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Modulation of STAT3 and STAT5 activity rectifies the imbalance of Th17 and Treg cells in patients with acute coronary syndrome

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Abstract The signal transducer and activator of transcription (STAT) activity plays an important role in the differentiation and imbalance of Th17 and Treg cells in acute coronary syndrome (ACS) patients. We determined that the basal STAT3 phosphorylation level was significantly increased and exhibited a positive relationship with Th17 cells but was negatively correlated with Treg cells in ACS patients. Opposite effects were observed for STAT5 activity. Using the pharmaceutical inhibitor TG101348 or knockdown of STAT3 reduced the number of Th17 cells while promoting the number and function of Treg cells via the Janus kinase2 (JAK2)/STAT3 pathway in ACS patients. Significantly more STAT5 bound to the *Foxp3* locus when STAT3 was knocked down, and overexpression of STAT5 led to an increased number of Treg cells but a decreased number of Th17 cells in ACS patients. Our findings demonstrate that modulation of STAT3/STAT5 activity rectifies the imbalance of Th17/Treg cells in ACS patients.

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1. Introduction

Acute coronary syndrome (ACS) is a leading cause of death and morbidity worldwide and may involve imbalanced lipid metabolism and maladaptive immune responses that lead to chronic inflammation of the arterial wall [1,2]. T cell activation and infiltration likely play an important role in the pathogenesis of ACS [3,4]. Th17 and Treg cells exhibit opposite functions in the adaptive immune system, and Th17/Treg subsets may therefore have evolved to induce or regulate inflammation. We and others have found that an increased ratio of Th17 to regulatory T (Treg) cells controls inflammation, and this process may contribute to the destabilization of atherosclerotic plaques and the onset of ACS [5,6].

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway plays a critical role in the signaling of a wide array of cytokines and growth factors and leads to a variety of cellular functions, including proliferation, growth, hematopoiesis, and immune responses [7,8]. IL-6, IL-23, and IL-21 signal through JAK-mediated phosphorylation of STAT3 and are required for Th17 cell generation. In addition, hyperactivation of STAT3 in Treg cells facilitates the conversion to Th17-like cells, leading to an uncontrolled inflammatory response [9,10]. STAT5-dependent Treg cells enhance FOXP3 expression and inhibit inflammatory responses. IL-2 is a potent inducer of STAT5; forced expression of STAT5 in donor T cells protects mice from acute graft versus host disease by enhancing the induction of Treg cells [11–13]. While STAT3 is an important inhibitor of FOXP3 expression, STAT5 is a key positive regulator of FOXP3 [14]. However, details related to the activity of STAT proteins in ACS patients, the relationships between these proteins and pathogenic Th17 and Treg cells, and the mechanisms by which the imbalance of Th17/Treg cells can be modified in ACS patients remain poorly understood.

Here, we observed STAT activity in ACS patients and determined that the basal STAT3 phosphorylation level was significantly increased, while the STAT5 phosphorylation level was reduced in ACS patients compared with healthy controls. STAT3 activity exhibited a positive relationship with Th17 cells and IL-17-producing Treg cells but was negatively correlated with Treg cells in ACS patients. However, STAT5 activity exhibited the opposite relationship. Modulation of the STAT3 activity of $CD4^+$ T cells from ACS patients using the pharmaceutical inhibitor TG101348 and small RNA interference promoted FOXP3 expression and Treg function but reduced Th17 cell differentiation. Significantly more STAT5 bound to the *Foxp3* locus when STAT3 was specifically knocked down, and overexpression of STAT5 in ACS patients led to an increased number of Treg cells and a decreased number of Th17 cells. The identification of the signaling pathways involved in this process suggests new target molecules for the control of ACS inflammation.

2. Materials and methods

2.1. Patients and controls

Eighty-two patients with coronary atherosclerosis disease (CAD) who underwent diagnostic catheterization at Xin Hua Hospital in the Shanghai Jiaotong University School of Medicine were randomly selected for inclusion. The CAD patients were classified into 2 subgroups based on disease severity. Twenty patients had stable angina (SA), with the following inclusion criteria: typical exertional chest discomfort associated with down-sloping or horizontal ST-segment depression greater than 1 mm in an exercise test and at least 1 angiographically documented coronary artery stenosis detected, with a 75% reduction of lumen diameter. Sixty-two patients had ACS, including unstable angina and acute myocardial infarction. All patients had experienced chest pain at rest within the preceding 12 h, ST-segment changes and/or T-wave inversions, myocardial infarction confirmed by a significant increase in creatine kinase MB (CK-MB) and troponin I (TnI) levels, and angiographic evidence of coronary lesions. Thirty blood samples from age- and sex-matched healthy controls (HC) with no clinical signs of CAD were obtained from the medical examination center of Xinhua Hospital. The exclusion criteria were as follows: previous myocardial infarction within 6 months, previous revascularization procedures, inflammatory conditions likely to be associated with an acute phase response, autoimmune disease, neoplastic disease, advanced liver disease, renal failure or severe heart failure (NYHA classes III–IV). The following parameters were measured in patients with CAD in the clinical chemistry laboratory of Xinhua Hospital: low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, CK-MB, highly sensitive C-response protein (Hs-CRP), N-terminal pro-brain natriuretic peptide (NT-proBNP) and TnI. All patients provided written informed consent for participation in the study, which was conducted in accordance with the principles of the Declaration of Helsinki and approved by the ethics committee of Xinhua Hospital.

2.2. Flow cytometric analysis and cell sorting

Human peripheral blood mononuclear cells (PBMCs) were freshly isolated using Ficoll density gradient centrifugation and re-suspended in phosphate-buffered saline (PBS) supplemented with 2% bovine serum albumin (BSA). For intracellular cytokine staining, cells were incubated for 5 h with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich) and 500 ng/ml ionomycin (Sigma-Aldrich) in the presence of GolgiPlug (BD Biosciences). The resulting cells were first surface-stained with an anti-human CD4 antibody (BD Biosciences) and then stained with antibodies specific for human IL-17 and FOXP3 (eBioscience) using the Fixation/Permeabilization Kit (eBioscience) according to the manufacturer's instructions. To stain phosphorylated STAT1, STAT3, STAT5 and STAT6, anti-phospho-STAT1 (Py701), anti-phospho-STAT3 (Py705), anti-phospho-STAT6 (Py641), and anti-phospho-STAT5 (Py694) antibodies and isotype controls (BD Bioscience) were used according to the manufacturer's instructions. Flow cytometric analysis was performed with FACS CantoII (BD Bioscience) using the FlowJo software (TreeStar). To purify $CD4^+CD25^{hi}CD127^{lo/-}$ cells, $CD4^+CD25^-$ T cells or $CD4^+CD45RA^+$ naïve T cells, PBMCs were surface-stained for CD4, CD25, CD127, and CD45RA and sorted using a BD Arial II. The purity of the cells was greater than 98%.