Laboratory Research

Upregulated voltage-gated potassium channel Kv1.3 on CD4+CD28null T lymphocytes from patients with acute coronary syndrome

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Objective The purpose of our study is to observe the voltage-gated potassium channel Kv1.3 expressed on CD4+CD28null T cells from the peripheral blood of acute coronary syndrome (ACS) patients by the patch clamp technique. Methods Kv1.3 potassium channels expression from 17 patients with ACS and 11 healthy age-match controls was detected in single cell (CD4+CD28null T cells and CD4+CD28null T cells) by fluorescence microscopy and patch clamp. Results The percentage of CD4+CD28null T cells was higher in the ACS patients (6.97±2.05%) than that in the controls (1.38±0.84%), P<0.05). The concentration of hsCRP was directly correlated with the number of the CD4+CD28null T cells in the ACS patients (r=0.52, P<0.05). The conductance (6.89±1.17ns vs 3.36±0.66ns), dens (1.95±0.80 μm2 vs 1.13±0.57 μm2) and numbers (574.5±97.6 n/cell vs 280.3±55.3 n/cell) of the Kv1.3 channels on the CD4+CD28null T cells were significantly higher than those on the CD4+CD28null T cells (all P<0.01) in ACS patients, but were similar on CD4+CD28null T cells between ACS patients and controls. Conclusion The CD4+CD28null T cells and the numbers of Kv1.3 channels on the CD4+CD28null T cells from patients with ACS are significantly upregulated and might contribute to the pathogenesis of ACS (J Geriatr Cardiol 2010; 7: 40-46).

Key words coronary disease; potassium channel, voltage-gated ;T lymphocyte

Introduction

Acute coronary syndromes (ACS), including unstable angina, non-ST and ST elevation myocardial infarction represent the major causes of death and disability in the world. It is increasingly clear that the recognition and treatment of common risk factors cannot fully explain and prevent the occurrence of ACS, and that optimal treatment of patients with ACS requires a better understanding of the events underlying the disease process. It is currently believed that plaque rupture/erosion, with the consequent superimposed thrombosis, represents a key event causing the sudden conversion of coronary syndromes from chronic to acute.1 Histological studies have confirmed that activated T cells and macrophages often accumulate in the shoulder region of the plaque, through the release of matrix-degrading enzymes such as matrix metalloproteinases, macrophages possibly being major effector cells in the plaque. 2 Plaque-infiltrating T cells are enriched for a particular subset, CD4+CD28null T cells.3 Such CD4+CD28null T cells are functionally distinct from classic CD4 helper T cells, besides their ability to release large amounts of interferon (IFN-γ) to activated the monocytes,4 but also effectively kill endothelial cells and smooth muscle cell in vivo without a need for antigen recognition.5 Therefore these T cells may directly contribute to plaque instability. In the peripheral blood the subset of CD4+CD28null T cells was expanded in patients with ACS but infrequent in patients with stable angina and the healthy individuals.3,6 T-cell activation is central to the acquired immune response. Activated T cells require high levels of intracellular calcium, which enters cells through calcium-release-activated calcium channels (CRAC) after intracellular stores are depleted of calcium.7 The voltage-gated K+ channel (Kv1.3) and the calcium activated K+ channel (IKCa1) regulate membrane potential and Ca2+ signaling in T lymphocytes.8,9 Kv1.3, which is the most thoroughly studied in immune cells are expressed abundantly in resting and activated human T cells, where they play an important physiological role in setting the cell resting membrane potential, cell mitogenesis and volume regulation.8 Kv1.3 channels are also involved in apoptosis of the T cell line and malignant lymphocytes.8 It also associate with the adhesion and migration of the activated T cells.10 Adoptive transfer of Kv1.3high rat memory T cells...
recognized as potential therapeutic targets for availability of specific and potent inhibitors, Kv1.3 are widely the restricted tissue distribution of these channels and the local institutional review board. formed consent, and the protocol was approved by each diabetes, thyroid disease, stroke. All patients provided in- any acute infection 4 weeks before enrollment, history of greater than 2 times); pregnancy or lactation, malignancy,hibited drug), vitamin E, severe anemia; renal failure requir-uent treatment with other lipid-regulating agents (statin, ure (New York Heart Association Class IIIb or IV); concur- preceding 3 months; percutaneous coronary interven- preceding 3 months; coronary artery bypass surgery within the preceding 3 months; percutaneous coronary interven- prior limit of normal.

infarction) required increased serum creatine kinase or myocardial infarction or ST elevated myocardial inf-

nosis of myocardial infarction (include non-ST-elevated angina required new or dynamic ST-segment or T-wave angina required new or dynamic ST-segment or T-wave

location of Ca

Selective targeting of these disease causing cells with Kv1.3 channel blockers leads to membrane depolarization, inhibition of Ca

T cells into native recipients causes severe experimental autoimmune encephalomyelitis (EAE), a model for MS. Selective targeting of these disease causing cells with Kv1.3 channel blockers leads to membrane depolarization, inhibition of Ca

channels to inves-

phagocyte lineages, trophoblasts and osteoclasts. Due to the restricted tissue distribution of these channels and the availability of specific and potent inhibitors, Kv1.3 are widely recognized as potential therapeutic targets for immunotherapy. The CD4+CD28null T cells from the ACS is the effector memory T cells. In the present study, we used the patch-clamp technique in combination with fluorescence microscopy and flow cytometry to determine whether the Kv1.3 channel high express on it. Maybe the Kv1.3 channel will be a new target for ACS.

Methods

Patients

The study included 17 patients admitted within 24h of onset of ACS without percutaneous coronary intervention and 11 healthy age-match controls. ACS was defined as high-risk unstable angina, non-ST elevated myocardial infarction (MI) or ST-elevated MI. The diagnosis of unstable angina required new or dynamic ST-segment or T-wave changes in at least 2 contiguous ECG leads or a new wall motion or myocardial perfusion abnormality. The diagnosis of myocardial infarction (include non-ST-elevated myocardial infarction or ST elevated myocardial infarction) required increased serum creatine kinase or its MB fraction concentration exceeding 2 times the upper limit of normal.

Patients were excluded if coronary revascularization was planned or anticipated at the time of screening. Other exclusion criteria were: evidence of Q-wave acute MI within the preceding 3 months; coronary artery bypass surgery within the preceding 3 months; percutaneous coronary intervention within the preceding 6 months; left bundle-branch block or paced ventricular rhythm; severe congestive heart failure (New York Heart Association Class IIIb or IV); concurrent treatment with other lipid-regulating agents (statin, clofibrate, probucol or analog, nicotinic acid, or other prohibited drug), vitamin E, severe anemia; renal failure requiring dialysis; hepatic dysfunction (alanine aminotransferase greater than 2 times); pregnancy or lactation, malignancy, any acute infection 4 weeks before enrollment, history of diabetes, thyroid disease, stroke. All patients provided informed consent, and the protocol was approved by each local institutional review board.

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of patients and healthy volunteers using Ficoll-Hypaque gradient procedure. We investigated hs-CRP plasma levels of them routinely. Peripheral blood mono-
nuclear cells (PBMCs) were stained with PE-labeled anti-
CD4, and FITC-labeled anti-CD28 mAbs (both BD Pharmingen). The cells were analyzed on flow cytometer (Becton Dickinson).

Immunostaining and electrophysiology

PBMCs were obtained from both patients and the volunteers, washed in phosphate-buffered saline (PBS) 3 times and incubation in the 5% CO2 gas incubator on a glass Petridish at 37°C. Glass adherent cells were removed after 2h. The cells were >95% viable determined by trypan blue dye exclusion. Channel expression was studied in single CD4+CD28null T and CD4+CD28+T cells. Cells were stained for CD4 and CD28 with PE-labeled anti-CD4, and FITC-labeled anti-CD28 mAbs (both BD Pharmingen). Cells were washed, and kept in the dark at room temperature for 10–30 minutes to attach. CD4+CD28null T and CD4+CD28+ T cells were visualized by fluorescence Microscopy (Olympus) and patch clamped immediately. During our electrophysiologi-

cal experiments, we visually selectively chose lymphocytes by their appearance and small diameter and in combination with the Two Colour Fluorescence. Membrane currents were measured using the whole-cell configuration of the patch clamp technique. An EPC-9 patch clamp amplifier was inter-
faced to an computer for pulse application and data recording. A standard protocol of depolarizing voltage stimuli was applied every 30 s from -60 mV to +40 mV (20 mV increment) or every second to visualize Kv1.3’s characteristic cumulative inactivation, pulse duration was 500 ms and holding potential, -80 mV. Patch electrodes of 3-5 MΩ were fabricated from borosilicate glass (Quanshui Teching Experymental instryment CO.LTO). The maximum uncompens-

ated series resistance was <10 MΩ during whole-cell recordings, so the voltage error was <5 mV for a current amplitude of 500 pA. whole-cell currents were allowed to stabilize for 10 min before the K+ current parameters were measured. This allowed for the shift in voltage dependence of the currents which occurs in the first few minutes and for diffusion of the pipette contents into the cell. For current recordings the electrodes were filled with Ca²+-free pipette solution containing (in mM): 145 KF,10 HEPES,10 EGTA,2 MgCl₂, pH7.3,290–310mOsm/L, The extracellular solution contained(in mM):160 NaCl,4.5 KCl,2 CaCl₂,1 MgCl₂,10 HEPES,10 Glucose,pH7.3,290–310mOsm/L. All recordings were done at room temperature (25-27°C). Voltage-gated currents were filtered at 3 kHz and stored on computer disk for subsequent analyses. Analyses were performed on computers with software Origin7.5. We use the drug 4-

aminopyridine (4-AP), the blocker of K+ channels to inves-
tigate whether the current we recorded was the delayed rectifier type potassium (K⁺) channels.

The chord conductance was calculated according to the definition: gK = Ip/V-Vrev, where: Ip = amplitude of the current, V = membrane potential, Vrev = reversal potential of the current, assumed to be -75 mV. Kv1.3 channel numbers per cell were determined by dividing the whole-cell Kv1.3 conductance by the single-channel conductance value for each channel (Kv1.3, 12 pS). Cell capacitance, a direct measure of cell surface area, was constantly monitored during recording. To normalize for cell size, we determined Kv1.3 channel densities per im2 cell surface area by dividing the average channel number/cell by the average cell surface area determined from capacitance measurements (1 pF = 100 μm²).

Western blotting

PBMC were obtained from consenting patients and the volunteers. Venous blood was collected in heparinized tubes and diluted 50% with phosphate-buffered saline (PBS). The suspension was centrifuged at 3000 rpm for 5 min at room temperature. The interface was removed, washed with PBS, and Adherent cells were removed by incubation in Petri dishes for 2 h at 37°C. FACS analysis of PBMC showed that CD4⁺ lymphocytes constitute 80% of the peripheral blood lymphocytes in the PBMC population. Cells were harvested by centrifugation (1,200 rpm for 2 min) and resuspended in lysis buffer containing (in millimolars): 50 Tris.HCl, 150 NaCl, 1 EDTA, 1 PMSF, and 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100, 2 μg/ml leupeptin, and 5 μg/ml aprotinin. After centrifugation, the protein content was measured using the Bradford Assay. Aliquots of cell proteins were fractionated on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride Membrane. Nonspecific protein-binding sites were blocked by incubation in TBST with 5% nonfat dry milk for 1 h at room temperature. The blots were incubated with the primary Abs (1/500 dilution for anti-Kv1.3 Ab) overnight at 4°C. After washing three to four times, the membranes were incubated for 2h at room temperature with affinity-purified HRP-conjugated rabbit anti-goat IgG (1:4000) secondary Abs (Pierce). Bands were visualized on X-ray film. And repeat 3 times for every samples. Anti-Kv1.3 were obtained from Santa Cruz. GAPDH Abs were obtained from KangChen Bio-tech Inc (Shanghai, CHI) respectively.

Statistical analysis

Data were expressed as mean±SD. Correlations were determined using Spearman’s rank correlation test. The non-parametric Mann-Whitney test was used to compare the groups. The remaining variables were compared using Student’s t test or one-way ANOVA. P<0.05 was considered significant. Data were analyzed with SAS.

Results

Clinical characteristics of the subjects

There was no difference in the age and the sex between the patients with ACS (8 men; mean age, 61.24±12.63 years) and the controls (5 men; mean age, 68.82±11.11 years). The percentages of risk factors (hypertension and hyperlipidemia) was higher in the group of ACS than those in the control, and there was no significant difference of the ratio of smoking between the two groups. The numbers of total white blood cell [(6.49±2.25×10⁹/L vs 8.25±4.35×10⁹/L] and lymphocytes [(1.52±0.60)×10⁹/L vs (1.33±0.54)×10⁹/L] were similar in the two groups (P>0.05). hs-CRP concentrations was higher in the ACS than the control [(1.49±1.12) vs (0.35±0.81), P<0.05] group after log-transformation.

CD4⁺CD28null T cell

CD4⁺CD28null T cells were infrequent in healthy individuals. To answer the question whether ACS patients carry CD4⁺CD28null T cells, PBMC were analyzed by 2-color cytomtery. Median frequencies of CD4⁺CD28null T cells were 6-fold higher in ACS than in control [(6.97±2.05)% vs 1.38±0.84]% (P<0.05, Fig.1). The frequency of CD4⁺CD28null T cells correlated with the concentration of hs-CRP (r=0.52, P<0.05).

Fig.1 Expression of CD4⁺CD28null T cells by flow cytomtery in ACS patients and control subjects. A:control; B:ACS.

The characteristics of Kv1.3 channels of human T cells

An example of the whole-cell currents recorded in a T cells applying a standard protocol of depolarizing stimuli described in Methods is presented in Fig.2A The currents were completely blocked upon application of 5 mM 4-aminopyridine (4-AP, Fig.2B), which selectively blocks K channels in T cells. Fig.2C show the current-voltage (I/V) relationship for current densities (pA/pF). When we repetitively stimulate the T cell every second, Kv1.3 currents exhibited a decrease in current amplitude, show the “cumulative inactivation” that is characteristic of this channel (Fig.2D).

These indicate that the recorded currents were predominantly due to the activation of Kv1.3 channels.
Up-regulation of Kv1.3 channel on the CD4<sup>+</sup>CD28<sup>nil</sup> T lymphocytes

We confirmed the Kv1.3 channel up-regulation on CD4<sup>+</sup>CD28<sup>nil</sup> T cells at the single-cell level by staining for CD4 and CD28 and patch clamping immunostained CD4<sup>+</sup>CD28<sup>nil</sup> T and CD4<sup>+</sup>CD28<sup>+</sup> T cells visualized by fluorescence microscopy (Fig.3A-F) from different groups. Because the CD4<sup>+</sup>CD28<sup>nil</sup> T is rarely in the healthy control, we didn’t record the current on CD4<sup>+</sup>CD28<sup>nil</sup> T cells from it. CD4<sup>+</sup>CD28<sup>+</sup> T cells appeared orange and green following immunostaining for CD4 (PE) and CD28 (FITC). CD4<sup>+</sup>CD28<sup>nil</sup> T cells stained only with PE-labeled anti-CD4 mAb’s exhibited orange fluorescence, but not green because it lost the CD28. Immunostaining did not affect patch-clamp seal formation, membrane capacitance, series resistance, or the biophysical properties of Kv1.3 currents. Larger Kv1.3 currents were observed in CD4<sup>+</sup>CD28<sup>nil</sup> T cells (Fig.3H) than in CD4<sup>+</sup>CD28<sup>+</sup> T cells but have no significantly different between CD4<sup>+</sup>CD28<sup>+</sup> T cells from the ACS (Fig.3I) and from the controls(Fig.3G). As shown in the Fig.4, I/V relationship of the different cells. Peak current densities (pA/pF) of the CD4<sup>+</sup>CD28<sup>nil</sup> T cells (269.41±119.78 pA/pF, n=10) was higher than the CD4<sup>+</sup>CD28<sup>+</sup> T cells from the ACS(105.46±65.10 pA/pF, n=13) and the control(156.22±78.60 pA/pF, n=12, P<0.05), but there was no difference in the CD4<sup>+</sup>CD28<sup>+</sup> T cells between the two groups. The number of channels per cell was computed by dividing the whole cell conductance by the measured single-channel conductance of 12pS. The conductance, channel number, channel density of Kv1.3 channels on the CD4<sup>+</sup>CD28<sup>nil</sup> T cells was higher (P<0.05), and the differences of them on the CD4<sup>+</sup>CD28<sup>+</sup> T cells between the two groups have no significantly(Table 1).

**Kv1.3 express in T lymphocytes**

The Kv1.3 polypeptide in total lysate of PBMCs was detected with the affinity-purified Ab anti-Kv1.3. Western blot analysis revealed a single band of 60 kDa. This band disappeared when the Ab was preabsorbed to the corresponding Ag, thus indicating specificity of the anti-Kv1.3. Kv1.3 protein expression significantly increase in the total lysate of the PBMCs from the patients with ACS. (Fig.5)

**Discussion**

The pathogenetic mechanisms that cause intimal ruptures in atherosclerotic plaques responsible for thrombosis
Atherosclerotic plaques with thin or virtually nonexistent fibrous caps showed a surface zone dominated by macrophages intermingled with T lymphocytes and lack of smooth muscle cells.

The current finding that an unusual subset of T lymphocytes, CD4\(^{+}\)CD28\(^{null}\) T cells, is expanded in patients with ACS but not in patients with SA and healthy individuals suggests that these cells are involved in the acute events complicating otherwise stable and slowly progressive coronary disease.

CD4\(^{+}\)CD28\(^{null}\) T cells from the plaque and the peripheral blood share the same TCR receptor sequences indicate that they are not fully understood. Atherosclerotic plaques with thin or virtually nonexistent fibrous caps showed a surface zone dominated by macrophages intermingled with T lymphocytes and lack of smooth muscle cells. The current finding that an unusual subset of T lymphocytes, CD4\(^{+}\)CD28\(^{null}\) T cells, is expanded in patients with ACS but not in patients with SA and healthy individuals suggests that these cells are involved in the acute events complicating otherwise stable and slowly progressive coronary disease.

Fig. 3  Fluorescent immunostained images of patch-clamped different T cell subsets. Fluorescent images of immunostained and patch-clamped CD4\(^{+}\)CD28\(^{+}\) T cells and CD4\(^{+}\)CD28\(^{null}\) T cells are shown in Fig (A–F) CD4\(^{+}\)CD28\(^{+}\) T cells from control subjects appeared orange and green following immunostaining for CD28(FITC)(A) and CD4 (PE)(D). CD4\(^{+}\)CD28\(^{null}\) T cells stained with PE-labeled anti-CD4(E), but no FITC-labeled anti-CD28(3b) exhibited orange fluorescence only. CD4\(^{+}\)CD28\(^{+}\) T cells from ACS stained with PE-labeled anti-CD4(C) and FITC-labeled anti-CD28(F) appeared orange and green. Substantially larger Kv1.3 currents were observed in CD4\(^{+}\)CD28\(^{null}\) T cells(H) than in CD4\(^{+}\)CD28\(^{+}\) T cells(G and I) (Fig.3, bottom panels).

Fig. 4  current-voltage (I/V) relationship for current densities (pA/pF) of difference cells.

Fig. 5  Immunoblotting analysis of Kv1.3 protein in PBMCs from patients (n=3) and healthy controls (n=3). A total of total lysate was loaded in each lane. GAPDH was used as a constitutive protein in these samples.
may came from the same clone. The activated CD4+CD28null T cells can produce high levels of interferon-γ, trigger neighboring macrophages to synthesize and secrete tissue-degrading enzymes, such as metalloproteinases, causing weakening of the fibrous cap before rupture. However, they express tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) capable of inducing apoptosis of vascular smooth muscle cells in the atherosclerotic plaque, through acquisition of stimulatory killer immunoglobulin like receptors (KIRs) and the adapter molecule DAP12, endows them with the ability to kill endothelial cells and smooth muscle cells in vivo, even in the absence of TCR triggering.

Furthermore, the detrimental effects of CD4+CD28null T cells appear to be amplified by CRP. Previous research show that the absolute significance of the CD4+CD28null T cells subset that increases to 10% of the circulating T cells in acute coronary syndrome patients. In this study we find that the percent of CD4+CD28null T cells in the circulation T cells increases to 6%, 6 times than the healthy controls. The number of the CD4+CD28null T cells is directly correlated with the concentration of hsCRP in the ACS, it indicate that the increase of the CD4+CD28null T cells may accompany with the enhancement of the inflammation. The interaction of them can increase the cytotoxicity of the CD4+CD28null T cells to urge the plaque rupture.

The T-cell-mediated immune response is initiated by recognition of processed antigenic peptide bound to major histocompatibility complex (MHC) proteins on antigen-presenting cells (APCs) by T-cell receptor (TCR) on T cells. Once activated, then the followed event is secretion of biologically active proteins, proliferation, differentiation, and migration of differentiated lymphocytes into tissues. Many of these processes depend on calcium signaling. Depletion of internal Ca2+ stores causes voltage-independent Ca2+ release activated Ca2+ (CRAC) channels to open in the membrane, and the ensuing Ca2+ influx sustains elevated levels of cytosolic Ca2+. Ca2+ influx through CRAC channels is reduced at depolarized potentials and consequently membrane depolarization attenuates the Ca2+ signal. The outward K+ current through Kv1.3 channels can clamp the membrane at a hyperpolarized potential and thus, allow sustained Ca2+ influx through CRAC channels required for gene expression. Resting human T lymphocytes express about 300-400 Kv1.3 channels. During activation, up-regulation of Kv1.3 channels, increasing the expression to-600 channels per cell. A very interesting, activation-induced subset-specific change in the K+ channel repertoire was described in human T-cells by Wullf et al., they find that activation of TEM is accompanied by an increase in the number of Kv1.3 channels to 1500 per cell. Selective blockade of Kv1.3 channels inhibit the cytokine production and cell proliferation, but did not influence native and TEM T cells. Therefore, selective blockade of Kv1.3 channels in chronically activated T cells, without affecting normal lymphocytes, could be a safer strategy for the treatment of autoimmune disorders. In this study, we used a combination of fluorescence microscopy and patch-clamp analysis to characterize the patterns of K+ channel expression in CD4+CD28null T cells from patients with ACS, we confirm that Kv1.3 channels high express to 600 channels per cell, but by contrast, there only 200-300 channels per cell in the CD4+CD28+T cells. So we presume that may be the activation of the CD4+CD28null T cells be correlate with the Kv1.3 channels on it, selective block the channels can inhibit proliferation and differentiation of the CD4+CD28null T cells. Western blot analysis also reveal that the Kv1.3 protein increase in the total lysate of PBMCs, so we think this protein may be came from the CD4+CD28null T cells.

Atherosclerosis as a chronic inflammatory disorder has been investigated extensively, cumulate evidence have proof that the atherosclerotic process is essentially a disease highlights the pivotal role played by the immune system. Recent research found that an unusual CD4+CD28null T cells expansion largely in the ACS. CD4+CD28null T cells are cytotoxic lymphocytes able to kill target cells via killer cell immunoglobulin receptors recognizing polymorphic regions on MHC class I molecules and independence with the TCR. The defect in CD28 cell surface expression result from chronic exposure to antigens. Therefore, the expansion of CD4+CD28null T cells in ACS patients may reflect a persistent immune response to microorganisms or autoantigens contained in atherosclerotic plaques, or circulating in peripheral blood. So there have the similarities between ACS and chronic inflammatory diseases with involvement of immune pathways such as rheumatoid arthritis and Multiple Sclerosis. This suggest that inhibit the activation of T cell may represent an adjunctive therapeutic strategy also in patients with ACS. Selectively inhibit the Kv1.3 channels may be a new target to treat the ACS in the future.

<table>
<thead>
<tr>
<th>groups</th>
<th>Conductance(nS)</th>
<th>Channels density(n/μm²)</th>
<th>Channels number(n/cell)</th>
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<tbody>
<tr>
<td>ACS</td>
<td></td>
<td></td>
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<tr>
<td>CD4+CD28nullT cells (n=10)</td>
<td>6.89±1.17ab</td>
<td>1.95±0.8ab</td>
<td>574.54±97.63ab</td>
</tr>
<tr>
<td>CD4+CD28nullT cells (n=13)</td>
<td>2.85±1.39</td>
<td>0.76±0.4</td>
<td>237.7±115.47</td>
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<tr>
<td>control</td>
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<tr>
<td>CD4+CD28+T cells (n=12)</td>
<td>3.36±0.66</td>
<td>1.13±0.57</td>
<td>280.25±55.25</td>
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Difference between ACS and health controls. P<0.01; difference between CD4+CD28null T cells and CD4+CD28null T cells in ACS. P<0.01
Study limitations

In this study we included only patients with ACS and the healthy subjects, but there were no patients with stable angina (SA). The CD4^+CD28^−null T cells we used was fresh and not activated with the specific antigen (ox-LDL, or HSP60) in vitro and the numbers of the cells is less, so it is possible that our result did not reflect the characteristic CD4^+CD28^−null T cells truly. Therefore, further studies are needed to clarify the role of the T cells in ACS, and in particular the importance of immune-modulation as innovative approach for prevention and treatment of ACS.

References