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Immunoparalysis: Clinical and immunological associations in SIRS and severe sepsis patients

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ABSTRACT

Introduction: This study was designed to identify changes in the monocytic membrane marker HLA-DR and heat shock proteins (HSPs) in relation to T-regulatory cells (T-regs) and other immunological marker changes in patients with systemic inflammatory response syndrome (SIRS) or sepsis/septic shock.
Methods: Healthy volunteers, intensive care unit (ICU) patients with SIRS due to head injury and ICU patients with severe sepsis/septic shock were enrolled in the current study. Determination of CD14+/HLA-DR+ cells, intracellular heat-shock proteins and other immunological parameters were performed by flow cytometry and RT-PCR techniques as appropriate. Univariate and multivariate analysis examined associations of CD14/HLA-DR, HSPs, T-regs and suppressor of cytokine signalling (SOCS) proteins with SIRS, sepsis and outcome.
Results: Fifty patients (37 with severe sepsis and 13 with SIRS) were enrolled, together with 20 healthy volunteers used as a control group. Compared to healthy individuals, patients with SIRS and severe sepsis showed progressive decline of their CD14/HLA-DR expression (0% to 7.7% to 50% within each study subpopulation, $p < 0.001$). Mean fluorescent intensity (MFI) levels of HSP70 and HSP90 on monocytes and polymorphonuclear cells were significantly higher in SIRS patients compared to controls and fell significantly in severe sepsis/septic shock patients ($p < 0.05$ for all comparisons). There was no statistically significant difference between subgroups for levels of T-regulatory cells or relative copies of Suppressor of Cytokine Signalling 3 (SOCS3) proteins. In univariate models percent of CD14/HLA-DR was associated with mortality (OR: 1.8 95%CI 1.02–3.2, $p = 0.05$), while in multivariate models after adjusting for CD14/HLA-DR only younger age and lower Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were associated with increased chances of survival (beta −0.05, OR 0.9, 95% CI 0.9–0.99, $p = 0.038$ for age and beta −0.11, OR 0.89, 95% CI 0.8–0.99, $p = 0.037$ for APACHE II score).
Conclusions: Significant associations with SIRS and sepsis were found for CD14/HLA-DR expression and monocyte and polymorphonuclear cell levels of HSP70 and 90. The role of these biomarkers in assessing the prognosis of sepsis needs to be further explored and validated in prospective studies.

1. Introduction

Treatment of sepsis is currently mainly based on prompt optimisation of physiological parameters and antibiotic use since the early goal-directed therapy has been shown to increase survival considerably [1]. In contrast, despite significant efforts our understanding of the pathophysiology of sepsis is still evolving. Moreover, no significant improvement in reducing death rates has

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been achieved over the last decade. It is clear that further elucidation of the biology of sepsis will lead future diagnostic strategies and guide therapeutic approaches.

A profound activation of the innate, non-specific immunity characterizes the onset of septic shock. An overwhelming release of pro-inflammatory mediators accompanies this early response, followed immediately by an immunosuppression state that further contributes to the adverse outcomes associated with sepsis [2]. The exact starting point, the intensity and extent of the hyper-inflammatory state versus a subsequent anti-inflammatory state are difficult to identify, above all in the clinical setting [3]. The discovery of novel biomarkers that could assist in the differentiation of these stages of sepsis as well as contribute to better understanding of the course of the sepsis syndrome will assist in guiding interventions and in the prediction of the overall outcome. Different molecules have been studied over the years to this effect.

The expression of the monocytic membrane marker HLA-DR has been shown to be negatively influenced by the presence of systemic inflammatory response syndrome (SIRS) and septic shock and has been suggested to correlate with sepsis mortality in the second (immunosuppressive) stage of sepsis [4,5].

Heat shock proteins (HSP) are cellular proteins that are highly upregulated to protect cells against elevated temperatures [6]. The heat shock proteins 70 and 90 (HSP70, HSP90) are ubiquitous chaperones with numerous roles, including cell protection against stress, anti-apoptotic effects independent of the Fas-ligand mediated pathway and other not fully understood, immunomodulatory functions [6–8]. HSP90 and HSP70 have been identified as functional receptors for the bacterial lipopolysacharide (LPS) on the surface of human monocytes/macrophages acting in a CD14-independent fashion [9]. They can both serve as novel biomarkers for the characterization of the different stages of sepsis [10–12].

The regulatory T cells, a specific CD4+, CD25+ T cell minor subpopulation, with important immunosuppressive regulatory effects, are also gaining increasing interest regarding their role during the second phase of sepsis [13–15].

Dysregulation of cytokine signalling may have a significant role in the immune modulation observed in all the phases of sepsis [16,17]. The suppressors of cytokine signalling (SOCS) proteins have a key negative regulatory role in the Janus kinase/signal transducer and activator of transcription pathway and are important regulators of adaptive immunity [18–21]. The downregulation of proinflammatory cytokines by SOCS proteins may be desirable to boost the immune responses [22,23].

In the current study, we attempted to identify the relation between the state of immunoparalysis and the expression of the above named biological markers, namely the HLA-DR surface marker, the HSP70, HSP90 and SOCS3 intracellular proteins, and the population of the T-reg cells. For this purpose, these immunological markers were studied both in healthy volunteers and patients with SIRS or severe sepsis/septic shock. The aim was to further identify and understand patterns of immune response in patients with SIRS and severe sepsis/septic shock.

2. Methods

2.1. Clinical criteria

Intensive care unit (ICU) hospitalized patients fulfilling the criteria of severe sepsis/septic shock and ICU patients with SIRS due to head injury were enrolled in the current study. Inclusion criteria for the participating patients were: (a) age $\geqslant$18 years; (b) presence of early severe sepsis/septic shock (48 h or less after the first symptoms) and (c) presence of SIRS in patients with head trauma. Severe sepsis/septic shock or SIRS were defined according to guidelines of the surviving sepsis campaign [24]. More specifically, presence of two or more of the following criteria at day 0, defined SIRS: (1) pyrexia >38 °C or temperature below 36 °C; (2) leukocytosis (>10,000/μL) or leukopenia (<4000/μL), or >10% bands; (3) tachycardia (>90 beats/min); and (4) increased respiratory rate (>20 breaths/min) or mechanical ventilation. Presence of an infection (either probable or proven) together with SIRS defined sepsis. Sepsis-induced hypoperfusion or organ dysfunction thought to be due to the infection defined severe sepsis. Shock was defined as presence of hemodynamic instability for at least 1 h despite adequate fluid resuscitation or requirement of vasopressors [24]. Shock was defined as septic shock if sepsis was present. Exclusion criteria were: (a) presence of malignancies; (b) presence of autoimmune diseases; (c) prior use of corticosteroids; (d) immunosuppressive illness; (e) late sepsis or SIRS. In addition blood was sampled from age- and gender-matched healthy volunteers. Twelve milliliters of blood were collected from every patient and control in a heparin tube (Becton Dickinson, Cockeysville, MD, USA) under sterile conditions at the time of study inclusion. Complete blood count, serum creatinine levels and maximum glucose levels at the day of observation were recorded. All blood samples were collected within 24 h of the development of sepsis. The reasons for this were: (a) to have comparable data reflecting early response to sepsis, since comparison of sepsis to SIRS/trauma patients is also more reasonable in an early time-window; (b) to have fewer external factors affecting patients' condition e.g. hospital-related events or complications. The classification systems for severity and prognosis in intensive care patients APACHE II and SOFA (Sequential Organ Failure Assessment) scores were calculated for each participating patient. For head injury/trauma patients we calculated the injury severity score (ISS). The study protocol was approved by the Ethics Committee of the ATTIKON University hospital (822/13-12-2011). Written inform consent was signed by the patient or their first-degree relative of each patient and by each healthy volunteer.

2.2. Determination of intracellular heat-shock proteins

Whole blood (100 μl) was incubated in the dark with monoclonal antibodies anti-CD33 (5 μl) at the flurochrome phycoerythrin-Cy5 (PeCy5, emission 667 nm, Biolegned, SanDiego, CA, USA) and anti-CD45(5 μl) at the fluorochrome phycoerythrin-Cy7 (PeCy7, emission 767 nm, Biolegend, London, UK). White blood cells were then fixed (Fixation Buffer, Biolegend) and permeabilized (Permeabilization Wash Buffer, Biolegend) and incubated with monoclonal antibodies anti-Hsp70/Hsp72 at the fluorochrome fluorescein isothiocyanate (FITC, emission 525 nm, Enzo Life sciences, NY, USA) and anti-Hsp90a at the fluorochrome phycoerythrin (PE, emission 575 nm, Enzo) for intracellular staining at a 1:10 dilution according to manufacturer's instructions. Afterwards blood was washed and red blood cells were lysed with NH_4Cl solution (Lysing solution, Beckman Coulter Co, Miami, FL, USA). White blood cells were washed, reconstituted in PBS and analyzed after running through the CYTOMICS FC500 flow cytometer (Beckman Coulter Co); 100,000 events were analyzed. Separate gating was used to determine the mean fluorescence intensity (MFI) of HSP70 and HSP90 on monocytes and polymorphonuclear cells according to their characteristic CD33/SS and CD45/SS scattering. Monocytes were defined as the cell population with lower granulation and high expression of CD33 and polymorphonuclear cells as the population with higher granulation and dim expression of CD33. CD45 was used as an internal control to verify the separation of the two populations. IgG isotype controls were used for each patient.