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1,25-Dihydroxyvitamin D_3 regulates T lymphocyte proliferation through activation of P53 and inhibition of ERK1/2 signaling pathway in children with Kawasaki disease

X.-L. QI, L.-L. CHEN, X.-G. SUN, X.-M. LI, L.-H. ZHAO, D.-J. KONG

Department of Pediatrics, Linyi Central Hospital, Linyi, Shandong, China

Abstract. – OBJECTIVE: To study the effect of $1,25(OH)_2D_3$ on T lymphocytes in Kawasaki disease and to further investigate its molecular mechanism.

PATIENTS AND METHODS: A total of 30 child patients was diagnosed as Kawasaki disease, 60 child patients were diagnosed as infectious fever, and 60 normal children, were selected. 4 mL peripheral blood was collected before treatment. Peripheral blood mononuclear cells were isolated and separated using the Facollin method and cultured. Flow cytometry was used to identify T cells. The cell sections were prepared for immunohistochemical staining. After T cells in each group were treated with $1,25(OH)_2D_3$, the proteins were extracted for Western blotting.

RESULTS: Peripheral blood T cells were successfully isolated and cultured, and the maximal atoxic concentration of $1,25(OH)_2D_3$ on T cells was 10-3 μmol/L. In T cells of child patients with Kawasaki disease, signal transducer and activator of transcription 3 (STAT3) were continuously activated, P53 apoptosis genes were inactivated, and nuclear factor κB (NF-κB) P65 pathway and extracellular signal-regulated kinase (ERK) pathway were activated. After the intervention with $1,25(OH)_2D_3$ *in vitro*, STAT3 and NF-κB P65 had no significant changes, the activation of ERK1/2 signaling pathway was inhibited and the P53 protein was activated.

CONCLUSIONS: Apoptotic T cells in peripheral blood in KD cannot initiate the normal apoptosis program, so they continue to proliferate and differentiate, eventually leading to the increase and abnormal activation of T cells and the immune imbalance in the body. $1,25(OH)_2D_3$ can inhibit the excess hyperplasia of T cells through adjusting partial signal transduction pathway.

Key Words:
$1,25(OH)_2D_3$, Kawasaki disease, T lymphocyte, P53, ERK1/2.

Introduction

Mucocutaneous lymph node syndrome, also known as Kawasaki disease (KD), is a kind of acute fever and rash disease in children, characterized by systemic vasculitis. The disease can lead to severe cardiovascular diseases, such as coronary aneurysm, coronary artery stenosis and myocardial infarction, among which the occurrence rate of coronary aneurysm is about 13%. In recent years, the incidence rate of KD has been on the rise and KD has replaced rheumatism as the main disease causing the acquired heart disease in children, seriously threatening the life safety of patients[1,2]. The pathogenesis and mechanism of KD have not been clear yet. Recent studies have shown that the significant systemic immune activation exists in KD in the acute phase; in particular, the abnormal activation of T cells plays an important role in the pathogenesis[3]. Imbalance of peripheral blood T cell subsets in the acute phase and the increased CD4/CD8 ratio make the immune system in an activated state[4]. The increased lymphokines secreted by CD4 promote B cells' polyclonal activation, proliferation and differentiation into plasma cells, leading to the increased serum immunoglobulins. These lymphokines and activin can induce endothelial cells to express and produce new antigens. However, the cause and mechanism of the above immune disorders remain unclear. $1,25(OH)_2D_3$ is the active form of vitamin D and belongs to the steroid hormone, which binds to its receptor (vitamin D receptor, VDR) to form the hormone-receptor complex, and then binds to vitamin D response elements in nuclei to play a biological role[5,6]. In recent years, many studies have found that $1,25(OH)_2D_3$ is not

Corresponding Author: Lingling Chen, MD; e-mail: chbell@163.com

only the calcium-phosphorus regulator, but also the T/B cell proliferation inhibitor. 1,25(OH)$_2$D$_3$ has effects on various immune cells, which can inhibit the functions of dendritic cells and macrophages, restrain the synthesis of immunoglobulins and suppress the transcription of many cytokines, such as IL-1, IL-2, IL -6, IL-12, tumor necrosis factor-α(TNF-α) and TNF-γ[7-9]. Gregori et al[10] also found that 1,25(OH)$_2$D$_3$ can up-regulate the CD4+CD25+Treg and induce the immune tolerance. There are a variety of receptors on the surface of immune cell membrane, and external informational molecules can specifically bind to them, stimulating cells to produce a certain physiological response process, namely the transmembrane signal transduction. The whole process of signal transduction and regulation in cell is complex, and different pathways interrelate and constrain another, showing a network shape. Therefore, four representative signaling pathways were selected for study: extracellular signal kinase pathway that is closely related to cell proliferation and differentiation; cytokine-activated signal transducer and activator of transcription (STAT) pathway; nuclear factor κB (NF-κB) signaling pathway with wide regulatory effects; apoptotic-related P53 pathway. This experiment studied the effect of 1,25(OH)$_2$D$_3$ on T lymphocytes in KD, and further investigated the roles of the above four important signaling pathways.

Patients and Methods

Patients

General information: KD group: 30 child patients diagnosed as KD in 2016-2017 from Linyi Central Hospital (in line with the diagnostic criteria of MCLS of Japan in 2002); infectious fever group: 60 child patients diagnosed as infectious fever in our hospital; normal group: 60 normal healthy children. Sample collection: 4 mL peripheral blood was collected before treatment. This study was approved by the Ethics Committee of Linyi Central Hospital. Signed written informed consents were obtained from all participants before the study.

T cell Isolation and Culture

Peripheral blood mononuclear cells were isolated and extracted using the Facollin method. Facollin solution with the volume as twice as blood was added and the blood was also slowly added without damaging the interface between Facollin solution and blood. The mixed solution was centrifuged for 15 min, and the intermediate floccules were collected. After floccules were washed, 2 mL red blood cell lysis buffer was added in a dark place for 15 min, followed by centrifuge at 1000 rpm. The supernatant was removed and the cells were mixed uniformly using the T cell column-passing solution. The cell suspension was prepared and the T cell column was placed. 2 mL column-passing solution was added to wash the column and the cell suspension was then added for incubation at room temperature for 15 min. Then, 8 mL column-passing solution was added to wash the column and the solution was collected, followed by centrifugation at 800 rpm to collect cells. Cells were cultured using RPMI-1640 and stimulated using 10 μg/mL phytohemagglutinin (PHA); the solution was replaced every other day.

Detection of Cell Viability via Methyl Thiazolyltetrazolium (MTT)

Cells were inoculated onto the 96-well plate (10^6 cells per well) and 20 μL MTT (5 mg/dL) was added to each well for incubation for 4 h. After centrifugation, the culture solution was abandoned and 150 μL dimethylsulfoxide was added to each well, followed by vibration for 10 min and crystal dissolution. OD values at 490 nm were measured for three times and the average was taken. OD value of test well= OD value per well - OD value of blank well.

Identification of T Cells via Flow Cytometry

T cells were collected and centrifuged at 1000 rpm for 5 min, and the supernatant was abandoned. Cells were dispersed using the vibrator and resuspended with 100 μL phosphate buffer saline (PBS). 2×10^6 cells were collected from each sample and centrifuged at 1000 rpm for 5 min. Next, 20 μL antibody was added for action for 30 min at room temperature in a dark place. Cells were centrifuged at 1000 rpm for 5 min, the supernatant was abandoned, and the constant volume was set with PBS to 500 μL, followed by detection on the machine.

Protein Preparation

Cell culture solution was centrifuged, the supernatant was abandoned and cells were collected. The cell precipitates were washed with PBS twice and cell lysis buffer was added. Then, the cells were incubated on ice for 15 min and centrifuged at 12000 rpm for 10 min. The protein in supernatant was taken and the protein concentration

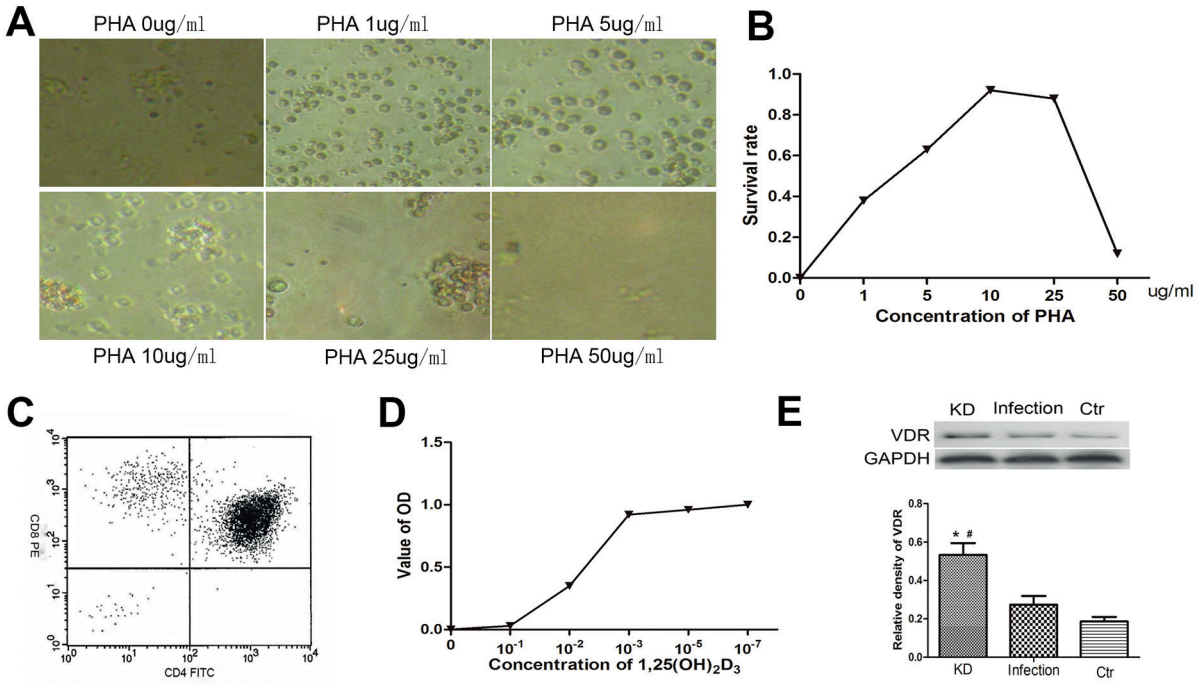

Figure 1. Culture and identification of T Lymphocyte. ***(A)*** Representative pictures of T cells culture. ***(B)*** Survival rate of T cell via MTT at different concentrations of PHA. ***(C)*** Identification of T cells via flow cytometry. ***(D)*** OD values of T cell via MTT at different concentrations of 1,25(OH)2D3. ***(E)*** Western blots analysis reveals the expression of VDR of T cells.

was measured using Bio Rad kit (Hercules, CA, USA). VDR, STAT3, p-STAT3, NF-κB, P65, and GAPDH were purchased from Abcam (Cambridge, MA, USA).

Western Blotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was prepared, and an appropriate amount of protein was taken for electrophoretic separation, transferred to the nitrocellulose membrane and sealed using 50 g/L skim milk powder. Next, the primary antibody and secondary antibody were incubated successively, followed by color development via the chemical kit and semi-quantitative analysis of gray value using Image Lab 3.0 software (Bio-Rad, Hercules, CA, USA)

Statistical Analysis

All quantitative data were expressed as mean ± standard deviation. SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Comparison between groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). p-values < 0.05 were considered statistically significant.

Results

Effective Concentration of PHA on Peripheral Blood Mononuclear Cells

PHA was added in the *in vitro* culture of T cells. The required concentration of PHA in different cells is not the same, and the too-high concentration will lead to cell death. The experimental results showed that cells died after 2 days of culture without adding cells. When PHA concentration was 50 μg/mL, the number of cells was decreased sharply, and many cells died. When PHA concentration was 1 μg/mL-10 μg/mL, cells could proliferate. When PHA concentration was 10 μg/mL, cells were gathered into the mass with obvious proliferation, so it should be the optimal effective concentration. When PHA concentration was 25 μg/mL, the cell death was greater than proliferation and the number of cells began to decrease. MTT assay further confirmed that the optimal effective concentration of PHA on peripheral blood mononuclear cells was 10 μg/ml (Figure 1 A-B).

T Cell Identification

T cells were isolated and obtained using the T cell separation column (R&D, Minneapolis, MN,

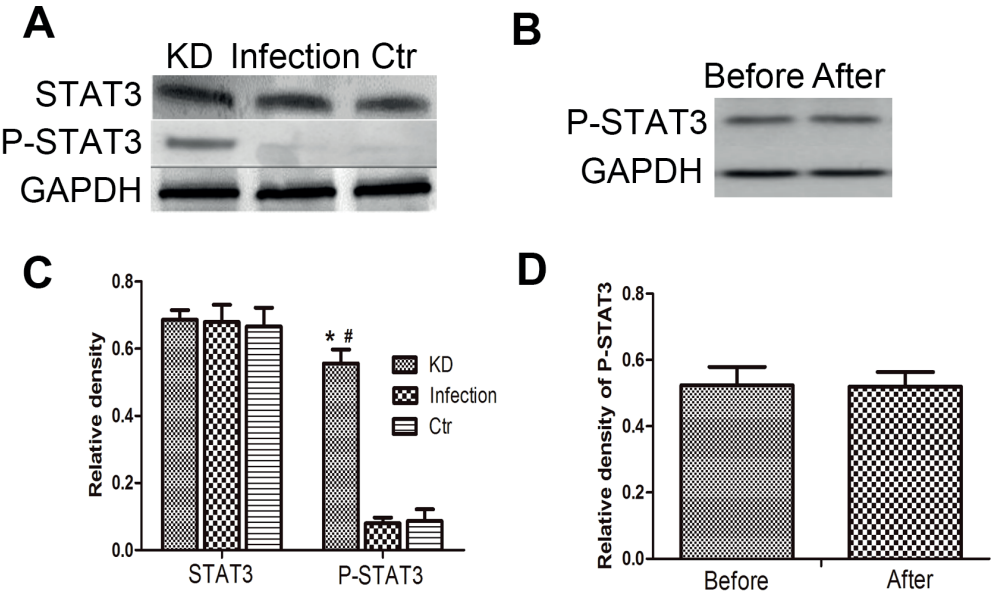

Figure 2. Effect of 1,25(OH)$_2$D$_3$ on STAT3 expression in peripheral blood T cells in KD. ***(A)*** Western blots analysis reveals the expression of STAT3 and P-STAT3. ***(B)*** Semiquantitative analysis of the expression of STAT3 and P-STAT3. ***(C)*** Western blots analysis reveals the expression of P-STAT3. ***(D)*** Semiquantitative analysis of the expression P-STAT3. $^{*}p<0.05$ KD *vs.* Infection, $^{\#}p<0.05$ KD *vs.* Ctr.

USA), and the expressions of CD4 and CD8 on the cell surface were detected via flow cytometry. It was found that the contents of CD4 and CD8 in cells extracted were up to 90%, proving that T cells were successfully isolated (Figure 1 C).

Measurement of Peripheral Blood T Cell Concentration via 1,25(OH)$_2$D$_3$

1,25(OH)$_2$D$_3$ was diluted into different concentrations (10^{-1} μmol/L, 10^{-2} μmol/L, 10^{-3} μmol/L, 10^{-5} μmol/L and 10^{-7} μmol/L), and T cells were added, respectively. After MTT staining for cells, OD values were measured via microplate reader. The Figure was drawn based on the above OD values. The results showed that the maximal atoxic concentration of 1,25(OH)$_2$D$_3$ on T cells was 10^{-3} μmol/L. Therefore, the following experiments were performed from 3 concentration gradients: 10^{-3} μmol/L, 10^{-5} μmol/L and 10^{-7} μmol/L (Figure 1 D).

Expression of VDR in T Cells

Western blotting was used to detect the expressions of VDR in T cells of KD group, infectious fever group and normal group. The results showed that the VDR expression in T cells in KD group was significantly higher than that in infectious fever group and normal group (Figure 1E).

Effect of 1,25(OH)$_2$D$_3$ on STAT3 Expression in Peripheral Blood T Cells in KD

The expressions of total STAT3 protein in T cells in KD group, infectious fever group and normal group, were consistent. The activated form of STAT3, P-STAT3, was significantly expressed in T cells of child patients with KD, but not expressed in T cells of normal children and child patients with infectious fever. 1,25(OH)$_2$D$_3$ (10^{-3} μmol/L) was used to stimulate T cells for 24 h to study the effect of 1,25(OH)$_2$D$_3$ on P-STAT3 expression in peripheral blood T cells in KD, and the results showed that P-STAT3 expression in T cells in child patients with KD did not change significantly (Figure 2).

Effect of 1,25(OH)$_2$D$_3$ on NF-κB Expression in Peripheral Blood T Cells in KD

The expressions of NF-κB protein in T cells in KD group, infectious fever group and normal group, were consistent. 1,25(OH)$_2$D$_3$ (10^{-3} μmol/L) was used to stimulate T cells for 24 h to study the effect of 1,25(OH)$_2$D$_3$ on NF-κB expression in peripheral blood T cells in KD, and the results showed that NF-κB expression in T cells in child patients with KD did not change significantly (Figure 3).

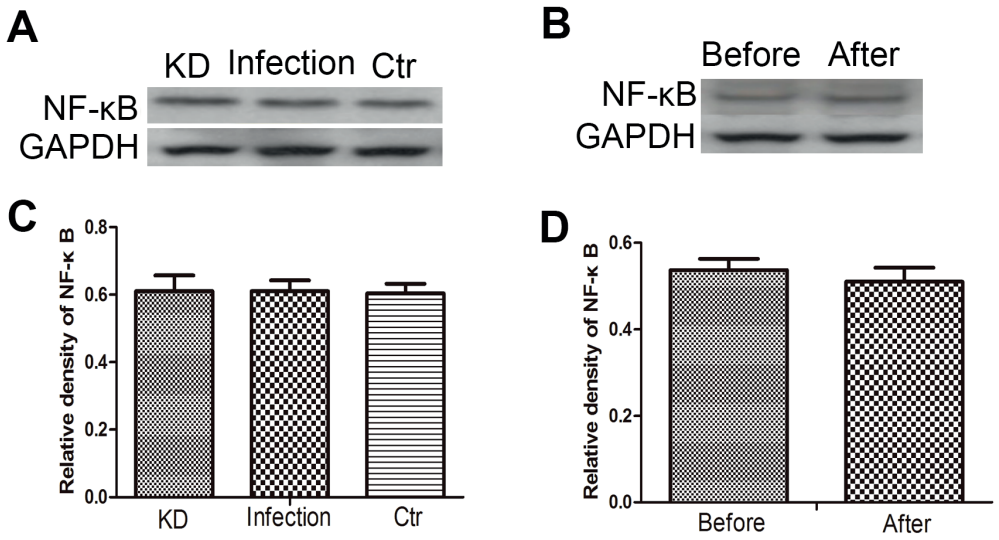

Figure 3. Effect of $1,25(OH)_2D_3$ on NF-κB expression in peripheral blood T cells in KD. ***(A)*** Western blots analysis reveals the expression of NF-κB. ***(B)*** Semiquantitative analysis of the expression of NF-κB. ***(C)*** Western blots analysis reveals the expression of NF-κB. ***(D)*** Semiquantitative analysis of the expression NF-κB.

Effect of $1,25(OH)_2D_3$ on P53 Expression in Peripheral Blood T Cells in KD

P53 protein was expressed in infectious fever group, but expressed weakly in KD group and normal group, especially in KD group. $1,25(OH)_2D_3$ was used to stimulate peripheral blood T cells in KD for 2 h. The results showed that P53 was expressed in T cells of child patients with KD (Figure 4).

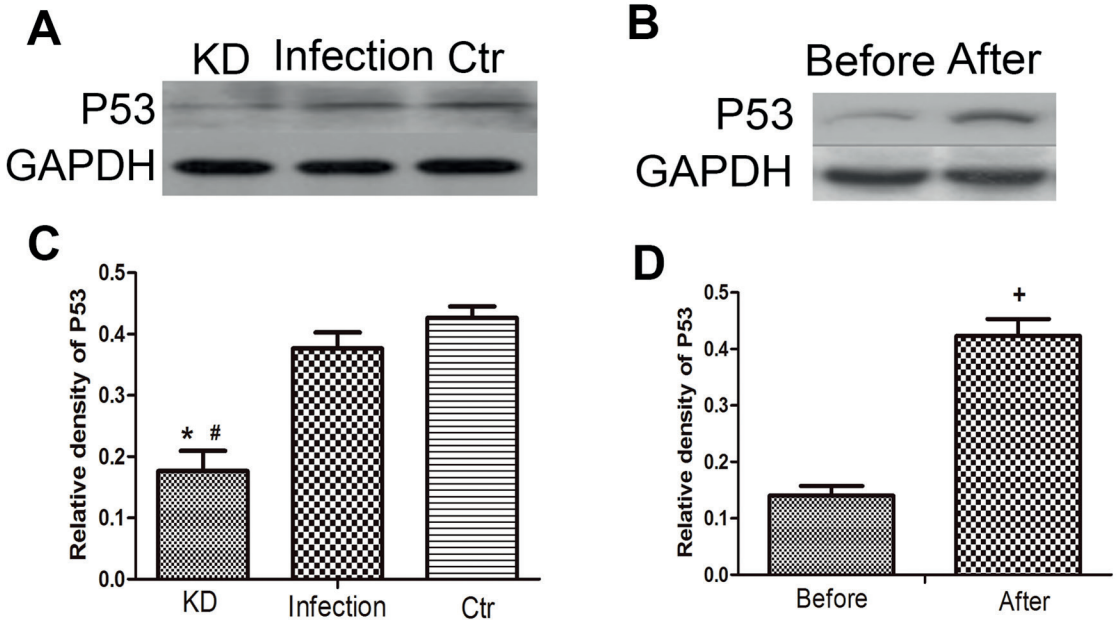

Figure 4. Effect of $1,25(OH)_2D_3$ on P53 expression in peripheral blood T cells in KD. ***(A)*** Western blots analysis reveals the expression of P53. ***(B)*** Semiquantitative analysis of the expression of P53. ***(C)*** Western blots analysis reveals the expression of P53. ***(D)*** Semiquantitative analysis of the expression P53. $^{*}p<0.05$ KD *vs.* infection, $^{\#}p<0.05$ KD *vs.* Ctr, $^{+}p<0.05$ before *vs.* after.

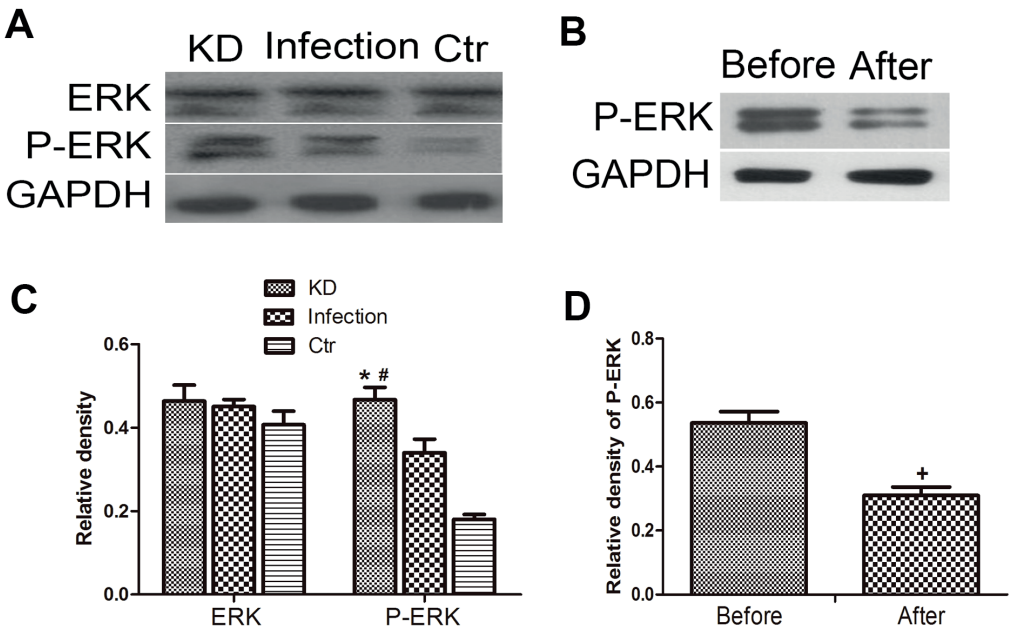

Figure 5. Effect of 1,25(OH)2D3 on ERK expression in peripheral blood T cells in KD. ***(A)*** Western blots analysis reveals the expression of ERK and P-ERK. ***(B)*** Semiquantitative analysis of the expression of ERK and P-ERK. ***(C)*** Western blots analysis reveals the expression of P-ERK. ***(D)*** Semiquantitative analysis of the expression P-ERK. $^{*}p<0.05$ KD *vs.* infection, $^{\#}p<0.05$ KD *vs.* Ctr, $^{+}p<0.05$ before *vs.* after.

Effect of 1,25(OH)$_2$D$_3$ on ERK Expression in Peripheral Blood T Cells in KD

The expressions of total STAT3 protein in T cells in KD group, infectious fever group and normal group were consistent. The activation level of the activated form of ERK, P-ERK, was higher in T cells of child patients with KD than that in another 2 groups. 1,25(OH)$_2$D$_3$ was used to stimulate peripheral blood T cells in KD for 24 h, and the results showed that the P-ERK expression in T cells in child patients with KD was significantly decreased (Figure 5).

Discussion

KD is a kind of vasculitis syndrome with unknown pathogenesis, accompanied by skin and mucous membrane and lymph node damage, which is common in children aged below 3 years old. Since the disease was discovered, people have been studying its pathogenesis, but there is no breakthrough so far. At present, there are two hypotheses about its pathogenesis in China and other countries. Some scholars believe that KD is a kind of abnormal immune activation caused by bacterial or viral superantigens[11], while some propose that the pathogenesis of KD may be related to the heat shock protein 65 in bacteria[12]. Both hypotheses have one thing in common: the abnormal activation of T cells in child patients with KD is the key to vascular injury. The mechanism leading to its imbalance has been a research hotspot in recent years. T lymphocytes are developed from the lymphoid stem cells in bone marrow in thymus gland, and the mature T cells settle in peripheral immune organs through the blood circulation and lymphatic transport recycle through the lymphatic vessels, blood circulation and tissue fluid, and play an immune function. T cells belong to small lymphocytes and apoptosis will occur after 24 h *in vitro*, and death and cracking into pieces after 72 h. Therefore, it is needed to add PHA, concanavalin A (Con A) or CD3 monoclonal antibody in the *in vitro* culture of T cells to stimulate the cell proliferation and avoid its apoptosis[13,14]. PHA is the most widely used and it is the stimulant of human lymphocyte mitosis, and the resulting proliferation of lymphocytes belongs to the non-specific transformation. Although T cells have different identification processes for the specific and non-specific antigens, the induced division and proliferation processes are identical

after being activated. T cell separation column was used to isolate T cells with the purity of up to 90%. Before separation, PHA was used to stimulate and culture peripheral blood mononuclear cells. The experiment proved that the optimal concentration of PHA was 10 μg/mL. The cells stimulated by PHA can be cultured for about 1 month, or cell apoptosis or death will occur in 48 h. On this basis, we successfully performed the subsequent experiments. It was found in this work that $1,25(OH)_2D_3$ receptor VDR was expressed in nuclei of T cells, and the VDR expression in T cells of child patients with KD was significantly higher than that in child patients with infectious fever and normal children, which might be closely related to the abnormal activation of T cells in child patients with KD. Child patients with infectious fever are often accompanied by the increased T cells, so the VDR expression in T cells of child patients with infectious fever is significantly higher than that in normal children. The experimental results also suggest that VDR is weakly expressed in T cells of normal children, which may be related to the slight activation of VDR when T cells were stimulated using PHA in the experiment. This is consistent with the report of Bhalla et al[15] that VDR is not expressed in the resting T cells, but can be activated by PHA. It was also found that P-STAT3 was expressed in peripheral blood T cells in KD but not expressed in T cells of normal children, because T cells in child patients with KD were activated and many cytokines were released. However, at the same time, the experimental results also suggest that P-STAT3 is not expressed in child patients with infectious fever. Various investigations in China and other countries have confirmed that STAT3 is transiently activated in many diseases, but continuously activated in blood cells of some diseases, such as myeloid leukemia and rheumatoid disease[16-19]. Krause et al[20] revealed that STAT3 is in an activated state in the synovial membrane of patients with rheumatoid arthritis, synovial fibroblasts are reproduced, and anti-apoptosis and other aspects are enhanced, while inhibiting STAT3 transcription factor will lead to apoptosis of synovial fibroblasts. Therefore, we suggest that the abnormal activation of T cells in child patients with KD is closely related to the continuous activation of STAT3 signaling pathway, and the activation of STAT3 pathway inhibits the apoptosis of T cells. STAT3 pathway was not activated after T cells were cultured with $1,25(OH)_2D_3$, so we think that $1,25(OH)_2D_3$ does not participate in the regulation of STAT3. At the same time, P53 protein was not expressed in T cells of child patients with KD, further confirming that the T cell apoptosis in child patients with KD is inhibited, because only DNA damage with sufficient intensity can activate P53. At this time, P53 also loses the function of repairing damaged DNA and can only induce the cell growth cycle inhibition or apoptosis, which also partially prevents the DNA mutation. The experimental results showed that T cell apoptosis in child patients with KD was inhibited, and after culture with $1,25(OH)_2D_3$, P53 pathway was activated and the apoptosis was initiated. Also, the activation of NF-κB showed that the anti-apoptotic ability of T cells in child patients with KD is enhanced[21,22]. NF-κB plays a key regulatory role in cytokine-induced gene expression, participating in a variety of biological processes, such as immune response, lymphocyte differentiation and growth, and playing an important role in T cell activation, especially the anti-apoptotic effect. At present, it is speculated that the anti-apoptotic mechanism of NF-κB is as follows: NF-κB is dissociated from inhibitory molecule IκB when cells receive the apoptotic stimulation, IκB is degraded and NF-κB enters the nuclei and activates apoptotic genes and other target genes. Wang et al[23] found that in the common forms of NF-κB, P65 subunit mainly has the anti-apoptotic ability. Therefore, NF-κB P65 protein was selected as the research object. The continuous activation of NF-κB P65 in T cells in child patients with KD showed that the pathway was playing an anti-apoptotic role, and there was no significant change in NF-κB after intervention with $1,25(OH)_2D_3$. The experimental data also showed that the activation level of ERK in T cells of child patients with KD was higher than that in another 2 groups, suggesting the T cell growth and proliferation. ERK kinase family is a member of MAPK, mainly two subtypes, 44 KD and 42 KD, namely ERK1 and ERK2. This kind of kinase is activated by Raf-MEK-ERK cascade signaling to exhibit its activity in the phosphorylated form. The activated ERK can be transferred to the nucleus, activating the transcriptional regulator to initiate gene transcription and leading to cell proliferation[24,25]. The expression of activated P-ERK1/2 protein was decreased and the cell proliferation was inhibited after intervention with $1,25(OH)_2D_3$. The above experimental findings were summarized and analyzed: the continuous activation of STAT3 in T cells of child patients with KD inhibited the cell apoptosis. At the same time, the inactivation

of P53 apoptotic gene and anti-apoptotic effect of NF-κB P65 realize the survival of T cells, and the activation of ERK pathway enhanced the cell growth, proliferation and differentiation, while the activation of ERK1/2 signaling pathway was inhibited, P53 protein was activated and the proliferation of T cells was regulated after the *in vitro* intervention with 1,25(OH)$_2$D$_3$.

Conclusions

We suggest that the molecular mechanism of abnormal activation of T cells in child patients with KD may be as follows: apoptotic T cells in peripheral blood in KD cannot initiate the normal apoptosis program, so they continue to proliferate and differentiate, eventually leading to the increase and abnormal activation of T cells and the immune imbalance in the body. 1,25(OH)$_2$D$_3$ can inhibit the excess hyperplasia of T cells through adjusting partial signal transduction pathway.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Petropoulos A, Ehringer-Schetitska D, Fritsch P, Jokinen E, Dalla PR, Oberhoffer R. Preventing cardiac diseases in childhood. Hell J Nucl Med 2015; 18 Suppl 1: 148.
2) Tian J, Lv HT, An XJ, Ling N, Xu F. Endothelial microparticles induce vascular endothelial cell injury in children with Kawasaki disease. Eur Rev Med Pharmacol Sci 2016; 20: 1814-1818.
3) Wakiguchi H, Hasegawa S, Suzuki Y, Kudo K, Ichiyama T. Relationship between T-cell HLA-DR expression and intravenous immunoglobulin treatment response in Kawasaki disease. Pediatr Res 2015; 77: 536-540.
4) Ding Y, Li G, Xiong LJ, Yin W, Liu J, Liu F, Wang RG, Xia K, Zhang SL, Zhao L. Profiles of responses of immunological factors to different subtypes of Kawasaki disease. BMC Musculoskelet Disord 2015; 16: 315.
5) Meyer MB, Benkusky NA, Onal M, Pike JW. Selective regulation of Mmp13 by 1,25(OH)$_2$D$_3$, PTH, and Osterix through distal enhancers. J Steroid Biochem Mol Biol 2016; 164: 258-264.
6) Ryynanen J, Carlberg C. Primary 1,25-dihydroxyvitamin D3 response of the interleukin 8 gene cluster in human monocyte- and macrophage-like cells. PLoS One 2013; 8: e78170.
7) Neme A, Nurminen V, Seuter S, Carlberg C. The vitamin D-dependent transcriptome of human monocytes. J Steroid Biochem Mol Biol 2016; 164: 180-187.
8) Martinesi M, Ambrosini S, Treves C, Zuegel U, Steinmeyer A, Vito A, Milla M, Bonanomi AG, Stio M. Role of vitamin D derivatives in intestinal tissue of patients with inflammatory bowel diseases. J Crohns Colitis 2014; 8: 1062-1071.
9) Chung BH, Kim BM, Doh KC, Cho ML, Kim KW, Yang CW. Protective effect of 1alpha,25-dihydroxyvitamin D3 on effector CD4+ T cell induced injury in human renal proximal tubular epithelial cells. PLoS One 2017; 12: e172536.
10) Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM, Adorini L. Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. J Immunol 2001; 167: 1945-1953.
11) Blankier S, McCrindle BW, Ito S, Yeung RS. The role of atorvastatin in regulating the immune response leading to vascular damage in a model of Kawasaki disease. Clin Exp Immunol 2011; 164: 193-201.
12) Chun JK, Jeon BY, Kang DW, Kim DS. Bacille Calmette Guerin (BCG) can induce Kawasaki disease-like features in programmed death-1 (PD-1) gene knockout mice. Clin Exp Rheumatol 2011; 29: 743-750.
13) Hong JQ, Gao Y, Song J, Zhuo WB, Sun HT, Ping BH. [comparison of biological characteristics and immunosuppressive activity between human amniotic mesenchymal stem cells and human bone marrow mesenchymal stem cells]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2016; 24: 858-864.
14) Bertran T, Brachet P, Vareille-Delarbre M, Falenta J, Dosgilbert A, Vasson MP, Forestier C, Tridon A, Evrard B. Slight Pro-Inflammatory immunomodulation properties of dendritic cells by gardnerella vaginalis: the “invisible man” of bacterial vaginosis? J Immunol Res 2016; 2016: 9747480.
15) Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. J Clin Endocrinol Metab 1983; 57: 1308-1310.
16) Zhu Z, Lu X, Jiang L, Sun X, Zhou H, Jia Z, Zhang X, Ma L. STAT3 signaling pathway is involved in decitabine induced biological phenotype regulation of acute myeloid leukemia cells. Am J Transl Res 2015; 7: 1896-1907.
17) Kumar H, Tichkule S, Raj U, Gupta S, Srivastava S, Varadwaj PK. Effect of STAT3 inhibitor in chronic myeloid leukemia associated signaling pathway: a mathematical modeling, simulation and systems biology study. 3 Biotech 2016; 6: 40.
18) Fan D, He X, Bian Y, Guo Q, Zheng K, Zhao Y, Lu C, Liu B, Xu X, Zhang G, Lu A. Triptolide modulates TREM-1 signal pathway to inhibit the inflammatory response in rheumatoid arthritis. Int J Mol Sci 2016; 17: 498.
19) Espigol-Frigole G, Planas-Rigol E, Ohnuki H, Salvucci O, Kwak H, Ravichandran S, Luke B, Cid MC, Tosa-

TO G. Identification of IL-23p19 as an endothelial proinflammatory peptide that promotes gp130-STAT3 signaling. Sci Signal 2016; 9: ra28.

20) KRAUSE A, SCALETTA N, JI JD, IVASHKIV LB. Rheumatoid arthritis synoviocyte survival is dependent on Stat3. J Immunol 2002; 169: 6610-6616.

21) MA L, GUAN YQ, DU ZD. Salvianolic acid b down-regulates matrix metalloproteinase-9 activity and expression in tumor necrosis factor-alpha-induced human coronary artery endothelial cells. Chin Med J (Engl) 2015; 128: 2658-2663.

22) MATSUDA A, MORITA H, UNNO H, SAITO H, MATSUMOTO K, HIRAO Y, MUNECHIKA K, ABE J. Anti-inflammatory effects of high-dose IgG on TNF-alpha-activated human coronary artery endothelial cells. Eur J Immunol 2012; 42: 2121-2131.

23) WANG WJ, CHANG CH, SUN MF, HSU SF, WENG CS. DPP-4 inhibitor attenuates toxic effects of indoxyl sulfate on kidney tubular cells. PLoS One 2014; 9: e93447.

24) RIZZO F, COFFMAN JA, ARNONE MI. An Elk transcription factor is required for Runx-dependent survival signaling in the sea urchin embryo. Dev Biol 2016; 416: 173-186.

25) LIU H, YAO S, DANN SM, QIN H, ELSON CO, CONG Y. ERK differentially regulates Th17- and Treg-cell development and contributes to the pathogenesis of colitis. Eur J Immunol 2013; 43: 1716-1726.