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Characteristics of the complement system gene expression deficiency in patients with symptomatic pulmonary embolism

Wei Lv, Lemin Wang *, Qianglin Duan, Zhu Gong, Fan Yang, Haoming Song, Yanli Song

Department of Cardiology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China

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ABSTRACT

Introduction: Pulmonary embolism (PE) is a disease with a high mortality and morbidity rate, and the pathogenesis of PE remains still unclear. We aimed to investigate the gene expression differences of the complement system in peripheral blood mononuclear cells (PBMCs) from patients with symptomatic PE and controls.

Methods: Twenty cases of PE patients and twenty sex and age matched controls were recruited into the study. Human cDNA microarray analysis was used to detect the gene expression difference of the complement system between the two groups.

Results: 1). Expression of twenty-one genes encoding complement components was detected. In PE patients, expression of the genes encoding C1qα, C1qβ, C4b, C5 and Factor P was significantly greater ($P < 0.05$) than controls, while C6, C7, C9, mannose-binding lectin (MBL) and mannan-binding lectin serine peptidase 1 (MASP1) mRNAs were lower ($P < 0.05$) than controls. 2). Expression of seven genes encoding complement receptors was examined. In PE patients, CR1, integrin αM, integrin αX and C5aR mRNAs were significantly up-regulated ($P < 0.01$) compared with controls. 3). Seven genes encoding complement regulators were examined. The mRNA expression of CD59 and CD55 was significantly up-regulated ($P < 0.05$), whereas Factor I mRNA was significantly down-regulated ($P < 0.05$) in PE patients than controls.

Conclusions: In PE patients, the mRNA expressions of complement components, receptors and regulators were unbalanced, suggesting dysfunction and/or deficiency of the complement system, which leads to decreased function of MAC-induced cell lysis in PE patients finally.

Introduction

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are collectively known as venous thromboembolism (VTE). PE is categorized as acute pulmonary embolism (APE) and chronic thromboembolic pulmonary hypertension (CTEPH). PE is a major health problem, with a high incidence, mortality, misdiagnosis and miss diagnosis rate [1]. VTE risk factors can be classified as either acquired or inherited, and most of them are acquired. The American College of Chest Physicians (ACCP) has published guidelines for the prevention, diagnosis, and treatment of VTE in surgical patients since 1995 [2]. Nine issues have been published so far [3]. However, the incidence of symptomatic VTE was increasing instead of reducing [4], probably because the pathogenesis of VTE is still unclear.

Smeeth et al. [5] have reported that acute infections were associated with an increased risk of VTE. Previously, we reported that VTE was found in multiple organs including the lungs, spleen, pancreas, kidneys, and adrenal glands from a patient who died of severe acute respiratory syndrome [6]. In addition, our previous study showed significantly down-regulated mRNA expression of genes associated with natural killer (NK) cells and T cells in patients with symptomatic PE [7], and declined cellular immune function in patients with acute PE and CTEPH respectively [8,9]. These previous studies indicate that the occurrence and progress of symptomatic PE are closely associated with both the innate and adaptive immunity.

The complement system plays an important role in both the innate and adaptive immune systems to defense against pathogens [10]. It is composed of more than 30 different proteins, including complement components, receptors and regulators. In clinical practice, it's hard to detect the levels of all the proteins in the complement system currently. Therefore, in the present study, human microarray analysis was used to examine the mRNA expression of the complement components, receptors and regulators in PBMCs isolated

* Corresponding author at: Department of Cardiology, Tongji Hospital of Tongji University, 389 Xincun Road, Shanghai 200065, China. Tel.: +86 21 66111289; fax: +86 21 66111329.
E-mail address: wanglemin@tongji.edu.cn (L. Wang).

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from symptomatic PE patients and controls. We designed this in vitro study to investigate the changes in the function of the complement system in patients with symptomatic PE.

Patients and Methods

Patients

Twenty patients with PE were recruited from Tongji Hospital of Tongji University from 2007 to 2008. A diagnosis of PE required any two of the following three criteria: 1) Selectivity pulmonary angiography shows pulmonary artery obstruction or filling defect; 2) Lung ventilation/perfusion scan shows single or multiple blood perfusion defect, normal or abnormal ventilation, and V/Q does not match. 3) Clinical diagnosis: there are risk factors for PE and other cardiovascular diseases can be excluded by clinical performance, electrocardiogram and chest film, arterial blood gas analysis suggests hypoxemia and hypocapnia, and D-dimer detection, echocardiography, chest computed tomography support PE diagnosis. We chose twenty patients admitted in our department of cardiology at the same time as control in the study. The patients were divided into two groups: 1) PE patient group: 20 patients (11 males and 9 females), with a mean age of 70 ± 14 (44 ~ 89) yr, include 3 cases of CTEPH; 2) Control group: 20 patients (11 males and 9 females) without PE, DVT, arterial thrombosis, and congenital coagulation abnormality, mean age with a mean age of 72 ± 14(44 ~ 91) yr, which were matched in sex and age with the PE group. There was no significant statistical difference between the age of the two groups ($P > 0.05$). The clinical trial has been approved by the Ethics Committee of Tongji University, and informed consent form was also obtained.

Gene Expression Profiling

Agilent G4112A Whole Human Genome Oligo Microarrays were purchased from Agilent (USA). A microarray is composed of 44,290 spots including 41675 genes or transcripts, 314 negative control spots, 1924 positive control spots and 359 blank spots. The functions of more than 70% of genes in the microarray have been known. All patients were subjected to microarray analysis.

Total RNA Isolation

5 ml of peripheral blood samples anti-coagulated with EDTA were drawn from patients suspected with PE immediately after admitting to the hospital and from those patients without PE, respectively. Mononuclear cells were obtained through density gradient centrifugation with Ficoll solution and remaining red blood cells were destroyed with erythrocyte lysis buffer (Qiagen, Hilden, Germany). Total mononuclear cell RNA was extracted with TRIzol(Invitrogen, Carlsbad, USA) and purified with Qiagen RNeasy column (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Isolated total RNA was testified and quantified by means of Nanodrop ND-1000 spectrophotometer (Nanodrop Technology, Cambrige, UK).

Detection of Gene Expression

About 1 μg of total RNA was reversely transcribed into double strand cDNA. After purification, *in vitro* amplification was performed with Agilent Low RNA Input Linear Amplification Kit (Agilent, Pal alto, USA) and modified UTP [aaUTP, 5-(3-aminoally1)-UTP] was used to replace UTP. The integrated aaUTP can interact with Cy3 NHS ester forming fluorescent products which are then used for hybridization. The integration rate of fluorescence can be determined with a NanodropND-1000 spectrophotometer. Then, hybridization mixture was prepared with Agilent oligonucleotide microarray in situ hybridization plus kit. About 750 ng of fluorescent products were fragmented at 60 °C and hybridization was conducted in Human Whole-Genome 60-mer oligo-chips (G4112F, Agilent Technologies) at 60 °C for 17 h at 10 rpm. After hybridization, the chips were washed with Agilent Gene Expression Wash Buffer according to manufacturer's instructions. Original signals were obtained Agilent scanner and Feature Extraction software. The standardization of original signals was carried out with RMA standardized method and standardized signal values were used for screening of differentially expressed genes.

RT-PCR

Three differential genes in the microarray were selected and their expressions were confirmed by RT-PCR. Among genes with differential expressions, 3 genes were randomly selected and these genes and house keeping gene (GAPDH) were subjected to RT-PCR. The relative expressions were expressed as the expressions of target genes normalized by that of GAPDH ($2^{-\triangle\triangle Ct}$). Melting curve and $2^{-\triangle\triangle Ct}$ method were used to compare the difference in the expressions between control group and PE group. Results from RT-PCR were consistent with microarray analysis.

Statistical Analysis

Independent-Samples T Test was used to compare mRNA levels in samples from PE patients and controls. Statistical tests were performed using SPSS 17.0, and p values <0.05 were considered significant. Before t test, test for equality of variances was performed, if variances were not equal, t test result would be corrected.

Results

Gene Expression of Complement Components

The results showed that mRNA expressions of complement early components including C1qα, C1qβ, C1qγ, C1r, C1s, C2, C3, C4b, Factor B, Factor D, Factor P, MBL, MASP1 and MASP2 in PBMCs from patients with PE and controls were detected (Fig. 1A). In PBMCs from PE patients, expression of the genes encoding C1qα, C1qβ, C4b and Factor P was significantly greater ($P < 0.01$) than that in controls. Gene expression of MBL and MASP1 was lower ($P < 0.05$) in PBMCs from PE patients compared with controls.

Gene expressions of complement late components including C5, C6, C7, C8α, C8β, C8γ and C9 in PBMCs from PE patients and controls were also detected (Fig. 1B). In PE patients, mRNA expression of C5 was significantly up-regulated ($P < 0.05$), whereas C6, C7 and C9 were significantly down-regulated ($P < 0.05$) compared with controls.

Gene Expression of Complement Receptors

The results showed that mRNA expressions of complement receptors including CR1, CR2, C3aR, integrin αM, integrin αX, integrin β2 and C5aR in PBMCs from PE patients and controls were examined (Fig. 2A). CR3 consists of integrin αM and integrin β2, and CR4 comprises integrin αX and integrin β2. In PE patients, expressions of all the seven genes mRNAs were up-regulated, and mRNA expressions of CR1, integrin αM, integrin αX and C5aR were significantly up-regulated ($P < 0.01$) compared with controls.

Gene Expression of Complement Regulators

Gene expressions of complement regulators C4b binding protein, α (C4BPα), C4b binding protein, β (C4BPβ), Factor H, Factor I, CD59, CD55 and CD46 in PBMCs from PE patients and controls were detected (Fig. 2B). CD59 and CD55 mRNAs were both significantly up-regulated ($P < 0.05$), while Factor I mRNA was significantly down-regulated ($P < 0.05$) in PBMCs from PE patients than controls.