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Decreased myeloid dendritic cells indicate a poor prognosis in patients with severe fever with thrombocytopenia syndrome

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SUMMARY

Objectives: Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerging infectious disease caused by a novel bunyavirus in which host immune system suppression is thought to be crucial in the development of disease. This study was designed to study the frequencies and activation status of dendritic cells (DCs) at different stages of SFTS and their association with disease severity.
Methods: All confirmed SFTS patients ($N = 115$) were recruited from the Wuhan Union Hospital in 2015; routine laboratory parameters were collected. The frequencies, phenotypes, and subsets of circulating DCs, including myeloid and plasmacytoid dendritic cells (mDCs and pDCs), were analyzed by flow cytometry. Serum levels of interleukin (IL)-6, IL-10, and tumor necrosis factor alpha (TNF-α) were detected by ELISA. The laboratory parameters and other clinical events related to mortality were assessed by multivariate logistic regression analysis and receiver operating characteristic (ROC) curves.
Results: The frequency of circulating mDCs, especially from day 9 after disease onset, could serve as a valuable prognostic biomarker for the outcome in SFTS patients (area under the curve = 0.929, $p < 0.0001$). In addition, persistent down-regulation of the co-stimulatory molecules CD80/CD86 on the mDCs was observed during the disease process. Moreover, levels of mDCs were inversely correlated with the production of IL-6, IL-10, and TNF-α and with viral load at admission.
Conclusions: The present results indicate that DCs might be functionally impaired in SFTS. A decreased level of circulating mDCs was closely correlated with the severity of SFTS.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging disease characterized by abrupt high fever, respiratory or gastrointestinal symptoms, leukocytopenia and thrombocytopenia, bleeding, and neurological symptoms, which may lead to multi-organ failure at the late stage of disease, or even death within 7–14 days after the onset of the disease.[1] SFTS virus (SFTSV) has been identified as the pathogen of this disease. Since 2009, SFTS has been reported in China, South Korea, and Japan, with a reported mortality rate varying between 2.5% and 30%.[2–5] It has become a substantial risk to public health worldwide. In addition, humans may also be infected through direct contact with an infected patient's blood,[6–8] and no specific treatment for SFTS is available. Therefore, an understanding of the pathogenesis of SFTSV infection is important.

Both viral and host factors are crucial for the pathogenesis of SFTSV. Previous studies have shown that old age, high viral RNA levels in the blood at admission, and a cytokine storm (with abnormal expression of the cytokines interleukin 1 receptor antagonist (IL-1RA), interleukin (IL)-6, IL-10, tumor necrosis factor alpha (TNF-α), granulocyte-colony stimulating factor (G-CSF), and interferon gamma-inducible protein 10 (IP-10)), play an important role in determining the severity and clinical outcome of this disease.[9,10] As reported previously, various immune cell populations, including T lymphocytes and natural killer cells, have been found to be abnormal in SFTSV-infected patients, especially during the acute phase and in severe cases.[11] Furthermore, elderly people are more susceptible to severe SFTSV infection, indicating that the

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impairment of host immunity is a contributing factor to the development and progression of the disease.[12] However, our understanding of the role of immune cells in SFTS patients is very limited.

Dendritic cells (DCs), in addition to their ability to control infections directly via innate immune functions, are key antigen-presenting cells that can orchestrate an effective immune response and are therefore responsible for the induction of adaptive immunity. DCs are thus recognized to be involved in various inflammatory diseases, including infections caused by Ebola virus (EBOV), influenza virus, and hepatitis C virus (HCV),[13–15] and are suggested to play an important role in disease processes. In addition, a recent study reported that human monocyte-derived DCs infected by dengue virus (DENV) showed a distinct activated phenotype, with increased production of proinflammatory cytokines and chemokines, except for type I interferons.[16] Furthermore, the infection of DCs by DENV may induce DC apoptosis and impair their ability to present antigens to T-cells, which contributes to the pathogenesis of dengue.[17] These studies suggest that functionally impaired DCs may mediate the suppression of host-specific T-cell immune responses and thus facilitate viral persistence and disease progression. However, the role of DCs in SFTSV infection has not yet been elucidated.

In this study, the frequencies and phenotypes of circulating DCs, including myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs), were examined dynamically to shed some light on the development of the disease and to facilitate a better understanding of the mechanisms underlying the progression of SFTS.

2. Materials and methods

2.1. Dynamic collection of clinical samples

The study protocol was approved by the Ethics Committee of Tongji Medical College of HuaZhong University of Science and Technology. Written informed consent was obtained from all patients prior to blood collection. The diagnosis was made according to the clinical guidelines on SFTS released by the Ministry of Health of the People's Republic of China in 2010. During the period April to November 2015, a series of blood samples was collected from SFTS patients every other day starting at the time of admission. In addition, 25 healthy volunteers were recruited for the study. Patient details, including their clinical history, physical examination findings, and routine hematology laboratory results (white blood cell (WBC) counts, platelet (PLT) counts, elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK)) were collected from the medical records to conduct a retrospective analysis. The basic characteristics and serum viral loads of these subjects at admission are listed in Table 1.

2.2. Isolation of peripheral blood mononuclear cells

Ethylenediaminetetraacetic acid (EDTA) anti-coagulated peripheral blood samples were collected from healthy subjects and SFTS patients. All samples were processed within the first 4 h after collection. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation with Ficoll–Paque Plus (DAKEW Biotech, China).

2.3. ELISA

The concentrations of TNF-α, IL-6, and IL-10 in the serum of SFTS patients were measured by ELISA using commercial kits (DAKEW Biotech, China) in accordance with the manufacturer's instructions. The detection limit for each cytokine was as follows: TNF-α, 8–1000 pg/ml; IL-10, 7.813–500 pg/ml; IL-6, 6.25–200 pg/ml.

2.4. SFTS viral load assay

The total RNA of every clinical patient's serum specimen was extracted using a viral RNA kit (DAAN Gene, Guangzhou, China) as per the manufacturer's instructions. The viral load of SFTSV RNA copies in the serum samples of SFTS patients was detected using a certified real-time PCR kit (SFDA Registration No. 340166, China) based on the detection of the SFTS viral S genomic segment with specific primers and probes. The detection sensitivity and specificity were demonstrated to be 98.6% and 99.1%, respectively.

2.5. Flow cytometry analysis

At least 200 000 events per tube were acquired by flow cytometry to determine the frequencies of mDCs and pDCs, as well as the expression of CD80/CD86. All antibodies were purchased from Biolegend (USA). CD80/CD86 levels were reported as the

Table 1
Differences in clinical and laboratory characteristics on admission between patients with severe fever with thrombocytopenia syndrome (SFTS) who survived and those who died[a]

Characteristic	Healthy controls ($n=25$)	All patients ($n=115$)	p-Value	All patients		p-Value
				Survived ($n=94$)	Died ($n=21$)	
Age, years	58 (28–70)	60 (28–91)	0.4821[b]	60 (28–83)	63.5 (48–91)	0.051[b]
Male, sex, n (%)	13 (53.3%)	45 (39.1%)	0.161[c]	34 (36.2%)	11 (52.4%)	0.169[c]
Days of hospitalization	N/A	10 (2–35)	N/A	11 (3–35)	4 (2–14)	<0.0001[b]
Plasma RNA, $\log_{10}$	N/A	4.37 (2.08–7.94)	N/A	4.10 (2.08–6.7)	5.81 (4.28–7.94)	<0.0001[d]
WBC, 10^9/l	5.45 (3.92–9.45)	2.84 (0.27–12.13)	<0.0001[b]	2.83 (0.27–9.75)	2.68 (1.11–12.13)	0.089[b]
PLT, 10^9/l	174.5 (126–319)	41 (11–191)	<0.0001[b]	45 (11–191)	35 (11–65)	0.0196[b]
ALT, U/l	13.5 (5–39)	76 (17–445)	<0.0001[d]	69 (17–384)	115.5 (35–445)	0.0003[d]
AST, U/l	20.5 (12–38)	188 (12–2672)	<0.0001[d]	150 (12–1318)	490 (74–2672)	<0.0001[d]
GGT, U/l	22.5 (9–47)	53 (14–2012)	0.0416[d]	47 (14–502)	99 (14–2012)	0.001[d]
ALB, g/l	39.8 (35.9–49.2)	30.95 (19.7–46.6)	<0.0001[d]	32.1 (19.7–46.6)	28.05 (22–37)	0.0019[d]
CK, U/l	62 (13–119)	516 (31–13 113)	<0.0001[d]	432.5 (31–6694)	1268 (250–13 113)	<0.0001[d]
LDH, U/l	191 (18.2–223)	709 (137–11 108)	<0.0001[d]	675 (138–2977)	1605 (392–11 108)	<0.0001[d]

N/A, not applicable; WBC, white blood cell count; PLT, platelet count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; ALB, albumin; CK, creatine kinase; LDH, lactate dehydrogenase.

[a] Data are the median (range) unless specified otherwise.

[b] By means of the t-test.

[c] By means of the Pearson Chi-square test.

[d] By means of the Mann–Whitney U-test.