Genomic polymorphisms in sepsis

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Objective: This article aims to review all relevant genetic polymorphism studies that may contribute to the pathogenesis of sepsis with emphasis on polymorphisms of the innate immunity, pro- and anti-inflammatory cytokines, and coagulation mediators.

Data Source: Published articles reporting on studies of associations between genetic polymorphisms, sepsis, septic shock, and other relevant infectious disease models.

Data Analysis: Research into the pathogenesis of sepsis has led to the development of many potential therapeutic strategies. Several therapeutic agents and treatment modalities have been shown to decrease mortality rates in large, prospective, and randomized clinical trials. However, although these advances have resulted in improved survival for certain patient populations, the overall mortality rate for septic patients remains high. With the rapid development of molecular and genetic techniques, substantial interests have developed in using genomic information to define disease-mediating genetic variants in sepsis. Combined with microarray technology, it is anticipated in the near future that one will be able to tailor drug selection and dosage and predict outcome by correlating genetic profile with disease presentation. Numerous genetic association studies in sepsis have already been reported and more are likely to be published.

Conclusions: Although studies examined in this review are of small heterogeneous populations, the identification of strong associations between certain genetic polymorphisms and increased mortality rate or susceptibility to severe sepsis is intriguing and supports further research using this approach. The establishment of these associations does not equal causation, and further research is required in both genetic and molecular aspect of sepsis.

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Sepsis is an infection-initiated, inflammation-induced syndrome. It is considered severe when it is associated with organ dysfunction (1). In the United States, it is estimated that approximately 666,000–750,000 cases of severe sepsis occur annually (2, 3). The mortality rates range from 15% to 80% (2, 4). This wide range reflects the complex pathophysiology and heterogeneity of sepsis and is perhaps the reason why effective treatment for sepsis has been slow in coming despite the myriad of clinical trials. With recent advancements in supportive care, mortality rates in subgroups of patients with sepsis-induced organ failure have been reduced (5–7). In addition, research into the pathogenesis of sepsis has led to the development of potential therapeutic strategies, and several agents have now been shown to decrease mortality rates in large, prospective, and randomized clinical trials (8–10). However, although these advances have improved survival for certain patient populations, the overall mortality rate for septic patients remains high. The rapid advancement of molecular biology leading to near completion of the Human Genome Project has resulted in substantial interest in using genomic information to define disease-mediating genetic variants in sepsis. It is anticipated that these new research techniques will lead to new therapeutic strategies and better prognostic indicators.

Multiple studies have supported genetic contributions to infectious disease susceptibility (11, 12). Although traditional linkage analysis was able to detect genetic loci in single-gene disorders, the linkage analysis to sepsis has been limited by the modest contribution of individual genetic variants, the lack of clear genetic segregation, and the susceptibility at extremes of age (13). Despite these difficulties, genome-wide based association studies are now viewed as essential for uncovering the genetic component of “complex diseases.” Because of the need for high-density genetic markers in studying multifactorial disease, single nucleotide polymorphisms (SNPs), as simple bi-allelic codominant markers, have gained popularity over the more traditional markers, such as restriction-fragment length polymorphisms and the abundant highly polymorphic microsatellite variable number tandem repeats (13). SNPs are single-base differences in the DNA sequence that can be observed between individuals in the population at an average frequency of approximately 1 per 1,000 base pair. A polymorphism has been defined as the least common allele occurring in ≥1% of the population (14).

The traditional approach for finding the genetic basis of disease has been the case-control designs in which a difference in allele frequency is sought between affected individuals and unrelated unaffected controls. Unfortunately, conventional case-control gene association studies are limited by the potential for confounding or spurious association resulting from correlation with the true risk factor (15). A major limitation in this approach is the potential for population stratification when inappropriate patient-to-control matching occurs. Another problem is that since each marker investigated would be essentially an independent test, screening millions of markers would lead to thousands of confounding chance associations. Furthermore, associations found between disease state and polymorphic alleles do not in themselves establish causation, as it may be in link-
age disequilibrium with the true functional locus. Linkage disequilibrium arises when two alleles at different loci occur together within an individual more often than would be predicted by random chance. Functional phenotypic assessment of candidate SNP polymorphism remains controversial because it remains unclear how to interpret the finding in which the apparently same variant has very different effects in different populations (13, 16). Despite these difficulties, properly designed association studies are a powerful approach for finding genetic determinants of complex disorders (15). Coupling this with the ease with which association studies can be conducted with SNPs and the recent publication of nearly 1.4 million SNPs in the human genome (17), a wealth of association studies in sepsis have already been reported and more are likely to be published.

The purpose of this article is to summarize all relevant polymorphisms that may contribute to the pathogenesis of sepsis. Emphasis will be placed on polymorphisms in innate immunity, pro- and anti-inflammatory cytokines, and coagulation mediators. Studies of polymorphisms in infectious disease within this framework are also included for possible future studies.

**INNATE IMMUNITY**

_Bacterial Cell Wall Recognition Molecules_. The rapid response to pathogens is mediated through innate immunity. Vital to this interaction is the activation of macrophages, which engulf invading pathogens and secrete cytokines that activate the immune and coagulation cascades. Several key molecules have been characterized as important mediators of bacterial recognition. Lipopolysaccharide (LPS) binding protein (LBP) and bacterial/cidal/permeability increasing protein (BPI) are functionally related lipid-transfer proteins with high affinity for LPS (18, 19). BPI is a cationic protein produced in polymorphonuclear leukocytes whereas LBP is a plasma protein produced by the liver constitutively and in increased amounts during the acute phase response (20). BPI is cytotoxic for Gram-negative bacteria (21) but inhibits LPS delivery to the CD14 marker on monocytes (22). In contrast, LBP binding LPS in conjunction with CD14 is important in macrophage activation by LPS (23). CD14 is a membrane glycoprotein mainly expressed on the surface of macrophages and monocytes. It is a receptor for a wide variety of microbial products including LPS (24), peptidoglycans, and lipoteichoic acid (25, 26). CD14 is also found in free soluble form in plasma (sCD14) and mediates LPS activation of CD14-negative cells such as endothelial cells (27). In animal models, LBP was not required for clearance of LPS from the circulation but was essential for the rapid induction of an inflammatory response by small amounts of LPS or Gram-negative bacteria (28). Overexpression of human CD14 in transgenic mice increased their susceptibility to endotoxin shock (29). In contrast, CD14-deficient mice are hypo-responsive to LPS (30). In humans, it appears that an increase in sCD14 is associated with increase mortality rate in both Gram-positive and Gram-negative shock (31, 32).

Associations between patients with sepsis and genomic polymorphisms in CD14, LBP, and BPI have been examined (Table 1). Three bi-allelic polymorphisms in BPI including amino acid exchanges at position 216 from lysine to glutamine...
A polymorphism consisting of C to T transition at base pair −159 (C−159T) inside the CD14 promoter was identified previously, and subjects carrying the T allele were shown to have significantly higher soluble and membrane CD14 concentrations than do carriers of C allele (34, 35). In a German study, C−159T polymorphism was not associated with sepsis development or mortality rate (36). In contrast, analysis of a French patient population showed that a significantly higher proportion of septic shock patients were homozygous for T allele. Furthermore, in a multiple logistic regression model, the TT genotype was associated with an increased relative risk of death (odds ratio 5.30) independent of tumor necrosis factor (TNF) allele (37). The difference in outcome between these two studies may be due to differences in genetic makeup; however, the possibility of confounding population stratification and high variability in sepsis phenotype remains possible.

Toll-Like Receptors. Downstream from the CD14-LBP interaction, the signal transduction pathway involved in the activation of the immune system also has been the subject of intense research. The Toll-like receptors (TLRs) appear to be very important in this process. To date, ten TLRs have been described and they appear to interact with different components of the bacterial cell wall (38). Of particular interest are TLR2 and TLR4, which recognize cell wall components from Gram-positive (peptidoglycan and lipoteptides) and Gram-negative bacteria (LPS), respectively (39, 40). Mouse strain C3H/HeJ and C57BL/10ScCr have functional mutation in the TLR4 gene (Pro712His) and a deletion of TLR4, respectively. Both are hyporesponsive to purified LPS but hyporesponsive to infection by certain Gram-negative bacteria (41). Generalizing from these animal models, it is hypothesized that mutations in human TLR4 could be associated with hyporesponsiveness to endotoxin and in turn have enhanced susceptibility to Gram-negative sepsis.

Arbour et al. (42) screened the coding region of TLR4 in 83 healthy adults (31 men and 52 women) and detected an A→G substitution at nucleotide 896 from the start codon of TLR4 complementary DNA. The allelic frequency of the A896G substitution was 6.6% in this study population. This substitution resulted in a missense mutation at the amino acid 299 position with the replacement of a conserved aspartic acid residue with glycine (Asp299Gly). An additional cosegregating missense mutation (replacing a nonconserved threonine with isoleucine at amino acid 399, Thr399Ile) in the extracellular domain of TLR4 also was found. Of the 83 subjects, ten were either heterozygous or homozygous for the Asp299Gly/Thr399Ile mutations. Using an inhaled LPS challenge, these subjects had a more blunted response than the subjects with wild-type genotype. A causal relationship was further supported by in vitro data showing that cells transfected with Asp299Gly and not Thr399Ile TR4 had significantly less nuclear factor-κB activity on stimulation with LPS.

The relevance of these TLR4 mutations was further examined in patients with Gram-negative septic shock (43). In a French study comparing 91 septic patients to 73 healthy controls, no difference in prevalence of TLR4 Asp299Gly/Thr399Ile mutants was identified. Most interesting is the identification of five septic patients with only Asp299Gly mutation and none in the control group, suggesting that TLR4 Asp299Gly may be associated with Gram-negative septic shock. In contrast, examination of 1,047 patients with microbiologically confirmed meningococcal disease and 879 healthy northern English controls did not show any association between Asp299Gly TLR4 polymorphism to susceptibility or severity of meningococcal infection (44). No differences in allelic frequency were observed even when data were stratified by age and serogroup, thus excluding them as potential confounding factors. This discrepancy may again reflect the greater variability in septic phenotype.

Different from TLR4, TLR2 appears to be involved in staphylococcal signaling (45, 46). A defective TLR2 gene may predispose susceptible individuals to infection by Gram-positive organisms leading to shock syndrome that is clinically indistinguishable from shock caused by Gram-negative organisms. Lorenz et al. (47) also examined polymorphism in TLR2 gene. They screened 110 healthy controls and found that 3% of people tested had a missense mutation that resulted in an arginine to glutamine substitution at residue 753 (Arg753Gln) of the human TLR2 gene. In vitro study confirmed that cells transfected with the Arg753Gln are significantly less responsive to bacterial peptide derived from Borrelia burgdorferi and Treponema pallidum. The same patient population from the TLR4 studies was again examined. Genotype screening of this population showed that two of 91 septic shock patients (22 of whom had documented Gram-positive infection) were heterozygous for TLR2 Arg753Gln mutation. No allelic frequency for the healthy control group was reported in the study. Together, these results suggest possible associations between mutations in TLR4 or TLR2 genes and septic shock, but further studies are required.

Mannose-Binding Lectin. Killing of encapsulated organisms and other pathogens is critically dependent on complement (48), which can be activated by the classic pathway with specific antibody or by protein constituents of the innate immune system. Mannose-binding lectin (MBL) is a member of the collectin family of proteins. MBL binds to an array of carbohydrate structures on the surface of microbes and mediates an antibacterial effect either by direct killing through complement-mediated lytic membrane attack complexes or by promoting phagocytosis (49). In human and animal models, MBL deficiency appears to increase susceptibility to infections (50, 51). The amounts of MBL in human plasma are genetically determined. Point mutations within exon 1 of the MBL gene at codon 52, 54, or 57 (variant B, C, and D, respectively) resulted in amino acid substitu-
tions that compromise assembly of functional oligomers (52). Individuals heterozygous for these mutations have reduced concentrations of MBL in serum, and MBL is almost absent in homozygotes and compound heterozygotes. In addition, there are three major polymorphisms in linkage disequilibrium within the promoter region of the MBL gene that also influence the expression of these proteins (53). Positive association of these variants and susceptibility to meningococcal disease in patients with malignancy has been demonstrated (54–56). Although no studies directly addressed patients fitting the definition of sepsis, the possibility of clinically relevant MBL deficiency contributing to a worse outcome in the context of sepsis-induced immunodeficiency remains.

Immunoglobulin-γ Receptors. Antibody receptors, with their ability to activate complement, facilitate phagocytosis, and mediate antibody-dependent cellular cytotoxicity, are essential in the defense against certain microbes. Receptors of immunoglobulin G (FcγR) expressed on leukocytes constitute a heterogeneous family of membrane-bound and soluble proteins. The various FcγR subclasses of this family differ in ligand affinity and specificity through changes in primary structure, glycosylation, association with signaling subunits, and interaction with serine proteases. Three major classes of leukocyte Fcγ exist in humans: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). Functional bi-allelic polymorphisms of FcγR on neutrophils (FcγRIIA, FcγRIIb) and natural killer cells, monocytes, and macrophages (FcγRIIA, FcγRIIB) are associated with the reduced binding of antibodies and an increase risk of bacteremia and meningitis (57). Two allelic forms of FcγRIIb are known, designated FcγRIIa-R131 and FcγRIIa-H131, because of either an arginine or a histidine at position 131 in the extracellular domain of the receptor (58). Neutrophils from homozygous FcγRIIa-R/ R131 are less effective than either FcγRIIa-H/H131 or heterozygous FcγRIIa-H/R131 at phagocytosis of meningococci (59). FcγRIIa has either valine or phenylalanine at amino acid 158, and this determines the efficiency of immunoglobulin-γ in triggering cellular effectors (60). FcγRIIb neutrophil antigen polymorphism has four amino acid substitutions in the distal loop of the receptor, and FcγRIIb-NA1 binds immunoglobulin-γ complex more efficiently than FcγRIIb-NA2 (59, 61).

Plantonov et al. (62) compared the distribution of FcγRIIa-R131 and FcγRIIa-H131 alleles in 98 complement-sufficient patients with meningococcal disease with that of the alleles in 107 healthy controls. They found a strong association between the FcγRIIa-R/R131 and the development of meningococcal disease after the age of 5 yrs (p < .03; odds ratio, 2.9). A severe course of meningococcal disease was observed in 68% of patients with FcγRIIa-R/R131 genotype, in 54% of patients with FcγRIIa-R/H131 genotype, and in only 31% of patients with FcγRIIa-H/H131 genotype (p < .02; odds ratio, 4.7), suggesting that individuals >5 yrs of age who have the FcγRIIa-H/H131 allele are less susceptible to severe meningococcal disease (62). A Dutch study examining 50 surviving meningococcal disease patients, 183 first-degree relatives of patients with meningococcal disease, and 239 healthy controls showed that a combination of FcγRIIa-R/R131, FcγRIIa-F/F158, and FcγRIIb-NA2/2 genotypes (low-efficiency binding phenotypes) was increased significantly in relatives of patients with meningococcal disease, compared with healthy control subjects (p < .05; odds ratio, 2.6). Interestingly, FcγRIIa-R131 allele was found more often with meningitis, whereas FcγRIIa-H131 allele was relatively increased in patients with sepsis. Also, the FcγRIIa-V158 allele was found more in patients with meningitis (63). The significance of these associations between patients with sepsis and meningitis remains to be determined; however, a strong linkage between susceptibility to meningococcal disease and FcγR genes on chromosome 1 was established.

Cytokines and Cytokine Receptors. Coordinated production of proinflammatory cytokines such as TNF, interleukin (IL)-1, and IL-6 and anti-inflammatory cytokines such as IL-4, IL-10, IL-13, and transforming growth factor-β play a major role in the pathophysiology of systemic inflammatory responses and disease (64, 65). Interests in genetic polymorphisms influencing the balance of pro- and anti-inflammatory responses in disease are enormous and are beyond the scope of this review. This section will focus on studies specific for sepsis. For an extensive list of cytokine polymorphisms in infectious disease, please refer to other reviews (66).

Tumor Necrosis Factor. TNF-α is by far the most well-studied cytokine in sepsis. Administration of TNF-α to experimental animals mimics the deleterious effects observed with endotoxemia and bacteremia, and prior administration of anti-TNF improves the outcome of lethal sepsis in such a model (67). A great variation in TNF-α production is noted from one patient to the next, and this difference was initially correlated with class II HLA genotype (68). Recently, two polymorphisms were identified within the TNF loci that are associated with variations in TNF-α production. A genomic bi-allelic polymorphism has been described at position −308 of the human TNF promoter region consisting of the alleles TNF1 (guanine at position −308) and TNF2 (adenine at position −308) (69). The presence of TNF2 has been associated with enhanced spontaneous and stimulated TNF-α production in vivo and in vitro (70). Examination for the association between −308(G/A) polymorphism and severe sepsis has yielded conflicting results (Table 2). In a German study of 80 severe sepsis patients and 153 healthy controls, no association between TNF2 polymorphism and severe sepsis was found (71). Similarly, in a Taiwanese study of 112 postoperative surgical patients with sepsis, TNF2 allele predisposed these patients to neither septic shock nor higher mortality rate. However, the allele frequency of TNF2 was significantly higher in this population over the general population (12% vs. 5.1%). In patients who developed septic shock, those with TNF2 allele had higher concentration of detectable TNF-α in their serum and a significantly higher mortality rate than those homozygous for TNF1 allele (72). Consistent with this data, Mira et al. (73) also found a higher percentage of septic shock patient with TNF2 allele (39% vs. 18%) compared with healthy French controls. In addition, among septic shock patients, TNF2 frequency was significantly higher in those who died (73). Conversely, the carriage of TNF1 haplotype had a significant protective effect against the development of sepsis in a Tennessee study (74). Together, these data establish an association but not causation between TNF2, high TNF-α production, and increased mortality rate in sepsis. These contradictory results also emphasize the influences that different local allelic frequencies may have on evaluating the significance
of gene polymorphisms and disease susceptibility.

Within the cluster of human leukocyte antigen class III genes on chromosome 6, near TNF-α gene, lies the gene coding for TNF-β, also known as lymphotxin-α. An additional bi-allelic NcoI restriction-fragment length polymorphism has been described at position 1069 in the first intron of the TNF-β gene, consisting of alleles TNF1B1 (guanine at position 1069 and susceptible to NcoI digestion) and TNF2B2 (adenine at position 1069). Similar to TNF2 allele, TNF2B2 homozygotes have been associated with a high TNF responder phenotype and several autoimmune diseases (75, 76). Studies in a German population have consistently demonstrated an association between a homozygous individual's disease susceptibility and increased mortality rate and higher TNF-α concentration in the setting of postoperative intensive care (71, 77) or postblunt trauma settings (78). However, despite this strong association, it appears that TNF2B2 allele is in linkage disequilibrium with TNF1 allele (71, 74), and the possibility of a yet to be identified causative gene within this region remains. This complex relationship between the TNF gene polymorphisms and sepsis outcome is further demonstrated by the reported lack of association between TNF2B2 and survival of women with severe sepsis (79).

**IL-1 and IL-1 Receptor Antagonist.**

Similar to TNF-α, IL-1 family cytokines, such as IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra), also play important roles in the pathogenesis of sepsis. IL-1α and IL-1β are proinflammatory gene products with crucial roles in the development of sepsis and shock in humans and animals. In contrast, IL-1Ra is an acute phase protein that competitively binds to IL-1 receptor and protects against the deleterious effects of bacterial toxins (64, 80). Genes encoding the IL-1 cytokine family are located within the q13-21 area of chromosome 2. Polymorphism in IL-1Ra gene is caused by variable number tandem repeats of an 86 base pair sequence in intron 2 (81). Correlation between increased frequency of allele 2 (A2) that contains two repeats and various chronic diseases such as ulcerative colitis, diabetic nephropathy, and systemic lupus erythematosus has been described (82–85). Biological relevance of this polymorphism is supported by the observations that healthy IL-1Ra A2 carriers have higher IL-1Ra plasma concentration than noncarriers and that their mononuclear cells produced higher amounts of IL-1Ra after in vitro stimulation (86, 87). There are two bi-allelic base exchange polymorphisms within the IL-1β gene: at −511 in the promoter region (Avai polymorphism) and at position +3953 in the fifth axon (Tagl polymorphism) (88, 89). It has been demonstrated that the presence of this rare polymorphism is associated with higher endotoxin-induced IL-1β production in vitro (88). In the Caucasian population, an IL-1α polymorphism consisting of 46 base pair variable number tandem repeats at intron 6 has been described (90). Different from IL-1β or IL-1Ra, the effect of IL-1α polymorphism on serum concentration has not been reported.

The relationship between these polymorphisms and sepsis has been examined in three populations. Fang et al. (91) examined the allele frequencies and genotype distributions of IL-1β Tagl and IL-1Ra polymorphisms in 93 severe sepsis patients and 263 healthy controls. They found IL-1Ra A2 allele occurs significantly more frequently in severe sepsis patients than in normal individuals (37% and 24%, respectively, p < .01), suggesting an association between this allele and susceptibility to sepsis. No association with patient outcome was observed for either IL-1β or IL-1Ra allele. A similar study by Ma et al. (92), comparing the allelic frequencies and genotype distribution of IL-1α, IL-1β, and IL-1Ra in 60 sepsis patients and 60 healthy controls, also found a significantly higher frequency of IL-1Ra A2 allele in septic patients than controls (34% and 23%, respectively, p < .05). The frequency of the IL-1α and IL-1β polymorphism did not vary between normal and septic patients. Interestingly, the presence of IL-1α2, IL-1β Avail, and IL-1Rα A2 polymorphism appears to be associated with the highest mortality rate in this population. A notable deficiency in these two studies is the lack of serum measurement for biological

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**Table 2. Summary of association studies on sepsis with cytokines gene and others miscellaneous polymorphisms**
correlation. By contrast, a study by Arnalich et al. (93) comparing 78 Spanish patients with severe sepsis (51 survivors and 27 nonsurvivors) with 130 healthy controls and 56 patients with uncomplicated pneumonia showed that homozygous IL-1RaA2 is strongly associated with decreased ex vivo production of IL-1Ra in patients with severe sepsis and is an independent risk factor for mortality (6.47-fold increase risk of death) but not susceptibility. Together, IL-1 family gene polymorphisms are promising markers for predicting sepsis susceptibility and mortality rate when larger population studies across different ethnicities are performed.

IL-6. IL-6 is a pleotropic cytokine with potent stimulatory effect on macrophages and B- and T-lymphocytes as well as induction of fever and acute phase response (94). Among the milieu of cytokines induced during sepsis, the best correlation of plasma cytokine concentration with mortality rate has been made with IL-6 (95, 96). Administration of anti-IL-6 antibodies was protective in a animal model of sepsis (97). A polymorphism at –174 position of IL-6 promoter region with a G to C substitution (G-174C) has been described. The C allele was associated with significantly lower IL-6 production in a reporter gene assay. Moreover, significant differences in basal plasma concentration of IL-6 were found in healthy individuals, with highest values in carriers of the GG genotype, intermediate values for heterozygous carriers, and the lowest levels for CC genotype (98). Schuler et al. (99) compared allele frequencies, genotype distributions, and plasma IL-6 concentrations in 326 surgical patients (of whom 50 developed sepsis) and 207 healthy German controls. Significant differences in allelic frequencies or genotype distribution were not identified between surgical patients and controls or between patients with and without sepsis, suggesting that IL-6 polymorphism is not associated with susceptibility to sepsis. In patients who succumbed to sepsis, significantly less GG homozygotes were observed compared with survivors, indicating an association of GG genotype and improved survival. When IL-6 plasma concentrations were compared, low IL-6 producers were significantly associated with survival, in agreement with previously published results that demonstrated plasma IL-6 as a prognostic marker in sepsis (95, 96). However, no associations between systemic IL-6 concentrations and genotypes were found. This lack of an association was confirmed in a separate German study comparing (G-174C) genotype and ex vivo IL-6 response to endotoxin in trauma patients (100). This discrepancy may be explained by the different assay used to determine IL-6 responsiveness in these studies. Also, the effects of IL-6 promoter polymorphism may be cell/tissue specific, and interaction with additional promoter polymorphism remains possible (101). Alternatively, the association of GG genotype and improved survival may represent yet another linkage disequilibrium.

Interferon Gamma. Interferon (IFN)-, produced by natural killer cells and T lymphocytes, has many immunostimulatory activities such as up-regulation of class II major histocompatibility complex expression and TNF- and IL-1 production (102). Interests in IFN- polymorphism in sepsis evolved from the observation that monocytes from nonsurviving trauma patients with infection had significantly lowered class II major histocompatibility complex expression than survivors with infection (103). IFN- adjuvant therapy also restored class II major histocompatibility complex expression but demonstrated only partial protection (104). Both IFN- receptor 1 (IFN-1R1) and IFN- gene polymorphisms have been examined using variable number tandem repeat markers in patients with infection following major trauma (105, 106). Davis et al. (106) found that development of major infection was significantly associated with certain IFN- alleles in 38 trauma patients with Injury Severity Scores of 16. Similarly, IFN- polymorphisms on chromosome 12 were examined in 61 post-trauma patients, and homozygotes for the D allele increased the chance of developing sepsis (105). Unfortunately, neither study provided phenotypic studies for biological correlation. Although these data suggest a possible role for IFN polymorphism in sepsis, studies with larger populations, clear ethnic backgrounds, and better controls are needed to confirm these preliminary findings.

ENDOTHELIAL-RELATED FACTORS AND OTHERS

Plasminogen Activator Inhibitor-1. The endothelium with its ability to regulate vascular tone, leukocyte transmigration, and coagulation homeostasis is another pivotal player in the pathogenesis of sepsis. It has been suggested that endothelial dysfunctions in specific organs may give rise to the phenotypic heterogeneity observed in sepsis-related multiple-organ failure (107). Intravascular fibrin deposition and microvascular thrombosis in various organs are such hallmarks of multiorgan dysfunction. Excess fibrinolytic inhibitors or deficiency in anticoagulants may lead to a procoagulant state. In meningococcal septic shock, inherited disorders in protein C, protein S, antithrombin, and the presence of the factor V Leiden mutation do not appear to play a major role in the severity of the disease course (108). In contrast, high plasma concentration of plasminogen activator inhibitor-1 (PAI-1) has been associated with adverse outcome in sepsis (109) and meningococcal septic shock (110). The 4G allele of a single base pair deletion/insertion (4G/5G) polymorphism in the promoter region of PAI-1 gene has been associated with higher plasma PAI-1 concentration (111).

Three studies on PAI-1 polymorphism in disease outcome have been reported. A meningococcal research group (112) (Table 3), comparing a combined cohort of 175 children with meningococcal disease from London and Rotterdam with 226 healthy controls, found that patients with the 4G/4G genotype had significantly higher PAI-1 plasma concentration and increased risk of death from sepsis than those with 4G/5G or 5G/5G genotypes (relative risk 2.0 [1.0–3.8] for the two cohorts combined and 4.8 [1.8–13] for the London cohort). No differences in allele or genotype frequencies were found, suggesting lack of an association between PAI-1 polymorphism and disease susceptibility. These data were confirmed by another Dutch study. Westendorp et al. (113) examined PAI-1 alleles and genotype frequencies in 50 patients who survived meningococcal infection, 131 controls, and 183 first-degree relatives of patients with meningococcal infection. They found that patients whose relatives were carriers of the 4G/4G genotype had a six-fold higher risk of developing septic shock than meningitis (odds ratio, 5.9; 95% confidence interval, 1.9–18) compared with all other genotypes. A similar study in posttrauma patients was performed. When 61 trauma patients were compared with 32 healthy volunteers, the 4G/4G genotype was significantly associated with higher IL-1, TNF- and PAI-1 plasma concentration (114). A correlation between these concentrations and
Angiotensin I Converting Enzyme. Angiotensin I converting enzyme (ACE) converts angiotensin I to angiotensin II and metabolizes kinins and many other biologically active peptides, including substance P, chemotactic peptides, and opioid peptides. ACE exists in both membrane-bound and soluble forms. It is found in varying amounts on the surface of epithelial and endothelial cells of various organs including brain, lung, testis, prostate, and kidney. The broad spectrum of substrates for ACE and its wide distribution throughout the body indicate that this enzyme, in addition to an important role in cardiovascular homeostasis, may be involved in additional physiologic processes such as atherosclerosis, kidney and lung fibrosis, myocardial hypertrophy, inflammation, and wound healing (115).

Genetic contribution to the variability of plasma ACE concentrations between individuals has been suggested (116). An insertion (I)/deletion (D) of 287 base pair alu repeat sequence restriction fragment length polymorphism was found in the noncoding region of human ACE gene (117). High plasma and tissue ACE concentrations were shown to be associated with DD genotype (118, 119). However, studies of associations between the D allele and the development and/or progression of cardiovascular disease (120), diabetic nephropathy (121), sarcoidosis (122, 123), and asthma (124) yielded both positive and negative results. Recently, associations between ACE polymorphism and severity of meningococcal disease in children and in patients suffering from acute respiratory distress syndrome (ARDS) were examined. Harding et al. (125) examined genotype distribution in 110 Caucasian children with meningococcal sepsis. ACE DD genotype is associated with significantly higher predicted mortality rate and worse disease severity. The observed mortality rate was approximately two-fold higher in the DD genotype group but without statistical significance (125). In a separate study, Marshall et al. (126) compared genotype distribution in 96 patients with ARDS to 88 patients with non-ARDS respiratory patients, 174 patients undergoing coronary artery bypass, and 1,906 healthy controls. Different from the meningococcal study, there was no significant difference in severity of illness scores or length of intensive care unit stay in the ARDS group by genotype. However, patients with ARDS had significantly increased DD genotype frequency and higher mortality rate compared with all of the control groups, suggesting a potential role for local (pulmonary) renin-angiotensin systems in the pathogenesis of ARDS (126). The lack of concurrent plasma or local ACE concentration measurements in either study makes it difficult to establish causation. Nonetheless, these two studies offer intriguing insight into new factors that may contribute to the pathogenesis of sepsis and multiple-organ failure.

With the rapid advancement of molecular biology, more genes and molecules are being discovered that may significantly contribute to the pathogenesis of sepsis. For example, the induction of heat shock proteins helps to ensure cellular survival during stress. It has been demonstrated that experimental expression of heat shock proteins in pulmonary epithelium is able to protect rats from ARDS in cecal ligation models (127). Although experimental evidence suggests that heat shock proteins may be an important mediator in ARDS, genetic polymorphisms of heat shock protein 70 genes are not associated with either susceptibility or outcome of sepsis (128). Genetic polymorphisms in other important molecules such as endothelial nitric oxide synthase, poly(adenosine ribose) synthetase, macrophage inhibitory factors, IL-10, IL-12, complements, and various coagulation related molecules will need to be examined in the context of sepsis.

**CONCLUSIONS**

Outcome from sepsis has improved with early-goal directed therapy, low-dose steroid supplementation, tight control of blood glucose, and therapy with activated protein C. Population studies have left little doubt that genetic differences contribute to the observed variation in susceptibility to and mortality from sepsis. Further advancements in strategies on sepsis prevention, diagnosis, and treatment may lie in understanding how a person's genetic makeup influences susceptibility and response to pharmacotherapy. Studies of genetic polymorphisms summarized in this article are just the beginning of this process.

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**Table 3. Summary of association studies on sepsis with endothelial related factors and other miscellaneous polymorphisms**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Polymorphisms</th>
<th>Study Populations</th>
<th>Study Origin</th>
<th>Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1</td>
<td>4G/5G</td>
<td>Meningococcal disease and healthy controls</td>
<td>Great Britain + Netherlands</td>
<td>Strong association of PAI-1 4G allele with poor outcome from sepsis (112–114)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meningococcal disease patients, relatives, and healthy controls</td>
<td>Great Britain</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posttrauma patients and healthy controls</td>
<td>Germany</td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>I/D</td>
<td>Meningococcal sepsis ARDS, pneumonia, CABG patients, and healthy controls</td>
<td>Great Britain</td>
<td>DD genotype is significantly associated with higher mortality rate (125, 126)</td>
</tr>
<tr>
<td>HSP70</td>
<td>HSP70-HOM</td>
<td>Severe sepsis and healthy controls</td>
<td>Germany</td>
<td>No associations (128)</td>
</tr>
<tr>
<td></td>
<td>HSP70-2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PAI, plasminogen activator inhibitor; ACE, angiotensin I converting enzyme; ARDS, acute respiratory distress syndrome; CABG, coronary artery graft bypass; HSP, heat shock protein.
Although studies examined in this review are of small heterogeneous populations, the identification of strong associations between certain genetic polymorphisms and increased mortality rate or susceptibility to severe sepsis is intriguing and supports further research using this approach.

Microarray technology provides a powerful new tool for rapidly analyzing large numbers of alleles at the same time. In the near future, one may have the ability to tailor drug selection and dosage and predict outcome using genetic information obtained at disease presentation. It is also anticipated that these advances can lead to better delineation of heterogeneity encountered in sepsis and improve our knowledge of the pathophysiology of organ dysfunctions encountered in sepsis and severe sepsis. However, as with any new technology or discoveries, healthy skepticism must accompany the process to prevent erroneous assumptions and misguided research. It is important to keep in mind that most studies included are of small populations with geographic distinction and are not necessarily ethnically distinct, thus making interpretation of either positive or negative results difficult. Furthermore, because of the limited number of ethnic groups examined, application of genetic information to random patients will need to be overcome by multiple-center and multinational, collaborative studies. Applying the recommendations recently published for future research in acute lung injury (129) to the field of sepsis is a good first step that ensures rapid progress in our understanding of sepsis.

REFERENCES

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