REVIEW

Contribution of genes polymorphism to susceptibility and outcome of sepsis

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Abstract  Sepsis and its sequelae are still a major cause of morbidity and mortality in intensive care units (ICU). The evidence that endogenous mediators actually mediate the individual’s response to infection has led to various approaches to assess the individual’s contribution to the course of the disease. The role of an individual’s genetic background and predisposition for the extent of inflammatory responses is determined by variability of genes encoding endogenous mediators that constitute the pathways of inflammation. Pro- and anti-inflammatory responses contribute to the susceptibility and outcome of patients with systemic inflammation and sepsis. Therefore, all genes encoding proteins involved in the transduction of inflammatory processes are candidate genes to determine the human genetic background that is responsible for interindividual differences in systemic inflammatory responses. Polymorphism of TNA α, TNFB, IL-10, IL-6, IL-1β, IL-1RN, HMGB1, TLR, PAI-1, DEFB1, HSP and MMP-9 has contribution to susceptibility and outcome of sepsis in some research. Examination of the association between genetic polymorphisms and sepsis promises to provide clinicians with new tools to evaluate prognosis, to intervene early and aggressively in treating high risk persons, and to avoid the use of therapies with adverse effects in treating low risk persons. Genomic information may be useful to use to identify groups of patients with a high risk of developing severe sepsis and multiple organ dysfunctions.

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1. Introduction

Sepsis is a systemic inflammatory response syndrome arising from infection. Severe sepsis is the sepsis associated with organ dysfunction, hypoperfusion abnormality or sepsis-induced hypotension [1]. Despite recent advances in antibiotic therapy, aggressive operative intervention and intravenous hyperalimentation, sepsis, severe sepsis, and its sequelae are still reported to contribute to significant high morbidity and mortality in the surgical intensive care unit. In various settings, mortality rates of patients suffering from severe sepsis have reached 20–70% [2]. Care of patients with sepsis costs as much as $50,000 per patient, resulting in an economic impact of nearly $17 billion annually in the United States [3]. Based on these findings, it is essential to determine the mechanism underlying the pathophysiology of sepsis in order to design better intervention therapy.

Both pro and anti-inflammatory responses contribute to the outcome of patients with systemic inflammation and sepsis. Therefore, all genes encoding proteins involved in the transcription of inflammatory processes are candidate genes to determine the human genetic background responsible for interindividual differences in systemic inflammatory responses to infection. This group of genes comprises, but is not restricted to, cytokines and involves numerous other effector molecules involved in inflammatory processes. Genes of the coagulation system, heat shock proteins, or signal transduction molecules contribute to the list of candidate genes for sepsis that show genomic variation. Cytokines released from immunocompetent cells are major players in the inflammatory response to infection. Primary proinflammatory cytokines, such as tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1), induce secondary pro and anti-inflammatory mediators such as IL-6 and IL-10 [4]. Genetic variation of this cytokines has been shown to play a large role in determining susceptibility to and outcome of sepsis.

This article will discuss the contribution of pro and anti-inflammatory cytokine gene polymorphism to susceptibility and outcome of patients with systemic inflammation and sepsis.

2. Genes polymorphism and sepsis

2.1. Tumor necrosis factor (TNF) α

Odwyer et al. [5] examined the association of TNFα promoter single nucleotide polymorphisms (SNP) and haplotypes with gene expression in terms of mRNA levels and with outcome in a cohort of patients with severe sepsis. There was a trend for patients homozygous for the G allele at position −308 to produce more TNFα mRNA on day 1 than those carrying an A allele. Carrier status for haplotype 1 (with A at position −863 and G at position −308) was associated with greater TNFα mRNA levels on day 1. Carrier status for haplotype 4 (with C at position −863 and A at position −308) was associated with a nonsignificant decrease in TNFα mRNA levels on day 1. When directly compared, haplotype 1 was associated with significantly greater levels of TNFα mRNA than with haplotype 4 on day 1. Patients homozygous for the A allele at position −308 were more likely to succumb to severe sepsis than those carrying the G allele.

These findings were supported by a previous study that shown the G → A SNP at the −308 position in the TNFα promoter increases the risk for severe sepsis after trauma [6], after burn injury [7], in patients with community-acquired pneumonia [8] and surgical infection [9].
2.2. TNF β gene (TNFB)

TNFβ (lymphotoxin-β) is a cytokine that orchestrates lymphoid neogenesis and the formation of germinal center reactions. In the first intron of the TNFB, there is an Nco1 polymorphism consisting of the allele TNFβ1 in the presence of the restriction site, and the allele TNFβ2 in its absence. Nco1 polymorphism was found to be correlated with an amino acid variation of the TNFβ sequence at position 26, which is asparagine for the TNFβ1 sequence and threonine for the TNFβ2 sequence. With regard to the functional consequences of TNF synthesis, it was found that phytohemagglutinin-stimulated peripheral blood mononuclear cells from TNFβ1-homozygous individuals showed a significantly increased TNFβ production, whereas phytohemagglutinin- and endotoxin-stimulated monocytes from TNFβ2-homozygous individuals showed a significantly higher production of IL-1β and TNFα.

A study conducted by Majetschak et al. [11] had shown that genotypes were related to the occurrence of severe posttraumatic sepsis and TNFα serum concentrations. Genotype distribution in patients with an uncomplicated clinical course was significantly different from that in patients with severe posttraumatic sepsis. Development of severe posttraumatic sepsis was significantly increased in patients homozygous for the allele TNFβ2. In patients with severe posttraumatic sepsis, TNFα serum concentrations were significantly higher in TNFβ2-homozygous individuals compared with heterozygous and TNFβ1-homozygous individuals. Thus in multiply injured patients, the Nco1 polymorphism within the TNFβ gene is associated with the development of severe posttraumatic sepsis and with increased TNFα serum levels when severe sepsis has occurred.

2.3. Tumor necrosis factor receptor (TNFR)

In the case of TNF, regulatory mechanisms include shedding into the circulation of two membrane-bound TNF receptors, TNFRSF1A (TNFR1) and TNFRSF1B (TNFR2). Cleavage of the extracellular portion of these receptors produces soluble molecules (sTNFRSF1A and sTNFRSF1B) in the blood that retain the ability to bind TNF and inhibit its acute activity. Evidence is gradually emerging that genetic variations within the TNF receptor gene loci may be important in the pathogenesis of various inflammatory conditions. Polymorphisms within the TNFRSF1B locus have also been associated with sepsis and genotype distribution of the IL-10 gene promoter region (−592, −819 and −1082 in the promoter region of the IL-10 gene are associated with increased incidence of severe sepsis. They found that patients with severe sepsis were more likely to have IL-10 −1082 allele 1, compared with controls. Genotype distribution of the IL-10 −1082 polymorphism significantly differed between patients and controls. However, the allele frequencies and genotype distribution of the IL-10 −1082 polymorphism did not differ between surviving and dead patients. No significant differences in the genotype distribution and allele frequencies of the IL-10 −592 and IL-10 −819 polymorphisms were observed between patients with severe sepsis and healthy controls, or between surviving and dead patients.

The other study [19] analyzed 284 adult patients presenting to the Emergency Department (ED) with community-acquired pneumonia (CAP). Subjects with either genotype C/T or T/T at IL-10 −819 were associated with a greater risk of progression to severe sepsis compared with the common homozygote C/C. This study did not find a consistent difference in plasma IL-10 levels between subjects with different genotypes. In this preliminary analysis of subjects with CAP, those with the IL-10 −819 C/T or T/T genotypes are more likely to develop severe sepsis compared with those with the usual homozygous C/C phenotype.

2.4. Interleukin-10

IL-10 is the most potent anti-inflammatory cytokine, as it downregulates the production of proinflammatory cytokines and chemokines secreted by activated monocytes, polymorphonuclear leucocytes and eosinophils, prevents antigen-specific T-cell activation, inhibits T-cell expansion, and potentiates the release of the inflammatory modulator IL-1ra. IL-10 may play a role in the pathogenesis of severe sepsis.

An anti-inflammatory cytokine profile of high IL-10 production has been observed to correlate with the development of meningococcal disease and community-acquired infection [14], while genomic polymorphisms within the IL-10 gene have been demonstrated as being associated with inter-individual differences in IL-10 production [15]. The plasma concentration of IL-10 in patients with sepsis has been shown to be correlated with both the severity and the outcome of sepsis [16,17].

Qiang et al. [18] conducted a study in China to investigate whether three biallelic polymorphisms at positions −592, −819 and −1082 in the promoter region of the IL-10 gene are associated with increased incidence of severe sepsis. They found that patients with severe sepsis were more likely to have IL-10 −1082 allele 1, compared with controls. Genotype distribution of the IL-10 −1082 polymorphism significantly differed between patients and controls. However, the allele frequencies and genotype distribution of the IL-10 −1082 polymorphism did not differ between surviving and dead patients. No significant differences in the genotype distribution and allele frequencies of the IL-10 −592 and IL-10 −819 polymorphisms were observed between patients with severe sepsis and healthy controls, or between surviving and dead patients.

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In other study, the influence of genetic polymorphisms of IL-6 gene promoter –174 G/C on the severity of systemic inflammatory response syndrome (SIRS) associated with CAP was studied by Martín-Loeches et al. [21]. This research showed that the distribution of the G/C 174 genotype was similar in CAP patients and controls. In patients who were admitted with CAP, no significant differences were observed compared with progression between groups. These findings demonstrate that the 174G/C polymorphism is not associated with risk and outcome of CAP in the Spanish white Caucasian population.

2.6. Interleukin 1β

IL-1β is a potent proinflammatory cytokine released by macrophages involved in the systemic inflammatory response. IL-1β is capable of inducing the symptoms of septic shock and organ failure in animal models and is regarded as a primary mediator of the SIRS [22]. Despite the finding that a homozygous failure in animal models and is regarded as a primary mediator is capable of inducing the symptoms of septic shock and organ failure in animal models and is regarded as a primary mediator, IL-1β secretion of the SIRS [22]. Despite the finding that a homozygous IL-1β population with risk and outcome of CAP in the Spanish white Caucasian population.

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2.7. Interleukin-1 receptor antagonist gene (IL-1RN)

The polymorphic region within intron 2 of the IL-1RN* gene contains a variable numbers of tandem repeats (VNTR) of 86 bp, five alleles of the IL-1RN* have been reported (*1–*5), corresponding to 2, 3, 4, 5 and 6 copies of the 86-bp sequence, respectively [24]. Ex vivo experiments suggested greater IL-1ra responses associated with alleles containing small numbers of the 86-bp repeat. Ex vivo studies also demonstrated higher levels of IL-1ra protein expression and protein release for A2 homozygous individuals, compared with heterozygotes, after stimulation with LPS [25]. There previous study shown that the allele frequency of the allele IL-1raA2 was increased in 93 patients with severe sepsis compared with normal individuals (p < .01) but no association with patients’ outcome was observed [26].

The research that conducted by Arnalich et al. [24], found that compared with patients homozygous or heterozygous for the allele *1, IL-1RN*2 homozygotes produced significantly lower levels of IL-1Ra from patient PBMC. This result provides evidence that homozygosity of the IL-1RN*2 is associated with a decreased production of IL-1Ra in PBMC and higher mortality risk during severe sepsis. These findings are consistent with the hypothesis that individuals producing lower amounts of IL-1Ra are afforded a lower level of protection against fatal outcome than subjects producing higher levels.

2.8. High mobility group box 1 protein (HMGB1)

HMGB1 is a pleiotropic cytokine, recently implicated in the pathophysiology of the SIRS and sepsis. HMGB1 is a fascinating and markedly complex nuclear and cytoplasmic protein that is readily measurable in the systemic circulation in response to severe injury. The protein has the propensity to bind to a variety of inflammatory mediators such as lipopolysaccharide and pro-inflammatory cytokines, including IL-1 [27]. HMGB1 functions as an alarmin or damage-associated molecular pattern molecule, and acts as an endogenous ligand for pattern recognition receptors of the innate immune system [28].

Kornblit et al. [29] reported the first evidence of the HMGB1 gene’s impact on the risk of SIRS and sepsis. These investigators performed a long-term, 4-year study comparing HMGB1 sequencing data in 239 ICU patients with HMGB1 blood levels and clinical outcomes. A promoter variant (–1377delA) was associated with a markedly reduced long-term survival rate after ICU admission in SIRS patients. They also observed a significant interaction with a polymorphism within the coding region of the HMGB1 gene at position 982 (C > T) in exon 4. Carriers of the polymorphism had an increased frequency of early death from infection along with higher Simplified Acute Physiology Score II compared with wild-type genotypes. Interestingly, this 982C > T variant was accompanied by significantly lower HMGB1 blood levels.

2.9. Toll like receptors (TLR)

More than half of the cases of sepsis are caused by gram-negative bacteria. Because TLR4 is required for innate immune responses to LPS, several research have investigated possible associations between the Asp299Gly polymorphism and sepsis [30]. Two of these studies demonstrated that this polymorphism increases the risk of gram negative infections [31,32], and another study linked this polymorphism to an increased incidence of SIRS [33]. However, it is likely that the impact of the Asp299Gly polymorphism on sepsis is restricted to gram negative infections, because the Asp299Gly polymorphism does not affect polymicrobial sepsis [34].

With regard to other pathogens, an association between the Asp299Gly polymorphism of TLR4 and severe respiratory syncytial virus-induced bronchiolitis was reported. Respiratory syncytial virus is a known ligand for TLR4. Taken together, these data suggest that human TLR4 has a critical role in the innate immune response to gram negative bacteria and respiratory syncytial virus, although the cellular and molecular events affected by the TLR4 polymorphisms in pathogenesis and adaptive responses to infection have not yet been identified.

2.10. Plasminogen activator inhibitor type 1 (PAI-1)

Plasminogen activator inhibitor type 1 (PAI-1) is a 50 kilodalton glycoprotein of the serine protease inhibitor family. The primary role of PAI-1 in vivo is the inhibition of both tissue and urokinase type plasminogen activators. In addition to this function, PAI-1 acts as an acute phase protein during acute inflammation. PAI-1 is a pivotal player in the pathogenesis of sepsis. In patients with sepsis, the levels of PAI-1 are positively related to poor outcome, increased severity of disease, and increased levels of various cytokines, acute phase proteins, and coagulation parameters.

Kornelisse et al. [35] have investigated the relation between PAI-1 and TNF-α, which is the principal stimulator, and have found a difference between survivors and nonsurvivors: the production of PAI-1 in nonsurvivors was 1.9 times higher for the same levels of TNFα. Hermans et al. [36] found that patients with the 4G/4G genotype had significantly higher PAI-1 concentrations than did those with the 4G/5G or 5G/5G genotype. Haralambous et al. [37] have recently confirmed in an independent study that white pediatric patients carrying the functional
PAI-1 4G/4G genotype are at an increased risk of developing vascular complications and dying from meningococcal disease. The 4G/5G insertion/deletion promoter polymorphism, which leads to differences in PAI-1 production, has been demonstrated to affect the risk of developing severe complications and dying from sepsis during meningococcal infection and multiple traumas [38]. Menges et al. [39] demonstrated that the genetic predisposition to produce high levels of PAI-1 (the 4G/4G genotype) is associated with poor prognosis and outcome of severe trauma. The PAI-1 4G allele is associated with high PAI-1 plasma concentrations and a poor survival rate in these patients.

2.11. Human β-defensin 1 (DEFB1)

DEFB1 is a multifunctional mediator in infection and inflammation, which has been largely explored in ex vivo studies. Fang et al. [40] conducted a research to investigate whether DEFB1 genomic variations are associated with incidence and outcome of severe sepsis. Six reported polymorphisms were detected. The −44G-allele and −44G-allele carrying genotypes were significantly associated with the incidence and outcome of severe sepsis. The −20G allele and GG genotype were associated with susceptibility to severe sepsis, while the −1816G-allele and −1816G-allele carrying genotypes influenced the outcome of severe sepsis. Haplotypes −20A/−44C/−25G showed a protective role against severe sepsis, whereas haplotype −20G/−44G/−25G served as a risk factor for fatal outcome of severe sepsis. The present findings have important implications in the understanding of the role of DEFB1 in the pathophysiology of severe sepsis, and DEFB1 genomic variations may offer a new means of risk stratification for patients with severe sepsis.

2.12. Heat shock protein (HSP)

HSPs are expressed in response to heat shock and a variety of other stimuli, including endotoxin and other mediators of severe sepsis. Three genes encoding members of the HSP family lie in the class III region of the MHC. Individuals carrying the polymorphism HSP70-2G exhibit lower levels of mRNA ex vivo. Schroeder et al. [41] hypothesized that individuals homozygous for the HSP70-2G allele should have greater susceptibility to and/or higher mortality from sepsis compared to other genotypes. They tested this hypothesis in 87 patients admitted to a surgical ICU with severe sepsis and found no association. They did find a linkage between HSP70-2A (the "protective" allele) and TNF-β2, an allelic variant previously shown to be associated with higher TNF-α levels and worse outcome from sepsis. The overall mortality in the group of HSP-2G homozygotes was not increased possibly because of linkage with the non-TNF-β2 haplotype. This finding highlights the importance of knowing all the polymorphisms relevant to the inflammatory response in individuals rather than interpreting a polymorphism in isolation.

2.13. Matrix metalloproteinase-9 (MMP-9)

MMP-9 is involved in extracellular matrix degradation and leukocyte migration. MMP-9 has been demonstrated to play an important role in organ dysfunction and outcome of sepsis in mouse [42]. This research found that using E. coli peritonitis, MMP-9−/− mice (MMP-9 gene-deficient) showed much higher peritoneal chemokine and cytokine levels compared with wild-type mice beside that MMP-9−/− mice displayed a diminished recruitment of leukocytes to the site of infection, indicating that cellular migration was impaired. Moreover, MMP-9−/− mice developed more severe distant organ damage during infection. However, genetic predisposition of MMP-9 to sepsis remained unknown.

Seven common SNPs within the functional regions of MMP-9 gene (rs17576, rs2274756, rs2250889, rs9509, rs3918240, rs3918241 and rs3918242) have investigated in human. Research that conducted by Chen [43] showed that the genotype distributions and allelic frequencies of the above seven SNPs were not significantly different between patients with severe sepsis and controls, as well as between surviving and nonsurviving patients with severe sepsis. Haplotype GCCCTTC, AGGTCTC, GGGCCTC, GACCTAT and AGCCCCCTC are the five most common haplotypes. The distribution of the haplotypes was also comparable among the defined groups. The median plasma levels of MMP-9 was 37.66 ng/ml in 32 patients within the first 24 h following the diagnosis of severe sepsis, and 30.15 ng/ml in 19 healthy controls. Compared with those in surviving patients with severe sepsis and healthy controls, the concentrations of MMP-9 appeared an increasing trend in nonsurviving patients with severe sepsis. This finding suggest that common polymorphisms within the function regions of MMP-9 gene may not play a major role in the predisposition to severe sepsis in the Chinese Han cohort but the plasma levels of MMP-9 may associate with the outcome of severe sepsis.

3. Conclusions

Polymorphisms of many inflammatory cytokine gene, innate immunity pathway gene, and coagulation cascade polymorphisms has been shown to play a large role in determining susceptibility to and outcome of such complex diseases as sepsis. Examination of the association between genetic polymorphisms and sepsis promises to provide clinicians with new tools to evaluate prognosis, to intervene early and aggressively in treating high risk persons, and to avoid the use of therapies with adverse effects in treating low risk persons.

3.1. Key messages

Confirmation in large, well conducted, multicenter studies is required to confirm current findings and to make them clinically applicable. Unbiased investigation of all genes in the human genome is an emerging approach. New, economical, high-throughput technologies may make this possible [44].

High-throughput techniques, such as genome-wide scans, will allow genotyping of a large number of SNP throughout the human genome. There is intense interest to apply this technology to understand genetics of severe sepsis. By performing genome-wide association studies, genome-wide scans of nonsynonymous SNP, and testing for differential allelic expression and copy number polymorphisms, we may yet be
able to tease out the complex influence of genetic variation on susceptibility and response to sepsis.

4. Disclosure statement

We declared that there is no conflict of interest.

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