Sepsis 2001;4:209–215
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Cytokine Polymorphisms and Susceptibility to Severe Infectious Diseases

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Abstract. **Cytokines are key regulators of the host response to infection, controlling the inflammatory reaction which is an essential component of the defense mechanisms. The major importance of these proteins in the pathogenesis and outcome of infectious diseases has been clearly demonstrated. In humans, there is increasing evidence that the host's cytokine response is genetically determined and that the genetic variability of cytokines underlies the complexity of interindividual differences in the immune response to micro organism invasions. We review the influence of host cytokine gene on the susceptibility to and the severity of parasitic, bacterial and viral infections. Proinflammatory cytokine polymorphisms are discussed in detail because of their importance in the course of severe infections such as meningococcal disease, cerebral malaria and septic shock. Genetic variants of the IL-10 gene appear to be responsible for an uncontrolled and intense CARS and may have also dramatic consequences as an overwhelming inflammatory response. Our greater understanding of the genetic factors that influence mortality and morbidity of infectious diseases will permit identification of genomic markers which may be required for risk stratification of patients targeted for novel immunomodulatory treatments. This will help clinicians to select the most appropriate treatment options for their patients.**

Key Words. **sepsis, inflammation-cytokines, polymorphisms, genetic predisposition to disease**

I. Introduction

Despite recent advances in understanding the pathophysiology of infectious diseases, sepsis remains the primary cause of death in intensive care units. Incorrect or late diagnosis, inadequate antimicrobial therapy or altered patient underlying condition all contribute to increase morbidity and mortality of severe sepsis [1]. Outcome has also been related to the number and duration of organ dysfunctions evolving during the course of sepsis. The development of multi-organ dysfunction in the critically ill depends upon the nature and magnitude of the inflammatory response. The inflammatory reaction is an essential component of the defense mechanisms of the body. Despite a tight regulation involving mediators that initiate and maintain inflammation and mediators that shut down the process, the inflammatory response may be greater than that required to control the initial insult [2]. Inflammation may consequently result in injury to various organs and tissues rather than promote resolution and healing.

Cytokines are the key protein regulators of the host response to infection and inflammation [3]. These small proteins, with molecular weights ranging from 8 to 40 kDa, initiate and orchestrate immune reactions as well as local and/or systemic intercellular regulatory factors. They can be functionally classified into pro-inflammatory (TNF-α, IL-1, IL-6, IL-8) and anti-inflammatory (IL-4, IL-10, IL-13, IL-1ra) molecules, since the latter share the ability to suppress expression of pro-inflammatory cytokines, chemokines or adhesion molecules. Pro-inflammatory cytokines are induced first and very rapidly after an infectious challenge, and this is followed by the production of the anti-inflammatory molecules [3]. However, some cytokines have pleiotropic effects and this classification should therefore be considered very carefully. For example, the anti-inflammatory cytokines IL-4 and IL-10 are also potent activators of B lymphocytes.

The major importance of cytokine networks in the pathogenesis and outcome of infectious diseases has been demonstrated by different approaches. Increased serum levels of both pro-inflammatory and anti-inflammatory cytokines have been measured in septic patients and numerous publications reported that the cytokine concentrations correlate with the severity and the outcome of sepsis [3]. The role of a particular cytokine in infection has also been established by experimental studies of genetically modified mice, in which the production of a specific cytokine is suppressed by the targeted deletion of its gene. In these so-called "knock-out" mice, the spontaneous development of an infectious disease and/or their susceptibility to an experimentally induced infection highlight the essential role of the missing protein in regulating the immune response to the pathogen [4]. For example, the in-

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creased susceptibility of IL-6-deficient mice to systemic candidiasis and tuberculosis demonstrates the crucial role of IL-6 in the efficient host defense against yeast and severe mycobacterial infections [5,6]. In contrast IL-10 knock-out mice possess an increased antimycobacterial immunity. Current experimental evidence suggest that cytokines and mediators are interrelated through complex potentiating and inhibiting pathways to orchestrate an inflammatory response that can determine the outcome of severe sepsis [7].

In humans, there is increasing evidence that the host's cytokine response is genetically determined. Studies in healthy individuals have shown stable and reproducible differences in the production of cytokines and have linked these differences with inherited variations in the genes encoding them. Most of the cytokine genes are polymorphic [8]. Single-nucleotide polymorphisms (SNPs) are substitutions of a single base at a particular site of the gene. They are stably carried by more than 1% of the population and can potentially affect the protein product when they are located in the promoter region of the gene to which they are related. Since cytokines are not expressed spontaneously and have to be synthesized *de novo* in response to any cell stressors such as pathogens, promoter SNPs for cytokines can have dramatic consequences. Hence, undoubtedly, the genetic variability of cytokines underlies the complexity of interindividual differences in the immune response to micro organism invasions.

In this review, we only describe cytokine gene polymorphisms that have been associated with a significant effect on transcriptional regulation and susceptibility, severity or clinical outcome of severe infectious diseases.

II. Pro-inflammatory Cytokine SNPs

II.1. Tumor necrosis factor

Tumor necrosis factor refers to two closely related cytokines: TNF-α and TNF-β (currently named lymphotoxin-α or LT-α). Both proteins interact with the same receptors and share *in vitro* very similar biological activities. However, they are derived from different cell types and have different induction kinetics: TNF-α (TNF) is secreted very rapidly (1–4 hours) by activated macrophages whereas TNF-β (LT) is secreted by T-lymphocytes 24–48 hours after mitogenic stimulation. Important insights into the respective functions of each TNF isoforms have been gained from studies performed in TNF- or LT-deficient mice. These studies have demonstrated that TNF and LT are functionally distinct cytokines *in vivo* without any functional redundancy or mutual compensation, and that TNF is the main cytokine in response to infection [9,10].

TNF-α is a pro-inflammatory cytokine that plays a pivotal role in many inflammatory diseases as well as in severe sepsis and septic shock. Produced by many different cell types, TNF is one of the first mediators to appear in response to a very diverse range of infectious stimuli. Once secreted TNF elicits a broad spectrum of immunological and inflammatory responses (synthesis of other inflammatory mediators, release of nitric oxide and oxygen free radicals, upregulation of adhesion molecules, etc.) resulting in fever, shock, and tissue injury [9,11]. 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 [12]. On the other hand, TNF may cause severe pathology when produced in excess. In vivo, injection of TNF produced clinical manifestations resembling those observed after injection of bacteria. Haemodynamic disturbances and mortality have been shown to be correlated to TNF plasma levels [13,14]. Hence, insufficient or excessive production of TNF may be associated with tissue injury, shock and death.

Since TNF is a central element in the host defense response, its production has to be tightly regulated to preserve cellular homeostasis. Interestingly, a marked inter-individual variability of TNF production in response to different stimuli has been reported in healthy subjects [15]. Family studies indicate that up to 60% of this variability is genetically determined [16]. Since the TNF response to infection is partly regulated at the transcriptional level, the role of polymorphisms of the TNF promoter in determination of inflammatory disease susceptibility or as a marker of severity has been the subject of intense research (more than 300 publications). The potential effects of promoter SNPs on transcriptional regulation may be assessed by reporter gene studies [17,18], by analyzing the consequences of the SNPs on protein-DNA interactions [19] or by comparing cytokine secretion *in vivo* [20].

The *TNFA* (TNF) and *TNFB* (LT) genes are closely linked and are located within the class III region of the major histocompatibility complex (MHC) on the short arm of chromosome 6 between 6p21.1 and 6p21.3. Various polymorphisms in the TNF genes have been linked to the variability of TNF production in different individuals [21]. *TNFA* has two biallelic polymorphisms that are responsible for elevated TNF production and have been linked to severe infectious conditions. Located at the position −308 upstream of the transcriptional start, the first one consists of a G (TNF1) to A (TNF2) substitution responsible for a 6- to 9-fold increase in transcription of TNF-α *in vitro* [17] and higher TNF plasma levels *in vivo* [20]. The second SNP in the *TNF* gene promoter resulting in high TNF-α basal expression by monocytes is a G to A transition located at position −376 of the *TNFA* gene promoter [19]. Both −308 and −376 polymorphisms are independent risk factors for cerebral malaria in large case-control studies of African populations [22,23]. Hence patients homozygous for the TNF2 allele have a relative risk of 7 for death or severe neurological sequel

due to cerebral malaria. TNF2 has also been associated with mucocutaneous leishmaniasis [24], scarring trachoma [25], lepromatous leprosy, nephropathia epidemica [26] and with death from meningococcal disease [27] or severe meliodosis [28]. In septic shock, we have shown that TNF2 allele increases the susceptibility for this syndrome (TNF2 allele frequency 39% vs. 18%, in septic shock and controls respectively) and represents a 3.7-fold higher risk of death (95% CI, 1.37–10.24) [29]. Moreover in the same study, the allele *TNFA*-376A was only detected in the non-survivor group of septic shock patients and could potentially represent another prognosis factor [29]. Our study has been confirmed recently in patients with severe surgical infections: TNF2 allele carriers with septic shock had a significantly higher mortality rate than patients carrying the homozygous TNF1 genotype (92% vs. 62%) [30]. In contrast to these results, Stüber et al. failed to show any association between the TNF2 SNP and outcome of patients with severe surgical sepsis [31]. Differences in study populations, in selection criteria or in the accuracy of genotyping techniques can all account for this apparent discrepancy. A polymorphism of the *TNFA* promoter (−806) associated with low plasma levels of TNF has also been published [32]. Since TNF is necessary to control bacterial invasion, it would be very interesting to assess the consequences of this genomic variant on the clinical course of severe sepsis.

In 1991, Pociot et al. reported a biallelic *Nco1* restriction enzyme fragment length polymorphism (RFLP) in the TNF gene locus that was associated with increased TNF-α production [33]. This site has been mapped to the first intron of the LT gene (*TNFB*) at position 1064–1069 and allows to define two alleles, TNFB1 and TNFB2. The latter does not possess the *Nco1* RFLP and seems to be associated with increased TNF-α plasma concentrations [34]. The precise mechanisms underlying these results remain unclear, and the *Nco1* polymorphism may not be directly related to TNF production but rather serve as a MHC marker because of its location in the class III region of the MHC [19]. TNFB2 has been associated with high mortality rates in severe sepsis and with the development of severe post traumatic infections in trauma patients. Hence, for the same age and injury score, the risk of developing a severe sepsis after trauma is 5.2-fold higher in the TNFB2 homozygous patient [34,35]. Finally, the TNFB1 allele, Nco1+/low TNFα genotype is strongly associated with postoperative infections after liver and kidney transplantations [36,37].

II.2. Interleukin-1

The interleukin-1 gene family consists of three members: IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra). The three corresponding genes, located on the human chromosome 2q13, are named respectively *IL1A*, *IL1B*, and *IL1RN*.

Besides TNF, IL-1α and IL-1β are very potent pro-inflammatory cytokines released by macrophages early in the course of sepsis. Both forms of IL-1 bind to the same receptors and have similar functions. *In vitro*, these cytokines amplify most of the biological effects of TNF. Various cells express receptors for IL-1 at their surface and release secondary molecules that mediate inflammation and shock when they are activated by this cytokine. Administration of IL-1α or IL-1β to humans result in inflammation, tissue injury and septic shock-like syndrome [38]. The IL-1 receptor antagonist (IL-1ra), another member of the IL-1 family, is an acute phase protein that binds to the IL-1 receptors but does not induce cell activation. [38]. Hence IL-1ra competes with the binding of IL-1 and represents the physiologic antiinflammatory counterpart of IL-1. *In vivo*, overexpression of IL-1ra protects mice from lethal endotoxemia challenge [38,39]. Balance between IL-1 and IL-1ra levels is obviously important in maintaining homeostasis and inter individual genetic differences in the secretion of the IL-1 family members could influence the severity of infection. The genes of the IL-1 complex are highly polymorphic but the different alleles seem to be in linkage disequilibrium, thereby creating various haplotypes. Intron 2 of *IL1RN* is a polymorphic region that contains a variable numbers of tandem repeats. Allele of this part of *IL1RN* are named according to the rank of frequencies in controls: allele 1 (IL-1RN*1) has a frequency of 0.74 and contains 6 repeats; allele 2 (IL-1RN*2) includes 4 repeats and has a frequency 0.24, allele 3 with 5 repeats has a low frequency of 0.02 (IL-1RN*3) [40]. Although the *IL1B* gene may contain two SNPs at position −511 and +3593 that seem to influence the production of IL-1β *in vitro* [41,42], the IL-1ra allele 2 IL1RN*2 is probably the strongest up-regulator of IL-1β levels *in vitro*. In contrast to the *IL1B* polymorphisms that do not influence incidence or outcome of severe sepsis, the IL1RN*2 has been associated with increased susceptibility to this syndrome [43]. ILIRN*2 is also associated with an adverse outcome of Helicobacter infection [44]. A recent report suggest that the composite genotype over *IL1B* (−511) and IL1RN*2 is strongly associated with fatal outcome of meningococcal disease, indicating that *IL1B* (−511) may be one of the genetic markers of poor prognosis in *N. meningitidis* infection [45].

II.3. Interleukin-6

Interleukin-6 (IL-6) is another potent pro-inflammatory cytokine whose gene contains functional SPNs. IL-6 has multiple biological activities on a broad variety of cells and is detected in serum and other biological fluids of patients with severe sepsis. Consequently, the use of IL-6 as a marker of the severity of infection has been suggested [3]. Experiments in IL-6-deficient

mice showed that IL-6 plays an important role *in vivo* in bacterial clearance and in the response to infection [46,47]. Inherited variations in the IL-6 gene have also been reported. The G to A substitution at position −174 in the IL-6 gene promoter is part of a complex haplotype (−579G; −572G; −373A; −174G) which increases *in vitro* transcription of IL-6 [48,49]. These data suggest that genetic polymorphisms in the IL-6 promoter influence cytokine transcription through complex interactions [49]. Despite the pivotal role of IL-6 in the pathophysiology of sepsis, this recently described IL-6 haplotype has not yet been associated with any infectious diseases.

II.4. Interleukin-8

IL-8 is a potent chemokine involved in different aspects of the inflammatory response, including neutrophil infiltration and recruitment after lung infection. Experimentally, infected airway epithelial cells secrete high levels of IL-8. In humans, IL-8 is considered as a major polymorphonuclear neutrophils chemoattractant cytokine in acute respiratory distress syndrome [50] as well as in bacterial pneumonia [51]. Although increased IL-8 fluid levels have been found in the bronchoalveolar lavage of patients with ARDS, it is still difficult to know whether IL-8 plays an important role in the pathophysiology of acute lung injury or increases as an epiphenomenon of the inflammatory process. Similarly, high levels of IL-8 have been found in the plasma and respiratory secretions of infants with respiratory syncytial virus (RSV) infection, correlating with the high proportion of neutrophils in their BAL fluid. A common SNP has been recently identified 251 bp upstream of the IL-8 transcription start site (allele frequency 0.44). The IL8-251A SNP has been associated with increased IL-8 production in response to LPS stimulation and constitutes a genetic predisposition for RSV bronchiolitis occurrence and severity [52]. Indeed, IL8-251A is more frequently found in infants requiring oxygen therapy for more than two days and in cases of RSV bronchiolitis with no other known risk factors. Incidence and consequences of IL8-251A in ARDS patients are still unknown.

III. Anti-inflammatory Cytokine SNPs

Sepsis induces an initial pro-inflammatory burst followed by an important release of anti-inflammatory cytokines (IL-4, IL-10, IL-13) that result in a down-regulation of humoral and cellular immunity called immunoparalysis or compensatory anti-inflammatory response syndrome (CARS) [53]. Genetic polymorphisms responsible for an uncontrolled and intense CARS may have the same dramatic consequences as an overwhelming inflammatory response.

Interleukin-10

IL-10 is an anti-inflammatory cytokine produced primarily by cells of the monocyte/macrophage lineage and to a lesser extent by activated T and B cells. It suppresses the function of macrophages (down-regulation of the Th1 cytokines) and indirectly inhibits the activity of B cells. The net effect of IL-10 release is immunosuppressive, and high IL-10 production has been shown to inhibit interferon-gamma expression and to delay clearance of intracellular pathogens such as Chlamydia. Accordingly, the risk for fatal outcome of meningococcal disease is increased in families with high IL-10 production [16]. Although both genetic and non genetic factors contribute to IL-10 production, twin studies suggested that the genetic factors could account for up to 75% of IL-10 production [16,54].

Several polymorphism sites resulting in interindividual differences in IL-10 production have been identified within the human interleukin-10 gene. Within the IL-10 proximal promoter, two CA-repeat microsatellites, and three SNPs located at −1082 (G/A), −819 (C/T), and −592 (C/A) upstream of the transcription start site have been described [55,56]. More SNPs in the distal IL-10 promoter have been recently identified with either a high- or a low IL-10 production phenotype, thereby creating eight distal promoter haplotypes [57]. *In vitro*, the IL10-1082G polymorphism has been associated with a high IL-10 producing capability by lymphocytes [58]. Within the Mandikas ethnic group, the IL10-1082G homozygote genotype was significantly more common among patients with trachoma than in controls (odds ratio 5.10; confidence interval, 1.24–24.2; $p = 0.009$) [59]. 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. 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 effect on all infections.

IV. Conclusions and Perspectives

The search for genetic variation associated with disease is a new and exciting area of medical investigation that has rapidly expanded in recent years. It is clear that inherited differences in cytokine DNA sequences contribute to phenotypic variations, risks of disease and responses to the environment including infectious challenge. Interindividual differences in cytokine profiles appear to be due mainly to allelic polymorphism within regulatory regions of the cytokine gene. Increasing numbers of cytokine SNPs are reported monthly and a web site featuring appropriate updates in this field is now available (*http://www.pam.bris.ac.uk/ services/GAI/cytokines4.htm*) [8]. The recent publication of a map of the human genome se-

quence variation containing 1.42 million SNPs will certainly add some complexity to this initial scheme. Such research may help intensivists to understand why some patients develop sepsis and die within a few hours when they encounter a pathogen, whereas others stay healthy and organize their defense system. Moreover, better understanding of the influence of cytokine SNPs may change the therapeutic strategies of sepsis. Hence, detection of genetically-predetermined high TNF secretors may permit to select patients who could benefit from anti-TNF strategies. On the other hand, as demonstrated by studies on transplant patients, it is possible that further inhibition of TNF-secretion by routine immunosuppressive agents such as corticosteroids may be harmful for low TNF-α producers.

In the near future, identification of genomic markers may be required for clinical use and risk stratification of patients targeted for novel immunomodulatory treatments. DNA microarrays allow the simultaneous measurement of the level of expression of thousands of genes and will facilitate mapping and identification of candidate disease genes for polygenic disorders such as susceptibility to severe infections. This technology may help to understand the genetic programs underlying immune responses and one can envision that gene profiling will help clinicians to select the most appropriate treatment options for their patients.

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