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Gene expression profiling reveals the defining features of monocytes from septic patients with compensatory anti-inflammatory response syndrome

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Summary *Objectives:* To characterize the expression profiles of genes in purified monocytes from septic patients during systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS), and then to investigate the potential mechanism of monocyte deactivation.
Methods: Lipopolysaccharides (LPS)-induced cytokine responses, phagocytosis assay and migration assay were performed in monocytes from SIRS patients, CARS patients and healthy volunteers ($n = 8$). After functional assays, each pair of samples from the same group was pooled into one for gene expression analysis. All new samples ($n = 4$) were hybridized on NimbleGen human gene expression 12 × 135 K microarrays, and selected genes were validated by real-time polymerase chain reaction. Pathway analysis and Gene Ontology analysis were performed on differentially expressed genes using Agilent GeneSpring (version 11.0).
Results: A set of genes related to pro-inflammation, phagocytosis, chemotaxis, antigen presentation, and anti-apoptosis were significantly down-regulated, while some genes associated with pro-apoptosis and anti-inflammation were up-regulated instead on monocytes from CARS patients compared with SIRS patients and healthy volunteers. Monocytes from CARS patients showed impaired production of TNF-α and IL-6, and increased release of IL-10 when stimulated by LPS. Functional analysis confirmed reduced phagocytosis and migratory activity of

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monocytes from CARS patients. Human leukocyte antigen-DR (HLA-DR) measurements demonstrated decreased expression of HLA-DR on monocytes from CARS patients.
Conclusion: Monocytes from CARS patients exhibited significant changes in mRNA expression of genes associated with phagocytosis, antigen presentation, inflammatory response, cell migration, and apoptosis, which might cause deactivation of monocytes during CARS.

Introduction

Sepsis is defined as a systemic inflammatory response to infection and it is the leading cause of death in the critically ill population with 750,000 cases per year resulting in 210,000 deaths annually in the United States.[1,2] Although overall survival rates improved between 1979 and 2000, the total sepsis-related mortality rose from 22 to 44 per 100,000 population, accounting for ~9% of the overall annual mortality in the United States alone.[2] Early studies suggested that the mortality and organ injury associated with severe sepsis were primarily due to an exaggerated innate immune and inflammatory response,[3] but anti-inflammatory therapies have for the most part not resulted in significant improvements in clinical outcome.[4] Given the substantial mortality and economic costs, a better understanding of the basic immune alterations in sepsis may help to direct therapy.

Due to improvements in resuscitation and supportive care, most patients survive early sepsis. After the initial hyper-inflammatory phase (systemic inflammatory response syndrome, SIRS), septic patients usually display features of immunosuppression (compensatory anti-inflammatory response syndrome, CARS), which is believed to contribute to the susceptibility of septic patients to nosocomial infections. This condition includes enhanced leukocyte apoptosis,[5] defective lymphocyte proliferation in response to recall antigens or mitogens,[6] marked elevation of the percentage of circulating regulatory $CD4^+CD25^+$ T cells,[7] and deactivation of monocyte functions. Monocytes are one of the main effectors of innate immunity against infection. They have the capacity to phagocytize microorganisms, to sense microbial products, and, in response, to release a large number of inflammatory mediators that contribute to defense against infection. As antigen presenting cells they represent a link with adaptive immunity through their capacity to induce specific T cell activation. However, after the onset of septic shock, monocytes have been shown to rapidly exhibit impaired production of proinflammatory cytokines in response to additional bacterial challenge (also known as endotoxin tolerance), and a reduced antigen presentation capacity likely due to their decreased expression of human leukocyte antigen-DR (HLA-DR).[8] Most importantly, it has been observed that these changes develop to a larger extent in non-surviving patients in comparison with survivors. Thus, a better understanding of the underlying molecular mechanisms responsible for monocyte deactivation may be an important step in identifying new strategies for restoring immune functions in this deadly clinical situation. To date, little work has been devoted to monocyte mRNA expression profiles in CARS patients with monocyte deactivation. Therefore, we sought to characterize the expression profiles of genes in purified monocytes from septic patients during SIRS and CARS using NimbleGen human gene expression microarrays which represent about 44,000 genes, and then investigate the potential mechanism of monocyte dysfunction using sophisticated statistical analysis and independent functional assays.

Materials and methods

Study populations

The present study protocol was approved by Changhai Hospital Ethics Committee and written informed consent was obtained from participating patients (or their relatives) and healthy volunteers on enrollment. Over an 8-month period, 11 consecutive patients with early sepsis were enrolled within 24 h of the onset of sepsis in the Intensive Care Unit (ICU) of Changhai Hospital (Second Military Medical University, Shanghai, P. R. China). Another 12 consecutive patients with septic shock were enrolled on day 3 after the onset of septic shock since immunoparalysis is believed to be established after the first 24 h of the syndrome.[9,10] Patients with trauma, human immunodeficiency virus-related disease, neutropenia or end stage hepatic disease, diabetes mellitus, subjects receiving immunosuppressive agents or glucocorticoid, subjects receiving neoadjuvant radiochemotherapy, and those with autoimmune disease were excluded. Patients with early sepsis and monocyte HLA-DR (mHLA-DR) expression of 60% or more were classified in the SIRS group. Patients with septic shock and mHLA-DR expression of 30% or less were classified in the CARS group. The clinical diagnosis of sepsis and septic shock was based on the diagnostic criteria of the American College of Chest Physicians/Society of Critical Care Medicine.[11] Patients were treated according to the standardized recommendations of our ICU. Evidence of illness severity was assessed using acute physiology and chronic health evaluation II (APACHE II). Eight healthy individuals similar in age and sex were also enrolled as controls. Mortality was defined as death occurring within 28 days after the onset of shock. Blood samples (20 mL) were obtained on the day of enrollment.

Monocytes detachment

After blood sampling, tubes were transported at 4 °C to the laboratory within 1 h for the monocytes detachment. First, PBMCs were separated from whole blood samples using standard gradient centrifugation with Lymphocyte Separation Medium (PAA Laboratories GmbH, Pasching, Austria). Second, monocytes were purified for functional analysis and microarray analysis using immunomagnetic beads coated with anti-CD14 monoclonal antibody (Miltenyi Biotec Bergisch Gladbach, Germany) according to the manufacturer's instructions and previously described by Saikh