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REGULAR ARTICLE

Thrombopoietin levels increased in patients with severe acute respiratory syndrome

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Abstract

Hematological changes in patients with Severe Acute Respiratory Syndrome (SARS) are common and frequently include thrombocytopenia. Using a ELISA method, we found an increase in thrombopoietin (TPO) levels in the plasma of convalesced SARS patients (290 ± 53 pg/ml) and active SARS patients (251 ± 23 pg/ml) comparing to that from normal control patients (228 ± 17 pg/ml). In addition, the plasma from active SARS patients had an inhibitory effect on CFU-MK formation, which could be neutralized by anti-TGF-β antibodies. In the experiment to determine whether SARS-CoV can directly infect hematopoietic stem cells and megakaryocytic cells, incubation of the cells with SARS-CoV did not show active infection. Our findings of increased TPO levels in the plasma of SARS patients provide a possible explanation for the genesis of thrombocytosis, which frequently develops from thrombocytopenia in SARS patients.

Introduction

Severe Acute Respiratory Syndrome (SARS) is a recently emerged human disease caused by the infection of a novel coronavirus (SARS-CoV) [1]. The

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sequence of the complete genome of SARS-CoV has been determined [2,3], which is 29,727 nucleotides in length and has at least 11 open reading frames. The genome organization is similar to those of other coronaviruses [2]. The most common presenting symptoms in SARS patients are fever, chills, rigor, myalgia, cough, headache, and dizziness [4]. Less common symptoms include sputum production, sore throat, coryza, nausea and vomiting, and diarrhea [4].

Hematological changes in patients with SARS are common. For example, thrombocytopenia is found in 55% of SARS patients [4–7]. Previously, a platelet count of $<150\times10^9$/l was documented in 44.8% of the SARS patients ($n=138$) in our hospital [4]. A prolonged activated partial-thromboplastin time greater than 38 s was noted in 42.8% of the SARS patients, whereas the prothrombin time remained normal in most cases. In 45.0% of the SARS patients, the D-dimer level was also elevated [4]. In one of our later reports, the common hematological changes found in SARS patients ($n=157$) included thrombocytopenia (55%), thrombocytosis (49%), and isolated prolonged activated partial-thromboplastin time >40 s (63%) [5]. The mechanism of how coronavirus causes thrombocytopenia and subsequent thrombocytosis have not been well understood, neither does the role TPO play in the process. In this study, TPO levels in plasmas from SARS patients, the effect of these plasmas on *in vitro* megakaryocytopoiesis, and whether SARS-CoV can directly infect hematopoietic stem cells and megakaryocytic cells are investigated.

Patients and methods

Patients

Plasma were collected from patients with SARS at the Prince of Wales Hospital, Hong Kong. These patients were divided into three groups: SARS patients in convalescence ($n=10$), active SARS patients ($n=7$), and normal control patients ($n=10$, laboratory staff and research students). The study was approved by the Committee on Clinical Research of the Institution.

ELISA

Plasma TPO levels were measured by an ELISA kit (R&D, Minneapolis, USA) following the manufacturer's instructions. Briefly, a monoclonal antibody specific for TPO was pre-coated onto a microplate. The standards and test samples were then pipetted into the wells and any TPO present was bound by the immobilized antibodies. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TPO was added to the wells. Following a wash that removed any unbound antibody–enzyme reagent, a substrate solution was added to the wells and color was developed, which is in proportion to the amount of TPO bound to the well. The optical density of each well was measured at the wave length of 450 nm by a microreader (Bio-tek, Instruments, Winooski, USA).

Human CFU-MK assay

Mononuclear cells from cord blood were separated by centrifugation using Ficoll–Hypaque (density 1.077) and added to a plasma clot culture system [8]. After incubation with plasma (5%) of SARS patients or normal control patients for 12 days, the megakaryocyte colonies were stained with an immunocytochemical method using biotin/alkaline phosphatase and detected with naphthol AS-MX phosphate and fast red. The cells were counterstained with haematoxylin and examined under an inverted-microscopy. The anti-TGF-β antibodies were obtained from R&D Systems (Minneapolis, MN) [9].

Infection of megakaryocytic cells and CD34+ cells by SARS-CoV

Human megakaryoblastic cell line Meg-01 cells were obtained from ATCC (Rockville, MD) [8]. Megakaryocytic progenitor cells were collected from human CFU-MK colonies. Haematopoietic CD34+ cells were purified from human cord blood [8]. These megakaryocytic cells and CD34+ cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) with 10% Fetal Calf Serum (FCS) (CSL, Australia), Penicillin (100 U/ml) and streptomycin (50 μg/ml) with or without SARS-CoV for 7 days at 37 °C in a humidified incubator with 5% CO2 [8].

Immunofluorescence methods

The expression of SARS-CoV antigens on cultured human megakaryocytic cells and CD34+ cells were investigated by an immunofluorescence method using serums of SARS-CoV patients [10].

Statistical analysis

The statistical significances of the TPO levels among different groups were evaluated by Tukey–Kramer HSD (honestly significant difference) test, an ANOVA multiple comparison test, using software package JMP (SAS Institute, Cary, North Carolina, USA). The data are presented as the mean ± SD. A p value less than 0.05 was considered statistically significant. The correlation of platelet counts and TPO levels in the three groups were analyzed by linear regression using JMP.

Results

Plasma TPO levels in SARS patients

The seven patients with active SARS were symptomatic patients admitted to the Prince of Wales Hospital in Hong Kong for acute management during the SARS outbreak from March to May in 2003. The diagnosis was serologically confirmed and fulfilled the WHO case definition of SARS. Their plasma were collected for laboratory investigation and stored in −80 °C for research use [5,11]. Convalescent plasma was obtained from 10 patients in the same hospital who had recovered from the SARS virus infection. Specifically, "recovery" was defined as having an afebrile status for at least seven days, showing radiographic improvement of 25%, having no further need of an oxygen supplement and being at least fourteen days following the onset of the symptoms [12]. In these groups of patients, the changes of platelet count have been observed in 55% of the active SARS patients with thrombocytopenia (platelet count of $<140\,000/mm^3$), and in 49% of the recovered SARS patients with thrombocytosis (platelet count of $\geq400\,000/mm^3$) have been documented [5].