Polymorphisms in Toll-like receptor 4 and the systemic inflammatory response syndrome

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Abstract

The systemic inflammatory response syndrome (SIRS) is a major cause of morbidity and mortality, and is thought to be due to an over-amplification of an inflammatory response. The Toll-like receptor 4 (TLR4) Asp-299 → Gly polymorphism has been shown to reduce lipopolysaccharide responsiveness. We examined whether this TLR4 polymorphism is associated with severity of SIRS. A trend was found between the minor allele and mortality in SIRS (odds ratio of 4.3; \( P = 0.076 \)), suggesting a role for TLR4 signalling in the severity of SIRS.

Introduction

The systemic inflammatory response syndrome (SIRS) is a major cause of morbidity and mortality throughout intensive care units (ICUs). In cases of SIRS where an infective organism is identified, this is labelled as sepsis. SIRS is thought to be due to an over-amplification of cytokine production by haematopoietic cells. There is much variability in the clinical outcome within patients who develop SIRS. Patients with SIRS can progress to develop multiple-organ dysfunction syndrome and septic shock, which are associated with a worse prognosis. The factors predisposing to disease progression or resolution in SIRS are unknown.

The innate immune system provides an immediate response against toxic insults, mediated by phagocytic cells and host-defence molecules, including pattern recognition receptors (PRRs). An important PRR is Toll-like receptor 4 (TLR4), a transmembrane receptor that recognizes a range of ligands, including lipopolysaccharide (LPS), which is found in the cell wall of Gram-negative bacteria.

A single nucleotide polymorphism (SNP) in the TLR4 gene, consisting of Asp-299 → Gly, has been shown to lead to hypo-responsiveness to an inhaled LPS challenge, reduced airway epithelial TLR4 density and reduced inflammatory cytokine response to LPS [1]. Given that variability in the natural history of SIRS may be influenced by genetic factors in host-defence pathways, we aimed to determine whether the Asp-299 → Gly polymorphism in the TLR4 gene is associated with clinical outcomes in SIRS.

Materials and methods

A cohort of patients with SIRS in the adult ICU of Southampton General Hospital was recruited. For inclusion, patients were required to have at least three of the following SIRS criteria: tachycardia, tachypnoea (or mechanical ventilation), abnormal white-blood-cell count, and an abnormal body temperature. Signs of at least one end organ failure were also required for inclusion: hypoxaemia, hypotension, acidosis, oliguria, thrombocytopenia, coagulopathy or reduced Glasgow coma scale [2]. Patients who fulfilled the criteria were recruited at anytime during their primary admission to ICU during the period of the study. The patients gave written informed consent, or if they were unable to consent, assent was given by relatives. This study was approved by the Southampton and South West Hampshire local research ethics committee.

DNA extraction was performed on an 8 ml blood sample in EDTA using a simple salt-extraction method. A tetra-primer amplification refractory mutation system (‘ARMS’) PCR was used to genotype the polymorphic region [3] (the primers are shown in Table 1).

The PCR cycling conditions were 95 °C for 5 min; then 9 cycles of 94 °C for 30 s, X ° C for 30 s (where ‘X’ was initially 72 °C, decreasing 1 °C per cycle to 64 °C), 72 °C for 30 s; then 31 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 30 s; and finally, 72 °C for 10 min.

To identify the genotype, PCR products were resolved by micro-array diagonal gel electrophoresis (‘MADGE’) and visualized using a fluoromager. Allele-specific PCR products were present (aspartate allele, 147 bp; glycine allele, 292 bp), together with a control band (385 bp). Two researchers independently scored the genotypes using the Phoretix software programme. The following phenotypes were extracted from data collected by ICU staff during the patient’s stay: mortality (during the ICU episode; \( n = 79 \) available), length of stay (LOS), acute physiological and chronic health evaluation (APACHE II) score and day 1 results for lung injury score (LIS), sequential organ failure score

Key words: inflammatory cytokines, lipopolysaccharide responsiveness, systemic inflammatory response syndrome (SIRS), Toll-like receptor 4 (TLR4).

Abbreviations used: SIRS, systemic inflammatory response syndrome; TLR4, Toll-like receptor 4; ICU, intensive care unit; PRR, pattern recognition receptor; LPS, lipopolysaccharide; SNP, single nucleotide polymorphism; LOS, length of stay; APACHE II, acute physiological and chronic health evaluation; LIS, lung injury score; SOFA, sequential organ failure score; \( P_{aO2} /FiO2 \), partial pressure of arterial oxygen/fraction of inspired oxygen.

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Table 1 | Primers used in this study
The lowercase letters in the primers indicate mismatches.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Outer upper</td>
<td>5′-CCTGACCCCTATGAACTTATCC-3′</td>
</tr>
<tr>
<td>Outer lower</td>
<td>5′-CCATTACCTATGAACTTATCC-3′</td>
</tr>
<tr>
<td>Inner upper (Asp allele)</td>
<td>5′-GCATATGATCAGATCTCTGGAAGA-3′</td>
</tr>
<tr>
<td>Inner lower (Gly allele)</td>
<td>5′-GTCGAAATTTACATTTCTC-3′</td>
</tr>
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(SOFA) and the partial pressure of arterial oxygen/fraction of inspired oxygen (PaO2/FiO2) ratio. Phenotypic outcomes for the Asp/Gly or Gly/Gly genotype group using, where appropriate, $\chi^2$ (or Fisher’s exact test) or Student’s $t$ tests (SPSS version 11). A $P$ value of <0.05 (two-tailed) was considered significant.

Results

Ninety-four patients (mean age 57 years; LOS 13 days, APACHE II score 20) were recruited. The primary reasons for their ICU admission were: sepsis, 55%; cardiovascular, 11%; pancreatitis, 7%; gastrointestinal, 6%; respiratory, 5%, post-operative, 5%; trauma, 2%; others/unknown, 6%. The genotype frequencies were: Asp/Asp, 86 (91.5%); Asp/Gly, 7 (7.4%); and Gly/Gly, 1 (1.1%), and did not deviate from the Hardy–Weinberg equilibrium. The frequency of the aspartate allele was 95.2% and that of the glycine allele was 4.8%, in agreement with previous reports in Caucasian populations. The frequency of the Asp/Gly or Gly/Gly genotype was increased in SIRS patients who died in ICU as compared with those who survived (19% cf. 5%; $P = 0.076$, Fisher’s exact test) (Table 2). The odds ratio of death during ICU admission, given the presence of Asp/Gly or Gly/Gly genotypes combined, was 4.3 (95% confidence interval, 0.9 to 21.2). There were no significant differences in the means between the genotype groups for LOS, APACHE II, LIS, SOFA or the PaO2/FiO2 ratio ($P > 0.05$).

Discussion and conclusions

We observed a trend towards increased mortality in SIRS patients carrying the glycine allele, possibly suggesting a role for TLR4 signalling in the severity of SIRS. No associations were observed with other measures of severity in SIRS. At present, the sample size of the cohort is small and further recruitment will increase power.

It is currently unresolved whether a hyporesponsive LPS signalling pathway is beneficial or detrimental to the host. Activation of TLR4 has been postulated to be responsible for the pathogenesis of SIRS. Through the activation of TLR4 receptors, inflammatory cytokines, including tumour necrosis factor $\alpha$ [4], are released that cause tissue destruction and microvascular injury.

In contrast with this, TLR4 has an important function in the timely recognition of Gram-negative bacteria [5]. The importance of an intact innate immune response was demonstrated by inability of C3H/HeJ TLR4-deficient mice to contain and eradicate invading pathogens [5]. Recent studies support the hypothesis that the low-functioning TLR4 polymorphism may result in a reduced inflammatory response, which is unable to eradicate the pathogen. Individuals with the Asp-299→Gly polymorphism have been shown to have reduced levels of circulating inflammatory cytokines [6], an increased risk of acute bacterial infections [6–8] and a trend towards increased mortality [7]. In concordance with these previous studies, we observed a trend towards a worse clinical outcome (mortality) associated with the Asp-299→Gly polymorphism in our cohort of SIRS patients.

TLR4 is a receptor for Gram-negative bacteria, and hence may be more important in patients suffering from bacterial-induced SIRS, as opposed to its other causes. However, even in the absence of bacteraemia, patients with SIRS are prone to an overgrowth of Gram-negative bacteria in their small bowel and an increase in mucosal permeability, facilitating the translocation of bacteria from the gastrointestinal tract into the portal venous and lymphatic circulations [9].

A number of other factors need to be considered in interpreting these results, including linkage disequilibrium with other SNPs in TLR4 or nearby genes which could themselves be causal, and gene–gene interaction with polymorphisms of other genes of the innate immune system, e.g. CD14. Other important confounders will be incorporated in the ongoing study. Our preliminary results suggest that TLR4 is a biologically plausible candidate disease-modifying gene. If confirmed, association studies of TLR4 and other genes involved in the innate immune response may identify patients at risk of developing more severe SIRS, who may need closer monitoring and may benefit from more aggressive therapy, e.g. recombinant human activated protein C.

References


Received 15 January 2003