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Genetic polymorphisms of innate and adaptive immunity as predictors of outcome in critically ill patients

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ABSTRACT

Sepsis and septic shock frequently cause the admission or complicate the clinical course of critically ill patients admitted in the intensive care units (ICU). Genetic variations disrupting the immune sensing of infectious organisms, could affect the ability of the immune system to respond to infection, and may influence both the genetic predisposition to infection and the diversity of the clinical presentation of sepsis. The aim of this study was to uncover possible associations between common functional immune gene polymorphisms (of both innate and adaptive immunity) and ICU-acquired sepsis and mortality. The *TLR4*-D299G (rs4986790), *TLR4*-T399I (rs4986791), *C2*-c.841_849+19del28 (rs9332736), *TACI*-C104R (rs34557412), *BAFFR*-P21R (rs77874543), and *BAFFR*-H159Y (rs61756766) polymorphisms were detected in a cohort of 215 critically ill patients, admitted in an 8-bed medical/surgical ICU. Interestingly, *TLR4*-D299G, *TLR4*-T399I and *BAFFR*-P21R carriage was associated with a lower risk of ICU-acquired sepsis. This association applied particularly in medical patients, while in trauma and surgical patients no significant associations were observed. Moreover, carriers of *TACI*-C104R displayed an undiagnosed mild to moderate hypogammaglobulinemia along with a significantly lower survival rate in the ICU, although lethal events were not attributed to sepsis. These findings further elucidate the role that host immune genetic variations may play in the susceptibility to ICU-acquired sepsis and ICU mortality.

Introduction

Severe sepsis and septic shock frequently cause the admission or complicate the clinical course of critically ill patients admitted in ICU, and they are the leading causes of death of such patients worldwide (Carson and Kunkel 2012; Martin et al. 2003). There is increasing evidence that the immune system is crucially implicated in the pathophysiology of sepsis, and represents the root cause during its progression to severe sepsis (sepsis with dysfunction of one organ), then to multiple organ dysfunction syndrome (MODS), and eventually to septic shock and death (Carson and Kunkel 2012; Opal and Esmon 2003). Sepsis is rather a complex syndrome that develops when the initial, appropriate host response to an infection becomes amplified (cytokine storm), and is then dysregulated resulting in immunosuppression (immune paralysis) (Boomer et al. 2005; Villar et al. 2004). However, genome-wide transcription profiling in human sepsis has revealed an interaction of pro- and anti-inflammatory mechanisms, occurring multiple times during a single septic event (Tang et al. 2010). This complex interaction, involving both innate and adaptive immune responses, results either in resolution of the septic event or in multiple organ dysfunction and death (Abraham 2005; Boomer et al. 2005).

It is worth of note that septic syndrome is characterized by a great interindividual variation. Considering also that death from infection is strongly heritable in human populations, it is now widely accepted that genetic variations disrupting the immune sensing of infectious organisms, could affect the ability of the immune system to respond to infection, and consequently may

Abbreviations: AIS, abbreviated injury scale; APACHE, acute physiology and chronic health evaluation; APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; BAFFR, BAFF receptor; BCMA, B cell maturation antigen; COPD, chronic obstructive pulmonary disease; CRRT, continuous renal replacement therapy; CVID, common variable immunodeficiency; ICU, intensive care unit; ISS, injury severity score; LOS, length of stay; LPS, lipopolysaccharide; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SOFA, sequential organ failure assessment; TACI, transmembrane activator and calcium modulator and cyclophilin ligand interactor; TNF, tumor necrosis factor; TLR4, toll-like receptor 4; wt, wild-type.

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influence both the genetic predisposition to infection and the diversity of the clinical presentation of sepsis. Such genetic variations may identify the patients who are at low or high risk for sepsis development and organ dysfunction during severe infections (Lin and Albertson 2004; Stüber 2001; Villar et al. 2004).

Single base variations known as SNPs, are the most commonly identified genetic variants. These alterations may alter protein structure or expression in a way that profoundly affects physiology. The rationale for studying such immune gene SNPs in critical illnesses seeks to identify potential markers of susceptibility, severity, and clinical outcome, as well as targets for therapeutic intervention. During the last decades, the analysis of such SNPs and their association with the phenotype has provided new insights in the concept of sepsis (Lin and Albertson 2004; Stüber 2001; Tabrizi et al. 2001).

The aim of this study was to explore potential associations of common innate and adaptive immune gene SNPs with ICU-acquired sepsis and ICU mortality. For this purpose we analyzed three genetic variants located into genes of innate immunity: two functional SNPs of TLR4 (dbSNP accession no. rs4986790, *TLR4*-D299G; rs4986791 *TLR4*-T399I), a member of the TLR family that recognizes endotoxin/lipopolysaccharide (LPS) of Gram-negative bacteria and intrinsic mediators, such as heat-shock proteins (Gao et al. 2008), and one mutation causing complement-C2 deficiency (rs9332736, *C2*-c.841_849+19del28), namely the most common complement deficiency in Western countries (Figueroa and Densen 1991; Johnson et al. 1992).

Moreover, we investigated the role of three SNPs of adaptive immunity that recently have been implicated in the pathogenesis of CVID and lymphomagenesis, namely rs77874543 (*TNFRSF13C/BAFFR*-P21R), rs61756766 (*TNFRSF13C/BAFFR*-H159Y) and rs34557412 (*TNFRSF13B/TACI*-C104R) (Hildebrand et al. 2010; Pieper et al. 2014; Salzer et al. 2009; Speletas et al. 2011). BAFFR (encoded by *TNFRSF13C*) and TACI (encoded by *TNFRSF13B*), along with BCMA (encoded by *TNFRSF17*), are B cell receptors of the TNF-superfamily ligands BAFF and APRIL, which play an important role in B cell homeostasis and differentiation (Mackay et al. 2003; Pieper et al. 2013). Interestingly, increased levels of BAFF and APRIL have recently been found in patients with a high inflammatory burden, such as critically ill patients with sepsis (Roderburg et al. 2013), or patients with acute pancreatitis (Pongrantz et al. 2013). On the other hand, decreased TACI expression has been implicated in susceptibility of mice newborns to infections with encapsulated bacteria and in impaired immunogenicity of polysaccharide vaccines (Kanswal et al. 2008). To the best of our knowledge, this is the first study analyzing the possible associations of the above-mentioned adaptive immunity SNPs with sepsis and ICU mortality.

Methods

Patients

Two hundred and fifteen patients (male/female: 148/67, mean age ± SD: 54.1 ± 19.7) admitted in the 8-bed medical/surgical ICU of Thriassion Hospital (Athens, Greece), were enrolled in the study. All patients were prospectively followed until their outcome (discharge from the ICU or death in the ICU). Patients with LOS shorter than 48 h and readmissions were excluded from the study.

Data recorded were patient demographics, medical history, admission category (medical, surgical, trauma), APACHE II and SOFA scores at admission in the ICU, Charlson's comorbidity index (Charlson et al. 1987), ward hospitalization before admission in the ICU, ICU-acquired infections, ICU day of the first infectious episode, need for CRRT in the ICU, duration of mechanical ventilation, maximum SOFA score in the ICU, LOS in the ICU, and ICU outcome (death/discharge). In trauma patients, the ISS was calculated by taking the highest AIS severity code in each of the three most severely injured ISS body regions (head or neck, face, chest, abdominal or pelvic contents, extremities or pelvic girdle, external), squaring each AIS code and adding the three squared numbers (Association for the Advancement of Automotive Medicine; http://www.unav.es/ecip/english/files/graph/aaam/index.html).

A cohort of 639 healthy individuals (male/female: 377/262, mean age: 52.7, range: 18–92), already analyzed by us (Liadaki et al. 2011; Pieper et al. 2014; Speletas et al. 2011) for the prevalence of the analyzed polymorphisms in Greek population was served as control groups. The study was approved by the Institutional Review and Ethics Board and written informed consent was obtained by the closest relative of each patient.

Molecular analyses

Molecular studies were performed on peripheral blood that was drawn for genetic analysis. Genomic DNA was initially extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Crawley, UK), according to manufacturer's instructions. The detection of the genetic polymorphisms rs4986790 (*TLR4*-D299G), rs4986791 (*TLR4*-T399I), rs9332736 (*C2*-c.841_849+19del28), and rs34557412 (*TACI*-C104R), was performed as previously described in detail (Johnson et al. 1992; Liadaki et al. 2011; Speletas et al. 2011).

The detection of rs77874543 (*BAFFR*-P21R), rs61756766 (*BAFFR*-H159Y) polymorphisms was performed by PCR amplification of exon 1 and 3 of *TNFRSF13C*, respectively, followed by PCR-RFLP analysis. The sequence of the utilized primers, derived from a previous study (Losi et al. 2005). For both PCRs, a total of 100–200 ng of DNA was amplified in a 30 μl reaction mixture using 62.5 μM of each deoxynucleoside triphosphate, 20 pmol of each primer, 1.5 mM $MgCl_2$, 10% DMSO and 0.8 U Taq polymerase (Bioline, London, UK) in a buffer supplied by the manufacturer. The thermocycler conditions for the amplification of exon 1 consisted of an initial denaturation at 94 °C for 2 min, followed by 32 cycles of PCR amplification (denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s, and elongation at 72 °C for 45 s) and a final elongation at 72 °C for 5 min. Afterwards, the 223 bp PCR product was subjected to digestion with the restriction enzyme EagI (New England Biolabs, UK) at 37 °C overnight. WT samples resulted in the digestion of PCR products to 110, 62 and 60 bp fragments, due to the presence of 2 EagI restriction sites, while the presence of the polymorphism resulted in the abolishment of a restriction site and the generation of 182 and 60 bp fragments. The thermocycler conditions for the amplification of exon 3 consisted of an initial denaturation at 94 °C for 2 min, followed by 35 cycles of PCR amplification (denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and elongation at 72 °C for 60 s) and a final elongation at 72 °C for 5 min. Afterwards, the 310 bp PCR product was subjected to digestion with either the restriction enzyme MsC I (New England Biolabs) or Eae I (New England Biolabs) at 37 °C for 4 h. Wild-type samples resulted in the digestion of the PCR products to 180 and 130 bp fragments using MsC I and 178, 70 and 62 bp fragments using Eae I. All PCR procedures were carried out in the PCR-engine apparatus PTC-200, MJ-Research (Watertown-Massachusetts, USA) and the PCR products were analyzed in 2.0% TBE agarose gels, stained with ethidium bromide and visualized under UV light.

For the confirmation of the PCR-RFLP results, randomly chosen PCR products positive and negative for the aforementioned polymorphisms were purified by use of a PCR purification system (Qiagen) and were directly sequenced using an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA) and a BigDye Terminator DNA sequencing kit (Applied Biosystems).