Host Genetics and Pediatric Sepsis

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Abstract: Susceptibility to, and outcome from, sepsis in children is highly variable due in part to genetic variation in genes coding for components of the innate immune response. This review article will discuss evidence for the influence of host genetic variability on the susceptibility to, and outcome from, sepsis in children and adults. Polymorphisms in genes coding for proteins involved in the recognition of bacterial pathogens (TLR4, CD-14, Fe⁺RIIa, and mannose binding lectin) and the response to bacterial pathogens (TNF-α, IL-1β, IL-1RIα, IL-6, IL-10, heat shock proteins, ACE, plasminogen activator inhibitor-1) can influence the amount or function of the protein produced in response to bacterial stimuli. Evidence is discussed suggesting that some of these genetic polymorphisms influence the susceptibility to, and outcome from, sepsis.

Conclusion: Host genetic variability in the regulatory and coding regions of genes for components of the innate immune system may influence the susceptibility to and/or outcome from sepsis. The disparate results observed in many studies of polymorphisms in sepsis emphasize the need for future studies to be larger, to include the analysis of multiple polymorphisms, and to be better designed with respect to control populations in order to identify the degree of influence that genetic variability has on sepsis.

Keywords: Sepsis, polymorphism, pediatrics, outcome, genetics.

INTRODUCTION

Sepsis remains a major global health problem with a high mortality despite major advances in the care of critically ill children and adults. Examining why certain patients continue to have a high mortality may provide clues to novel therapeutic interventions. Recent research has explored the hypothesis that common genetic variations within each of us may either contribute to or influence the severity of infections, thereby contributing to the high mortality in sepsis.

The sequencing of the human genome has revealed the enormous degree of genetic variation that exists in the human population. Indeed, most genes are polymorphic meaning that they exhibit small differences in their nucleotide sequence. These variations account for the hereditary differences among us all, including how we respond to illnesses. Recent studies have suggested that clinical presentation, treatment, and outcome from critical illnesses are influenced in part by some of these genetic variations. These genetic variations may contribute to why one child with viral or bacterial pneumonia presents to the emergency department and is well enough to go home, while another child with the same pathogen presents in fulminate septic shock and respiratory failure. Thus, host genetic variations may influence the response to infection and severity of disease [1-3]. That host genetics may play a role in the outcomes from sepsis is supported by the observation that an early death of a biologic parent from infection is associated with a much greater risk of death of a child from infection than the death of an adoptive parent from infection on the risk of death of an adopted child from infection [4]. An understanding of the host factors which contribute to the susceptibility to, and outcome from, sepsis is crucial given the major impact of sepsis on the morbidity and mortality in neonatal, pediatric, and adult intensive care units.

The innate immune response enables the host to recognize pathogens and provide a rapid inflammatory response that includes the production of cytokines, chemokines, and effector molecules and allows for the interaction with the adaptive immune response [5]. This innate immune system, therefore, must first differentiate self from pathogen by identifying the presence of pathogen-associated products and, second, respond by activating a number of signaling pathways whose end result is the synthesis of inflammatory cytokines and counterbalanced by the production of anti-inflammatory cytokines. Indeed, severe sepsis is thought to be perpetuated by the exaggerated systemic production of inflammatory cytokines not adequately counterbalanced by the production of anti-inflammatory cytokines [6-9]. These two components of the innate immune system, recognition and response, utilize a large number of receptors and accessory proteins, signaling molecules, and transcription factors involved in protein synthesis. Any part of the recognition and response components that is altered in quantity or functional activity may ultimately influence the final host response. Thus, the number of possible genes in which variations might
influence the innate immune response, and thereby the susceptibility to, and outcome from, sepsis is considerably large. This review will examine the evidence of the influence of host genetic variability in patients with sepsis and Table 1 summarizes many of the studies.

GENETIC VARIATION IN PATHOGEN RECOGNITION MOLECULES

The host response to infection requires recognition of the presence of pathogen-associated compounds, also termed pathogen-associated molecular patterns (PAMPs), and the subsequent initiation of a number of signaling pathways. The following section reviews the evidence that genetic polymorphisms in genes coding for recognition of PAMPs influence the susceptibility to and outcome from sepsis.

Toll-Like Receptors

The demonstration that the Drosophila protein Toll was crucial for the protection of the fly against fungal infections [10] greatly enhanced our understanding of the innate immune system at the molecular level. The human toll-like receptors (TLR) are a family of cell surface and intracellular receptors that are involved in pathogen recognition [11-14]. These receptors recognize a variety of diverse bacterial, viral, and fungal pathogens and allow for the induction of the appropriate signaling pathways in order initiate a pathogen specific response.

Perhaps the best studied of the TLRs is TLR4 which along with the accessory proteins CD-14 and MD-2, binds LPS, one of the major components of the cell wall of gram negative bacteria and a powerful stimulator of the innate immune response. Several studies in mice demonstrate that TLR4 is required for response to LPS [15] and that a single amino acid change can significantly reduce response to LPS [16, 17] and enhance susceptibility to infection. Several SNPs have been identified in the promoter and coding regions of the human TLR4 gene [18-20]. The SNP identified in the fourth exon of the TLR4 gene results in the replacement of a conserved aspartic acid at amino acid residue 299 with glycine. This SNP is in linkage disequilibrium with a second SNP at amino acid 399 which changes a threonine to an isoleucine. The Gly299Ile399 variant appears to be expressed at lower levels in human airway epithelia [18], and a number of studies have demonstrated an association of this variant with a reduced airway reactivity in response to inhaled LPS [18, 19, 21]. This variant is associated with a decreased response to LPS in in vitro studies utilizing primary human epithelial cells heterozygous for the variant as well as in studies using a transfected cell system [18].

Do individuals with the TLR4 genetic variants associated with a decreased responsiveness to LPS have a greater likelihood of gram negative bacterial infection and/or sepsis? Human studies have demonstrated an association of these variants with susceptibility to gram negative bacterial infections and septic shock [22-24] and mortality in patients with systemic inflammatory response syndrome [25]. In addition, a TLR4 polymorphism was found to be associated with an increased risk of severe sepsis in adult burn patients [26]. However, not all studies have confirmed this finding [27, 28], nor does the variant appear to be associated with either susceptibility to, or severity of, meningococcal disease specifically [29]. This apparent lack of an association of the variant with meningococcal disease is complicated by findings demonstrating that Neisseria meningitides is capable of eliciting an inflammatory response via TLR2 in the absence of LPS thereby bypassing any genetic variation in the TLR4 gene that may influence the host’s response [30, 31]. Thus, there is good evidence for the biologic plausibility that genetic variations in the TLR4 gene may influence the response to infections, but further studies are needed to assess whether these genetic variations influence the severity of sepsis in humans.

The TLR2 receptor recognizes a variety of PAMPs from gram positive and gram negative bacteria as well as fungus. Several genetic variations have been described in the promoter region and in the coding sequence of the gene for TLR2. One particular variation results in an arginine to glutamine at amino acid position 753 (arg753gln) that does not appear to increase the susceptibility to Staphylococcus aureus infections in humans [32]. In contrast, another study suggests that a promoter SNP in the TLR2 gene is associated with an increased prevalence of gram positive bacteremia and sepsis but not mortality [33] yet the mechanism by which this variation alters TLR2 levels or function is not known.

Variations in Components of the TLR Complex and Signal Transduction Pathways

The TLR signaling pathways are complex, and many details of the pathways remain to be determined. Fig. (1) demonstrates a simplified version of the TLR4 signaling pathway (for reviews see [34-36] and should help understand the notion that genetic variability in not only the TLRs themselves, but also their accessory proteins and signaling pathway components may also influence the susceptibility to, and outcome from, infection. The TLR4 receptor complex consists of two accessory proteins, CD14 and MD-2. LPS fails to elicit a response in both CD14 [37] and MD-2 [38] knockout mice demonstrating their crucial role in the recognition of gram negative pathogens. A polymorphic site in the promoter region of the gene coding for CD-14 in humans has been identified 159 nucleotides upstream of the transcription start site (C to T) [39-41] with the T allele demonstrating increased transcriptional activity [42]. Individuals homozygous for the -159T allele have increased levels of CD14 [41-43]. While some human studies demonstrate an increased risk of sepsis or septic shock [33, 44], others have not demonstrated an association with outcome in adults with confirmed gram negative bacteremia [28] or sepsis [45, 46]. The disparity in results may be due to inherent differences between the studies, such as the number of patients analyzed, origin of the infection (surgery, trauma, pneumonia), or heterogeneity of the patient populations.

Several genetic variations in the gene coding for MD-2 have been identified. One such variation alters a threonine at position 35 to an alanine and is associated with reduced TNF-α secretion in response to LPS in an in vitro stimulation assay [47]. Additional SNPs in the promoter region of the MD-2 gene have been identified (-1625C/G, -1064A/G, and -475A/T) with the -1625 SNP appearing to increase MD-2 expression [48]. In a cohort of adult Chinese
with trauma, those with the -1625 G allele were more likely to develop sepsis and organ dysfunction yet no affect on mortality is mentioned [48].

The TLR signaling pathways also include a number of adapter molecules (e.g., TIRAP/Mal, MyD88, and TRIF/TICAM-1) and intracellular kinases (e.g., IRAK-1, IRAK-4, and IκB) that transmit the signal to the nucleus. Some of these adapter molecules and kinases have functionally significant genetic variations that alter the signaling of the pathway and, thereby, the response. A polymorphism in the gene coding for TIRAP/Mal results in a ser180leu amino acid change and impaired TLR2 signaling. This variation is associated with a decreased risk of severe pneumococcal infection in those patients who are heterozygous for the polymorphism [49]. IRAK-1 is an intracellular kinase involved in TLR signaling. A specific haplotype containing a T to C polymorphism at nucleotide position +1595 within exon 12 is associated with increased nuclear translocation of NF-κB and increased risk for pneumococcal sepsis and higher mortality [50]. In addition, two polymorphisms in NF-κB are associated with protection against invasive pneumococcal disease in adults [51]. Thus, genetic variations within the genes coding for the TLR receptors and components of their signal transduction pathways influence the final host response to infections and may account for some of the variation observed in sepsis.

Mannose Binding Lectin

Mannose binding lectin (MBL) is a circulating lectin that recognizes polysaccharide moieties of various pathogens [52-54]. MBL is involved with 1) the opsonization of bacteria by virtue of its ability to bind bacterial surface oligosaccharides [55], and, 2) the binding of MBL-associated serine proteases leading to activation of complement [56]. The MBL protein exists as a heterotrimer with each peptide containing a carbohydrate domain involved in pathogen recognition and a helical tail domain involved in polymerization of the 3 peptides [57]. Several lines of evidence support the critical role of MBL in innate immunity including an increased mortality with *Staphylococcus aureus* infections [58] and increased postburn infections with *Pseudomonas aeruginosa* [59] in MBL-knockout mice.

Three genetic polymorphisms in the gene coding for human MBL result in changes in amino acids at positions 52, 54 and 57, (referred to as variants D, C, and B, respectively). These amino acid changes diminish the ability of the helical tails to polymerize resulting in an increased degradation of MBL [52, 60, 61] and reduced serum levels [60, 62]. Two additional polymorphisms at nucleotides -550 and -221 in the promoter region appear to be associated with lower serum levels of MBL such that the final circulating levels of
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The 3 MBL structural variants D, C, and B are associated with an increased risk for meningococcal infections [64, 65], pneumonia and sepsis in neonates [66], acute respiratory infections in children [67], hospitalizations due to infections in children [68], recurrent respiratory infections [69], and viral co-infections in adults with pneumococcal pneumonia [70]. Three studies have examined MBL polymorphisms in patients with invasive pneumococcal disease [71-73], with one of these three studies finding a significant association. However, a recent meta-analysis of these three studies demonstrated a significant association between homozygosity and presence of disease [74]. The frequency of each of the 3 variants in patients who were homozygotes (DD, BB, CC) or heterozygotes (BC, BD, CD) was significantly higher in patients than in a healthy control population suggesting an increased susceptibility to invasive pneumococcal disease in individuals with at least one copy of the variant polymorphism.

GENETIC VARIATION IN GENES INVOLVED IN THE HOST RESPONSE TO PATHOGENS

The host response to pathogens involves a number of cytokines and chemokines that coordinate and balance the overall response. Disruption of the various components can lead to an overwhelming inflammatory response that is detrimental to the host. Indeed, it is believed that an imbalance between the pro- and anti-inflammatory cytokines in favor of an overzealous response leads to the clinical picture of severe sepsis. Pro-inflammatory cytokines, notably TNF-α, IL-1 and IL-6, are usually elevated early after an infection followed by an elevation of other mediators, such as chemokines. Anti-inflammatory cytokines such as IL-10 generally follow and lead to a return to baseline of cytokine and chemokine levels and the start of tissue repair [75-77]. Genetic variability within the genes coding for these components of the response could potentially disrupt this delicate balance and influence the severity of and outcome from sepsis.

Tumor Necrosis Factor α (TNF-α)

As a pro-inflammatory cytokine, TNF-α plays a key role in the pathogenesis of the acute inflammatory response. TNF-α also appears to play a central role in development of the harmful effects of the inflammatory response such as hypotension, capillary leak, ARDS, and multi-organ failure [78-82]. An over-exaggerated pro-inflammatory response resulting in an imbalance between the pro-inflammatory cytokines such as TNF-α and the anti-inflammatory cytokines results in the clinical manifestation of sepsis and septic shock. One of the mechanisms by which an imbalance can occur is the presence of genetic variations that lead to increased levels of the pro-inflammatory cytokines or decrease levels of the anti-inflammatory cytokines.

Several single nucleotide polymorphisms within the regulatory region of the gene coding for TNF-α have been identified that are associated with both basal and stimulated TNF-α production in vitro and/or in vivo [83-90]. A well studied SNP is the G to A transition at nucleotide position -308 upstream from the transcriptional start site. In vitro studies have demonstrated that the rarer TNF-α-308A allele is associated with increased transcription as compared with the wild-type TNF-α-308G allele [89]. Furthermore, the TNF-α-308A allele is associated with increased secretion of TNF-α from macrophages after LPS stimulation in vitro [86]. This polymorphism lies near putative DNA binding sites for several transcription factors. Eletrophoretic mobility shift assays (EMSA) have demonstrated differential binding of nuclear proteins to DNA fragments containing either an A or a G at the TNF-α-308 position [91]. A second polymorphism at position -238 lies in a putative repressor binding site in the promoter region [92]. The less common A allele that substitutes for the more common G allele is associated with lower levels of transcription and secretion of TNF-α in in vitro studies [93]. A third polymorphism that appears to influence levels of TNF-α is approximately 250
bp downstream from the transcriptional start site for the gene coding for lymphotixin alpha (LT-α, also known as TNF-β). This site is approximately 3.2 kilobases upstream from the TNF-α gene and may be acting as an enhancer region, though the exact mechanisms by which this polymorphism results in higher TNF-α levels are unknown. An A at this position is associated with higher TNF-α production in vitro as well as higher serum levels in adults with sepsis and children with bacteremia [83, 84, 90]. Thus, there is convincing evidence that genetic variation within regulatory regions of the gene coding for TNF-α influences TNF-α production.

Associations between TNF-α regulatory polymorphisms that result in higher TNF-α levels and the severity of, and outcome from, sepsis are observed in several studies. For example, among adults with septic shock, the frequency of the less common A allele at the TNF-α-308 position is higher in those who died, and the risk of death was 3.7-fold greater in those patients with at least one copy of the TNF-α-308A allele even after controlling for age and severity of illness [94]. Other studies demonstrating an association of “high-secretor” genotypes and more severe disease include children with meningococcal infections [95] and bacteremia [90], severe sepsis in adults [26, 83, 85, 96], and community acquired pneumonia in adults [97, 98]. However, these results have not been uniformly observed [28, 99-103], and further studies are needed. Thus, individuals in which genetic variations result in a hyper-response during sepsis (as with those with the TNF-α-308A allele) or hypo-response (as with those with the TNF-α-238A allele) may have worse outcomes with sepsis.

**Interleukin 1 and Interleukin 1 Receptor Antagonist (IL-1/IL-1Ra)**

IL-1α and IL-1β are also key pro-inflammatory cytokines secreted early in the response to infection and play an important role in the pathogenesis of sepsis and septic shock. These molecules stimulate the production of prostaglandins and nitric oxide, two mediators of the vasodilatation observed in sepsis [104]. IL-1Ra is produced to balance the specific effects of IL-1β by competing with IL-1 for binding to its receptor [105, 106]. Serum levels of IL-1β and IL-1Ra are elevated in patients with meningococcal disease with the highest levels in those with more severe disease [107].

The genes coding for IL-1α, IL-1β, and IL-1Ra are clustered together on chromosome 2, and several polymorphisms have been described in this locus. Three types of polymorphisms have been described in the gene coding for IL-1α: a variable number of a 46 bp repeats in intron 6 with unknown functional significance [108]; a C to T transition at position -889 in the regulatory region that appears to increase transcriptional activity of the IL-1α gene [109] and increase levels of IL-1α protein [110]; and several polymorphisms in the 3′ untranslated region (UTR) some of which have been shown to be associated with higher IL-1α levels in response to LPS in an in vitro system [111]. Several polymorphisms in the IL-1β gene are located both in the promoter and coding region with several associated with higher levels of IL-1β in in vitro stimulation assays [112-114]. Polymorphisms have also been identified in the gene coding for IL-1Ra. One such polymorphism consists of a SNP within exon 2 at nucleotide position +2018 [115] while a well studied variation consists of a variable number of tandem repeats (VNTR) in intron 2 that is associated with variability in circulating levels of IL-1Ra and IL-1β [116-119]. In regards to the VNTR variation, most healthy individuals have either 4 copies (53%) (referred to as the A4 allele) or 2 copies (34%) (referred to as the A2 allele) of the 86 bp repeat [120]. The A2 allele is associated with higher serum levels of IL-1Ra and lower levels of IL-1α. In addition, mononuclear cells isolated from individuals with the A2 allele produce more IL-1Ra in vitro [116, 117].

Several of the IL-1 polymorphisms have been examined for association with susceptibility to, and outcome from, sepsis. The IL-1Ra VNTR polymorphism is associated with a number of diseases in which inflammation plays a key role [121-125]. A higher frequency of the IL-1Ra A2 allele was demonstrated in Caucasian adults with severe sepsis compared with the frequency in a healthy Caucasian population [120], but there was no association with higher mortality within the population with sepsis. Similarly, a higher frequency of the IL-1Ra A2 allele in a cohort of Chinese adults with sepsis was observed [126] as well as a higher mortality in adults with severe sepsis [122]. In contrast, a higher frequency of the A2 allele was not observed in a cohort of children with meningococcal disease compared with a healthy control population [127]. In a large cohort of predominantly pediatric patients with meningococcal disease, those with the IL-1Ra +2018 polymorphism had a higher mortality [128], however, this polymorphism is in linkage disequilibrium with the IL-1Ra gene A2 allele and, thus, may be a marker for the A2 allele which appears to be associated with poor outcome, as described above. Finally, our group has found an association between the presence of the A2 allele and more severe lung disease in a large cohort of children with community-acquired pneumonia [125]. Thus, despite some conflicting findings, genetic variability in the IL-1 locus, particularly the IL-1Ra A2 allele, appears to place patients at greater risk for the development of sepsis and perhaps at greater risk for mortality.

**Interleukin 6 (IL-6)**

IL-6 is another pro-inflammatory cytokine induced by TNF-α that stimulates both B- and T-lymphocytes and is involved in the induction of fever and the hepatic acute phase protein synthesis. Along with TNF-α and IL-1, it is believed to be a key mediator in the response to stress and pathophysiology of multi-organ failure. High serum levels of IL-6 have been correlated with severe sepsis and worse outcome from sepsis [129-137].

The gene coding for IL-6 has several genetic variations in the promoter region [138] and coding sequences. A common polymorphism located at position -174 in the promoter region is a G to C substitution, and in vitro studies demonstrate an association of the G allele with increased expression of IL-6 [139]. In addition, serum levels of IL-6 are lower in adults with sepsis with the C allele [140] and in healthy adults, the G allele is associated with higher basal serum levels of IL-6 [139]. In contrast, monocytes isolated from neonates with the C allele and stimulated with LPS
produce more IL-6 than monocytes isolated from neonates with the G allele [141], the opposite of what is observed in adults. In this age group, the C allele also appears to be associated with a greater rise in IL-6 levels during an acute inflammatory response. Such differences may be explained by changes in the immune response which occur during development.

Genetic association studies have demonstrated mixed results in regards to the influence of IL-6 polymorphisms in patients with sepsis. In adult surgical patients with sepsis, no association between the genotypes and serum levels of IL-6 is demonstrated, nor are there significant differences in genotype distribution between critically ill patients and healthy controls, or between patients with or without sepsis [131]. A significantly lower frequency of the -174 GG genotype is associated with higher mortality in septic patients compared with surviving septic patients, suggesting that the GG genotype is in some fashion protective, or conversely carriagge the C allele places individuals at risk. Indeed, in cohort of adult septic patients, the CC genotype was more frequent in those with shock [140]. Other SNPs also exist in the gene coding for IL-6 allowing for the analysis of haplotypes or combinations of various SNPs. One such group of haplotypes consists of polymorphisms at the -174 (G/C), +1753 (C/G), and +2954 (G/C) nucleotide positions. In a cohort of critically ill adult patients with SIRS, while no association between each of the SNPs individually and worse clinical outcomes is observed, individuals with the C/C/G, G/G/G or G/C/C haplotype demonstrate a higher mortality and more organ dysfunction [142]. In addition to suggesting that genetic variations within the IL-6 gene influence the severity of sepsis, these observations support the concept that in some instances haplotype analysis may be more valuable in identifying genetic associations compared with individual SNP analysis.

Studies in premature infants on the influence of the IL-6 polymorphisms in patients with sepsis find quite results. The -174 G allele associated with low levels of IL-6 appears to be associated with sepsis in Caucasian premature neonates [143, 144] while in an African-American cohort, it is the C allele that demonstrates this association [145]. Furthermore, a recent meta-analysis suggests that the IL-6 -174 polymorphism is not strongly associated with the risk of sepsis in this age group [146].

Interleukin-10 (IL-10)

The anti-inflammatory cytokines provide a counterbalance to the pro-inflammatory cytokines, dampening the inflammatory response and helping to protect the host from the detrimental effects of the inflammatory mediators. In addition to the anti-inflammatory cytokine IL-1RA discussed previously, they include IL-10 and transforming growth factor (TGF) [9, 147, 148]. IL-10 is produced primarily by monocytes and down regulates the expression of a wide range of cytokines [149, 150]. Neutralization of IL-10 results in an exaggerated pro-inflammatory response and death in animal models, while administration of IL-10 confers protection [9, 151, 152]. However, over-expression of IL-10 may induce immunosuppression in bacterial sepsis and increase mortality by inhibiting bacterial clearance [153, 154].

IL-10 production appears to be regulated primarily at the transcriptional level. Three SNPs located in the promoter region upstream from the transcriptional start site, at positions -1082 (G/A), -819 (C/T), and -592 (C/A), have been shown to affect IL-10 expression [155-157]. The -1082G/A substitution occurs within a putative Ets transcription factor binding site, the -819C/T lies within a putative positive regulatory region, and the -592C/A polymorphism lies within a putative STA 3 binding site and a negative regulatory region [156, 158]. There is significant linkage disequilibrium between the alleles at the -819 and -592 sites. Therefore, only 4 possible haplotypes of these 3 polymorphisms can occur (-1082/-819/-592): GCC, ACC, GTA, and ATA. The GCC/GCC haplotype is associated with higher IL-10 production in stimulated peripheral blood mononuclear cells [157]. Likewise, in studies in which only single SNPs were examined, stimulated whole blood from individuals with the G allele at the IL-10-1082 site [159] or the C allele at the IL-10-592 site [160] resulted in higher amounts of IL-10 compared with whole blood from individuals lacking these alleles. In addition, when the promoter regions from individuals homozygous for the 3 haplotypes GCC, ACC, and ATA were cloned into a luciferase vector and transiently transfected into cells, the GCC construct demonstrated significantly increased transcriptional activity compared to the ATA construct, while the ACC construct demonstrating an intermediate transcriptional activity [161]. Thus, in vitro evidence demonstrates that genetic variation in the promoter region of the gene coding for IL-10 influences the amount of IL-10 produced.

Excess levels of IL-10 may play a role in immunosuppression in bacterial sepsis [153], and high levels are associated with higher mortality in pneumococcal pneumonia [154] and more severe disease in adults with community-acquired pneumonia [162]. Given the associations between the IL-10 promoter haplotypes and higher IL-10 secretion, and high IL-10 levels and more severe disease, it follows that the IL-10 “high-secretor” haplotypes would be associated with more severe disease. However, genetic association studies have yielded conflicting results. The “high secretor” G allele at position -1082 was associated with more severe disease when compared with the “low secretor” A allele in a cohort of adults with community-acquired pneumonia [163]. In a cohort of patients with culture proven pneumococcal disease, 54% of those patients who developed septic shock had the “high secretor” G allele while only 16% of those patients who did not develop septic shock had this allele [159]. At the IL-10-592 site, there was no increased risk of sepsis in a cohort of critically ill adults with the “low secretor” A allele at the -592 site, but the mortality was higher in patients with the genotype [160]. In contrast, no association was observed in a cohort of adult critically ill patients between the IL-10 haplotypes and either serum IL-10 levels or mortality [164]. The conflicting results in these studies may be due to the types of patients enrolled in the studies as some cohorts had ongoing infectious processes [159], while other cohorts had a variety of illnesses not restricted to infectious etiologies [164]. Thus, additional association studies between the IL-10
regulatory polymorphisms and outcome are warranted to further delineate the influence of these genetic variations in sepsis.

**EFFECOR MOLECULES INVOLVED IN THE IMMUNE RESPONSE**

A number of other molecular and cellular systems enhance the host response to severe infections. The following section reviews the evidence that genetic polymorphisms in genes coding for other effector molecules influence the susceptibility to and outcome from sepsis.

**F \( \gamma \) \( \rho \) \( \chi \) \( \epsilon \) \( \pi \) \( \eta \) **

F \( \gamma \) receptors are glycoproteins located on the cell membrane of leukocytes that link the humoral and cellular components of the immune system by binding to the constant region of IgG and triggering a number of effector functions including phagocytosis of IgG-coated bacteria and induction of an inflammatory response [165, 166]. Three classes of human F \( \gamma \) receptors exist: the FcRI class consisting of the FcyR1a receptor, the FcRRI class consisting of FcyR1a, FcyR1b, FcyR1c receptors, and the FcRRII class consisting of FcyRIIa and FcyRIIib receptors. The cellular distribution and affinity of these receptors for the various subclasses of IgG vary.

Genetic variations including single nucleotide polymorphisms and copy number variants (CNV) that influence receptor function have been described in the genes coding for F \( \gamma \) receptors [166-168]. The FcyRIIa gene has a T to G substitution at nucleotide 559 that results in a valine (V) or phenylalanine (F) at amino acid 158, which in turn affects its affinity for IgG1, IgG3, and IgG4 [167, 169]. NK cell activity is increased in individuals homozygous for the V at this position compared with those individuals with the F. The FcRIIib gene has a polymorphism in the extracellular domain that results in a four amino acid substitution in the FcR domain that results in a four amino acid substitution in the FcRIIb protein (alleles FcyRIIib-NA1 or -NA2). These amino acid substitutions result in differences in FcRIIib receptor glycosylation [170] and efficacy of phagocytosis of IgG1 and IgG2-opsonized particles. Individuals homozygous for the FcRRIIib-NA1 allotype appear to have more efficient phagocytosis than individuals with the FcRRIIib-NA2 allotype [171, 172]. The gene coding for the FcRRIa receptor displays a G to A nucleotide substitution that changes an arginine at amino acid position 131 (R131 allele) in the extracellular domain of the receptor to a histidine (H131 allele). The functional significance of this change is that the FcRI-I131 allele binds the Fc portion of IgG2 with lower affinity than the more common FcRIIa-H131 allele [173-175]. The FcRRIa receptor is the only F \( \gamma \)R able to bind efficiently to the IgG2 subclass; hence, the FcRRIa genotype determines an individual's ability to bind IgG2. The functional consequence of this genetic variation is demonstrated in in vitro studies that demonstrate reduced phagocytosis of IgG2 opsonized particles in cells from individuals homozygous for FcRRIa-R131 compared to cells from individuals homozygous for FcRRIa-H131 [176, 177]. As IgG2 is the main antibody subtype directed against encapsulated bacteria such as Streptococcus pneumoniae, Haemophilus influenzae type b, and Neisseria meningitides and plays an important role in their phagocytosis, one could speculate that individuals with the FcRIIa-R131 polymorphism may have more severe infections due to these bacteria.

Genetic association studies have suggested that the FcRIIa-R131 and/or the FcRIIib-NA2 polymorphisms are associated with an increased susceptibility to infections by encapsulated bacteria. Most studies have demonstrated higher frequencies of the FcRIIa-R131/R131 or FcRIIib-NA2/NA2 genotypes in patients with meningococcal disease [178-184] particularly in patients with severe meningococcal disease or fulminant meningococcal septic shock. The FcRIIa polymorphism has also been demonstrated to be associated with infections due to other encapsulated bacteria [185, 186]. However, not all such studies have reported an association [187, 188].

**Bactericidal Permeability Increasing Protein (BPI)**

Several proteins bind bacterial toxins and play important roles in intracellular signaling that result in an appropriate response to pathogens. One example is BPI, a component of the azurophilic granules of neutrophils that binds the lipid A portion of LPS thereby attenuating the LPS-stimulated response [189]. BPI is also bactericidal against gram-negative bacteria [190] and increases the permeability of the bacterial cell membrane [191]. Administration of LPS to healthy adults increases BPI, and elevated serum levels of BPI are found in adults with sepsis [192]. The gene coding BPI contains a number of genetic polymorphisms including a G to C substitution at position 545 and an A to G substitution at position 645. The G545C variation is silent in that there is no change in the amino acid sequence of the final BPI protein. The A645G, however, changes a lysine at amino acid position 216 to glutamic acid. The gene coding for LBP also contains several genetic variations including a T to G at nucleotide position 292 and C to T at nucleotide position 1306 thereby changing the amino acids cysteine at position 98 to glycine and proline at position 436 to leucine, respectively [193].

Genetic association studies include a large cohort of critically ill children with either gram-negative or gram-positive infections in which the G allele at 545 but not the variation at position 645 was associated with sepsis when compared with healthy controls. When only those children with gram-negative infections were analyzed, a significant association between children with the GG genotype at 545 and the AG or GG genotype at 645 with the development of sepsis was identified [194]. A similar study in adults with sepsis did not find such an association in the gene coding for BPI but did find that the rarer alleles in the LBP gene were associated with sepsis; furthermore, males who were homozygous for either of the rarer LBP had a worse outcome [195]. It should be pointed out that despite the G545C polymorphism not resulting in a change in the amino acid sequence of the BPI protein, it may be in linkage disequilibrium with some as of yet unidentified variation that may be the causative or function variation. No similar studies have been performed in children with sepsis.

**Heat Shock Proteins (HSP)**

Heat shock proteins (HSP) are a highly conserved family of stress-inducible proteins that play a key role as intracellular chaperones to a variety of proteins; that is, they
prevent proteins from aggregating into nonfunctional complexes during stress. They are produced in response to heat, endotoxin, and other noxious stimuli [196]. These proteins are essential for cell survival during stress [197] and are involved in a number of important cellular functions including the folding, assembly, and translocation of proteins across membranes [198, 199]. One such HSP, HSP70, also appears to act as an inhibitor of cytokines and other inflammatory mediators produced via the NFκB pathway [200]. The 3 genes coding for family of HSP70 genes (HSPA1B, HSPA1A, and HASA1L) lie in the major histocompatibility complex III region on chromosome 6 near the genes coding for TNF-α and complement [201, 202]. Polymorphisms have been described in the gene coding for the HSPA1B that appear to influence the amount of HSP produced [203]. The A allele at the +1267 position appears to be in strong linkage disequilibrium with the C allele at position -179, and the -179C/+1267A haplotype is associated with significantly lower production of HSPA1B and TNF-α in response to LPS when compared with other haplotypes [204, 205]. While no association between the genotypes at the +1267 site and susceptibility to, or outcome from, severe sepsis was demonstrated in adult surgical patients [206], we have demonstrated that in adults with community-acquired pneumonia, those individuals with the AA genotype at the +1267 site were at greater risk for septic shock but not increased mortality than those individuals who were either heterozygous or homozygous for the more common G allele [98]. Further studies examining genetic variations in the HSP family of genes in children with sepsis are currently being performed.

**Dimethylarginine Dimethylaminohydrolase (DDAH)**

Endothelium-derived nitric oxide (NO) is a potent vasodilator synthesized from L-arginine by the enzyme nitric oxide synthase (NOS) and has been implicated in the pathophysiology of the hypotension observed in severe sepsis [207]. DDAH metabolizes asymmetrical dimethyl arginine (ADMA), a naturally occurring inhibitor of NOS, to citrulline [208]. Variation in the gene coding for DDAH may, therefore, alter the amount of the inhibitor available for NOS. Elevated ADMA is associated with more severe organ failure in patients with sepsis [209, 210]. Genetic variations the gene coding for DDAH have been found including polymorphisms in the promoter region that appear to influence transcription of DDAH [211]. In a small cohort of adults with severe sepsis, the frequency of the G allele at position -449 in the promoter region was greater in those adults with severe sepsis compared with healthy controls, and serum levels of the inhibitor, ADMA, were elevated [212].

**Angiotensin I Converting Enzyme**

Angiotensin I converting enzyme (ACE) is found on both endothelial and epithelial cells of various organs and is primarily responsible for converting angiotensin I to angiotensin II. ACE is also involved in the metabolism of chemotactic peptides suggesting that it may play a role in the inflammatory response. Individuals have been shown to have variable plasma and tissue levels of ACE, and evidence suggests that these variable levels are due in part to genetic factors [213]. Specifically, an insertion (I)/deletion (D) of a 287 base repair repeat sequence in the noncoding intron 16 of the gene coding for ACE [214] is associated with variable plasma levels; individuals homozygous for the deletion genotype (DD) have higher plasma and tissue levels of ACE compared with individuals who are homozygous for the insertion sequence (II) or are heterozygous (DI) [215, 216]. The mechanism by which the deletion of this sequence is associated with increased ACE levels is still controversial [217, 218] but may involve transcriptional regulation [219]. The deletion of the sequence may increase transcription by removing a binding site for a transcription repressor.

The association of the ACE I/D polymorphism with clinical outcomes in sepsis has been examined in a few studies. The D/D polymorphism was associated with more severe meningococcal disease in children as measured by a higher predicted risk of mortality, greater prevalence of inotropic support and mechanical ventilation, and longer intensive care unit stay [220]. In addition, the frequency of the D/D genotype was more frequent in those children who died compared with survivors, though this did not reach statistical significance. In contrast, no significant difference was observed either in the susceptibility to blood stream infection or sepsis-related mortality in of mechanically ventilated infants [221] or mortality from sepsis in adults [222].

**Other Mediators and Factors Involved in the Inflammatory Response**

Many other factors are involved in the host response to infections, and investigators are beginning to examine whether genetic variations in the genes coding for these factors also influence the susceptibility to or outcome from sepsis. Some of these are briefly mentioned here.

**Macrophage migration inhibitory factor (MIF)** is produced by a number of cell types and plays an important role in the pathogenesis of acute and chronic inflammatory and autoimmune disorders. In addition to stimulating both TNF-α and IL-8, it is able to override the anti-inflammatory and immunosuppressive effects of glucocorticoids [223-225]. Serum MIF levels are elevated in sepsis and correlate with severity of disease [226, 227]. Haplotypes in the gene coding for MIF but not individual SNPs are associated with sepsis in both a Caucasian and African American population of adults [228].

While interferon-gamma (IFNγ) is primarily involved in the host defense against viral infections, it also modulates components of the inflammatory response in response to bacterial toxins such as LPS. Co-stimulation of peripheral blood mononuclear cells with TNF-α and INFγ increases expression of complement factor B which is involved in activating the alternative complement pathway that plays a role in inflammation, bacterial cytotoxicity, and phagocytosis [229]. Genetic variations in the gene coding for INFγ are associated with elevated levels of INFγ [230, 231] and the development of sepsis in adult trauma patients [232].

Mitochondrial energy production is crucial during sepsis in order to maintain normal cellular functions as well as mount an effective immune response. Recently, a genetic variation in mitochondrial gene NADH dehydrogenase 1 (ND1) believed to impair ATP production [233] has been associated with susceptibility to infectious complications and
mortality in a cohort of adult trauma [234] and burn patients [235].

GENETIC VARIATION IN THE HOST COAGULATION SYSTEM

Derangement of the coagulation system leading to intravascular fibrin deposition is a key contributor to the pathogenesis of multi-organ failure in sepsis [236]. Plasma levels and activity of procoagulants such as tissue factor and inhibitors of fibrinolysis such as plasminogen activator inhibitor 1 (PAI-1) are elevated [237-239] while levels and activity of anticoagulants such as antithrombin III and protein S are low and activation of protein C is impaired [240, 241]. Thus, a procoagulant environment exists within the intravascular space.

There is significant crosstalk between inflammation and coagulation. Activation of the coagulation cascade, leads to inflammatory events. Coagulation of blood in vitro increases the concentrations of IL-6, IL-8, and TNF- by release of these cytokines from peripheral blood monocytes and endothelial cells [242-245]. In animal models of septic shock, lack of tissue factor, an endogenous activator of coagulation, leads to decreased mortality [246], whereas deficiency of thrombomodulin [247] and components of the protein C pathway, which is an endogenous regulator of coagulation, leads to increased mortality. Common genetic variations within genes of the coagulation cascade and the protein C pathway have been well characterized and a number of single nucleotide polymorphisms (SNPs) and haplotypes in these genes are known to alter levels of their respective mediators [248]. Therefore, it is biologically plausible that some of these polymorphisms are associated with clinical outcomes in sepsis. A few examples demonstrating the influence of genetic variation on the severity of sepsis in genes coding for components of the coagulation pathway will be discussed below.

Protein C

Endogenous protein C levels are depressed in patients with severe sepsis, and plasma protein C levels inversely correlate with morbidity and outcome of sepsis patients, regardless of age, infecting microorganisms, presence of shock, or severity of illness. Low plasma protein C levels have been correlated with increased severity of illness and poor outcome in adults and children with meningococcal sepsis [249, 250]. Administration of activated protein C leads to a reduction in mortality in adults with sepsis [251].

Among patients with meningococcaemia, those carrying the CG genotype had higher odds of developing sepsis, lower systolic blood pressure, and higher need for adrenergic support [255]. Therefore, the protein C promoter SNPs were not only associated with increased susceptibility to meningococcal infection, but also with severity of the disease.

Other studies also support the influence of these genetic polymorphisms on the severity of sepsis. In a cohort of Han Chinese patients with severe sepsis, the CA haplotype of the protein C promoter has been demonstrated to be associated with increased risk for death and organ dysfunction [256]. In contrast, the haplotypes of these two polymorphisms were not associated with the severity of sepsis in a Caucasian adult cohort. The AA genotype of the -1641AG polymorphism was associated with decreased 28-day survival in an initial derivation cohort, and in a larger replication cohort. In addition, the protein C -1641 AA genotype was also associated with significantly more organ dysfunction and more clinical evidence of systemic inflammation in patients undergoing cardiopulmonary bypass [257]. A study of North American East Asians with severe sepsis demonstrated that the C allele of a C/A SNP at the 673 position was found to be associated with increased mortality and organ dysfunction [258]. Interestingly, the SNP is in complete linkage disequilibrium with the CA haplotype [258].

Thus, genetic variations in protein C gene appear to be associated with clinical outcomes in sepsis. The differences in the specific haplotypes and genotypes associated with outcomes in each of these studies can be attributed to the varying allele frequencies of these SNPs among the ethnic groups studied. The results of these studies also suggest that the association of decreased plasma protein C levels with clinical outcomes in sepsis may be partly due to the underlying genetic differences among individuals. It is therefore plausible that the response to treatment with activated protein C among patients with sepsis may be governed in part by the underlying genotype. Studying these genotype-treatment interactions in future trials of activated protein C in sepsis may lead to identification of the patient groups most likely to benefit from activated protein C treatment.

Fibrinogen

Fibrinogen and fibrinogen degradation products are potent chemo-attractants and induce release of interleukin-8 from neutrophils [259-261]. Fibrinogen also increases binding of neutrophils to endothelial cells through ICAM-1, thereby increasing neutrophil migration [262, 263]. Genetic factors are believed to contribute up to 50% of the total variability in fibrinogen plasma levels [264] and appear to be associated with more severe sepsis. For example, the GAA haplotype in the gene coding for the fibrinogen beta chain defined by SNPs at positions -854, -455, and +9006 is associated with a lower mortality and trend towards less organ dysfunction in Caucasian patients with sepsis. The association with lower mortality is independent of age and APACHE II score at admission [265]. The association with reduced mortality may be related to the increased fibrinogen
<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphisms</th>
<th>Consequence of polymorphism</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR4</td>
<td>asp299Gly</td>
<td>299Gly/399Ile associated with decreased expression of TLR4 and response to LPS, and increased risk of sepsis and mortality</td>
<td>[18, 19, 21-26]</td>
</tr>
<tr>
<td></td>
<td>thr399Ile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD-14</td>
<td>-159 C/T</td>
<td>T allele associated with increased levels of CD-14, increased susceptibility to sepsis, and increased mortality in adults with sepsis</td>
<td>[33, 41-44]</td>
</tr>
<tr>
<td>MD-2</td>
<td>+103 A/G (thr35ala) -1625 C/G</td>
<td>+103G allele associated with increased TNF-α; -1625G allele associated with increased MD-2 mRNA and TNF-α and increased risk of sepsis and multiple organ dysfunction in Chinese adults;</td>
<td>[47, 48]</td>
</tr>
<tr>
<td>TLR2</td>
<td>-16933 T/A</td>
<td>A associated with increased gm+ bacterial sepsis</td>
<td>[33]</td>
</tr>
<tr>
<td>TIRAP/Mal</td>
<td>ser180leu (rs8177374)</td>
<td>180leu associated with decreased risk of severe pneumococcal infection</td>
<td>[49]</td>
</tr>
<tr>
<td>IRAK-1</td>
<td>+1595 T/C</td>
<td>C associated with increased NF-KB translocation, increased risk of pneumococcal sepsis and mortality</td>
<td>[50]</td>
</tr>
<tr>
<td>NK-1KB</td>
<td>rs3138053</td>
<td>Less common alleles associated with decreased risk of invasive pneumococcal disease</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>rs2233406</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBL</td>
<td>variant B, C, D</td>
<td>variants associated with decreased levels and activity and increased risk of meningococcal and pneumococcal infections</td>
<td>[64, 66-69, 71, 74, 278]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-308 G/A, -238 G/A, LT-α+250 G/A</td>
<td>-308A and +250A associated with increased TNF-α levels, increased mortality in sepsis and meningococcal disease, increased sepsis in adults with pneumonia, and increased mortality in bacteremia and sepsis; -238A associated with decreased TNF-α levels and increased mortality in adults</td>
<td>[83, 90, 94, 95, 97, 279, 280]</td>
</tr>
<tr>
<td>IL-1α</td>
<td>-889 C/T</td>
<td>T associated with increased IL-1α transcription and protein levels</td>
<td>[109, 110]</td>
</tr>
<tr>
<td>IL-1β</td>
<td>-3737 C/T</td>
<td>Various haplotypes associated with variable IL-1β levels [112] (see for review)</td>
<td>[112-114]</td>
</tr>
<tr>
<td></td>
<td>-1464G/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-511 C/T</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-31 T/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1ra</td>
<td>variable 86-bp repeat +2018 “2 allele”</td>
<td>A2 allele associated with variable levels of IL-1ra; A2 associated with increased risk of in adults but not children with sepsis; A2 associated with increased mortality in adults with sepsis; +2018 “2 allele” associated with increased mortality in children with meningococcal disease and more severe lung disease in children with pneumonia</td>
<td>[120, 122, 125-128]</td>
</tr>
<tr>
<td>IL-6</td>
<td>-174 G/C</td>
<td>G associated with increased IL-6 levels in adult patients but decreased levels in neonates; C associated with increased levels in monocytes from neonates, sepsis in neonates, and severe sepsis and organ dysfunction in children</td>
<td>[130, 142-146, 281]</td>
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</tbody>
</table>
### Table 1. Contd...

<table>
<thead>
<tr>
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<th>Polymorphisms</th>
<th>Consequence of polymorphism</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>IL-10</td>
<td>-1082 G/A -819 C/T -592 C/A</td>
<td>GCC haplotype associated with increased levels and risk of sepsis but not mortality</td>
<td>[157, 159, 282]</td>
</tr>
<tr>
<td>FcRHa</td>
<td>H131R</td>
<td>R associated with decreased affinity to IgG; and opsonization and increased risk of infection and septic shock</td>
<td>[178-181, 183-186]</td>
</tr>
<tr>
<td>BPI</td>
<td>545 G/C 654 A/G</td>
<td>545 G and 645 G associated with increased risk of sepsis in children with gm- infections;</td>
<td>[194]</td>
</tr>
<tr>
<td>HSP70A1B</td>
<td>-179 C/T +1267 G/A</td>
<td>-179C/+1267A associated with decreased HSPA1B and TNF-α; -1267A associated with septic shock in adults with CAP</td>
<td>[98, 204, 205, 283]</td>
</tr>
<tr>
<td>DDAH</td>
<td>-449 G/C (rs805305) -871 6G/7G variant</td>
<td>-871 7G associated with increased transcription; -449 G alleles associated with increased severity of sepsis</td>
<td>[211, 212]</td>
</tr>
<tr>
<td>ACE</td>
<td>287 base pair I/D</td>
<td>DD associated with increased serum and tissue levels and more severe meningococcal disease in children; no association of sepsis-related mortality in neonates or adults</td>
<td>[215, 216, 220-222]</td>
</tr>
<tr>
<td>MIF</td>
<td>specific haplotypes</td>
<td>specific haplotypes associated with sepsis and acute lung injury in adults</td>
<td>[228]</td>
</tr>
<tr>
<td>IFNγ</td>
<td>+874 T/A variable length CA repeat</td>
<td>+874T and allele 2 associated with increased IFNγ; specific alleles of the CA repeat associated with increased risk of sepsis</td>
<td>[230-232]</td>
</tr>
<tr>
<td>HSP70A1B</td>
<td>-179 C/T +1267 G/A</td>
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<td>[230-232]</td>
</tr>
<tr>
<td>ND1</td>
<td>+4216 T/C</td>
<td>C allele associated with increased risk of sepsis and mortality in adults with trauma and burns</td>
<td>[234, 235]</td>
</tr>
<tr>
<td>Protein C</td>
<td>-1641 A/G -1654 C/T</td>
<td>AA at -1641 associated with decreased survival and increased organ dysfunction in adults with sepsis; GC haplotype associated with more severe sepsis in children with meningococccemia; CA haplotype in Chinese adults associated with increased mortality in sepsis</td>
<td>[255-257]</td>
</tr>
<tr>
<td>Fibrinogen-beta</td>
<td>-854 G/A -455 G/A +9006 G/A val34leu</td>
<td>GAA haplotype associated with lower 28 day mortality and less severe organ dysfunction; val34leu variant associated with increased risk of sepsis in neonates</td>
<td>[265, 266]</td>
</tr>
<tr>
<td>PAI-1</td>
<td>4G/5G</td>
<td>4G associated with increased levels and septic shock in meningococcal disease</td>
<td>[269-274]</td>
</tr>
<tr>
<td>CXCL2</td>
<td>short tandem repeat in promoter</td>
<td>associated with increased mortality in adults with sepsis</td>
<td>[284, 285]</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>-717 A/G +1059 G/C +1444 C/T GT repeat in first intron</td>
<td>-717A associated with increased mortality; GT repeat associated with susceptibility to invasive pneumococcal disease</td>
<td>[286, 287]</td>
</tr>
</tbody>
</table>
levels among patients with the GAA haplotype since the ~455 A allele within the haplotype is associated with increased transcription. Another polymorphism in the fibrinogen gene, the Val34Leu polymorphism is known to alter the fibrin meshwork with variable permeation characteristics. In a multi-center study, carriage of the factor XIII-Val34Leu polymorphism was associated with a higher rate of sepsis among very low birth babies of mixed European descent [266]. While the exact mechanism for this association is not known, the authors suggest that the alteration in fibrinogen structure may have potentially resulted in alterations in blood viscosity and increased chemo attraction of inflammatory cells.

**Plasminogen Activator Inhibitor-1 (PAI-1)**

Plasminogen activator inhibitor 1 (PAI-1) is the primary inhibitor of plasminogen activator, the proteolytic activator of plasminogen; hence, PAI-1 is a potent inhibitor of fibrinolysis. A single nucleotide insertion/deletion polymorphism exists within the promoter region of the PAI-1 gene that is involved in the regulation of PAI-1 gene expression. In *in vitro* studies have demonstrated that the allele consisting of 5 guanines (5G) is associated with 6 times more PAI-1 mRNA than the allele consisting of 5 guanines (5G) in response to IL-1β [267]. The higher PAI-1 expression associated with the 4G allele appears to be due to the inability of a transcriptional repressor protein to bind to the 4G allele [268]. Individuals homozygous for the 4G allele produce more PAI-1 than either individuals heterozygous (4G/5G) or homozygous for the 5G allele [268]. In children with meningococcal disease, those with the 4G/4G genotype had higher plasma levels of PAI-1 [269] and a higher mortality compared with children with either the 4G/5G or 5G/5G genotypes [269-271]. Other adult and pediatric studies also demonstrate a higher mortality in those individuals homozygous for the 4G allele genotype in a variety of infectious diseases [272-274]. In contrast, other studies have not demonstrated a higher mortality in children with meningococcal disease [275] or adults with various gram negative infections [28]. Thus, there appears to be a strong association between the 4G/4G genotype in the PAI-1 gene, high plasma concentrations of PAI-1, and higher mortality in sepsis.

**LIMITATIONS OF GENETIC ASSOCIATION STUDIES**

Genetic association studies have several limitations that should be noted and care should be taken when assessing the findings of such studies [276, 277]. One concern that appears in many studies examining whether polymorphisms increase the risk of sepsis, is that the frequency of the polymorphism in the cases (those patients with sepsis) is compared with the frequency of the polymorphism in a healthy control population. However, the control population may not have been exposed to the same pathogens to which the patients with sepsis were exposed. One cannot conclude from such a comparison that the group that developed sepsis was at an increased risk of developing sepsis per se without the control group being similarly exposed. Rather, a more appropriate control group for comparison would be a group of patients with a similar infection who did not develop sepsis. Furthermore, the control group should be of the same ethnicity as the cases. It is now well known that the frequency of many of these polymorphisms varies between ethnic groups and so comparisons should only be made within the same ethnic groups. A second point to keep in mind is that in most cases, investigators describe the association between a specific genetic polymorphism and susceptibility to, or outcome from, sepsis. However, the specific nucleotide variation being investigated may in fact not be involved but rather is in strong linkage disequilibrium with the actual genetic variation causing the association. Third, most studies fail to correct for multiple comparisons; that is, if a study assessed the association of more than 1 genetic variation, then there should be a correction for the increased risk of a false-positive error occurring. Finally, many studies do not discuss the validity of the genotyping technique utilized, what quality control measures were used, and whether those individuals performing the genotyping assays were blinded to the clinical outcomes data of the subjects.

**CONCLUSIONS**

In summary, genetic variability in the host appears to play an important role in the severity of sepsis. By altering the ability of the host to recognize a pathogen, or by altering the intensity of the inflammatory response, genetic polymorphisms influence the clinical presentation and outcome of sepsis. In some types of infectious diseases common in intensive care units, genetic polymorphisms may increase the risk of developing more severe sepsis and shock. However, there are few studies in children with sepsis that have been performed to support this notion. These types of studies may not only help better understand why the mortality in sepsis remains high but they may also help identify novel therapeutic targets and identify individual children who are at greater risk for more severe sepsis.

**ACKNOWLEDGEMENTS**

Supported in part by the Children’s Research Institute and the Medical College of Wisconsin.

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The Open Inflammation Journal, 2011 Volume 4


