Journal of Critical Care (2013) **28**, 881.e7–881.e13

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Journal of Critical Care

Dendritic cells in childhood sepsis☆

Khalid I. Elsayh[a], **Asmaa M. Zahran**[b,*], **Ismail Lotfy Mohamad**[a], **Sanaa Shaker Aly**[c]

[a]*Pediatric Department, Faculty of Medicine, Assiut University, Assiut, Egypt*
[b]*Oncological Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Assiut, Egypt*
[c]*Clinical Pathology Department, South Valley University, Qena, Egypt*

Keywords:
Dendritic cells;
Flow cytometry;
Sepsis;
CD86;
CD83

Abstract
Purpose: Our aim was to investigate the level and the maturation status of dendritic cells (DCs) in pediatric patients with sepsis and its relation to prognosis.
Materials and methods: The study included 16 children with sepsis, 24 children with complicated sepsis, and 40 healthy control children. The patients were investigated within 24 hours of intensive care unit admission and after 28 days. Flow cytometric detection of DCs was done.
Results: Within 24 hours, the levels of both plasmoid DCs and monocytoid DCs and the expression of CD86 and CD83 on DCs were significantly lower in patients than in controls, and the difference was marked in patients with complicated sepsis. The amount of CD86 and CD83 per cell was significantly lower in patients with complicated sepsis. The baseline numbers of monocytoid DCs and plasmoid DCs were higher in the survival patients than in nonsurvival patients. In addition, the expression of CD86 and CD83 on the entire DCs was significantly higher in the survival patients with sepsis.
Conclusion: Sepsis is associated with reduced level of DCs and decreases their maturation. The estimation of DCs number and maturation state may be used as prognostic makers of sepsis.

1. Introduction

Sepsis refers to the disseminated inflammatory response elicited by microbial infections. It remains a current challenge. It is increasing in frequency, expensive to treat, and lethal, with an associated rate of death as high as 70%. There is growing evidence that sepsis develops when the appropriate initial host response to systemic infection becomes amplified and then unregulated, finally leading to the paralysis of the immune system [1]. Host defense against pathogenic microorganisms requires the coordinated actions of the innate and acquired immune system. However, dysregulation of the immune system occurs during severe sepsis, leading to either a rapid death caused by the development of multiorgan failure or an increase in complications caused by long-term immunosuppression [2-7]. Several defects in both innate and acquired immunity had been described in sepsis [6]. Apoptosis of B and T lymphocytes and reduced numbers of DCs were observed in patients with sepsis [8,9].

Dendritic cells (DCs) are the most potent antigen-presenting cells and play a key role in linking innate and adaptive host immune responses to microorganisms and the

☆ There is no conflict of interest.

* Corresponding author. Oncological Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt. Tel.: +20 0201003884329; fax: +20 0882368371.
E-mail addresses: elsayh23@yahoo.com (K.I. Elsayh), asmaa.zahran@yahoo.com (A.M. Zahran).

0883-9441/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcrc.2013.05.007

initiation of specific immune responses [10]. Dendritic cells serve 2 general functions in controlling T-cell immunity. The first is to process and present antigens to T cells, which is essential for T-cell activation and expansion. Second, DCs secrete cytokines that condition the extracellular milieu and determine the nature of the T-cell response [11]. Infectious microbes contact with the immature DCs and promote maturation, which is characterized by an increased capacity to present antigens and stimulate T cells and secretion of proinflammatory cytokines, which promote differentiation of type 1 T cells that efficiently clear the infectious agent [12]. Distinct subsets of circulating DCs can be identified in peripheral blood (PB), including myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) [13]. Although arising from common precursor cells in the bone marrow, mDCs and pDCs are phenotypically and functionally different [14]. Myeloid DCs secrete interleukin-12, which directly induces differentiation of type 1 T cells [12]. Plasmacytoid DCs secrete interferon-α, which is well known for its potent antiviral properties [15]. As a result of the pivotal role of DCs in immune activation, the modification of DC system during sepsis is becoming an area of active investigation [16].

The aim of this study was to investigate the level and the maturation status of DCs in pediatric patients with sepsis and its relation to prognosis, through flowcytometric analysis of PB DCs and their expression of CD83 and CD86.

2. Patients and method

The present study is a case-control prospective study conducted in the pediatric intensive care unit (ICU) of Children Hospital, Assiut University, during the period between June 2012 and December 2012. Forty children were included in this study and classified according to their diagnoses as follows: sepsis, which included 16 children (group 1), and complicated sepsis, which included 24 children (group 2). Forty healthy children with matchable age and sex were included in the study as a control group (group 3). An informed written consent was obtained from their guardians, and the study was approved by the Faculty of Medicine Ethic Committee for the Scientific Research Conduct. The patients were investigated within 24 hours of ICU admission (on admission) and after 28 days from admission and treatment.

Sepsis was characterized by confirmed infection (positive culture) or highly suspected infection (evidence of infectious focus) combined with 2 or more of the conditions considered for systemic inflammatory response syndrome, which are as follows: (*a*) (axillary) temperature higher than 37.5°C or lower than 36°C; (*b*) heart rate more than 160 beats/min in infants and more than 150 beats/min in children, or >2 SD above the reference values for age; (*c*) respiratory frequency greater than 60 movements/min in infant and greater than 50 movements/min in children, or >2 SD above the reference values for age; and (d) total leukocyte count more than 12 000 cells/mm^3, less than 4000 cells/mm^3, or more than 10% band forms [17]. Complicated sepsis included patients with severe sepsis and septic shock and was characterized as sepsis associated with organ dysfunction, hypoperfusion, or hypotension (systolic blood pressure <10th percentile for age), even after appropriate volume resuscitation, plus the presence of systemic perfusion disorders [18]. Sepsis-related Organ Failure (SOFA) score was calculated as an evaluation of the degree of severity of organ dysfunction/failure in 6 systems including respiratory, coagulation, liver, cardiovascular, central nervous, and renal systems. This evaluation represented by points ranged from 0 to 4 [19]. The Surviving Sepsis Protocol (international guidelines for management of severe sepsis and septic shock) was used in the treatment for patients [20]. The *nonsurvival* of patients was defined as death within 28 days after onset of sepsis. Seventeen patients died, 3 with sepsis and 14 with complicated sepsis. This study included children aged 1 month to 16 years presented by symptoms suggestive of sepsis, severe sepsis, or septic shock. Cases with systemic inflammatory response syndrome alone without criteria suggesting sepsis or complicated sepsis were excluded from this study.

The patients and controls were subjected to the following laboratory investigations: complete blood count (Celltac E automated hematology analyzer, Tokyo, Japan), blood culture, serum electrolytes (by AVL 9180 analyzer; Roche Diagnostics GmbH, Mannheim, Germany), blood urea and serum creatinine (Cobas Integra 400 Chemistry Analyzer; Roche Diagnostics GmbH), C-reactive protein (Atlas C-reactive Protein latex Reagent Kit; Atlas Medical, Cambridge, UK), and flow cytometric detection of DCs.

2.1. Flow cytometric detection of DCs numbers and phenotype

Dendritic cells in whole PB samples were enumerated using fluoroisothiocyanate (FITC)-conjugated monoclonal antibodies against lineage markers (includeing anti-CD3, CD14, and CD19), phycoerythrin (PE)-conjugated anti-CD123, PE-conjugated anti-CD11c, and peridinium-chlorophyll-protein (Per-CP)–conjugated anti–HLA-DR. All monoclonal antibodies were purchased from Becton Dickinson Biosciences, San Jose, Calif.

To determine DCs numbers, 100 μL of blood sample was stained with 10 μL of FITC-conjugated lineage-specific markers (CD3, CD14, and CD19), 10 μL of Per-CP–conjugated anti–HLA-DR, and 10 μL of PE-conjugated DCs markers (anti-CD11c or anti-CD123). To detect the expression of CD86 and CD83 on DCs, 100 μL of blood sample was stained with 10 μL of PE-conjugated lineage-specific markers (CD3, CD14, and CD19), 10 μL of Per-CP–conjugated anti–HLA-DR, and 10 μL FITC-conjugated CD86 or CD83. The tubes were incubated for 15 minutes at room temperature in the dark. Red blood cell lysis was done.