Genetics and Severe Sepsis

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Introduction

Despite significant advances in understanding the molecular basis of host-pathogen relationships and associated immunological responses, severe sepsis remain a problem world-wide, associated with multiple organ dysfunctions and elevated mortality [1]. Annually, more than 100,000 people in the USA die from septic shock, the most severe form of sepsis, which thereby represents the most common cause of death in the intensive care unit (ICU). Morbidity and mortality of severe sepsis are usually ascribed to incorrect or delayed diagnosis, inadequate antimicrobial therapy and underlying illnesses [2,3]. More recently, the host-specific immune response has been shown to be another important determinant of outcome of infectious diseases [4]. Genetically-determined differences in immune responses might explain why some people get sick and die when they encounter a pathogen whereas others stay perfectly healthy. The aim of this chapter is to review current knowledge regarding genetic variability associated with increased susceptibility to severe sepsis with emphasis on selected polymorphisms associated with a poor outcome. More extensive reviews have been recently published [4–9].

Rationale for Genetics in Sepsis and Infectious Diseases

The influence of genetic factors in determining susceptibility and resistance to severe infectious diseases has long been suspected. Numerous reports in animal models, ethnic groups, familial cases, twin and adoptee studies have definitively proved the importance of genetics in severe infections [10].

The use of animal models, which mimic human severe sepsis, is important in elucidating the molecular mechanisms of sepsis. Genetic factors differentiate inbred strains, and epigenetic factors elicit variations within a strain. In this regard, the prevalence of genetic strain differences, contributing to susceptibility to microbial infections has been well recognized in rodents. These models, essentially mice, are genetically well defined and may be easily genetically-modified (using genetically-engineered strains such as knock-outs) to demonstrate the physiological importance of a suspected gene [4,11,12]. The interest in studies of mice lies in the fact that nearly all of the murine genes involved in the response to sepsis have human homologs. Analysis of susceptibility to certain infectious diseases in mice

has led to the mapping and identification of candidate genes for human studies. Hence, some groups have shown that Toll-like receptor 2 (TLR2) knock-out mice do not respond to *Staphylococcus aureus* infection. After bacterial challenge, these mice have decreased production of cytokines, increased concentration of bacteria in blood and kidneys, and a higher mortality rate than wild-type mice [13,14]. Similarly, when infected by *Mycobacterium tuberculosis*, TLR2 knock-out mice have deficient bacterial clearance and develop chronic pneumonia [15]. Interestingly, similar susceptibility to *S. aureus* infection and tuberculosis have been reported in human populations carrying TLR2 polymorphisms [16–20]. Identification of the effects in such human states validates the use of murine knockout models to identify key pathways controlling predisposition to infection.

Studies in twins have also provided arguments for 'genetically programmed' susceptibility to infection, when homozygous twins who have the same genome are compared with heterozygous twins who are genetically different. Such studies clearly demonstrate that, in case of infection of the first twin, the risk for the second one to be infected by the same pathogen was higher for homozygous pairs versus heterozygous pairs [21–23].

Estimates of genetic predisposition, independent of environmental effects, have been obtained also from adoptee studies. Sorensen et al. [24] reported a large study of etiologies of premature death in 1,000 families with children adopted early in life. Adoptees with a biological parent who died before the age of 50 from an infectious disease had a 5.8-fold increase in the relative risk of dying from an infection. In contrast, the death of an adoptive parent from an infectious cause had no significant effect on the adoptee's risk of such a death, clearly indicating that host genetic factors are major determinants of susceptibility to infectious diseases [24].

Genetic Predisposition to Severe Sepsis: Mendelian or Non-Mendelian Genetics?

Genetic predisposition to severe sepsis may be either a monogenic or a complex multifactorial disorder.

Single Gene Defects

In monogenic diseases, mutation in a single gene is necessary and sufficient to produce the clinical phenotype. More than 100 rare major genetic defects of the immune system have been identified [25–31]. They are most commonly associated with unusual and recurrent bacterial infections detected in childhood. Recent genetic defects have been shown to be responsible for lethal tuberculosis or severe bacterial infections [27]. Thus, predisposition to rare and atypical mycobacteria (*M. cheloniae, M. fortuitum, M. avis*) or disseminated Bacille Calmette-Guerin (BCG) vaccine infections have been described in children that lack either chain of

interferon-gamma (IFN-γ) IFN-γ receptor or the interleukin (IL)-12 receptor [32]. Single gene defects provide valuable insights into the molecular and cellular basis of host immunity against specific pathogens.

Even a single mutated locus may generate a large spectrum of phenotypes in terms of disease severity. Cystic fibrosis is a classical example of such a monogenic trait with more than 1,000 identified mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [33]. Each of these mutations has been associated with the development of clinical signs of cystic fibrosis, but large variations in the severity of the phenotype exist for each genotype. Indeed, modifying the effects of other genes may result in marked variations in the symptoms of patients with the same disease [34].

Complex Multifactorial Disorders

Common diseases, such as diabetes, asthma or hypertension, are thought to result from a combination of diverse genetic and environmental factors [35]. Genetic predisposition to severe sepsis is also considered to be a non-Mendelian disease [10]. These complex diseases differ dramatically from illnesses associated with single-gene defects. The complexity of common diseases results from the fact that penetrance (the frequency at which a genotype gives rise to a disease) is highly variable. Hence, even if an identical twin has a multi-factorial disease, the second twin may not develop the trait.

Additional definitions are necessary to understand the molecular basis of genetic predisposition to severe sepsis. A *polymorphism* is a region of the genome that varies between individual members of a population and is present in more than 1% of the population. A *single nucleotide polymorphism* (SNP) is a polymorphism caused by the change of a single nucleotide. The difference may be an inversion (G to C or A to T), a transition (G/C to A/T or inverse), an insertion or a deletion of one base. Most genetic variations between individual humans are believed to be due to SNPs, but other variants are important, such as duplicate genes or repeat DNA sequences. Humans carry two sets of chromosomes, one from each parent. Equivalent genes in the two sets might be different, because of SNPs or other polymorphisms. An *allele* is one of the two (or more) forms of a particular gene. A particular combination of alleles or sequence variations that are closely linked on the same chromosome is named *haplotype*.

Complex diseases, such as sepsis, are characteristically caused by interacting genetic and environmental determinants. To identify genes that might confer susceptibility or resistance to severe sepsis, different approaches may be used depending on historical evidence, ease of recruiting study populations, and cost of genotyping [36]. Currently, most studies in the field of sepsis are association genetic studies. These involve a binary disease trait (such as development of septic shock, acute respiratory distress syndrome [ARDS], multiple organ failure [MOF], or mortality) and a functional gene with two alleles. They require an adequate number of unrelated individuals to have been typed for the gene of interest and

classed as having, or not having, the trait and have to fulfill all recommended criteria from published guidelines [37, 38].

The validity of genetic association studies relies on basic rules [39]. Studied populations have to be homogeneous: allele frequencies and frequency-dependent measures like linkage disequilibrium can only be estimated accurately from properly identified and sampled populations. Control groups should be in Hardy-Weinberg equilibrium. Sample design is crucial and an adequate study size and study power are also necessary to exclude false conclusions. Definition of the phenotype is a key issue in the design of any genetic study whose goal is to detect gene(s) involved in the course of the disease. For example, selecting more severely ill septic patients without significant comorbidities may help to identify the candidate genes responsible for septic shock. Inclusion of patients with severe co-morbidities or who received treatment that can contribute to mortality, such as inappropriate antibiotics, can lead to false negative studies. Despite these limitations, association study design is simple and provides high power to detect common genetic variants that confer susceptibility to sepsis. However, interpretation of their results is complex (Table 1).

Table 1. Interpretations of genetic association studies

1) Significant association:
a) True positive association
Variant is causal
Variant is in linkage disequilibrium with causal variant
b) False positive association
False positive due to multiple testing
False positive due to systematic genotyping error
False positive due to population stratification or other confounder
2) Reasons for lack of replication
a) Original report is a false positive
b) False negative
Phenotypes differ across studies
Study populations differ in genetic or environmental background
Replication study is under powered

Genetic Polymorphisms in Severe Sepsis and Septic Shock

Antimicrobial host defense is a complex process that relies both on innate and adaptive components [40, 41]. The generation of a large repertoire of antigen-recognition receptors and immune memory, hallmarks of acquired immunity, depends on the presence of an efficient innate immunity. Hence, innate immunity represents the first-line of host defense necessary to limit infection in the early hours after pathogen invasion and controls adaptive immune responses. Early

protection against microorganisms involves three mechanisms: 1) recognition of the pathogen; 2) phagocytosis and elimination of invading microorganisms; and 3) development of an inflammatory response necessary for resolution of the infection. Each step of this immune reaction may be affected by gene polymorphisms of individual components of the immune system which lead to susceptibility or resistance to infection and have been associated with organ failure and/or risk of death [42].

Gene Polymorphisms Altering Pathogen Recognition (Table 2)

Table 2. Gene polymorphisms modifying pathogen recognition receptors

Gene	Polymorphisms	Type of Infection
MBL	Codon 52, 54, 57	Respiratory infections, meningococcal disease, pneumococcal disease, sepsis in ICU
Fc-γRIIA	H131R	Meningococcal disease, pneumococcal disease, SARS infection, cerebral malaria
CD14	C159T	Septic shock
TLR5		Legionnaire's disease
TLR4	D299G	Gram negative sepsis, malaria
TLR2	R753Q	Gram positive sepsis, Borrelia sepsis, tuberculosis, Leprosy
CCR5	CCR5-Δ32	HIV-1 'resistance'

Throughout evolution, innate immunity has developed a very efficient system that recognizes invariant molecular constituents of infectious agents called pathogen-associated molecular patterns (PAMPs) [40]. This system of detection is currently referred to as pattern recognition receptors (PRR) and can be divided into three classes: 1) soluble receptors, such as mannose binding lectin (MBL) and the components of the complement system; 2) endocytic receptors, such as Fcγ receptors and scavenger receptors (including MARCO and DC-SIGN); and 3) and signaling receptors such as TLR and nucleotide-binding oligomerization domain (NOD) receptors. Almost all of these receptors have functional polymorphisms that have been associated with increased susceptibility to severe infections primarily through decreased clearance of pathogens. However, only MBL and CD14 variants are potentially associated with the severity of, and mortality from, septic shock.

Mannose Binding Lectin

MBL is a member of the collectin family of proteins. This calcium-dependent plasma lectin binds to sugars and possibly endotoxin on microbial surfaces, and then activates complement, acting as a so-called ante-antibody [9]. MBL can also directly act as an opsonin and bind to specific receptors expressed on the cell surface of various cell types, including monocytes, thereby potentiating TLR responses.

Thus, MBL clearly appears to be a pluripotent molecule of the innate immune system.

For maximal efficacy, proteins of the innate immune system have to be present at physiologically significant levels. The concentrations of MBL in human plasma are genetically determined and are profoundly reduced by either structural gene mutations or by promoter gene polymorphisms [43]. Three different alleles, resulting in structurally variant proteins, have been identified in codons 52, 54 and 57 of the exon 1 of the MBL gene. Structural variants within the MBL gene are common, with frequencies ranging between 0.11 and 0.29, and reduce complement activation independent of the MBL plasma level. Whereas MBL deficiencies can be explained by these three mutations, these structural gene mutations do not explain why MBL serum levels vary so widely between individuals. Genetic variations have also been detected in the promoter region of the MBL gene. These variations have been reported to control the plasma levels of structurally normal MBL [43]. In particular, G to C inversions at position −550 or −221 in the promoter region are associated with varying expression levels of MBL. Furthermore, these SNPs are always linked with the structural variants in most populations creating relevant haplotypes. As an example, the median serum concentrations of MBL for Caucasians were found to be 1,630 ng/ml for wild-type genotype; 358 ng/ml in patients heterozygous for the codon 54 mutation; and 10 ng/ml in patients homozygous for the codon 54 mutation.

A large number of studies have attempted to define the role of MBL in predisposing to severe infection [9]. Hibberd et al. reported a large cohort of patients with meningococcal disease admitted to a pediatric ICU and a second cohort of children who had survived meningococcal disease in the UK [44]. Both studies showed a clear association between MBL polymorphisms and susceptibility to meningococcal disease, with an odds ratio (OR) of 6.5 for the homozygous patients in the hospital study and of 4.5 in the national study. Heterozygous patients were also at increased risk of meningococcal infection, but to a lesser degree since the OR ranged from 1.7 in the hospital study to 2.2 in the national study. Using the population attributable fraction assessment, it is possible to calculate that gene variants could account for as many as a third of meningococcal disease cases. Similarly in the UK, adult patients homozygous for MBL structural variants, who represent about 5% of northern Europeans and North Americans, have a substantially increased risk of developing invasive pneumococcal disease [45]. Furthermore, in 272 prospectively monitored critically ill patients with systemic inflammatory response syndrome (SIRS), the presence of MBL variant alleles was associated with the development of sepsis, severe sepsis, and septic shock. An increased risk of fatal outcome was observed in patients carrying variant alleles [46]. All these data show that genetic variants contributing to inadequate MBL levels play an important role in the susceptibility of critically ill patients to the development and progression of severe sepsis and confer a substantial risk of fatal outcome.

Fcγ Receptor Polymorphism and Encapsulated Bacteria Infections

Antibodies, antibody receptors, and complement are essential components in defense against invasive encapsulated bacteria (*S. pneumoniae, Haemophilus influenzae, Neisseria meningitidis*). Fcγ receptors are located on the phagocytic cell surface, bind the Fc region of IgG, and mediate binding, phagocytosis, and destruction of bacteria opsonized with IgG. Certain genetically determined variations of IgG receptors on neutrophils (FcγIIa, FcγIIIb) as well as monocytes and macrophages (FcγIIa, FcγIIIa) are associated with reduced binding of antibodies and an increased risk of bacteremia and meningitis. In a study of 50 surviving meningococcal disease patients, 183 first-degree relatives of patients with meningococcal disease, and 239 healthy controls, the combination of low affinity polymorphisms of FcγIIa, FcγIIIa, and FcγIIIb was present significantly more often in relatives of patients than in the healthy control group [47]. Moreover, the distribution of FcγIIa and FcγIIIa differed between patients presenting with sepsis and those presenting with meningitis.

LPS Complex Receptor

Lipopolysaccharide (LPS) recognition by TLR4 on the cell surface is achieved in cooperation with several protein components, including LPS-binding protein (LBP), CD14, and MD-2, and leads to the activation of nuclear transcription factors, such as nuclear factor-kappa B (NF-κB) [40]. Modulation of cytokine expression as a result of the initial host–microbial interaction is important in the pathophysiology of sepsis. TLR4, CD14, and MD-2 have been reported to have polymorphic sites associated with altered functioning of the LPS receptor complex and with susceptibility to severe sepsis [27, 48].

In 2000, Arbour et al. identified two polymorphisms of the TLR4 gene (Asp299Gly and Thr399Ile), associated with hyporesponsivness to inhaled LPS in humans [49]. In 2002, Lorenz and colleagues studied the association between these two mutations and the outcome of patients with septic shock. First, these authors genotyped 91 patients with septic shock and 73 healthy controls. They found that the TLR4 Asp299Gly allele was present exclusively in patients with septic shock and also that patients with the TLR4 Asp299Gly/Thr399Ile co-mutation had a higher prevalence of Gram-negative infections [50]. Other studies have confirmed this result and shown that these variants are associated with mortality in SIRS [51]. Interestingly, these two frequent SNPs showed no association with susceptibility to, or severity of, meningococcal disease, although rare TLR4 mutations have been implicated in meningococcal susceptibility [52]. Despite these reports and the central role played by TLR4 in the development of Gram-negative sepsis, additional controlled studies, with increased numbers of patients are required to determine whether TLR4 SNPs are associated with risk or severity of Gram-negative sepsis.

The CD14 gene contains a promoter polymorphism (–159C/T) that has been reported to modulate both the density of CD14 expression on the membrane of monocytes and circulating levels of soluble CD14. CD14-159C/T polymorphisms

have been reported to be associated with susceptibility to septic shock and with the mortality rate from this condition [53–55]. However, evidence against this association has been found in trauma patients with severe sepsis as a secondary complication [56]. As mentioned above, the disparity in the results from these studies may be due to study differences, including the number of patients analyzed, the types of patients included (trauma, pneumonia, surgery), and heterogeneity in the patient populations (ethnicity or co-morbidities).

Gene Polymorphisms Modifying the Inflammatory Immune Response

The inflammatory reaction is an essential component of host defense mechanisms. Inflammation is tightly regulated by mediators that initiate and maintain the inflammatory process as well as others required for its resolution [57]. Cytokines are key protein regulators of inflammation. These small proteins, with molecular weights ranging from 8 to 40 kDa, are primarily involved in host response to infection and inflammation. Cytokines initiate and orchestrate immune reactions as local and/or systemic intercellular regulatory factors. Within minutes of an infectious challenge, pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-1, and IL-6, are secreted leading to strong activation of monocytes, chemokine-recruited polymorphonuclear cells, and endothelial cells. This initial pro-inflammatory state is followed by release of anti-inflammatory cytokines, such as IL-10, and inhibitory proteins, such as IL-1 receptor antagonist (IL-1ra), which are able to suppress the expression or actions of pro-inflammatory cytokines, chemokines, or adhesion molecules. Both pro-inflammatory and anti-inflammatory cytokines co-exist in infected sites and in the bloodstream in markedly increased amounts. Their relative concentrations correlate with the severity and the outcome of septic shock [40, 57, 58].

In humans, most cytokine genes are polymorphic and there is increasing evidence that the host's cytokine production is genetically determined [59]. Since most cytokines are not expressed spontaneously and have to be synthesized *de novo* in response to pathogens, functional promoter variants of their genes can have dramatic consequences. Hence, genetic variability of cytokines underlies the complexity of interindividual differences in the immune response to microbial invasion.

Pro-inflammatory Cytokines: TNF-α

TNF-α is a pro-inflammatory cytokine with a central role in many inflammatory diseases, including severe sepsis and septic shock. TNF may be produced by many different cell types and is one of the first mediators to appear in response to a diverse range of infectious stimuli. Once secreted, TNF-α elicits a wide spectrum of immune and inflammatory responses responsible for fever, shock, and tissue injury, and induces the release of additional inflammatory mediators, including other cytokines, nitric oxide (NO), and free oxygen radicals, and up-regulates adhesion molecule expression. Neutralization of TNF production by anti-TNF

antibodies or in TNF-knock-out mice has been associated with increased mortality in several models of infection, demonstrating that TNF is a critical mediator of host defense against infection [60]. However, TNF may cause severe pathology when produced in excess. *In vivo* injection of TNF produces clinical manifestations mimicking those observed after injection of bacteria. Hemodynamic disturbances and mortality have been shown to be correlated with TNF plasma levels. Hence, excessive production of TNF may be associated with tissue injury, shock, and death due to an imbalance between pro-inflammatory and anti-inflammatory cytokines.

Given TNF's role as a central element in the host defense response, its production has to be tightly regulated to preserve cellular homeostasis. Interestingly, marked inter-individual variability in TNF production in response to different stimuli has been reported in healthy subjects. Since the TNF response to infection is partly regulated at the transcriptional level, TNF promoter polymorphisms have been the subject of intense research and are probably the most extensively studied of all cytokines involved in sepsis pathophysiology (more than 25 publications).

Two polymorphisms in the TNF-α locus have been linked to variability in TNF production. The first TNF-α polymorphism consists of a G (called TNF1) to A (called TNF2) 308 base pairs upstream from the transcriptional start of *TNFA*. TNF2 was associated with higher TNF-α secretion than TNF1. The second TNF-α polymorphism is located within the *TNFB* gene, but still affects TNF-α synthesis. It was identified in 1991 by Pociot et al. who reported a biallelic *Nco1* restriction enzyme fragment length polymorphism (RFLP) in the TNF gene locus that was associated with increased TNF-α production [61]. This site has been mapped to the first intron of the LT gene (*TNFB*) at position +250 and allows the definition of two alleles, TNFB1 and TNFB2. The latter does not possess the *Nco1* RFLP and seems to be associated with increased TNF-α plasma concentrations. The precise mechanisms underlying this result remain unclear; the *Nco1* polymorphism may not be directly related to TNF production, but rather serve as a major histocompatibility complex (MHC) marker because of its location in the class III region of the MHC. Significant linkage disequilibrium between the two TNF SNPs has been reported with almost all individuals homozygous for TNFB2 (high TNF producer) also being homozygous for TNF1 (low TNF producer), adding some complexity to the final schema of TNF production [62].

Both TNF2 and TNFB2 polymorphisms have been associated with greater severity and worse outcome in a variety of infectious diseases. For example, TNF2 was described as an independent risk factor for cerebral malaria in large case-control studies of African populations [63]. Homozygosity for the TNF2 allele is associated with a relative risk of 6.8 for death or severe neurological sequelae due to cerebral malaria. A strong association has also been reported between TNF polymorphisms and mucocutaneous leishmaniasis, scarring trachoma, lepromatous leprosy, nephropathia epidemica, and with death from meningococcal disease, severe meliodosis, community-acquired pneumonia, and septic shock [64]. In septic shock, the TNF2 allele increases the risk of death by 3.7 fold even after controlling for age and severity of illness [65]. TNF2 is also clearly associated

with increased mortality from sepsis in neonates and ventilated, very low birth weight infants [66]. However, other studies have failed to demonstrate associations between either TNF2 or TNFB2 and mortality [67]. This discordance may arise, at least in part, from methodological problems such as incorrect genotype assignment and differences in study populations or inclusion and exclusion criteria [68].

Anti-inflammatory Cytokine SNPs: IL-10

Sepsis induces an initial pro-inflammatory response followed by an important release of anti-inflammatory cytokines (IL-4, IL-10, IL-13) responsible for a down-regulation of humoral and cellular immunity that has been called immunoparalysis or compensatory anti-inflammatory response syndrome (CARS). Genetic polymorphisms responsible for uncontrolled and intense CARS may have the same dramatic consequences on outcome from sepsis as an overwhelming inflammatory response.

IL-10 is expressed and secreted by a variety of cell types, including T and B cells, monocytes/macrophages, and epithelial cells, usually after an activation stimulus such as infection. It suppresses the function of macrophages (down-regulation of Th1 cytokines) and indirectly inhibits the activity of B cells. High IL-10 production also inhibits IFN-γ expression and delays clearance of intracellular pathogens, such as Chlamydia [69]. The potent anti-inflammatory effects of IL-10 indicate that this cytokine might play a crucial role in both the resolution and pathogenesis of severe sepsis and septic shock. Concentrations of IL-10 correlate with the severity of the inflammatory response as assessed by the APACHE score, MOF, or death [70, 71]. The risk of fatal outcome from meningococcal disease is increased in families with high IL-10 production. Although both genetic and non-genetic factors contribute to IL-10 production, twin studies suggest that genetics could account for up to 75% of the variability in IL-10 production [72, 73].

The human IL-10 gene demonstrates several polymorphisms resulting in interindividual differences in cytokine production. Within the IL-10 proximal promoter, two CA-repeat microsatellites, and three SNPs at −1,082 (G/A), −819 (C/T), −592 (C/A) upstream of the transcription start site, have been reported [69]. More SNPs in the distal IL-10 promoter have been identified recently with either a high- or a low IL-10 production phenotype, thereby creating eight distal promoter haplotypes [74]. *In vitro*, the IL10-1082G polymorphism has been associated with high IL-10 production by lymphocytes. Within the Mandikas ethnic group, the IL10-1082G homozygous genotype is significantly more common among trachoma patients than controls (odds ratio 5.1; confidence interval, 1.24–24.2; $p = 0.009$) [75]. In contrast, the IL10-1082G allele appears to be more common in persons with mildly symptomatic or asymptomatic Epstein-Barr Virus (EBV) diseases than in patients with EBV infections requiring hospitalization [76]. These findings suggest that high IL-10 producers are partially protected from severe EBV infection and show clearly that changes at the level of a given cytokine do not exert the same

effects on all infectious agents. This may explain why the results from genetic association studies of IL-10 polymorphisms in sepsis are contradictory.

The IL-10 −1,082 G/G genotype, which is linked with greater expression of IL-10, has been associated with higher severity scores and worse outcome in patients with community-acquired pneumonia [77]. Similarly, another IL-10 polymorphism (the −592 A allele, associated with low levels of IL-10) was associated with death both in patients with sepsis and in critically ill patients without sepsis [78]. In that study, although the IL-10 −1,082 allele frequencies were significantly different between cases and controls at admission to an ICU, no association was observed between the IL-10 −1,082 allele and the risk of death from sepsis. Recently, a new IL-10 haplotype, −592C/734G/3367G, has been associated with increased mortality and organ dysfunction in critically ill patients with sepsis secondary to a pulmonary source of infection, but not in similarly ill patients with extrapulmonary sepsis [79]. Overall, the data suggesting a role for genetic variation in the IL-10 gene on death due to severe sepsis remain inconsistent.

Hemostatic Gene Polymorphisms and Severe Sepsis

The inflammatory response observed during severe sepsis leads to a strong activation of coagulation and fibrinolysis. However, early increases in the anticoagulant tissue plasminogen activator are rapidly followed by sustained elevations in plasminogen-activator-inhibitor-1 (PAI-1) leading to a prolonged antifibrinolytic and a net procoagulant state. Activation of coagulation together with inhibition of fibrinolysis are responsible for the development of fibrin deposition and microthrombi that cause extensive endothelial damage associated with MOF [57]. High plasma concentrations of PAI-1 have been associated with an adverse outcome in patients with sepsis and septic shock [8]. Several polymorphisms have been described within the human PAI gene, which is located on chromosome 7, including a common single-base-pair polymorphism (four or five guanine bases) in the promoter region of the gene, 675 bp upstream of the transcriptional start site (4G/5G). The 4G allele (or deletion polymorphism) has been associated with higher plasma concentrations of PAI-1. Individuals homozygous for the 4G allele have higher basal and inducible concentrations of PAI-1 than those with one or two copies of the 5G allele that contains an additional G at location –675 of the PAI-1 promoter gene (insertion polymorphism) [52]. In addition to its antifibrinolytic properties, the 4G PAI-1 variant also seems to influence pro-inflammatory cytokine production. The 4G/4G patients not only had higher PAI-1 concentrations, but also demonstrated significantly higher plasma levels of TNF-α and IL-1 compared to the other genotypes [80]. Emonts et al. confirmed, in a population of 175 children with meningococcal disease and 226 controls, that those with the 4G/4G genotype had significantly higher PAI-1 concentrations compared to those with the 4G/5G or 5G/5G genotype (1051 [550-2440] versus 370 [146-914] ng/ml, $p < 0.0001$). In addition, the 4G/4G patients had an increased relative risk of death (2.0; 95% CI 1.0–3.8) [52]. Three studies reported similar results, indicating that

the PAI-1 'deletion' promoter polymorphism influences the prognosis of meningococcal disease and severely injured patients [80–82]. The latter study investigated the relationship between outcome from severe trauma and the PAI-1 genotype; it found that 58% of injured patients with the 4G/4G genotype died, whereas only 28% with the heterozygous genotype 4G/5G and 15% of patients with genotype 5G/5G did not survive [80].

Perspectives and Conclusions

Severe sepsis is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual's genetic makeup, co-morbidities (such as diabetes mellitus, obesity, cardiac failure), and environmental factors, such as the invasive microorganism responsible for the infection. In recent years, several studies have correlated genetic variations with the risk of, or outcome from, severe sepsis. However, the results of these studies are too inconsistent to enable useful conclusions to be drawn. This inconsistency can be attributed to the heterogeneity of the selected patients, the methods used to select cases and controls, study sizes, the genetic (racial) makeup of the populations studied, and the variability of the microorganisms causing the infections. As more and more polymorphisms are reported, the real multigenic scope of severe sepsis will emerge, and the polymorphisms present in an individual will have increasingly complex clinical implications. The development of technologies that allow high-throughput, fast, and low-cost genotyping will lead to greater insights into host susceptibility at the level of the individual patient.

Genetic markers are not like most biological markers that have wide ranges of values that overlap in people with and without a disease; rather, they are either present or absent. However, the interactions between environmental effects and the molecular mechanisms that influence outcome from sepsis remain poorly understood. An inherited predisposition to sepsis may remain clinically silent until an additional environmental factor occurs. Large-scale association studies that examine many polymorphisms simultaneously are required to allow reliable predictions to be made concerning the risks incurred by genetic factors in severe infection.

As genetic screening to evaluate the individual risk factors for infectious diseases becomes available, insights into the molecular interaction between a pathogen and its host will reveal novel molecular targets for drugs or vaccines. Increased understanding of molecular medicine will shift clinical practice from empirical treatment to therapy based on specific cellular mechanisms of infectious disease. Such approaches are already used in oncology, in which genetic testing can clearly identify persons at high risk, allowing for targeted intervention while sparing the personal and economic cost of unnecessary intervention in those who do not carry a relevant mutation. Detection of the genetic differences which affect drug response, commonly referred to as pharmacogenomics, may also result in fur-

ther classification of diseases, and consequently, the development of 'personalized' therapies.

Another important consequence of the development of genomics will be to begin incorporating genetic markers into severity scores and the design of clinical trials. A diagnosis that lacks sufficient power often results in treatment failure. Other factors such as the genetic characteristics of the host (polymorphisms in genes regulating drug bioavailability or in genes regulating production of the target) can also contribute to a heterogeneous response to therapy in a group of patients. Genetic screening and improved understanding of host–pathogen interactions will allow selection of the best treatment option for a given patient.

The last, but not the least important, point to consider concerns the ethical implications of research on the human genome. When the Human Genome Project was launched in 1990, a parallel program named ELSI (Ethical, Legal, and Social Implications) was established, to identify the various consequences of genetic information being available. Among its goals, ELSI includes practical ethical issues, such as preparation of guidelines for clinicians and enhancing public awareness of the ethical issues related to the human genome project. Whereas research into the genetic predisposition to severe sepsis could have beneficial effects, it also carries with it important ethical issues, such as the use of presymptomatic screening, as well as possible subsequent social discrimination due to 'at-risk polymorphisms'. Genetic data should not be used to predict outcomes or limit treatments; rather, identification of high-risk patients should help us to look for new preventive and therapeutic interventions for those who need them most [83, 84].

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