

## Original Article

# Changes of peripheral blood V $\delta$ 1 T cells in patients with atherosclerotic cerebral infarction

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**Abstract:** To observe the ratio of peripheral blood V $\delta$ 1 T cells in patients with atherosclerotic cerebral infarction (ACI) and their function changes, and preliminarily explore the mechanism of change in ratio of peripheral blood V $\delta$ 1 T cells in ACI patients. 30 ACI patients enrolled in the neurology department in our hospital from January 2016 to December 2016 were selected, and 30 healthy subjects enrolled in the hospital during the same period were selected as healthy controls. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation. The ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients was detected by flow cytometry, and the correlations between the ratio of V $\delta$ 1 T cells and the neurological deficits and infarction size in ACI patients were analyzed. A high proportion of V $\delta$ 1 T cells were obtained by in vitro amplification, and high-purity V $\delta$ 1 T cells and Naïve CD4 T cells were obtained by flow cytometry and magnetic bead sorting respectively. The effect of V $\delta$ 1 T cells on the proliferation of Naïve CD4 T cells and the secretion of IFN- $\gamma$  were investigated by CFSE staining method; the correlation between the ratio of V $\delta$ 1 T cells in peripheral blood and the Ox-LDL level in peripheral blood of ACI patients was analyzed. The V $\delta$ 1 T cells in peripheral blood were treated by Ox-LDL, and the effect of Ox-LDL on V $\delta$ 1 T cell apoptosis was determined by apoptosis staining method. Compared with the healthy control group, the ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients was significantly decreased ( $P < 0.0001$ ). The ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients was not significantly correlated with age, sex, hypertension, diabetes and dyslipidemia ( $P > 0.05$ ). However, with the gradual aggravation of neurological deficit and gradual increase of infarct volume, the ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients decreased gradually. Besides, the functional studies showed that the immunosuppressive functions of V $\delta$ 1 T cells in peripheral blood of ACI patients were also significantly decreased ( $P < 0.0001$ ). The ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients was negatively correlated with the Ox-LDL level in peripheral blood ( $r^2 = 0.1691$ ;  $P = 0.0240$ ); the Ox-LDL treatment of V $\delta$ 1 T cells induced apoptosis of V $\delta$ 1 T cells, and with the increased Ox-LDL concentration, the ratio of V $\delta$ 1 T cell apoptosis gradually increased. The decreased ratio of V $\delta$ 1 T cells in peripheral blood and loss of functions in ACI patients lead to the occurrence of immunoinflammatory reactions, which may be one of the possible causes of ACI. In addition, this study also showed that, Ox-LDL could induce V $\delta$ 1 T cell apoptosis and lead to decrease in ratio of V $\delta$ 1 T cells in peripheral blood, which may be one of the reasons for decreased ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients. In summary, this study can further help the understanding of the pathogenesis of ACI.

**Keywords:** Atherosclerosis, cerebral infarction, peripheral blood, T cells, V $\delta$ 1 T cells

## Introduction

In China, cerebrovascular disease is one of the most common causes of population death [1]. ACI, also known as atherosclerotic cerebral infarction, is the most common form of brain stroke. ACI is the brain atherosclerosis and thrombosis, making cerebrovascular stenosis or occlusion, leading to acute cerebral ischemia and ischemic necrosis of local brain tissues. The patients may have such symptoms as hemiplegia, aphasia and other brain focal

lesions, belonging to ischemic cerebrovascular disease, mainly occurring in elderly patients. The incidence is high in patients with high-fat diets, diabetes, smoking, etc. A number of studies have shown that, inflammation plays an important role in the formation of atherosclerosis and ischemic brain injury [2-4].

Regulatory T cells (Treg) are a group of cells with immunosuppressive functions, which play an important role in maintaining the body's inflammatory balance. The decreased ratio of

Treg cells or decreased functions may cause different degrees of inflammatory responses of the body. Different Treg cell subsets have different functions, and Treg cells of different phenotype and functions mediate different mechanisms of immune suppression. CD4<sup>+</sup>CD25<sup>+</sup> Treg is an important cell for maintaining self-tolerance and regulating the pathophysiological immune responses [5, 6]. At present, it has been confirmed that the ratio of Treg cells in peripheral blood of ACI patients is significantly abnormal [7, 8].

However, in addition to CD4 Treg, there are a number of other immunosuppressive cells in peripheral blood, such as regulatory B cells, regulatory DC cells and V $\delta$ 1 T cells, etc. [9-11].

T cells are divided into  $\alpha\beta$ T cells and V $\delta$ 1 cells according to different receptors [12, 13]. V $\delta$ 1 cells are newly discovered T cell subsets with immunosuppressive function in recent years. The main distribution range is thymus, mucosal epithelium and peripheral blood, and participation in infection, cancer, immune regulation, injury repair and other physiological processes extensively [14]. At present, the study of V $\delta$ 1 cells is concentrated in infection, immunity, tumor and so on [15-17], the role of ACI is almost no literature reported. In this study, we observed the ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients and their functional changes, and investigated the correlation between the ratio of V $\delta$ 1 T cells and ACI risk factors and patient's conditions, to provide theoretical basis for the study of pathogenesis of ACI.

In addition, we also focused on the study of the mechanism of the change of V $\delta$ 1 T cells ratio in peripheral blood in ACI patients. Ox-LDL (Oxidized low-density lipoprotein) is known to be a key factor in the pathogenesis of atherosclerosis (AS) and it can lead to plaque rupture and thrombosis through different mechanisms [18]. It has been reported that Ox-LDL can induce the apoptosis of Treg cells [7, 8]. At present, it has not been reported whether Ox-LDL can affect the change of the V $\delta$ 1 T cells ratio in peripheral blood of ACI patients by affecting the apoptosis of V $\delta$ 1 T cells. Studies on the mechanism will be conducive to the understanding of the pathogenesis of ACI. In this study, we investigated the correlation between Ox-LDL level and ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients, and by treating V $\delta$ 1 T cells with different concentrations of Ox-LDL, we studied the effect of Ox-LDL on apoptosis of V $\delta$ 1 T cells.

### Materials and methods

Thirty ACI patients enrolled in the neurology department in our hospital from January 2016 to December 2016 and 30 healthy controls (after physical examination in the hospital) during the same period were collected. Among these ACI patients, there were 15 male cases and 15 female cases, mean age of (57.81  $\pm$  7.04) years. Those patients without autoimmune diseases, severe heart and kidney dysfunction and metabolic diseases, blood diseases, systemic active infection, a history of acute myocardial infarction, vascular occlusive disease, cardiogenic cerebral embolism and cerebral hemorrhage in recent 3 months and other serious systematic diseases. In the healthy control group, there were 15 male cases and 15 female cases, with an average age of (57.92  $\pm$  7.52) years. ACI patients underwent neurological function deficit scale according to the National Institutes of Health Stroke Scale (NIH Stroke Scale, NIHSS) [13] (mild:  $\leq$ 15 points; moderate: 16-30 points; severe: 31-45 points). The infarct volume (small infarct:  $<$ 5 cm<sup>3</sup>; medium infarct: 5-10 cm<sup>3</sup>; large infarct:  $>$ 10 cm<sup>3</sup>) was counted according to the Pullicino formula (length  $\times$  width  $\times$  the number of MRI positive scanning layers/2). Informed consent was obtained from all subject, and the study was approved by the Ethics Committee of Ningbo NO. 2 Hospital.

### Main reagents

Bovine serum albumin (BSA) was purchased from Sigma; RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Gibco; phosphate buffered saline (PBS) was purchased from Hyclone; Lymphocyte separating medium, PE-anti-CD3 antibody, FITC-anti-TCR V $\delta$ 1 antibody, APC-anti-IFN- $\gamma$  antibody and apoptotic staining kit were purchased from Biolegend; the Purified anti-TCR V $\delta$ 1 antibody used for amplification was purchased from Beckman; the Naïve CD4 T cell sorting kits were purchased from Miltenyi Biotec; Purified anti-CD3 antibody and Purified anti-CD28 antibody for amplification were purchased from BD; the CFSE staining solution was purchased from Thermo.

### PBMC separation method

In the morning, 10 mL of peripheral venous blood was collected from ACI patients and healthy controls under fasting state. A 50 mL of

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centrifuge tube was taken, added with 10 mL of RPMI-1640 medium, and then blood sample was added to dilute according to the ratio of 1:1, and then a 50 mL of centrifuge tube was taken, added with 10 mL of lymphocyte separating medium, then the diluted blood sample was fetched with a pipette carefully to the separating medium (to ensure that the blood is at above the lymphocyte separating medium), centrifuged 18 min at 800  $\times$  g; after centrifugation, the middle circular milky white lymphocyte layer was fetched with a pipette carefully to another 15 mL of centrifuge tube, and 10 mL of serum-free RPMI 1640 medium cleaning solution was added to the centrifuge tube; after cells were mixed well, centrifuged at 400  $\times$  g for 10 min; and then the supernatant was discarded, the cell precipitate was resuspended in 10 mL serum-free RPMI 1640 medium and centrifuged at 250  $\times$  g for 8 min. The cells were re-suspended in 1 mL of RPMI-1640 complete medium containing 10% fetal bovine serum. After trypan blue staining and counting, it was prepared into cell suspension at a concentration of 2  $\times$  10<sup>6</sup> cells/mL.

### *Determination of the ratio of V $\delta$ 1 T cells in PBMC*

1  $\times$  10<sup>6</sup> PBMCs obtained by the density gradient centrifugation method were added to a 1.5 mL of Eppendorf tube, and 1 mL of PBS cleaning solution containing 1% BSA was added to mix well, then centrifuged at 250  $\times$  g for 8 min to discard the supernatant, and then the above procedure was repeated. Cells were re-suspended with 0.1 ml of PBS containing 1% BSA, and PE-anti-CD3 antibody and FITC-anti-TCR V $\delta$ 1 antibody were added to the solution, incubated in the dark at 4°C for 30 min; after washed twice with PBS containing 1% BSA, cells were re-suspended in 0.1 ml PBS for flow cytometry.

### *V $\delta$ 1 T cell amplification method*

0.2 ml of RPMI-1640 medium containing 0.125  $\mu$ g of anti-TCR V $\delta$ 1 monoclonal antibody was added to each well of a 48-well plastic plate, and incubated in a saturated wet environment (37°C, 5% CO<sub>2</sub>) for 2 h. The PBMC suspension resuspended with complete medium (RPI-1640 + 10% FBS) was added to a 48-well plate (1.0 ml per well) coated with anti-TCR V $\delta$ 1 monoclonal antibody and cultured in a saturated wet environment (37°C, 5% CO<sub>2</sub>). The liquid was

changed or the wells were divided every 1-3 days according to the cell growth state, to culture 2 W, and then V $\delta$ 1 T cells with purity greater than 90% were obtained by flow cytometry sorting.

### *Detection of naïve CD4 T cell proliferation*

Naïve CD4 T cells were washed once with 10 ml of serum-free RPMI 1640 medium stock solution, and then added to CFSE dye at a final concentration of 5 mmol/L, incubated for 10 min in a saturated wet environment (37°C, 5% CO<sub>2</sub>). Then 5 ml of pre-cooled RPMI 1640 medium containing 5% FBS (CFSE staining stop solution) was added to the centrifugal tube immediately, placed on the ice for 5 min to stop staining, centrifuged 8 min at 400  $\times$  g, then washed once with 10 ml of RPMI 1640 medium stock solution. After cells were re-suspended in RPMI-1640 complete medium containing 10% FBS, V $\delta$ 1 T cells and above Naïve CD4 T cells (at a ratio of 1:1) were added to a 48-well plate coated by 1  $\mu$ g/ml CD3 antibody and 2  $\mu$ g/ml CD 28 antibody, after incubated for 5 days, cells were harvested and detected by flow cytometry.

### *IFN- $\gamma$ secretion test of naïve CD4 T cells*

V $\delta$ 1 T cells and Naïve CD4 T cells (1:1) were added to a 48-well plate coated by 1  $\mu$ g/ml CD3 antibody and 2  $\mu$ g/ml CD 28 antibody, after incubation for 5 days, 100  $\times$  PMA + Ion was added to a culture plate to continue culture for 6 h at 37°C, then cells were collected, added with 0.5 ml of membrane rupture solution, placed at room temperature in the dark for 30 min, and cells were washed twice with penetrating solution. APC-anti-IFN- $\gamma$  antibody was added, placed for 30 min in the dark at room temperature, and then cells were washed twice with the penetrating solution, and then resuspended in 0.1 mL PBS for testing.

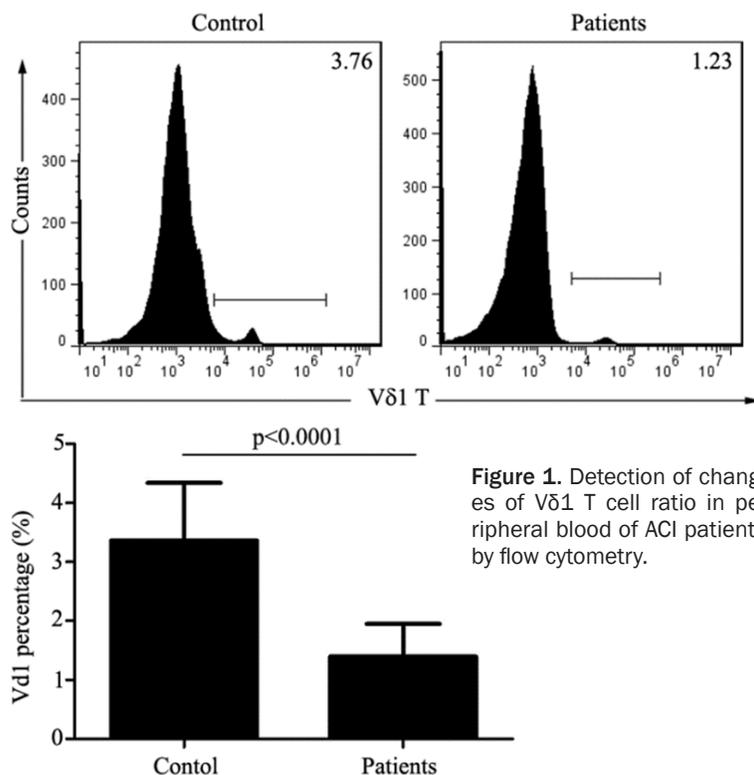
### *Treatment of V $\delta$ 1 T cells with Ox-LDL*

V $\delta$ 1 T cells were treated in vitro with different concentrations of Ox-LDL (0, 10, 50 and 100  $\mu$ g/L respectively) for 48 h, and then apoptosis detection was performed.

### *Detection of apoptosis*

The cell apoptosis was detected by Annexin V/7-AAD method. The specific procedure: Cells were washed twice with PBS, and re-suspend-

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**Figure 1.** Detection of changes of Vδ1 T cell ratio in peripheral blood of ACI patients by flow cytometry.

to gently mix cells well and incubate at room temperature in the dark for 15 min; and finally 400 μl of Annexin V Binding Buffer was added for flow cytometry analysis.

### Statistical processing

All data were expressed as mean ± SD. Data analysis was performed by SPSS 16.0 statistical software. Data between multiple groups were compared using One-Way-ANOVA, and data between two groups were compared using t test; the spearman correlation analysis was adopted.

### Results

#### Changes of Vδ1 T cell ratio in peripheral blood of ACI patients

As shown in **Figure 1**. The Vδ1 T cell ratio in peripheral blood of healthy controls was (3.36 ± 0.98)% and that of ACI patients was (1.39 ± 0.55)%; compared with the healthy controls, the Vδ1 T cell ratio in peripheral blood of ACI patients was significantly decreased (P<0.0001).

#### Correlation between the Vδ1 T cells in peripheral blood and the risk factors of ACI patients

As shown in **Table 1**. The ratio of Vδ1 T cells in peripheral blood of ACI patients was not significantly correlated with age, sex, hypertension, diabetes mellitus and dyslipidemia (P>0.05).

#### Correlation between the ratio of Vδ1 T cells in peripheral blood of ACI patients and the conditions of patients

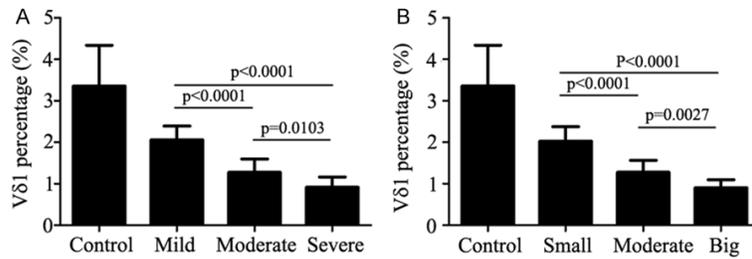
As shown in **Figure 2A**. The ratios of Vδ1 T cells in peripheral blood of ACI patients with mild, moderate and severe neurological deficits were (2.06 ± 0.33)%, (1.28 ± 0.32)% and (0.92 ± 0.25)% respectively. With the aggravation of neurological deficits, the ratio of Vδ1 T cells in peripheral blood of patients was gradually decreased. As shown in **Figure 2B**, the ratios of Vδ1 T cells in peripheral blood of ACI patients with small, medium and large infarcts were (2.03 ± 0.35)%, (1.285 ± 0.29)% and (0.91 ±

**Table 1.** The correlation between Vδ1 T cells in peripheral blood and the risk factors of ACI patients

| Item         | n  | Vδ1 T (%)   | P value |
|--------------|----|-------------|---------|
| Age (year)   |    |             |         |
| <60          | 16 | 1.31 ± 0.48 | 0.4029  |
| ≥60          | 14 | 1.48 ± 0.63 |         |
| Sex          |    |             |         |
| Male         | 15 | 1.38 ± 0.60 | 0.8826  |
| Female       | 15 | 1.41 ± 0.53 |         |
| Hypertension |    |             |         |
| Yes          | 18 | 1.44 ± 0.56 | 0.5524  |
| No           | 12 | 1.32 ± 0.56 |         |
| Diabetes     |    |             |         |
| Yes          | 16 | 1.36 ± 0.63 | 0.7077  |
| No           | 14 | 1.43 ± 0.46 |         |
| Dyslipidemia |    |             |         |
| Yes          | 17 | 1.52 ± 0.63 | 0.1610  |
| No           | 13 | 1.23 ± 0.40 |         |

ed with Annexin V Binding Buffer in the test kit to the suspension at the concentration of  $0.25-1.0 \times 10^7$  cells/mL; 100 μl of above cell suspension was transferred to a 15 mL centrifuge tube, then 5 μl of APC Annexin V and 5 μl of 7-AAD Viability Staining Solution were added,

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**Figure 2.** The correlation between the ratio of Vδ1 T cells in peripheral blood and patient's condition. A: Correlation between the ratio of Vδ1 T cells and neurological deficits; B: Correlation between the ratio of Vδ1 T cells and the infarct size.

0.19)% respectively; with the increased infarct volume, the ratio of Vδ1 T cells in peripheral blood of patients was gradually decreased.

### *Inhibition of Vδ1 T cells in peripheral blood on naïve CD4 T cell proliferation and cytokine secretion in ACI patients*

As shown in **Figure 3A**. In the healthy controls, the ratio of proliferation of Naïve CD4 T cells alone was ( $83.91 \pm 15.04$ )%, and after Naïve CD4 T cells were co-cultured with Vδ1 T cells, the ratio of proliferation of Naïve CD4 T cells was ( $47.35 \pm 9.03$ )%; in the ACI patients, the ratio of proliferation of Naïve CD4 T cells alone was ( $83.52 \pm 14.93$ )%, and after Naïve CD4 T cells were co-cultured with Vδ1 T cells, the ratio of proliferation of Naïve CD4 T cells was ( $70.19 \pm 11.37$ )%. Compared with the healthy controls, the inhibition ability of Vδ1 T cells in peripheral blood on Naïve CD4 T cell proliferation in ACI patients was significantly decreased ( $P < 0.0001$ ). As shown in **Figure 3B**, in the healthy controls, the percentage of IFN- $\gamma^+$  Naïve CD4 T cells in peripheral blood was ( $33.01 \pm 5.16$ )%, after Naïve CD4 T cells were co-cultured with Vδ1 T cells, the percentage of IFN- $\gamma^+$  Naïve CD4 T cells was ( $13.95 \pm 4.05$ )%; in ACI patients, the percentage of IFN- $\gamma^+$  Naïve CD4 T cells in peripheral blood was ( $31.52 \pm 4.91$ )%, and after Naïve CD4 T cells were co-cultured with Vδ1 T cells, the percentage of IFN- $\gamma^+$  Naïve CD4 T cells in peripheral blood was ( $22.94 \pm 3.81$ )%. Compared with the healthy controls, the inhibition ability of Vδ1 T cells in peripheral blood on the secretion of cytokines of Naïve CD4 T cells in ACI patients was significantly decreased ( $P < 0.0001$ ).

### *Correlation between the ratio of Vδ1 T cells and Ox-LDL level in peripheral blood of ACI patients*

As shown in **Figure 4**. The percentage of Vδ1 T cells was negatively correlated with the Ox-LDL level in peripheral blood of ACI patients ( $r^2 = 0.1691$ ;  $P = 0.0240$ ).

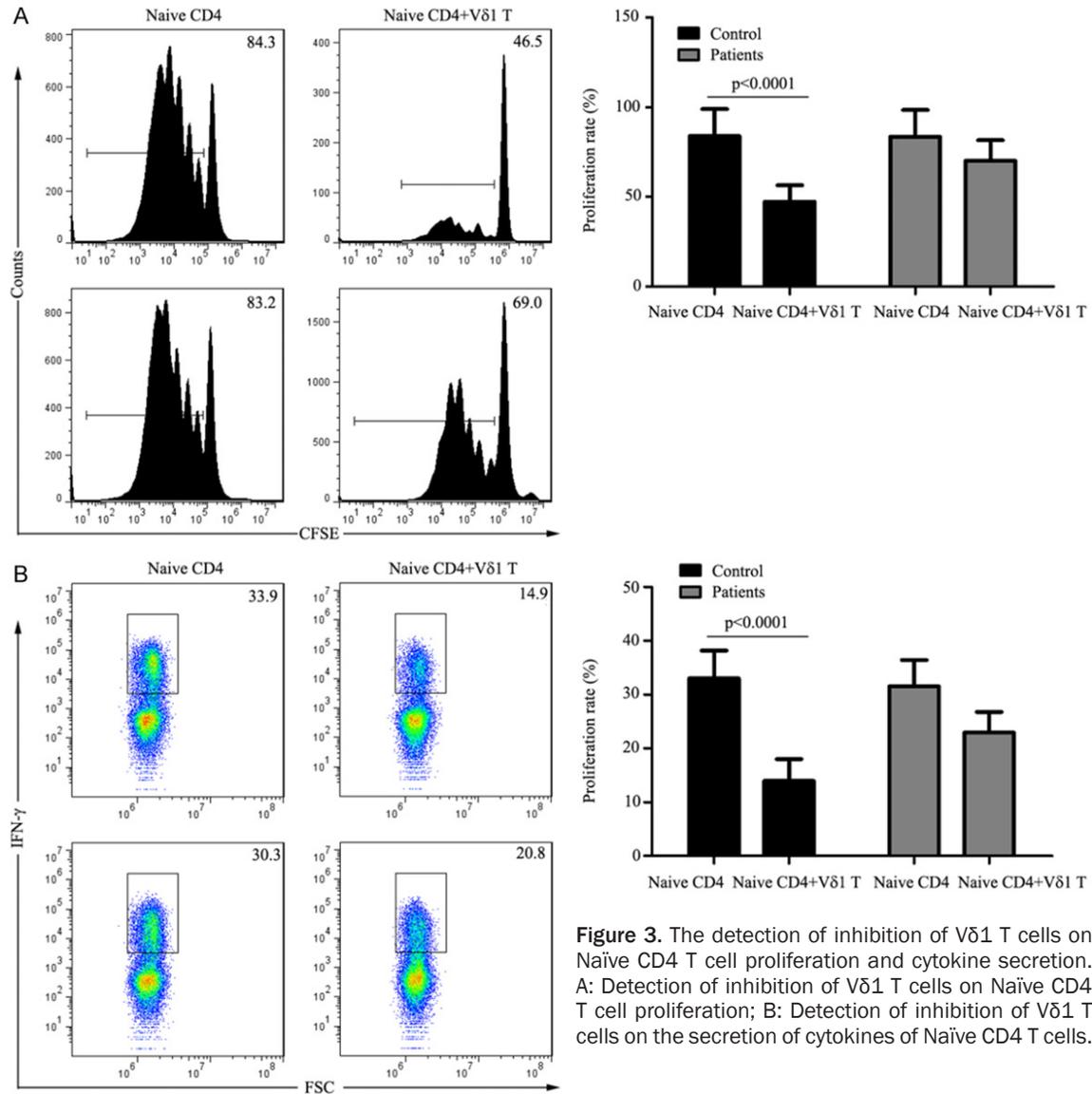
### *Effect of Ox-LDL on apoptosis of Vδ1 T cells*

As shown in **Figure 5**. The percentage of apoptotic cells in the control group was ( $1.28 \pm 0.37$ )%, and percentages of apoptotic cells in the 0  $\mu\text{g/L}$  group, 10  $\mu\text{g/L}$  group and 100  $\mu\text{g/L}$  group were ( $3.01 \pm 1.16$ )%, ( $7.39 \pm 1.26$ )% and ( $12.95 \pm 2.51$ )% respectively. With the increase of Ox-LDL concentration, the percentage of apoptotic cells increased gradually.

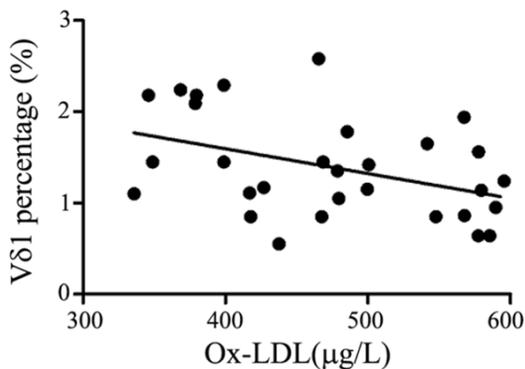
## Discussion

In this study, the flow cytometry showed that the ratio of Vδ1 T cells in peripheral blood of ACI patients was significantly lower than that of healthy controls ( $P < 0.0001$ ). Further studies showed that the ratio of Vδ1 T cells in peripheral blood was not significantly correlated with the patient's age, sex, hypertension, diabetes mellitus and dyslipidemia ( $P > 0.05$ ). However, with the gradual aggravation of neurological deficit and gradual increase of infarct volume, the ratio of Vδ1 T cells in peripheral blood of ACI patients decreased gradually, suggesting that the decreased ratio of Vδ1 T cells in peripheral blood was closely associated with the occurrence of ACI. Besides, the functional studies showed that, the inhibition ability of Vδ1 T cells in peripheral blood on the naïve CD4 T cell proliferation and the secretion of cytokines of naïve CD4 T cells in ACI patients was significantly decreased compared with that in healthy controls ( $P < 0.0001$ ), suggesting that the immunosuppressive functions of Vδ1 T cells in peripheral blood of ACI patients were significantly decreased ( $P < 0.0001$ ). These results suggested that, the decreased ratio and the functional changes of Vδ1 T cells in peripheral blood of ACI patients caused damage to the in vivo immune balance of the patients, ultimately leading to the occurrence of ACI.

## Peripheral blood Vδ1 T cells and ACI patients



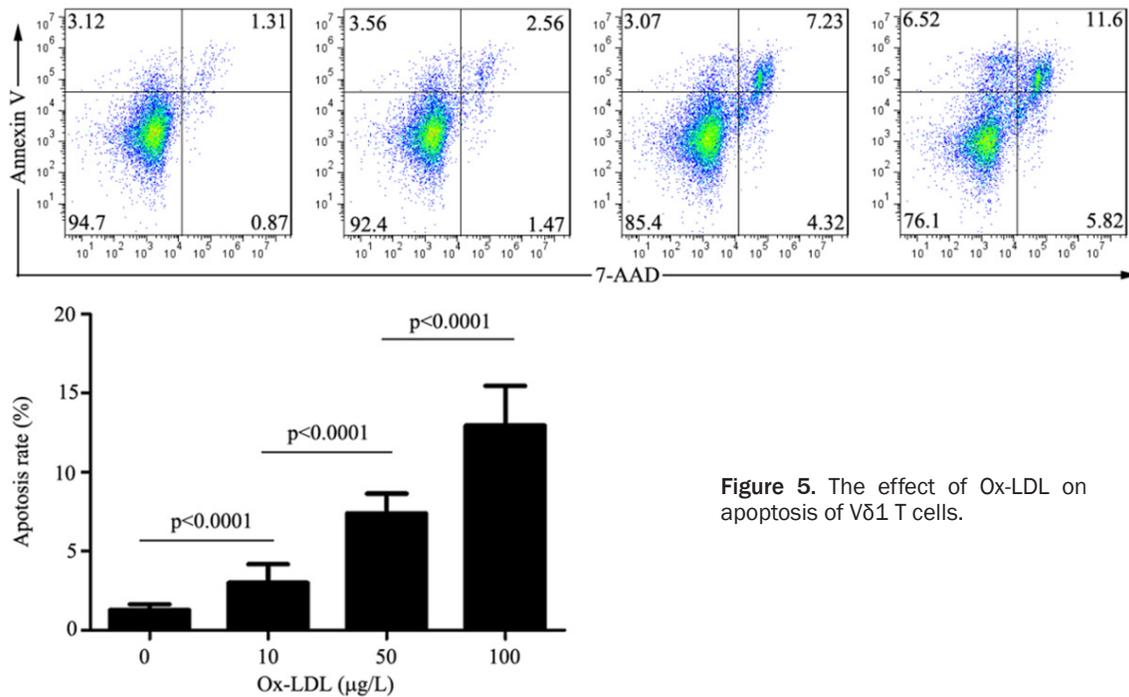
**Figure 3.** The detection of inhibition of Vδ1 T cells on Naive CD4 T cell proliferation and cytokine secretion. A: Detection of inhibition of Vδ1 T cells on Naive CD4 T cell proliferation; B: Detection of inhibition of Vδ1 T cells on the secretion of cytokines of Naive CD4 T cells.



**Figure 4.** The correlation between the ratio of Treg cells and the Ox-LDL level in peripheral blood of ACI patients.

Vδ1 T cells are a subset of peripheral blood γδ T cells [19]. It is well known that T cells can be classified into two subsets according to the expression of T cell receptors (TCR), namely, αβ T cells and γδ T cells [20]. Vδ1 T cells have a wide range of effects, which can secrete a variety of cytokines, chemokines, produce cytotoxic activity, cleavage of infected target cells, presenting antigen, induced maturation of dendritic cells, raise macrophages, start the cellular immune response, assisted B cells for humoral immunity, there are irreplaceable effects in the infection, immunity, cancer, repair damage and many other aspects [21-23]. γδ T cells are further classified into two subsets: Vδ1 T cells

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**Figure 5.** The effect of Ox-LDL on apoptosis of V $\delta$ 1 T cells.

(mainly distributed in epithelial related lymphoid tissue) and V $\delta$ 2 T cells (mainly distributed in peripheral blood) [24, 25]. V $\delta$ 1 T cells and V $\delta$ 2 T cells have different functions, V $\delta$ 2 T cells are mainly involved in inflammatory response, and V $\delta$ 1 T cells are mainly involved in immune regulation [26]. The latest studies by He et al. [27] showed that, the ratio of  $\gamma\delta$  T cells with the function of IL-6 secretion was increased significantly in the peripheral blood of ACI patients.  $\gamma\delta$  T cells could induce the formation of Th17 cells by secreting IL-6 to destroy the in vivo immune balance of the patients, leading to the onset of ACI. In this study, we mainly focused on V $\delta$ 1 T cell subsets whose role in the pathogenesis of ACI has not been reported yet. The existing studies on V $\delta$ 1 T cells is mainly focused on infection, autoimmune diseases and tumors [28, 29]. It has been reported that the number of V $\delta$ 1 T cells was reduced in autoimmune diseases [30], while the number of V $\delta$ 1 T cells in tumors was increased [11]. This study showed that, the ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients was reduced and their functions were changed significantly, which was consistent with the important role of inflammation in the formation of atherosclerosis and ischemic brain injury. In this study, we revealed the role of V $\delta$ 1 T cells in the pathogenesis of ACI for the first time. The results expand the study of V $\delta$ 1 T

cells to a new disease and deepen the understanding of the pathogenesis of ACI.

LDL can promote a series of in vivo complex pathophysiological processes after oxidized and modified to Ox-LDL, playing an important role in the occurrence and progression of AS [31]. Guo et al. [32] demonstrated that lectin-like Ox-LDL receptor-1 (LOX-1) interacts with Ox-LDL to play an important role in the occurrence of ACI. At the same time, Wang et al. [33] also confirmed that Ox-LDL has an important role in the occurrence and progression of acute myocardial infarction. In addition, it has been reported that, Ox-LDL could break the balance of Treg cells and Th17 cells in peripheral blood of ACI patients by changing the apoptosis of Treg cells [7, 8] and involve in the occurrence of ACI. However, it has not been reported whether Ox-LDL can change the ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients by affecting the apoptosis condition of V $\delta$ 1 T cells. In this study, we firstly studied the correlation between the percentage of V $\delta$ 1 T cells and the Ox-LDL level in peripheral blood of ACI patients. Results showed that the percentage of V $\delta$ 1 T cells was negatively correlated with the Ox-LDL level in peripheral blood of ACI patients ( $r^2=0.1691$ ;  $P=0.0240$ ), suggesting that the increased in vivo Ox-LDL level was closely associated with

the decrease in the ratio of V $\delta$ 1 T cells in ACI patients. In this study, V $\delta$ 1 T cells were treated with different concentrations of Ox-LDL, results showed that, Ox-LDL could induce apoptosis of V $\delta$ 1 T cells, and with the increase of Ox-LDL concentration, the percentage of apoptosis of V $\delta$ 1 T cells increased gradually.

In summary, the study results suggest that, the decreased ratio of V $\delta$ 1 T cells in peripheral blood and loss of functions in ACI patients lead to the occurrence of immunoinflammatory reactions and the occurrence of ACI. In addition, Ox-LDL could induce V $\delta$ 1 T cell apoptosis and lead to decrease in ratio of V $\delta$ 1 T cells in peripheral blood, which may be one of the reasons for decreased ratio of V $\delta$ 1 T cells in peripheral blood of ACI patients.

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### Disclosure of conflict of interest

None.

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