original draft preparation. YH: involved in methodology, validation, and data curation. DW: involved in formal analysis and software. ND: involved in formal analysis and conceptualization. XD: involved in conceptualization and supervision.

Funding

The work was supported by the National Key Research and Development Program of China (2016YFA0101100) and National Natural Science Foundation of China (31330029).

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to the manuscript.

Acknowledgements

We are thankful to Dr. Chuanlei Yang for his excellent technical assistance during the study.

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