IL-17 Upregulates MCP-1 Expression via Act1 / TRAF6 / TAK1 in Experimental Autoimmune Myocarditis

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Abstract

Myocarditis is a myocardial inflammatory infiltration heterogeneous disease. At present, various interventions are not effective in the treatment of myocarditis. IL-17, an important pro-inflammatory factor secreted mainly by Th17 cells, can promote the expression of multiple cytokines. MCP-1 is an important cytokine that mediates mononuclear cell infiltration. Studies have found that IL-17 could stimulate the expression of MCP-1 to mediate inflammatory infiltration. But the mechanism by which IL-17 induces MCP-1 expression in experimental autoimmune myocarditis (EAM) remains unclear. The purpose of this study is to establish an EAM model to explore the role of Act1/TRAF6/TAK1 cascade in the induction of MCP-1 by IL-17. In the present study, we found that in EAM, IL-17 could stimulate the expression of MCP-1 by activating Act1/TRAF6/TAK1 cascade. After interfering TAK1 with si-TAK1, myocardial tissue inflammation was greatly alleviated, and both MCP-1 mRNA and protein expression were downregulated. In conclusion, IL-17 can activate AP-1, NF-κB via Act1/TRAF6/TAK1 upregulation of MCP-1 expression in EAM.

Introduction

Myocarditis is a myocardial disease resulting from many causes, including microbial infection, autoantigens, drugs. The incidence of myocarditis is increasing year by year[1]. EAM is a myocardium inflammation mediated by $CD4^+$T cells. The pathological features of EAM were inflammatory cell infiltration, myocardial cell necrosis and collagen deposition in cardiac fibroblasts and myofibroblasts[2]. Studies have shown that persistent inflammation of the myocardium can lead to myocardial remodeling, which could evolve into dilated cardiomyopathy (DCM) with heart failure[1–3].

Interleukin-17(IL – 17), because of its HVS13 herpes virus gene showed high homology, originally called CTLA-8. It was renamed IL-17, when it was first cloned in 1993 from the mouse activated T cell hybridoma[4]. Th17 cells, as a newly discovered subset of $CD4^+$T cells, are involved in many inflammatory diseases and are the main source of IL-17 [5]. IL-17 could bind to IL-17R to promote the expression of several chemokines and participate in several physiopathological processes, including immunologic defense, inflammatory infiltration and the progression of cancer[6]. Studies have shown that treatment of mice with viral myocarditis using IL-17-neutralizing antibody significantly reduced myocardial inflammatory infiltration[7]. Monocyte chemokine protein 1 (MCP-1) is a major chemokine of mononuclear cells in vivo, which can drive monocytes or macrophages to gather in the direction of inflammation[8]. Our previous study found that MCP-1 was upregulated by IL-17 in cardiomyocytes, however, how IL-17 regulated MCP-1 expression remains unclear.[9].

It is found that after IL-17 is combined with IL-17R, IL-17R could recruit Act1 with the same domain through its SEFIR domain[10–11]. In addition, Act1 has a TRAF6 binding domain, which can bind downstream TRAF6 molecules and cause their ubiquitin activation[11–12]. Tang et al. found that the activation of TRAF6 then activated the P38 MAPK signaling pathway of human periodontal membrane

cells[13]. TGF-β activated kinase 1 (TAK1) is a member of the MAP3K family, is considered to be an important regulatory downstream regulator of TRAF6 and can mediate the activation of downstream IKK kinases and MAPK signaling pathways. IKK can regulate the activation of NF-κB through IκB phosphorylation, and MAPK signal transduction pathway is the key pathway to activate transcription factor AP-1.[14–15]. Huang et al. demonstrated that IL-17A could activate Act1/TRAF6/TAK1 axis to mediate NF-κB activation, which regulates CXCL2 expression in airway epithelial cells.[16]. Meanwhile, studies showed that IL-17 could activate AP-1 in chondrocytes to stimulate collagenase 3 expression[17]. Furthermore, activated AP-1 can upregulate the expression of MCP-1 in carcinoma cells[18]. Therefore, we speculate that IL-17 can mediate the expression of MCP-1 in EAM by activating Act1/TRAF6/TAK1 signal pathway. Based on the above findings, we intend to establish an EAM mouse model to explore the role of IL-17 in regulating MCP-1 expression through Act1/TRAF6/TAK1 in the pathogenesis of EAM.

Material And Methods

Establishment of the EAM model

Male BALB/c mice (18-22g) were purchased from Henan Experimental Animal Research Center. The animal experiments were approved by the Animal Experiment Committee of Zhengzhou University. For induction of EAM, the mice were subcutaneously injected with 200μg of the mixture of cardiac myosin polypeptide (MyHC-$_{\alpha 614-629}$:SLKLMATLFSTYAS)(GL Biochem, China) with CFA (Sigma-Aldrich, USA) on day 0 and day 7[19]. The control group was given an equal volume of saline and CFA mixture.

Histology and Immunohistochemistry

The hearts were immersed in 4% paraformaldehyde, covered in paraffin and cut into slices. Then hematoxylin-eosin staining was used to observe and take pictures under the microscope. Double-blind scoring was performed according to the area percentage of inflammation. The score of myocarditis was divided into 4 grades. 0 grade was no inflammatory cell infiltration; in grade 1, the inflammatory area was less than 25%; in grade 2, the inflammatory area was 25–50%; in grade 3, the inflammatory area was 50% – 75%; in grade 4, the inflammatory infiltration was more than 75%. For immunohistochemistry (IHC), paraffin sections were dewaxed, hydrated and blocked. Then MCP-1 and IL-17 antibodies (Affinity, China) were incubated at 4 ℃. On the next day, the primary antibodies were washed off with PBS, then incubated with secondary antibody for 1 hour. After washing, DAB was used as dyeing substrate, and sections were re-stained with hematoxylin. The IHC sections were observed with a microscope and analyzed with Image J software.

Flow cytometry

The spleens cells of mice were analyzed by flow cytometry on day 14, day 21, day 28 post the first immunization. Mice spleen cells were prepared according to the previous procedure[20]. To find Treg cells, we first stained the surface with FITC-CD4 antibody and Percy-CY5.5-CD25 antibody (Ebioscience, USA). For Th17 cells, we stimulated the cells with 50ng/mL phorbol ester (Solarbio, China) and ionomycin

(Solarbio, China) of 1mg/mL, and then added the Golgi blocker monensin (Solarbio, China) 0.7ug / mL, incubated at 5% CO2 at 37 ℃ for 4 hours, and stained the surface with FITC-CD4 antibody (Ebioscience, USA). Then, the cells were fixed and broken according to the instructions of the kit (Multi science, China). Next, Treg cells were labeled with PE-Foxp3 antibody and Th17 cells were labeled with APC-IL-17 antibody (Ebioscience, USA). After incubation, the samples were measured by Navios (Beckman, USA).

Enzyme-linked immunosorbent assay

The expression of IL-17 and MCP-1 in mice serum was tested by enzyme-linked immunosorbent assay (ELISA). After taking blood from mice eyeballs, the serum was extracted and detected by IL-17 and MCP-1 ELISA kits (Elabscience, China).

Quantitative RT-PCR

Total RNA was isolated according to the procedure of the RNA extraction kit (Takara, Japan) and transformed into cDNA by the reverse transcriptase kit. (TOYOBO, Japan). The PCR primer sequences are shown as below:

Table 1
PCR Primer Sequences

Gene	Primer sequences
IL-17	Forward 5'-GAGCTTCATCTGTGTCTCTGAT-3'
	Reverse 5'-GCCAAGGGAGTTAAAGACTTTG-3'
Act1	Forward 5'-CCAGAGCATCGGTTCACACTTACG-3'
	Reverse 5'-CCACTGAAGGAGTCTGCCAAAGC-3'
TRAF6	Forward 5'-GAAAATCAACTGTTTCCCGACA-3'
	Reverse 5'-ACTTGATGATCCTCGAGATGTC-3'
TAK1	Forward 5'-CCCTTCAATGGAGGAAATTGTG-3'
	Reverse 5'-CTCCAAGCGTTTAATAGTGTCG-3'
MCP-1	Forward 5'-TTTTTGTCACCAAGCTCAAGAG-3'
	Reverse 5'-TTCTGATCTCATTTGGTTCCGA-3'
NF-κB p65	Forward 5'-TGATGTGCATCGGCAAGTG-3'
	Reverse 5'-TTCTGATCTCATTTGGTTCCGA-3'
c-JUN	Forward 5'-AGAAGTTGAGTTTCGGGTAG-3'
	Reverse 5'-CTTAGGGTTACTGTAGCCGTAG-3'
GAPDH	forward 5'-CTGCACCACCAACTGCTTAG-3'
	Reverse 5'-GTCTGGGATGGAAATTGTGA-3'

Table 2
Myocardial pathological score of mice(X ± s)

Group	EAM group	Control group
14 Day	0.8±0.273*	0
21 Day	2.7±0.447*	0
28 Day	1.1±0.223*	0
*P < 0.05 vs Control group; #P < 0.05 vs EAM group on 21 day.		

Table 3
The IHC density of IL-17 and MCP-1 in the myocardium of each group(X ± s)

Group	IL-17	MCP-1
Control	0	0
14 Day	591.6676±92.4477*#	721.3977±123.424*#
21 Day	3049.732±318.2843*	2860.553±222.0973*
28 Day	922.6171±206.5938*#	911.9893±93.98956*#
*P < 0.05 vs Control group; #P < 0.05 vs EAM group on 21 day.		

The relative expression of genes was expressed as a ratio of gene expression and GAPDH expression. Each assay was performed three times.

Western Blotting

Total protein was extracted with RIPA lysate (Solarbio, China) containing phenyl methane sulfonyl fluoride (PMSF) (Solarbio, China) and quantified. Then, the protein was separated by 10% SDS-PAGE gel electrophoresis and transferred to membranes (Epizyme, China). The membrane was incubated with specific antibodies at 4°C. On the next day, the membrane was washed off by TBST, then incubated with secondary antibodies, washed and colored. The optical density of the strips was analyzed by Image J software and calculated the average gray value of each strip.

RNA interference

According to the instructions of the transfection reagent (Engreen, China), the EAM mice were transfected with 0.5mg/kg nanoparticle encapsulated si-NC or si-TAK1. From the 0 days of immunization, si-NC or si-TAK1 was injected every 3 days until 21 days. The sequence for si-TAK1 was 5'-CCAGCACAGGCTCATTCAT-3'; the sequences for si-NC was: 5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3'.

Statistics

The results were analyzed by SPSS 22.0 software. Data were presented as the mean±SD. Comparisons among multiple groups were performed by one-way ANOVA, and comparisons among groups were performed by LSD-t test, with a test level of $\alpha = 0.05$.

Results

Establishment of EAM model

In order to explore the progression of EAM, we set three different time points. We found that the heart weight of EAM mice was greatly increased on days 14, 21 and 28 post induction ($P < 0.05$); the heart weight/body weight ratio of the EAM was also higher than the control ($P < 0.05$). Then we analyzed the results of HE staining in heart and found that the inflammatory cells began to infiltrate on 14 day, reached the peak on 21 day, and began to decline on 28 day; no inflammatory cell was observed in the control group. At the same time, we made a double-blind score on the degree of inflammation. We found that the pathological score of the EAM group was greatly higher than that of the control group ($P < 0.05$), and reached the highest value on day 21.

Expression of IL-17 and MCP-1 in EAM

Subsequently, we explore the expression of IL-17 and MCP-1 in EAM. First, we performed IHC of the heart. As shown in Fig. 3, positive cells began to appear in the myocardium of EAM mice on 14 day. A high number of positive cells appeared in myocardial inflammatory lesions and stroma on 21 day, and decreased on 28 day. No positive cells were found in the control group at all times. Next, we analyzed the optical density and found that the EAM group had higher optical density of IL-17 and MCP-1 compared with the control group ($P < 0.05$), and the optical density of IL-17 and MCP-1 expressed on 21 day was higher than those on 14 day and 28 day($P < 0.05$). Then, we extracted myocardial mRNA for qRT-PCR and found that the expression of IL-17 and MCP-1 mRNA in EAM mice increased on 14 day, reached the peak on 21 day, and decreased on 28 day($P < 0.05$). Consistent with the IHC results, the expression levels of IL-17 and MCP-1mRNA in group EAM were greatly higher than those in the control group ($P < 0.05$). Meanwhile, we used ELISA kit to detect the levels of IL-17 and MCP-1 in serum. We found that the levels of IL-17 and MCP-1 were significantly increased in the EAM group ($P < 0.05$).

The proportions of Th17 cells and Treg cells in EAM

In order to further investigate the relationship between IL-17 and EAM, we tested the proportion of Th17 cells in the spleen of mice. In line with IL-17 expression, the proportion of Th17 cells greatly increased in the EAM group ($P < 0.05$), and reached the peak on 21 day. In parallel, we also analyzed the proportion of Treg cells. As the antagonistic cells of TH17 cells, the proportion of Treg cells in EAM group decreased significantly ($P < 0.05$). Then, we analyzed the correlation between Th17 cells, Treg cells and the inflammatory infiltration of EAM. The results further confirmed that Th17 cells were positively correlated with the inflammatory infiltration of EAM, and Treg cells were negatively correlated with the inflammatory infiltration of EAM($P < 0.05$).

The signaling pathway activated by IL-17 in EAM

In order to study the signal transduction mechanism and transcription factors of MCP-1 secreted by myocardial tissue induced by IL - 17, mRNA was extracted from mouse myocardial tissue to detect the expression of Act1, TRAF6, TAK1, p65 and c-jun. As shown in Fig. 7, the mRNA levels of Act1, TRAF6, TAK1, p65 and c-JUN were upregulated in the EAM group ($P < 0.05$). Interestingly, we found that the change trend at the gene expression was consistent with the change trend in inflammation. At the same time, we tested all the molecules from the protein level, and found that compared with the control group,

the protein expressions of Act1, TRAF6, TAK1, p-p65 and p-c-Jun were greatly increased ($P < 0.05$). Those suggested that IL-17 could activate Act1/TRAF6/TAK1 axis to up-regulate the expression of MCP-1 in EAM.

Blocking TAK1 diminishes immune cell infiltration during EAM

Studies have shown that TAK1 could activate NF-κB and AP-1, and then mediate the expression of the target gene.[21]. To assess the role of Act1/TRAF6/TAK1 pathway on IL-17-mediated inflammatory infiltration, we treated EAM mice with si-TAK1 and chose 21 day as the analysis time point. We found that the inflammatory infiltration was reduced in the si-TAK1 group, but it was still higher than that in the control group ($P < 0.05$). We next tested the mRNA expression and protein content of MCP-1. The results showed that the mRNA and protein levels of MCP-1 was down-regulated after blocking the signaling pathway ($P < 0.05$). Later, we tested the mRNA and protein levels of NF-κB and AP-1, and found that the levels of NF-κB and AP-1 was significantly inhibited in EAM mice treated with TAK1 siRNA ($P < 0.05$).

Discussion

Myocarditis is an inflammatory disease of the cardiovascular system with a predilection for adolescents and children that may eventually lead to DCM and heart failure. Chronic inflammation caused by virus infection or autoimmune reaction is the main cause of DCM[1, 22]. MCP-1 is an important inflammatory factor in vivo, which could mediate the infiltration of mononuclear cells. [23]. For example, in patients with bronchial asthma, MCP-1 can chemotaxis monocytes and macrophages to the alveoli, leading to airway inflammation and remodeling[24–25]. Our previous study also found that MCP-1 can lead to a large number of inflammatory cell aggregation through its receptor CCL2 in viral myocarditis[26]. Therefore, it is speculated that reducing the expression of MCP-1 is of great significance for the occurrence and development of myocarditis.

In previous studies, we found that IL-17 could up-regulate MCP-1 and aggravate cardiomyocyte injury[9]. In this study, we explored the relationship between IL-17, MCP-1, and myocarditis. We performed IHC of mouse hearts by constructing an EAM mouse model. The results showed that with the development of EAM, both IL-17 and MCP-1 levels increased and peaked on 21 day, and decreased on 28 day. Meanwhile, we also measured its mRNA expression and its content in serum, and consistent results were obtained. It is suggested that IL-17 and MCP-1 are involved in the inflammatory infiltration process of EAM.

Th17 cells are helper T cells that differentiate in response to stimulation by IL-6 and IL-23[27]. Evidence has proved that Th17 cells are associated with many autoimmune diseases, including Sjogren's syndrome, rheumatoid arthritis, Scleroderma, and so on[28–29]. Conversely, Treg cells, as indispensable cells for immune homeostasis, can play suppressive roles in a variety of autoimmune diseases[29–30]. From our analysis, we found that the proportion of Th17 cells in EAM group increased greatly, while the opposite result was found for Treg cells. We further performed a correlation between the proportion of

Th17 cells, Treg cells and the degree of inflammatory infiltration. The analysis showed that the severity of EAM was closely related to the proportion of Th17 cells and Treg cells. Those results suggested that the Th17 cells/Treg cells ratio may be used as a new therapeutic target for EAM to ameliorate myocardial inflammatory infiltration, and also further proved that IL-17 plays a key role in the inflammatory infiltration of EAM. But in EAM, by which signal IL - 17 up-regulates MCP - 1 expression in myocardial tissue, it remains unclear.

It has been documented that the intracellular region of IL-17R contains a SEFIR domain. Interestingly, Act1 contains both TRAF6 domains and a SEFIR domain. And Act1 is also the only one to contain a SEFIR domain in addition to the IL-17R family. Act1 can bind to a variety of TRAFs, and at the same time, it acts on cell receptors containing the SEFIR domain, such as IL-17R, through the interaction of the SEFIR-SEFIR domain[11,31–32]. Liao et al reported that TRAF6 could aggravate cardiac hypertrophy through TAK1 dependent signaling[33]. Tadashi et al found that IL-17A could activate the Act1/TRAF6/TAK1 signaling pathway to regulate cardiomyocyte contractility and death[34]. Therefore, we speculated that IL-17 regulates MCP-1 expression by activating Act1/TRAF6/TAK1 axis in EAM. In the present study, our data also support the hypothesis that the up-regulation of MCP-1 by IL-17 is dependent on the Act1/TRAF6/TAK1 signaling pathway. Promoters are DNA sequences that play an initiating role during transcription. Our previous studies revealed that MCP-1 promoter sequence contains NF-κB, AP-1 binding site[16,35–36]. Therefore, we further explored whether TAK1 activates NF-κB and AP-1 to up-regulate MCP-1 expression. We found that the levels of NF-κB and AP-1 increased significantly in EAM group, and the elevation was correlated with the degree of inflammatory infiltration. This suggests that in EAM, IL-17 may regulate MCP-1 expression through the Act1/TRAF6/TAK1 cascade to mediate inflammatory cell infiltration.

In the present study, we investigated the effect of IL-17 in the pathogenesis of EAM by regulating MCP-1 expression via Act1/TRAF6/TAK1 cascade. Other proinflammatory factors, such as TNF-α, IL-1, and IL-6, are also a key contributor to the occurrence of inflammatory diseases. Studies have shown that TNF-α can activate NF-κB, AP-1 to up-regulate MCP-1 of glomerular endothelial cells[37]. Meanwhile, Yan et al found that IL-1β may activate IKK / NF-κB, JNK/c-Jun, C/EBPβ to regulate MCP-1 expression in alveolar epithelial cells[38]. In addition, IL-6 can also stimulate MCP-1 secretion by activating downstream pathways through binding to IL-6R[39]. Interestingly, IL-6 can be regulated through RORγt to promote Th17 cell differentiation synergistically enhance MCP-1 expression[40]. It follows that IL-17, TNF-α, IL-1, and IL-6 synergistically promote MCP-1 expression.

In conclusion, this study shows that IL-17 regulates the inflammatory infiltration of EAM by regulating the expression of MCP-1 through Act1 / TRAF6 / TAK1, which will help to clarify the pathological mechanism of myocarditis and provide a new direction for clinical practice.

Declarations

Conflicts of interest

The authors declare no conflicts of interest.

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Availability of data and material

All data generated or analysed during this study are included in this published article.

Code availability

Not applicable for this section.

Authors' contributions

Xiao Huang designed the study, analyzed the data and revised the manuscript. Zhuolun Li analyzed the results and revised the manuscript. Xinhe Shen revised the manuscript. Na Nie performed the ELISA. Yan Shen designed the study, wrote the manuscript, and revised it.

Ethics approval

The animal experiments were approved by the Animal Experiment Committee of Zhengzhou University.

Consent to participate

Not applicable for this section.

Consent for publication

Not applicable for this section.

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References

1. Cooper, L. T., and Myocarditis. *The New England journal of medicine.* 2009;360(15):1526–1538.

2. Imanaka-Yoshida, K. Inflammation in myocardial disease: From myocarditis to dilated cardiomyopathy. *Pathology international.* 2020;70(1).
3. Rose, N. R. Viral myocarditis. *Current opinion in rheumatology.* 2016;28(4):383–389.
4. Yao, Z., W. C. Fanslow, and M. F. Seldin, et al. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity.* 1995;3(6):811–821.
5. Patel, D. D., and V. K. Kuchroo. Th17 Cell Pathway in Human Immunity: Lessons from Genetics and Therapeutic Interventions. *Immunity.* 2015;43(6):1040–1051.
6. Kuwabara, T., F. Ishikawa, M. Kondo, and T. Kakiuchi. The Role of IL-17 and Related Cytokines in Inflammatory Autoimmune Diseases. *Mediators of inflammation.* 2017;2017:3908061.
7. Fan, Y., W. Weifeng, Y. Yuluan, K. Qing, P. Yu, and H. Yanlan. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of coxsackievirus b3-induced viral myocarditis reduces myocardium inflammation. *Virology journal.* 2011;8:17.
8. Conti, P., and M. DiGioacchino. MCP-1 and RANTES are mediators of acute and chronic inflammation. *Allergy and asthma proceedings.* 2001;22(3):133–137.
9. Huang, K. D., Y. Shen, X. Wei, F. Q. Zhang, Y. Y. Liu, and L. Ma. Inhibitory effect of microRNA-27b on interleukin 17 (IL-17)-induced monocyte chemoattractant protein-1 (MCP1) expression. *Genetics and molecular research: GMR.* 2016;15(2).
10. Liu, C., S. Swaidani, and W. Qian, et al. A CC' loop decoy peptide blocks the interaction between Act1 and IL-17RA to attenuate IL-17- and IL-25-induced inflammation. *Science signaling.* 2011;4(197):ra72.
11. Herjan, T., L. Hong, and J. Bubenik, et al. IL-17-receptor-associated adaptor Act1 directly stabilizes mRNAs to mediate IL-17 inflammatory signaling. *Nature immunology.* 2018;19(4):354–365.
12. Shi, P., S. Zhu, and Y. Lin, et al. Persistent stimulation with interleukin-17 desensitizes cells through SCFβ-TrCP-mediated degradation of Act1. *Science signaling.* 2011;4(197):ra73.
13. Tang, L., X. Li, Y. Bai, P. Wang, and Y. Zhao. MicroRNA-146a negatively regulates the inflammatory response to Porphyromonas gingivalis in human periodontal ligament fibroblasts via TRAF6/p38 pathway. *Journal of periodontology.* 2019;90(4):391–399.
14. Cao, A. T., S. Yao, B. Gong, C. O. Elson, and Y. Cong. Th17 cells upregulate polymeric Ig receptor and intestinal IgA and contribute to intestinal homeostasis. *Journal of immunology (Baltimore, Md.: 1950).* 2012;189(9):4666–4673.
15. Atsaves, V., V. Leventaki, G. Z. Rassidakis, and F. X. Claret. AP-1 Transcription Factors as Regulators of Immune Responses in Cancer. *Cancers.* 2019;11(7).
16. Huang, F., C.-Y. Kao, S. Wachi, P. Thai, J. Ryu, and R. Wu. Requirement for both JAK-mediated PI3K signaling and ACT1/TRAF6/TAK1-dependent NF-kappaB activation by IL-17A in enhancing cytokine expression in human airway epithelial cells. *Journal of immunology (Baltimore, Md.: 1950).* 2007;179(10):6504–6513.

17. Benderdour, M., G. Tardif, and J.-P. Pelletier, et al. Interleukin 17 (IL-17) induces collagenase-3 production in human osteoarthritic chondrocytes via AP-1 dependent activation: differential activation of AP-1 members by IL-17 and IL-1beta. *The Journal of rheumatology.* 2002;29(6):1262–1272.
18. Lee, C.-H., P.-F. Hung, and S.-C. Lu, et al. MCP-1/MCPIP-1 Signaling Modulates the Effects of IL-1β in Renal Cell Carcinoma through ER Stress-Mediated Apoptosis. *International journal of molecular sciences.* 2019;20(23).
19. Wang, Y., W. Ma, and S. Lu, et al. Androgen receptor regulates cardiac fibrosis in mice with experimental autoimmune myocarditis by increasing microRNA-125b expression. *Biochemical and biophysical research communications.* 2018;506(1):130–136.
20. Duriancik, D. M., and K. A. Hoag. The identification and enumeration of dendritic cell populations from individual mouse spleen and Peyer's patches using flow cytometric analysis. *Cytometry. Part A: the journal of the International Society for Analytical Cytology.* 2009;75(11):951–959.
21. Endale, M., T.-H. Kim, and Y.-S. Kwak, et al. Torilin Inhibits Inflammation by Limiting TAK1-Mediated MAP Kinase and NF-B Activation. *Mediators of inflammation.* 2017;2017:7250968.
22. Pollack, A., A. R. Kontorovich, V. Fuster, and G. W. Dec. Viral myocarditis–diagnosis, treatment options, and current controversies. *Nature reviews. Cardiology.* 2015;12(11):670–680.
23. Yoshimura, T. The chemokine MCP-1 (CCL2) in the host interaction with cancer: a foe or ally? *Cellular & molecular immunology.* 2018;15(4):335–345.
24. Schneider, D., J. Y. Hong, and E. R. Bowman, et al. Macrophage/epithelial cell CCL2 contributes to rhinovirus-induced hyperresponsiveness and inflammation in a mouse model of allergic airways disease. *American journal of physiology. Lung cellular and molecular physiology.* 2013;304(3):L162-L169.
25. Ichinose, T., H. Takano, and K. Sadakane, et al. Differences in airway-inflammation development by house dust mite and diesel exhaust inhalation among mouse strains. *Toxicology and applied pharmacology.* 2003;187(1):29–37.
26. Shen, Y., W. Xu, Y.-W. Chu, Y. Wang, Q.-S. Liu, and S.-D. Xiong. Coxsackievirus group B type 3 infection upregulates expression of monocyte chemoattractant protein 1 in cardiac myocytes, which leads to enhanced migration of mononuclear cells in viral myocarditis. *Journal of virology.* 2004;78(22):12548–12556.
27. Wu, B., and Y. Wan. Molecular control of pathogenic Th17 cells in autoimmune diseases. *International immunopharmacology.* 2020;80:106187.
28. Yasuda, K., Y. Takeuchi, and K. Hirota. The pathogenicity of Th17 cells in autoimmune diseases. *Seminars in immunopathology.* 2019;41(3):283–297.
29. Lee, G. R. The Balance of Th17 versus Treg Cells in Autoimmunity. *International journal of molecular sciences.* 2018;19(3).
30. Göschl, L., C. Scheinecker, and M. Bonelli. Treg cells in autoimmunity: from identification to Treg-based therapies. *Seminars in immunopathology.* 2019;41(3):301–314.

31. Kanamori, M., C. Kai, Y. Hayashizaki, and H. Suzuki. NF-kappaB activator Act1 associates with IL-1/Toll pathway adaptor molecule TRAF6. *FEBS letters.* 2002;532(1–2):241–246.
32. Wu, L., J. Zepp, and X. Li. Function of Act1 in IL-17 Family Signaling and Autoimmunity. *Current Topics in Innate Immunity II*2012:223–235.
33. Liao, H.-H., N. Zhang, and Y.-Y. Meng, et al. Myricetin Alleviates Pathological Cardiac Hypertrophy via TRAF6/TAK1/MAPK and Nrf2 Signaling Pathway. *Oxidative medicine and cellular longevity.* 2019;2019:6304058.
34. Yoshida, T., N. A. Das, and A. J. Carpenter, et al. Minocycline reverses IL-17A/TRAF3IP2-mediated p38 MAPK/NF-κB/iNOS/NO-dependent cardiomyocyte contractile depression and death. *Cellular signalling.* 2020;73:109690.
35. Du, S., Z. Li, and X. Xie, et al. IL-17 stimulates the expression of CCL2 in cardiac myocytes via Act1/TRAF6/p38MAPK-dependent AP-1 activation. *Scandinavian journal of immunology.* 2020;91(1):e12840.
36. Ping, D., P. L. Jones, and J. M. Boss. TNF regulates the in vivo occupancy of both distal and proximal regulatory regions of the MCP-1/JE gene. *Immunity.* 1996;4(5):455–469.
37. Park, S.-K., W. S. Yang, and N. J. Han, et al. Dexamethasone regulates AP-1 to repress TNF-alpha induced MCP-1 production in human glomerular endothelial cells. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association.* 2004;19(2):312–319.
38. Yan, C., B. Li, and X. Liu, et al. Involvement of multiple transcription factors in regulation of IL-β-induced MCP-1 expression in alveolar type II epithelial cells. *Molecular immunology.* 2019;111.
39. Zegeye, M. M., M. Lindkvist, and K. Fälker, et al. Activation of the JAK/STAT3 and PI3K/AKT pathways are crucial for IL-6 trans-signaling-mediated pro-inflammatory response in human vascular endothelial cells. *Cell communication and signaling: CCS.* 2018;16(1):55.
40. Ju, X., T. Ijaz, and H. Sun, et al. Interleukin-6-signal transducer and activator of transcription-3 signaling mediates aortic dissections induced by angiotensin II via the T-helper lymphocyte 17-interleukin 17 axis in C57BL/6 mice. *Arteriosclerosis, thrombosis, and vascular biology.* 2013;33(7):1612–1621.

Figures

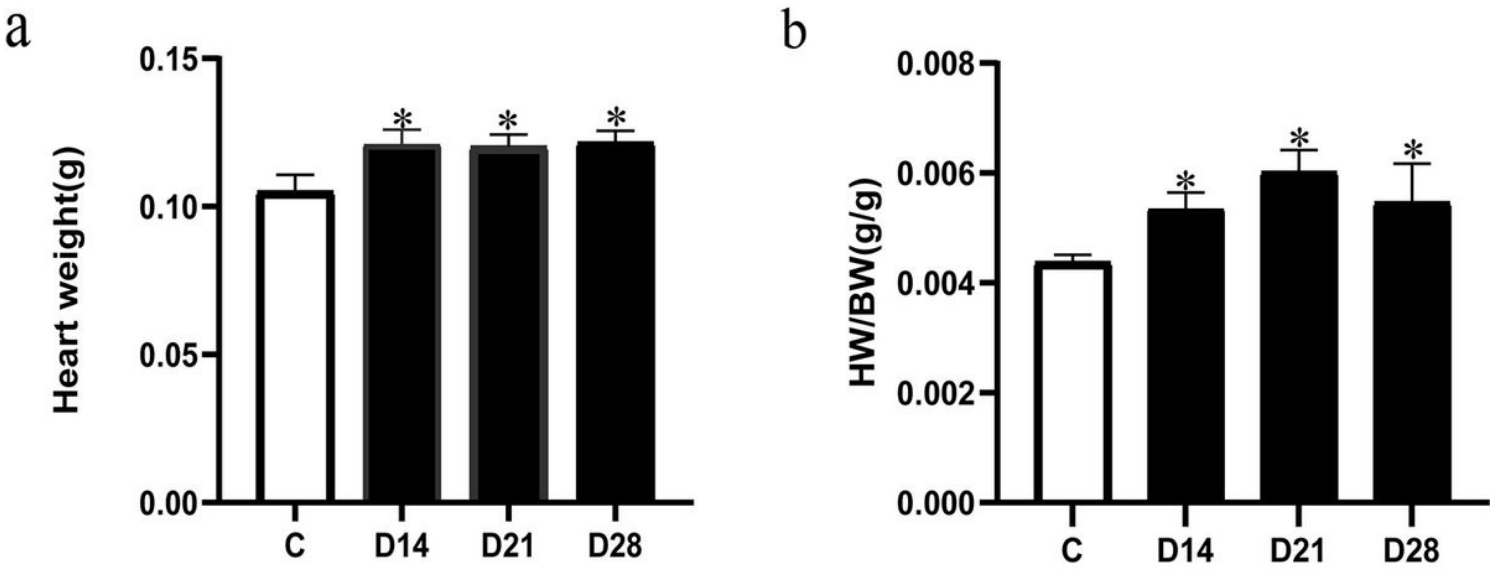

Figure 1

The heart weight and the ratio of heart weight to body weight; C, D14, D21 and D28 represent the control, 14 day, 21 day and 28 day respectively. a Heart weight; b The ratio of heart weight to body weight, HW / BW. (*P<0.05 vs Control)

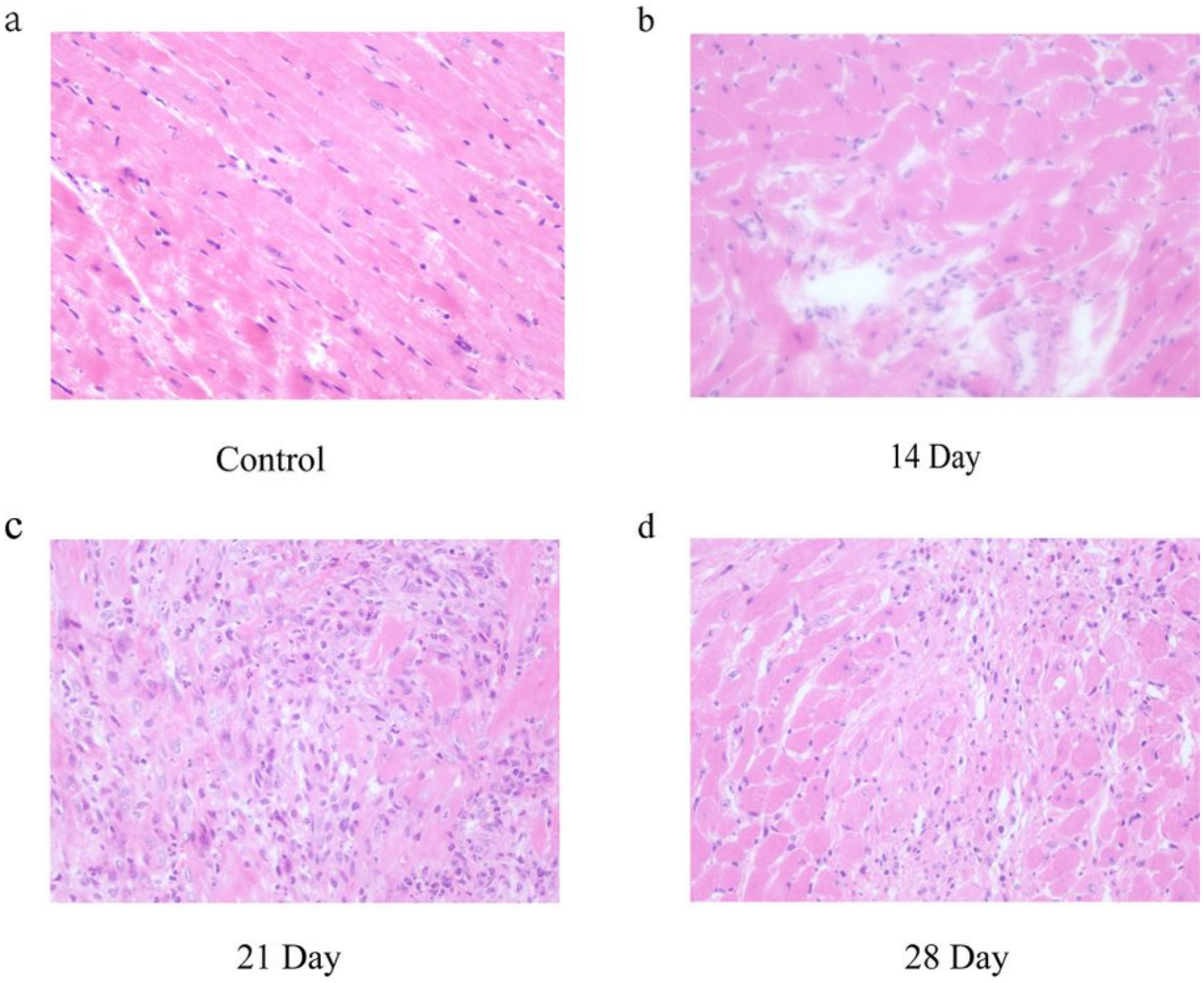

Figure 2

Histopathological analysis of HE staining in mice heart. a)Control group; b-d) EAM group in different time points.

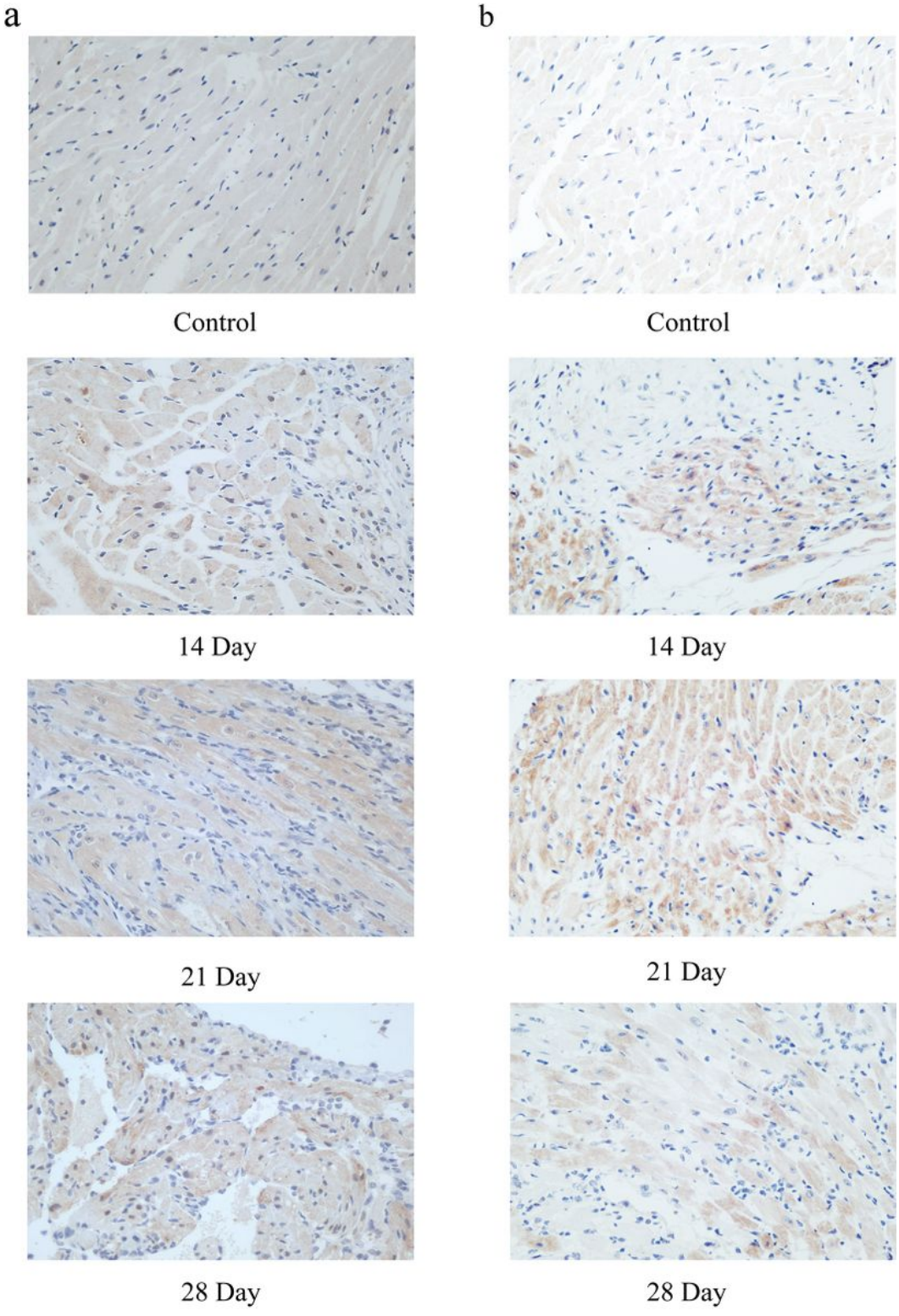

Figure 3

The IHC analysis of IL-17, MCP-1 expression in the myocardium. a) IHC for IL-17; b) IHC for MCP-1.

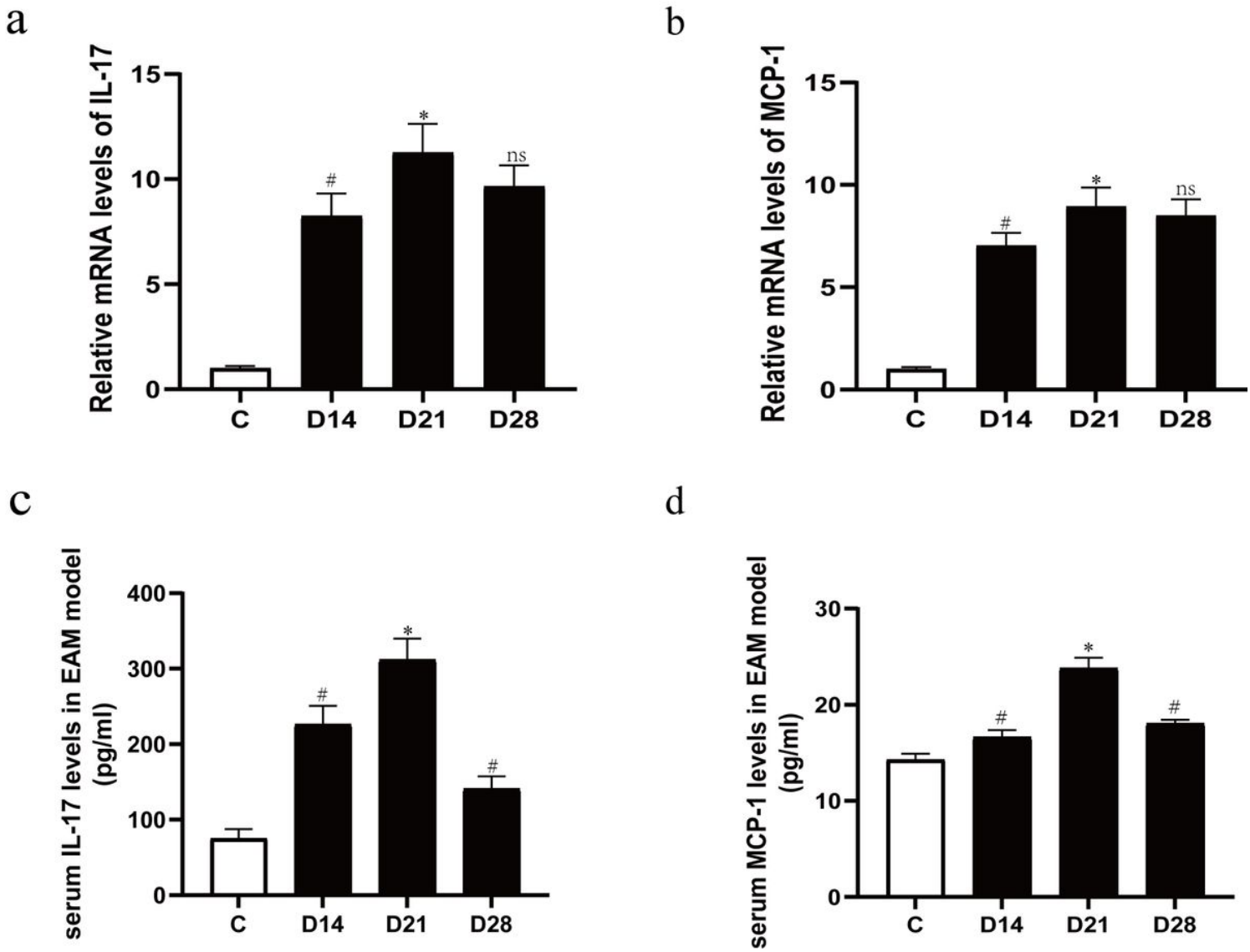

Figure 4

IL-17 and MCP-1 mRNA expression levels and serum levels; C, D14, D21 and D28 represent the control, 14 day, 21 day and 28 day respectively. a) IL-17 mRNA relative expression; b) MCP-1 mRNA relative expression; c) Serum IL-17 levels; d) Serum MCP-1 levels. (* P <0.05 vs Control□#P<0.05 vs EAM group on 21 day; ns P>0.05 vs EAM group on 21 day□

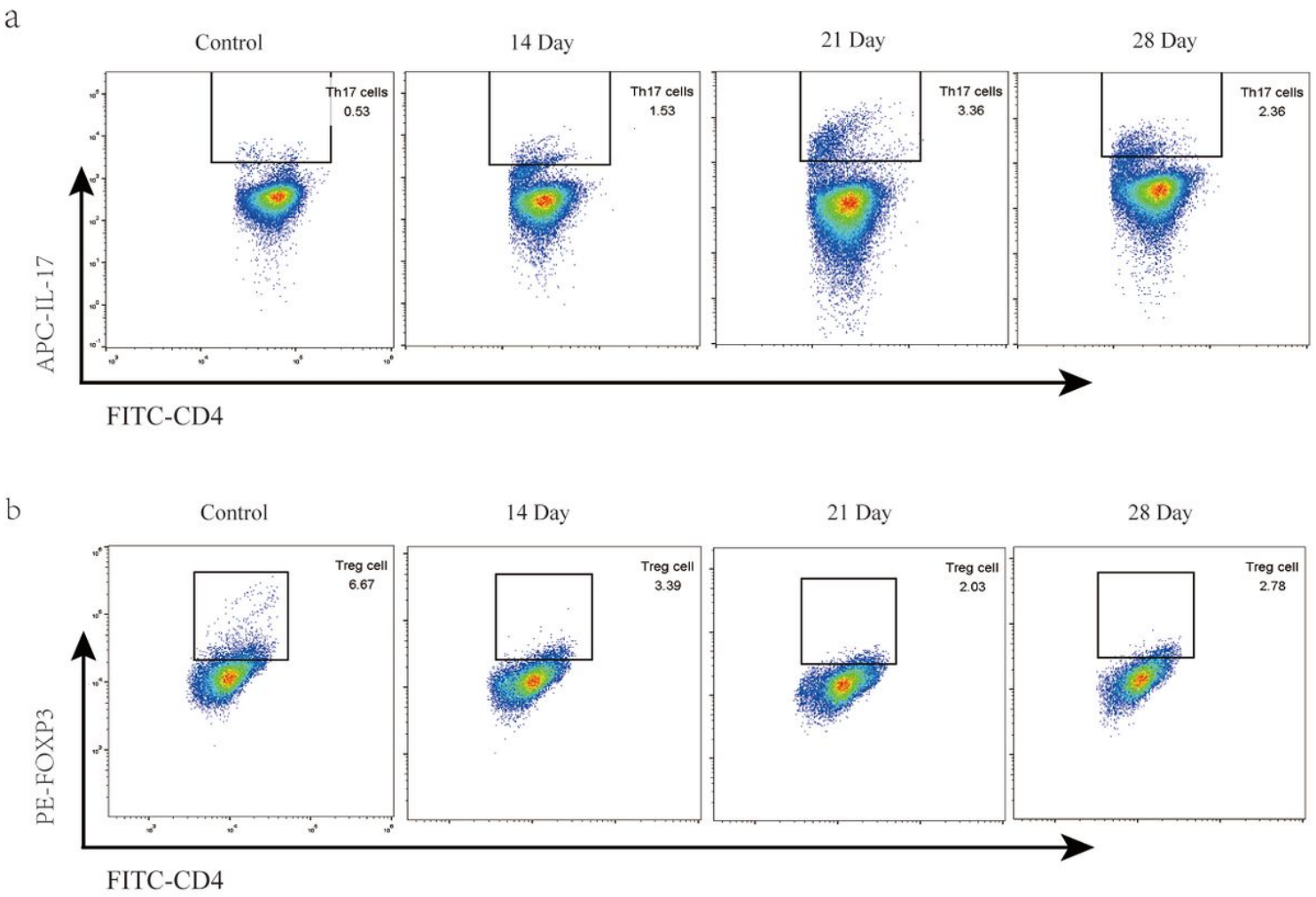

Figure 5

Expression of Th17 cells and Treg cells in EAM. a) the proportion of Th17 cell; b) the proportion of Treg cell.

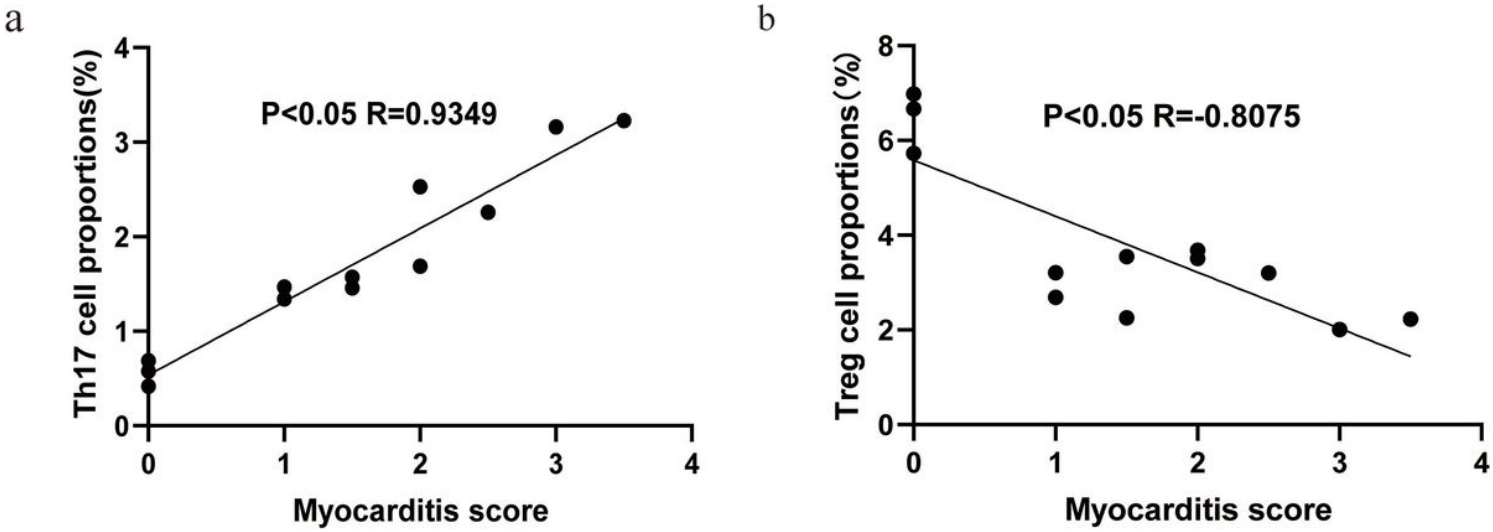

Figure 6

The correlation between Th17 cells, Treg cells and myocarditis score. a) correlation between Th17 cells and myocarditis score; b) correlation between Treg cells and myocarditis score.

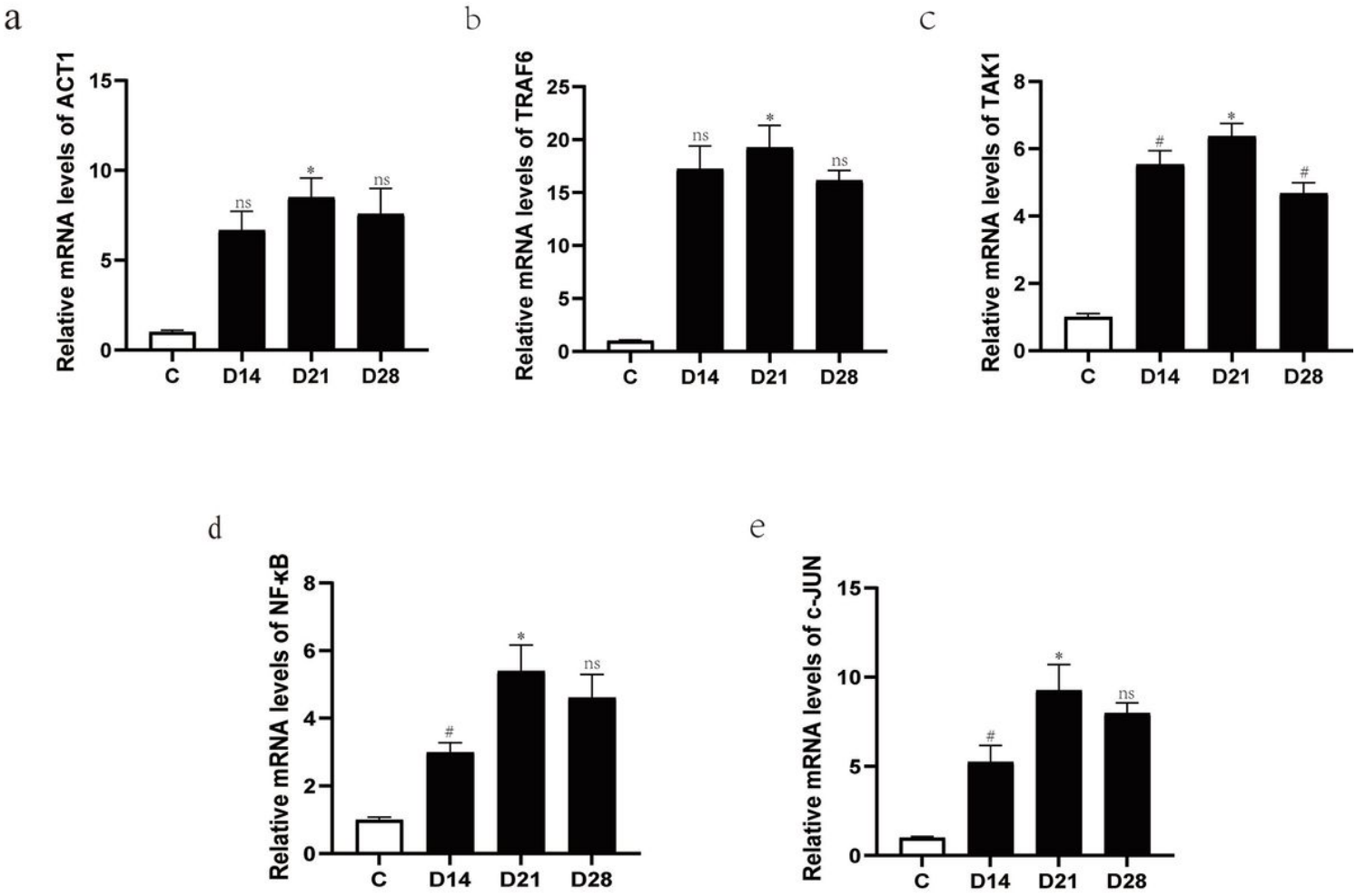

Figure 7

The mRNA expression of signal pathway molecules activated by IL-17 in EAM. C, D14, D21 and D28 represent the control, 14 day, 21 day and 28 day respectively. a) The relative expression of Act1 mRNA; b) The relative expression of TRAF6 mRNA; c) Relative expression of TAK1 mRNA; d) The relative expression of NF-κB mRNA; e) Relative expression of c-JUN mRNA. (*P <0.05 vs Control□#P<0.05 vs EAM group on 21 day; ns P>0.05 vs EAM group on 21 day)

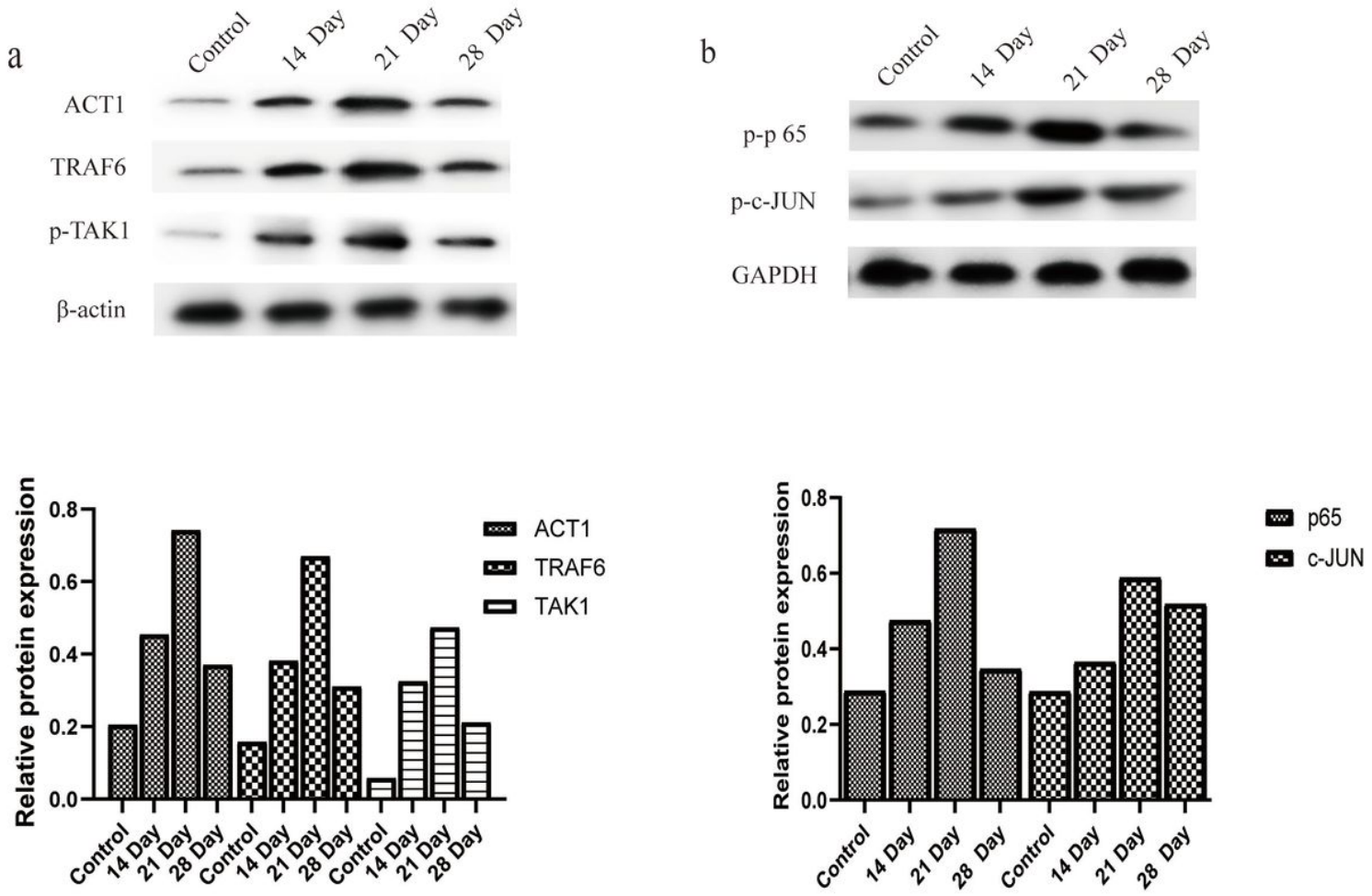

Figure 8

The expression of molecular proteins of signal pathway activated by IL-17 in EAM. a) The expression of Act1, TRAF6 and TAK1 protein; b) The expression of p-p65, p-c-JUN protein.

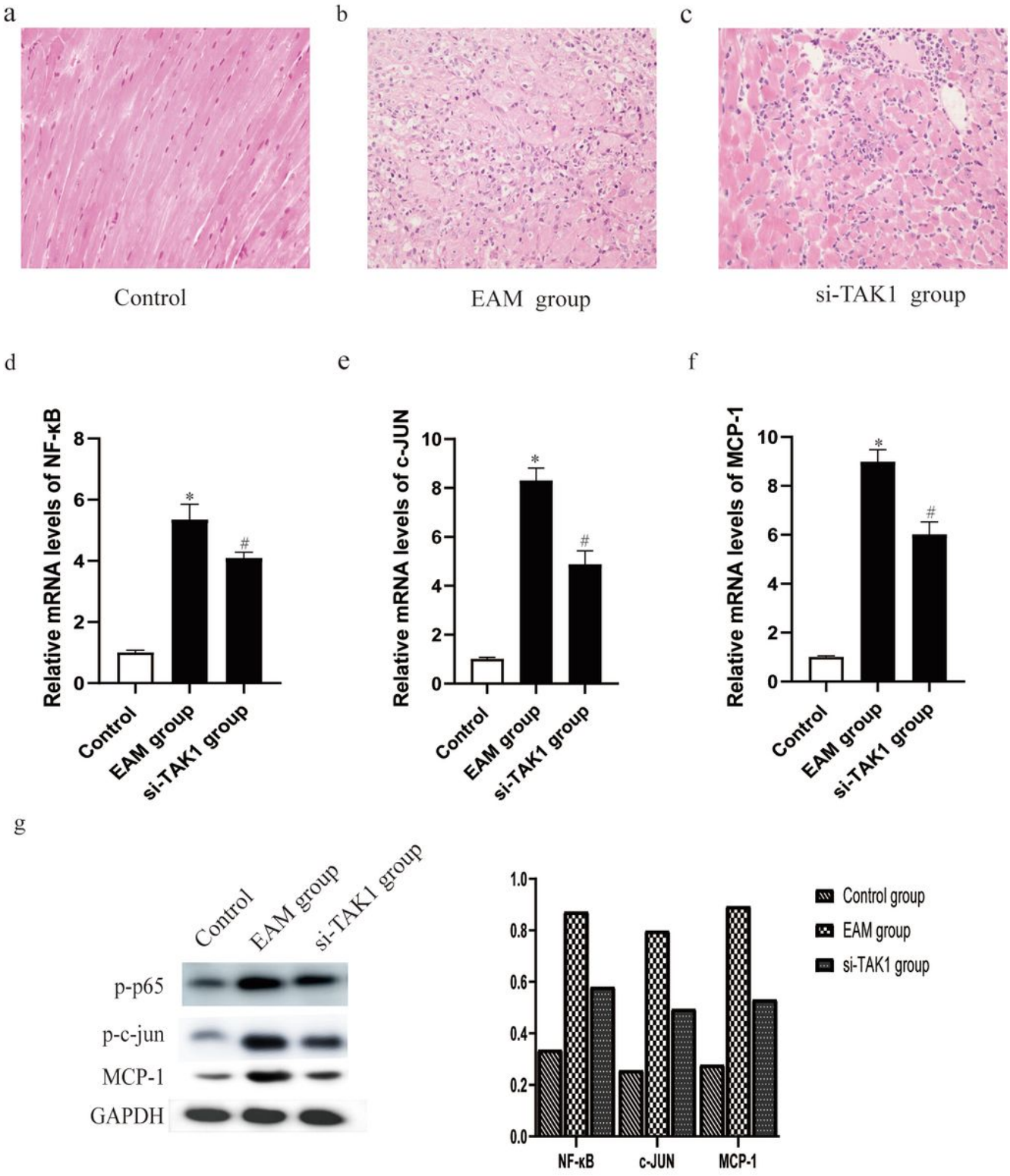

Figure 9

The effects of blocking TAK1 on myocardial immune infiltration in EAM. a-c) HE staining of cardiac tissue sections; d) The relative expression of NF-κB mRNA; e) The relative expression of c-JUN mRNA; f) The relative expression of MCP-1 mRNA; g) The expression of p-p65, p-c-JUN, MCP-1 protein.