The Th17/Treg imbalance in patients with acute coronary syndrome

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Abstract Atherosclerosis is a chronic inflammatory disease regulated by T lymphocyte subsets. Recently, CD4+CD25+Foxp3+ regulatory T (Treg) cells and Th17 cells have been described as two distinct subsets from Th1 and Th2 cells and have the opposite effects on autoimmunity. Th17/Treg balance controls inflammation and may be important in the pathogenesis of plaque destabilization and the onset of acute coronary syndrome [ACS, including unstable angina (UA) and acute myocardial infarction (AMI)]. To assess whether this balance was broken in patients with coronary heart disease, we detected Th17/Treg functions on different levels including cell frequencies, related cytokine secretion and key transcription factors in patients with AMI, UA, stable angina (SA) and controls. The results demonstrated that patients with ACS revealed significant increase in peripheral Th17 number, Th17 related cytokines (IL-17, IL-6 and IL-23) and transcription factor (RORγt) levels and obvious decrease in Treg number, Treg related cytokines (IL-10 and TGF-β1) and transcription factor (Foxp3) levels as compared with patients with SA and controls. Results indicate that Th17/Treg functional imbalance exists in patients with ACS, suggesting a potential role for Th17/Treg imbalance in plaque destabilization and the onset of ACS.

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KEYWORDS
Acute coronary syndrome; Th17; Regulatory T cells; Inflammation; Atherosclerosis

Introduction

Atherosclerosis is a chronic inflammatory disease involving various immune cells, particularly T lymphocytes [1–3]. The up-regulation of T helper (Th)1 response has been shown in the local atherosclerotic lesions and circulating lymphocytes in an atherosclerotic animal model and patients with acute coronary syndrome [ACS, including unstable angina (UA) and acute myocardial infarction (AMI)], suggesting that Th1/Th2 imbalance plays an important role in plaque rupture and the onset of ACS [4–7].

Recently, CD4+CD25+ regulatory T (Treg) cells and Th17 cells have been described as two distinct subsets from Th1 and Th2 cells. Treg cells expressing the forkhead/winged helix transcription factor (Foxp3) have an anti-inflammatory role and maintain tolerance to self components by contact-
dependent suppression or releasing anti-inflammatory cytokines [interleukin (IL)-10 and transforming growth factor (TGF)-β1] [8], while Th17 cells expressing retinoic acid-related orphan receptor-γt (RORγt) play critical roles in the development of autoimmunity and allergic reactions by producing IL-17 and, to a lesser extent, tumor necrosis factor (TNF)-α and IL-6 [9]. So the balance between Th17 and Treg may be important in the development/prevention of inflammatory and autoimmune diseases [10].

The objectives of this study were to evaluate whether the Th17/Treg balance was broken in patients with ACS.

Methods

Patients

This study conformed to the approved institutional guidelines. Informed consent was obtained from each patient. We studied 85 patients who underwent diagnostic catheterization (63 men and 22 women, mean age ± SD 59 ± 8 years). Patients were classified into 4 groups: (1) AMI group (20 men and 6 women, mean age 60 ± 7, inclusion criteria: myocardial infarction confirmed by significant rise of creatine kinase MB and troponin I levels); (2) UA group (12 men and 5 women, mean age 58 ± 9, inclusion criteria: chest pain at rest with definite ischemic electrocardiographic changes: ST-segment changes and/or T-wave inversions); (3) stable angina (SA) group (16 men and 6 women, mean age 59 ± 10, inclusion criteria: typical exertional chest discomfort associated with downsloping or horizontal ST-segment depression > 1 mm in an exercise test); (4) and the chest pain syndrome (CPS) group (15 men and 5 women, mean age 58 ± 12, inclusion criteria: chest pain without being accompanied by electrocardiographic changes, coronary arterystenosis, or coronary spasm when acetylcholine was given through intracoronary injection during coronary arteriography [11]).

No patient was treated with anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs, steroids, etc. None had collagen disease, thromboembolism, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant disease, other inflammatory disease (such as septicemia, pneumonia, etc.), valvular heart disease, atrial fibrillation or was using a pacemaker.

Blood samples

Blood samples were obtained from all the patients in the recumbent position with a 21-gauge needle for clean venipuncture of an antecubital vein after admission in a fasting state on the following morning of the admission day. Thus, the time interval between symptom onset and blood sampling was < 24 h in all cases. The samples were collected into collection tubes containing 0.2 ml sodium heparin. In the AMI group, 20 patients received reperfusion therapy after blood collection, 6 patients received reperfusion therapy before blood drawing. In the rest of the three groups, no one got reperfusion therapy before blood collection. Peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll density gradient for analysis of flow cytometric and real time-polymerase chain reaction (PCR). Plasma was obtained after centrifugation and stored at – 80 °C for the measurement of the cytokines and heat-sensitive C-reactive protein (hsCRP).

Flow cytometric analysis of Th17 and Treg

Cell preparation

For analysis of Th17, PBMCs were suspended at a density of 2 × 10⁶ cells/ml in complete culture medium (RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM glutamine and with 10% heat-inactivated fetal calf serum, Gibco BRL). The cell suspension was transferred to each well of 24-well plates. Cultures were stimulated with phorbol myristate acetate (PMA, 50 ng/ml) plus ionomycin (1 μM) for 4 h, in the presence of monensin (500 ng/ml, all from Alexis Biochemicals, San Diego, CA) The incubator was set at 37 °C under a 5% CO₂ environment. After 4 h of culture, the contents of the well were transferred to 5-ml sterile tubes. The cells were then centrifuged at 1500 rpm for 5 min. For analysis of Treg, PBMCs were aliquoted into tubes for further staining.

Surface and intracellular staining

Cells were aliquoted into tubes and washed once in phosphate-buffered saline (PBS). For Th17 analysis, the cells were incubated with phycoerythrin (PE) anti-human CD4 and Fluorescein isothiocyanate (FITC) anti-human CD25. After the surface staining, the cells were stained with FITC anti-human IL-17A for Th17 detection or PE anti-human Foxp3 for Treg detection after fixation and permeabilization according to the manufacturer’s instructions. Isotype controls were given to enable correct compensation and confirm antibody specificity. All of the Abs were from eBioscience. Stained cells were analyzed by flow cytometric analysis using

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical data of patients with AMI, UA, SA and CPS</th>
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<tbody>
<tr>
<td>Characteristics</td>
<td>AMI (n=26)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>20/6</td>
</tr>
<tr>
<td>Number of diseased vessels</td>
<td>1.8 ± 0.9</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>12 (46)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>6 (23)</td>
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<tr>
<td>Smoking, n (%)</td>
<td>8 (31)</td>
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<tr>
<td>Obesity, n (%)</td>
<td>5 (19)</td>
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<td>Hyperlipidaemia, n (%)</td>
<td>16 (62)</td>
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<td>Medications</td>
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<tr>
<td>Aspirin, n (%)</td>
<td>14 (54)</td>
</tr>
<tr>
<td>Beta-blockers, n (%)</td>
<td>12 (46)</td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>15 (58)</td>
</tr>
<tr>
<td>Calcium blockers, n (%)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>ACEI, n (%)</td>
<td>13 (50)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD or number. AMI: acute myocardial infarction; UA: unstable angina; SA: stable angina; CPS: chest pain syndrome; ACEI: angiotensin-converting enzyme inhibitor.
a FACScan cytometer equipped with CellQuest software (BD Bioscience PharMingen).

**RORγt and Foxp3 expression determined by real time-PCR**

Total RNA was extracted with TRIzol extraction (Invitrogen) according to the manufacturer’s instructions. cDNA was synthesized using random hexamer primers and RNase H-reverse transcriptase (Invitrogen). TaqMan primers and probes for human RORγt and Foxp3 were purchased from Applied Biosystems, and samples were analyzed utilizing the ABI Prism 7900 Sequence Detection System (Applied Biosystems). The following primer pairs were used: RORγt: F: 5'-TGAGAAGGACAGGGAGCACA-3', R: 5'-CCACAGATTATTGCAAGGGA TCA-3' and Foxp3: F: 5'-GAGAGCTGAGTCCCATGCA-3', R: 5'-AGACCCCTTGTCCGGATGAT-3'. For each sample, mRNA expression level was normalized to the level of GAPDH housekeeping genes.

**ELISA detection of plasma IL-17, IL-6, IL-23, TGF-β1, and IL-10**

The plasma levels of IL-17, IL-6, IL-23, TGF-β1, and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA), following the manufacturer’s instructions (IL-17 and IL-10 ELISA
kits, both from Biosource, Nivelles, Belgium; IL-23 ELISA kits, from Bender MedSystems, Burlingame, Calif; IL-6 and TGF-β1 ELISA kits, both from R&D system, Minneapolis, MN, USA). The minimal detectable concentrations were 2 pg/ml for IL-17, 0.7 pg/ml for IL-6, 78 pg/ml for IL-23, 5 pg/ml for TGF-β1, and 7.8 pg/ml for IL-10. Intra-assay and inter-assay coefficients of variation for all ELISA were <5% and <10%, respectively. All samples were measured in duplicate.

**Immunoturbidimetry measurement of plasma hsCRP**

hsCRP was measured using latex particle-enhanced Immunoturbidimetry (Beijing O&D Biotech Company Ltd, Cox Bio China, Beijing, P.R. China). The lower limit of detection for hsCRP was 0.06 mg/l.

**Statistical analysis**

Values are expressed as mean ± SD in the text and figures. The data were analyzed by ANOVA. If significance was found, Newman–Keuls test was performed for post-hoc analysis to detect the difference among groups. Spearman’s correlation was used as a test of correlation between two continuous variables. A probable value of P<0.05 was considered to be statistically significant.

**Results**

**Basic clinical characteristics of patients**

There were no significant differences in age, gender, number of diseased vessels, risk factors, medications among patients with AMI, UA and SA (Table 1).

**Circulating Th17 and Treg frequencies**

**Circulating Th17 frequencies increased in patients with ACS**

As shown in Figure 1, the frequencies of Th17 (CD4+IL17+/CD4+ T cells) were markedly higher in patients with AMI (2.3±1.3%) and UA (2.1±1.1%) than those with SA (0.5±0.3%) and CPS (0.3±0.2%) (P<0.01), while there was no obvious difference between the SA and CPS group (P>0.05).

**Circulating Treg frequencies decreased in patients with ACS**

As shown in Table 2 and Figure 2, the frequencies of CD4+CD25+ T lymphocytes were similar in the four groups (13.1±3.5% in the AMI group; 14.5±2.4% in the UA group; 12.5±2.9% in the SA group and 12.7±3.1% in the CPS group, P>0.05).

The frequencies of Treg (CD4+CD25+Foxp3+/CD4+ T cells) were significantly lower in the AMI group (1.3±0.8%), UA group (1.5±0.9%) and SA group (3.3±1.2%) as compared with the CPS group (5.3±1.9%) (P<0.01), while Treg frequencies in the AMI and UA groups were markedly lower than that of the SA group (P<0.01).

**Expression of RORγt and Foxp3 in PBMCs from patients with ACS**

RORγt is an important transcription factor for the differentiation of Th17, while Foxp3 is the master transcription factor in Treg. We thus investigate the expressions of RORγt and Foxp3 in PBMCs from patients with AMI, UA, SA and CPS.

As shown in Figure 3, the levels of RORγt expression were much higher in the AMI (32.7±8.2) and UA (31.8±7.1) groups than those in the SA (4.2±2.3) and CPS (3.9±1.5) groups (P<0.01), while there was no significant difference between the SA and CPS group (P>0.05). In contrast, the expression of Foxp3 was markedly lower in the AMI group (1.19±0.33), UA group (1.24±0.43) and SA group (4.5±2.1) as compared with the CPS group (9.3±2.9) (P<0.01), while Foxp3 levels in AMI and UA groups were significantly lower than that of the SA group (P<0.01).

**Plasma cytokines and CRP concentrations in patients with ACS**

Plasma IL-17, IL-6, IL-23, TGF-β1, and IL-10 were detected within 24 h after onset of symptoms in each groups (Table 3). The IL-17, IL-6 and IL-23 concentrations in patients with AMI (IL-17: 72.3±54.2 pg/ml; IL-6: 45.6±24.9 pg/ml; IL-23: 615±254 pg/ml) and UA (IL-17: 64.6±38.7 pg/ml; IL-6: 40.4±21.2 pg/ml; IL-23: 571±252 pg/ml) were significantly higher compared with concentrations in patients with SA (IL-17: 24.2±15.3 pg/ml; IL-6: 20.2±9.0 pg/ml; IL-23: 138±67 pg/ml) and CPS (IL-17: 20.8±13.3 pg/ml; IL-6: 16.5±7.6 pg/ml; IL-23: 128±70 pg/ml), while plasma TGF-β1 and IL-10 concentrations in the AMI (IL-10: 6.1±4.2 pg/ml; TGF-β1: 322±235 pg/ml) and UA (IL-10: 5.6±3.8 pg/ml; TGF-β1: 471±273 pg/ml) were significantly lower than those of the SA (IL-10: 15.3±7.7 pg/ml; TGF-β1: 735±357 pg/ml) and CPS groups (IL-10: 12.4±6.7 pg/ml; TGF-β1: 1013±564 pg/ml). In addition, TGF-β1 concentrations in the SA group were markedly lower as compared with the CPS group. Besides, TGF-β1 concentrations showed positive correlation with the frequencies of Treg cells (r=0.598, P<0.01, Fig. 4A) and negative correlation with IL-17 concentrations in the four groups (r=-0.352, P<0.01, Fig. 4B). As shown in Figure 4C, IL-17 concentrations were positively correlated with plasma concentrations of IL-23 (r=0.631, P<0.01) in four groups.

**Table 2** CD4+CD25+Foxp3+ Treg decreased in patients with ACS

<table>
<thead>
<tr>
<th></th>
<th>AMI (n=26)</th>
<th>UA (n=17)</th>
<th>SA (n=22)</th>
<th>CPS (n=20)</th>
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</thead>
<tbody>
<tr>
<td>CD4+CD25+/CD4+ T cells (%)</td>
<td>13.1±3.5*</td>
<td>14.5±2.4*</td>
<td>12.5±2.9*</td>
<td>12.7±3.1</td>
</tr>
<tr>
<td>CD4+CD25+Foxp3+/CD4+ T cells (%)</td>
<td>1.3±0.8#</td>
<td>1.5±0.9#</td>
<td>3.3±1.2#</td>
<td>5.3±1.9</td>
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</tbody>
</table>

*Values are expressed as mean±SD. AMI: acute myocardial infarction; UA: unstable angina; SA: stable angina; CPS: chest pain syndrome.

*P>0.05 vs. CPS. †P<0.01 vs. CPS. ‡P<0.01 vs. SA.
The levels of hsCRP were markedly increased in AMI and UA groups compared with those in SA and CPS groups.

Discussion
Th17/Treg balance controls inflammation and may be important in the pathogenesis of plaque destabilization and the onset of ACS. To assess whether this balance was broken in patients with ACS, we detected Th17/Treg functions on different levels including cell frequencies, related cytokine secretion and key transcription factors. The results demonstrated that patients with ACS revealed significant increase in peripheral Th17 number, Th17 related cytokines (IL-17, IL-6 and IL-23) and transcription factor (RORγt) levels and obvious decrease in Treg number, Treg related cytokines (IL-10 and TGF-β1) and transcription factor (Foxp3) levels as compared

![Diagram A](image1)

![Diagram B](image2)

**Figure 2** Circulating Treg frequencies decreased in patients with ACS. PBMCs from patients with ACS, SA, and CPS subjects were stained with labeled antibodies as described in Methods. A: CD4+CD25+ T subsets were gated by flow cytometry. Plots in intern box represented CD4+CD25+ T cells. B: Representative Foxp3 expression in CD4+CD25+ T subsets from each group was shown. AMI: acute myocardial infarction; UA: unstable angina; SA: stable angina; CPS: chest pain syndrome. The percentage of positive cells was shown in each panel.
with patients with SA and normal coronary artery subjects.

Results above indicate that Th17/Treg functional imbalance exists in patients with ACS, suggesting a potential role for Th17/Treg imbalance in plaque destabilization and the onset of ACS.

ACS occurs as a consequence of coronary plaque erosion or rupture and it is thought that inflammation plays an important role in these coronary events [1–3]. The major lineage of T lymphocytes presented in atherosclerotic lesions is CD4+ T lymphocytes including Th1 and Th2 subsets. Th1/Th2 imbalance plays an important role in plaque rupture and becomes a target for the treatment on atherosclerosis and ACS [4–7,12].

Recently, CD4+CD25+Foxp3+ Treg cells have been demonstrated efficiently in the control of autoimmune disease [8]. The number of Treg cells decreases in several autoimmune diseases [13–16], and adoptive transferring of purified Treg cells improves the autoimmune disorders [17–20]. Several studies have reported that Treg frequencies decreased and their functional properties compromised in patients with

Figure 3  Expression of RORγt and Foxp3 in PBMCs from patients with ACS. A: The ratios of RORγt/GAPDH mRNA were compared in 4 groups. *P<0.01 vs. SA and CPS; #P>0.05 vs. CPS. B: The ratios of foxp3/GAPDH mRNA were compared in 4 groups.* P<0.01 vs. CPS; #P<0.01 vs. SA. AMI: acute myocardial infarction; UA: unstable angina; SA: stable angina; CPS: chest pain syndrome.

Table 3  Plasma levels of cytokines and CRP in four groups

<table>
<thead>
<tr>
<th></th>
<th>AMI (n=26)</th>
<th>UA (n=17)</th>
<th>SA (n=22)</th>
<th>CPS (n=20)</th>
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<tr>
<td>Interleukin-17 (pg/ml)</td>
<td>72.3±50.2#</td>
<td>64.6±38.7#</td>
<td>24.2±15.3*</td>
<td>20.8±13.3*</td>
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<td>Interleukin-6 (pg/ml)</td>
<td>45.6±24.9#</td>
<td>40.4±21.2#</td>
<td>9.0±7.6</td>
<td>16.5±6.7</td>
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<tr>
<td>Interleukin-23 (pg/ml)</td>
<td>615±254#</td>
<td>571±252#</td>
<td>138±67</td>
<td>128±70</td>
</tr>
<tr>
<td>Interleukin-10 (pg/ml)</td>
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<td>5.6±3.8#</td>
<td>15.3±7.7#</td>
<td>12.4±6.7#</td>
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<tr>
<td>Transforming growth factor-β (pg/ml)</td>
<td>322±235*</td>
<td>471±273*</td>
<td>735±357*</td>
<td>774±564*</td>
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<tr>
<td>C-reactive protein (mg/l)</td>
<td>5.3±2.8*</td>
<td>5.1±2.2*</td>
<td>2.2±1.8*</td>
<td>1.7±1.1*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. AMI: acute myocardial infarction; UA: unstable angina; SA: stable angina; CPS: chest pain syndrome. *P>0.05 vs. CPS. #P<0.01 vs. SA or CPS. *P<0.05 vs. SA or CPS. †P<0.05 vs. CPS.

Figure 4  Spearman correlation of circulating cytokines and Treg cells. A: TGF-β1 concentrations positively correlate with a proportion of Treg/CD4+ T cells (P<0.01 and r=0.598). B: TGF-β1 concentrations negatively correlate with IL-17 concentrations (P<0.01 and r = -0.352). C: IL-23 concentrations positively correlate with IL-17 concentrations (P<0.01 and r=0.631). AMI: acute myocardial infarction; UA: unstable angina; SA: stable angina; CPS: chest pain syndrome.
Adaptive transferring of natural or antigen-specific induced Treg cells can attenuate atherosclerosis in an animal model [24–26]. In this study, we found that the frequencies of Treg cells and the expression of Foxp3 were significantly lower in patients with coronary heart disease as compared with CPS patients, and Treg cells frequencies and Foxp3 expression markedly decreased in patients with ACS compared to patients with SA, which conformed to the results demonstrated by Mor et al. and Han et al. [21,22], suggesting again that Treg cells have a potentially protective role in the progression and stability of the atheroma.

Treg cells exert their function partly by secretion of anti-inflammatory cytokines (IL-10 and TGF-β1), while TGF-β1 can promote expression of Foxp3 and induce differentiation of Treg cells which originate from CD4+CD25− T cells [27,28]. In this study, we measured the levels of plasma IL-10 and TGF-β1 and found that these two cytokines both decreased in the ACS group, while TGF-β1 concentrations positively correlated with the frequencies of Treg cells in the four groups (r = 0.598, P < 0.01). The positive correlation between TGF-β1 and Treg cells suggested TGF-β1 may play a protective role in the onset of ACS partly through inducing the production of Treg cells and acting as one of the effective factors of Treg cells.

The Th17 cell is a distinct lineage different from Th1 and Th2 cells which regulates tissue inflammation and emerges from the discovery of a new type of cytokine, IL-17 [9]. Th17 cells expressing RORγt play critical roles in the development of autoimmunity and allergic reactions by producing IL-17 and, to a lesser extent, TNF-α and IL-6 [9,29]. IL-17 acts in vitro and in vivo as a proinflammatory cytokine [30]. It has pleiotropic activities including inducing the expression of proinflammatory cytokines (such as IL-6 and TNF-α), chemokines [such as macrophage chemotactant protein (MCP)-1 and macrophage inflammatory chemokine-2 (MIP-2)] and matrix metalloproteases, which mediate tissue infiltration and destruction [30]. Expression of IL-17 increased in autoimmune conditions including rheumatoid arthritis (RA) [31], multiple sclerosis (MS) [32] and inflammatory bowel disease (IBD) [33], suggesting the contribution of IL-17 to the induction and development of these diseases. The development of collagen induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE), the animal models for RA and MS, respectively, was suppressed in IL-17−/− mice [34,35]. Therefore, Th17 cells are likely to play critical roles in autoimmunity and allergic reactions.

Hashmi et al. have reported that IL-17 and IL-17 induced cytokines (IL-6 and IL-8) significantly increased in ACS patients [36]. In this study, we found that patients with ACS exhibited a significant increase in peripheral Th17 number, Th17 related cytokines (IL-17, IL-6 and IL-23) and RORγt levels as compared with patients with SA and normal coronary artery subjects, suggesting that Th17 may participate in the inflammatory process in plaque destabilization and the onset of ACS. As we know, this is the first report on the relationship between Th17 cells and patients with coronary heart disease from cell and transcription factor levels.

Some animal studies link IL-17 with atherosclerosis which may help us explain the possible effect of elevated Th17 cells on patients with coronary heart disease. Owing to up-regulation of IL-17 receptor in the local vascular wall, IL-17 is shown to be involved in the attraction and activation of macrophages in atherosclerotic lesions [37]. IL-17 activating macrophages may contribute to lesion progression by release of proteolytic factors such as matrix metalloproteases, possibly via IL-17-related mechanisms [38]. In proatherogenic conditions such as aging, vascular production of IL-17 seems to increase several-fold [39]. Increased vascular TNF-α and IL-17 levels can act synergistically to create a proinflammatory microenvironment, which promotes the development of atherosclerotic vascular disease [40].

Mouse Th17 cells are differentiated from naive T cells in response to IL-6 plus TGF-β and need the presence of IL-23 for their expansion and/or maintenance [41,42]. However, TGF-β plus IL-6 can not induce differentiation of human Th17 from naive CD4+ T cells, while IL-23 is an important inducer for differentiation of human Th17 cells [43,44]. Consequently IL-23 is the inducer and TGF-β is the inhibitor of Th17 differentiation in human beings [44]. In the present study, the results showed that IL-23 concentrations were higher in the ACS group and positively correlated with IL-17 concentrations (r = 0.631, P < 0.01), while TGF-β1 concentrations were lower in the ACS group and negatively correlated with IL-17 concentrations (r = −0.352, P < 0.01), suggesting IL-23 may induce, while TGF-β1 may inhibit Th17 differentiation and production of IL-17 to participate in the onset of ACS.

Some investigations have demonstrated that reperfusion treatments including emergent PCI and thrombolytic therapy have an effect on inflammatory factors [45,46]. In our experiments, 6 patients in AMI group got reperfusion therapy before blood collecting. We analyzed the 20 patients in the AMI group who received reperfusion therapy after blood collecting and found no obvious change in the AMI group happened on Th17 number [(2.3 ± 1.4)%], ratio of RORγt/GAPDH mRNA (32.7 ± 8.2), plasma Interleukin-17 (67.8 ± 40.2 pg/ml), ratio of Foxp3/GAPDH mRNA (1.1 ± 0.3), Treg frequencies [(1.1 ± 0.7)%] and plasma TGF-β1 (302 ± 232 pg/ml). The statistical results compared with the other groups were similar to the previous analysis which do not exclude the six patients (data not shown). Whether reperfusion therapy has an impact on the balance between Th17 and Treg needs further study.

In summary, our data demonstrate that Th17/Treg functional imbalance exists in patients with ACS, suggesting a potential role for Th17/Treg imbalance in plaque destabilization and the onset of ACS. Because of the reciprocal developmental pathway for the generation and the opposite effects of Th17 and Treg cells, Th17/Treg subsets may therefore have been evolved to induce or regulate tissue inflammation, analogous to the dichotomy of Th1/Th2 T-cell subsets [10,42]. We need to prove our conclusion in a larger scale of the population and ongoing efforts should be made to identify the precise effect and mechanism of Th17/Treg imbalance in plaque progression and destabilization in atherosclerosis animal models. Th17/Treg balance will provide a new target for the treatment on atherosclerosis and ACS.

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