Preface

Cytokines and chemokines are an important class of effector molecules that play a fundamental role in orchestrating the innate and acquired immune responses needed to eliminate or wall off invading pathogens. In vitro and in vivo studies have been instrumental in revealing the complexity of the cytokine network and the many facets of cytokine biology, such as pleiotropism (i.e., the capacity for a given cytokine to stimulate several cell types) and redundancy (i.e., the ability of different cytokines to exert similar effects). However, the development of sensitive reagents to detect and measure human cytokines and chemokines has provided opportunities to investigate the role of these important mediators in human inflammatory and infectious diseases. Despite many similarities, important differences in cytokine responses and mode of action between human and animal models became evident. A shift in focus from animal to clinical studies was, therefore, inevitable.

In recent years, we have witnessed an outpouring of information on the role of cytokines and chemokines in human infectious diseases. These studies have led to a deeper understanding of the pathogenesis of infectious diseases, an appreciation for differences of cytokine and chemokine production profiles in response to various pathogens, and a realization that genetic host factors influence the type and magnitude of cytokine and chemokine responses to a given microorganism. Our understanding of the immunopathogenesis of specific infections has become much more profound and thorough, and has thus contributed to the design of better and more effective therapeutic interventions for the management of patients with infectious diseases.

While playing a pivotal role in host defense against infection, cytokines also contribute to pathology when released in excessive amounts. Much work, both in academic institutions and in the biotechnology and pharmaceutical industries, has been devoted to the development of cytokine or anticytokine treatment strategies in infectious diseases. Although some strategies have failed, there have been numerous successes that have led to effective interventions for inflammatory and infectious diseases. One reason for the failure of cytokine-based therapies in infectious diseases may have stemmed from a lack of understanding of important differences in cytokine biology in infections caused by different pathogens.

Cytokines and Chemokines in Infectious Diseases Handbook is meant to provide a unique and up-to-date reference on the role of cytokines and chemokines in a variety of human infectious diseases. International leaders in the field present a comprehensive overview of cytokine and chemokine responses in bacterial, viral, fungal, and parasitic infections. Readers will gain a better appreciation for the differences in cytokine profiles in distinct infectious diseases and will see how this knowledge has led to a deeper understanding of host–pathogen interactions, as well as the pathogenetic basis of infectious diseases. In addition, *Handbook of Cytokines and Chemokines in Infectious Diseases* is intended to provide a critical evaluation of the use of cytokines and anticytokines in the treatment of infectious diseases and to demonstrate how knowledge of cytokine pleiotropic effects, redundancy, and the complexity of the cytokine network has impacted the use of cytokines as therapeutic tools.

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2

Cytokine Gene Polymorphism and Host Susceptibility to Infection

Frank Stüber

1. INTRODUCTION

The individual susceptibility to infection of any organism is determined by a variety of factors such as environmental conditions of the host, pathogenicity of infecting microbes, and the effectiveness of the host's defense systems. The induction of specific immune responses such as antibodies released by β-cells and effector T-cells directed against antigens of invading microbes rely on antigen-presenting cells such as macrophages and dendritic cells. These "smart weapons" guarantee the elimination and clearance of antigens and invaders from the body without harming the host's own cellular and organ structure and function. In contrast, molecules as part of the evolutionary older innate immune system like defensins or cytokines may prove to be harmful for the host if released in excessive amounts into systemic circulation. On the other hand, low levels of these molecules locally released at the site of infection may result in insufficient clearance of invading microbes.

The host's inflammatory response to infection contributes as a main factor to morbidity and mortality in today's intensive care units, and it displays a high interindividual variation *(1)* that is not sufficiently explained by single factors such as gender *(2)*. Comparable amounts of infectious units of microbial organisms induce a wide range of severity of infectious diseases. The role of an individual's genetic background and predisposition to the extent of inflammatory responses is also determined by genetic variants of endogenous mediators that constitute the pathways of endogenous mediators of host responses to infection. Important candidate genes for host susceptibility to infection are cytokine genes.

Primary responses in inflammation are mediated by pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin 1 (IL-1) *(3)*. Recent evidence suggests that anti-inflammatory mediators have important effects on the host's immune system *(4)*. Anti-inflammatory mediators induce a state of immunosuppression in sepsis that has also been named "immunoparalysis" *(5)*. Pro-inflammatory and anti-inflammatory responses contribute to the outcome of patients with systemic inflammation and sepsis in humans. The genetically determined capacity of cytokine production and release may contribute to a wide range of clinical manifestations of inflammatory disease: a patient with peritonitis, for example, may present without symptoms of sepsis and recover within days or may suffer from fulminant septic shock, resulting in death within hours.

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2. CYTOKINE GENE POLYMORPHISM: CANDIDATE GENES

2.1. Tumor Necrosis Factor

Primary proinflammatory cytokines like TNF and IL-1 induce secondary proinflammatory and anti-inflammatory mediators like IL-6 and IL-10. They have been shown to contribute substantially to the host's primary response to infection. Both TNF and IL-1 are capable of inducing the same symptoms and the same severity of septic shock and organ dysfunction as endotoxin in experimental settings as well as in humans *(6)*. Genetic variations in the TNF and IL-1 genes are of major interest concerning genetically determined differences in the susceptibility and response to infection.

Tumor necrosis factor is considered one of the most important mediators of endotoxin induced effects. Interindividual differences of TNF release have been described *(7,8)*.

The TNF locus consists of three functional genes. TNF is positioned between lymphotoxin β (LTβ) in the upstream direction and lymphotoxin α (LTα) in the downstream direction. Genomic polymorphisms within in the TNF locus have been under intense investigation.

Genetic variation within the TNF locus is rare, as the TNF gene is well conserved throughout evolution *(9)*. Especially, the coding region is highly conserved.

The main interest has been focused on the genomic variations of the TNF locus: Biallelic polymorphisms defined by restriction enzymes (NcoI, AspHI) or other single base-changes (–308, –238) as well as multiallelic microsatellites (TNFa–e) have been investigated in experimental in vitro studies and also in various diseases in which TNF is considered as an important or possible pathogen. Functional importance for regulation of the TNF gene has been suggested for two polymorphisms within the TNF promoter region. Single-base changes have been detected at positions –850, –376, –308, and –238 *(10–13)*. A G to A transition at position –308 has been associated with susceptibility to cerebral malaria *(14)*. These results could not be confirmed by another malaria study that showed fewer fever episodes in heterozygous carriers of the allele TNF2 *(15)*. In contrast, more recent findings link altered OCT-1 binding in the TNF promoter with susceptibility to severe malaria *(11)*. Further evidence for the association of quantitative cytokine responses with susceptibility to parasitemia has been reported very recently *(16)*. Even susceptibility to *Helicobacter pylori* infection has been examined and shows a correlation of the rare allele TNF2 of the –308 polymorphism with infection with the cagA subtype in Korean patients with gastric disease *(17)*. Studies linking TNF genomic variability to the incidence or severity of viral hepatitis C infection or response to antiviral therapy could not be confirmed by Rosen et al. *(18)*, whereas allele TNF2 might display protective effects in cytomegalovirus infection *(19)*.

The rare allele TNF2 (A at position –308) was suggested to be linked to high TNF promoter activity *(14)*. Autoimmune diseases like diabetes mellitus or lupus erythematosus did not show differences of allele frequencies or genotype distribution between patients and controls *(20,21)*. In addition, patients with severe sepsis and a high proportion of Gram-negative infection also did not display altered allele frequencies concerning both biallelic promoter polymorphisms (positions –238 and –308) *(22)*. Analysis of the TNF promoter by means of reporter gene constructs revealed contradictory results. A first report supposed a functional importance of the –308 G to A transition *(14)*. Two articles could not confirm differences of the TNF promoter activity in relation to the –308 polymorphism *(22,23)*. A recent article reported a possible influence on TNF promoter activity by the –308 G to A transition in a B-cell line *(214)*. Data demonstrating an impact of this genomic polymorphism on transcription are rather weak, as reports predominantly derive from one group *(25)* (*see* Table 1), findings seem to be restricted to few cell lines, and impaired or enhanced binding of transcription factors has not been shown. In addition, the difference in transcription rates in the responsive cell line seems to require a specific stimulus (PMA plus retinoic acid) *(26)*.

Table 1
Actual Evidence that Association of TNF Polymorphisms with Gene Function is Weak

TNF promoter polymorphisms	Promoter activity	Association with Protein expression	Susceptibility to infection
–238	Uncertain	Uncertain	Uncertain
–308	In B-Lymphocytes	In septic shock	Uncertain
TNF locus polymorphisms			
NcoI	Unknown	In peritonitis	In trauma
TNFa–e microsatellites	Unknown	Uncertain	Unknown

Studies trying to associate incidence or severity of infectious disease with TNF polymorphisms have been published on a variety of pathogens. Positive associations of the biallelic TNF –308 as well as LTα polymorphisms with susceptibility to mucocutaneous leishmaniasis and leprosy have been reported *(27,28)*.

Genotyping of this polymorphism in patients with severe sepsis or septic shock still shows controversial results. In contrast to the negative findings in sepsis are the results of two recent studies that suggest an association of the rare allele TNF2 with nonsurvivors of septic shock *(29,30)*. These publications again open the discussion about functionality of the –308 TNF promoter polymorphism and its possible relevance for routine clinical use. In addition to the discussion about the relevance of association, formal standards of genotyping techniques have to be established. Are there typing techniques like allele-specific amplification that imply overestimation or underestimation of certain alleles and genotypes?

In contrast to genomic variations located in the promoter region, intronic polymorphisms are more difficult to associate with a possible functional relevance. Two biallelic polymorphisms located within intron 1 of LTα have been studied in autoimmune disease *(31,32)*. One polymorphism is characterized by the absence or presence of a NcoI restriction site. First reports demonstrated genomic blots revealing characteristic 5.5- or 10.5-kb bands after genomic NcoI digest, which hybridize to TNF-specific probes *(33)*. These bands correspond to presence and absence, respectively, of a NcoI restriction site within intron 1 of lymphotoxin α.

The allele TNFB2 of this NcoI polymorphism (10.5-kb band) has been shown to be associated with high TNF release ex vivo *(34)*. Other studies showed no differences between genotypes in other models of ex vivo TNF induction, whereas another study suggests an increased LTα response in TNFB2 homozygotes *(8)*. The question of which genotype is clearly associated with a high proinflammatory response in the clinical situation of severe Gram-negative infection and severe sepsis cannot yet be answered by ex vivo studies. Different conditions of cell culture and cytokine induction contribute to differing results. In addition, the genomic NcoI polymorphism within intron 1 of the LTα gene may represent a genomic marker without evidence for own functional importance in gene regulation. This genomic marker may coincide with so far undetected genomic variations that are responsible for genetic determination of a high proinflammatory responses to infection. Results from studies in patients with severe intra-abdominal sepsis suggest TNFB2 homozygotes to be associated with a high TNF response. In contrast, genotyping for another biallelic polymorphism within intron 1 of LTα (AspHI) did not show significant association to TNF plasma levels (data not shown).

Several studies in chronic inflammatory autoimmune diseases suggest an association between TNFB2 and incidence or severity and outcome of the disease *(31,32,35)*. Studies in acute inflammatory diseases like severe sepsis in patients in surgical intensive care units showed a correlation between

TNFB2 homozygosity and mortality *(22)* or incidence of septic states in traumatized patients *(36)*. TNFB2 homozygotes displayed a relative risk of 2.9 of dying from severe sepsis when compared to corresponding genotypes.

2.2. Interleukin-1

In addition to TNF, IL-1 is another potent proinflammatory cytokine released by macrophages in the systemic inflammatory response. IL-1 is capable of inducing the symptoms of septic shock and organ failure in animal models and is regarded as a primary mediator of the systemic inflammatory response. Antagonizing IL-1 in endotoxin challenged animals including primates abrogates the lethal effects of endotoxin *(37)*. A biallelic TaqI polymorphism has been described within the coding region (exon 5) of IL-1β *(38,39)* Despite the finding that a homozygous TaqI genotype correlates with high IL-β secretion *(38)*, genotyping of patients with severe sepsis did not reveal any association with incidence or outcome of the disease. In contrast, the allele T of the IL-1BC-31T polymorphisms has been linked to susceptibility to persistent *H. pylori* infection in Japanese patients following an eradication program *(40)*.

2.3. Interleukin-1 Receptor Antagonist

Proinflammatory mediators comprise the hyperinflammatory side of the host's response to infection. At the same time, anti-inflammatory mediators are induced by proinflammatory cytokines and try to counterbalance the increased inflammatory activity. This physiologic process of limiting the extent of inflammation by release of anti-inflammatory proteins may escape physiologic boundaries of local and systemic concentrations of these mediators. Proteins like IL-4, IL-10, IL-11 or IL-13, or IL-1ra contribute to a very powerful downregulation of cellular and humoral proinflammatory activities. This downregulation results in decreased expression of class II molecules in antigen presenting cells as well as in low ex vivo responses of immuncompetent cells to inflammatory stimuli. This state of imunosuppression has also been termed "immunoparalysis" *(5)*. It results in a situation of anergy and diminished capabilities of fighting infectious pathogens. A new term for this status, which is a consequence of the systemic inflammatory response, is "compensatory anti-inflammatory response syndrome" (CARS) *(41)*. The outcome of patients with, for example, severe sepsis is not only influenced by hyperinflammation in fulminant situations of progressing organ dysfunction but may also be limited by immunosuppression and lack of restoration of immune function. In this view, an overwhelming anti-inflammatory response with a possible genetic background of interindividual differences in the release of anti-inflammatory mediators following infection contributes to the human systemic inflammatory reaction to a similar extent as proinflammatory responses.

A genomic polymorphism of the anti-inflammatory cytokine IL-1ra is located within intron 2 and consists of variable numbers of a tandem repeat (VNTR) of a 86-bp motif. This 86-bp motif contains at least three known binding sites for DNA-binding proteins *(42)*. Ex vivo experiments suggest that higher IL-1ra responses combined with alleles containing low numbers of the 86-bp repeat. Ex vivo studies also demonstrate a higher level of IL-1ra protein expression and protein release of A2 homozygous individuals compared to heterozygotes following stimulation with lipopolysaccharide *(43)*.

The allele A2 has been associated with the incidence of autoimmune diseases like lupus erythematosus and insulin-dependent diabetes mellitus *(44,45)*. In acute systemic inflammation, there is no difference between surviving or nonsurviving patients with severe sepsis. This finding is in contrast to the results concerning the biallelic NcoI polymorphism within intron 1 of LTα: Homozygotes for the TNFB2 genotype revealed a high mortality when compared to heterozygotes and TNFB1 homozygotes. The overall group of patients with severe sepsis did not show an increase in the TNFB2 allele frequency. For the IL-1ra polymorphism, however, an increase of the allele A2 in the patients with severe sepsis was detected. Patients carrying the haplotype TNFB2 homozygous and A2 homozygous did not survive in this study.

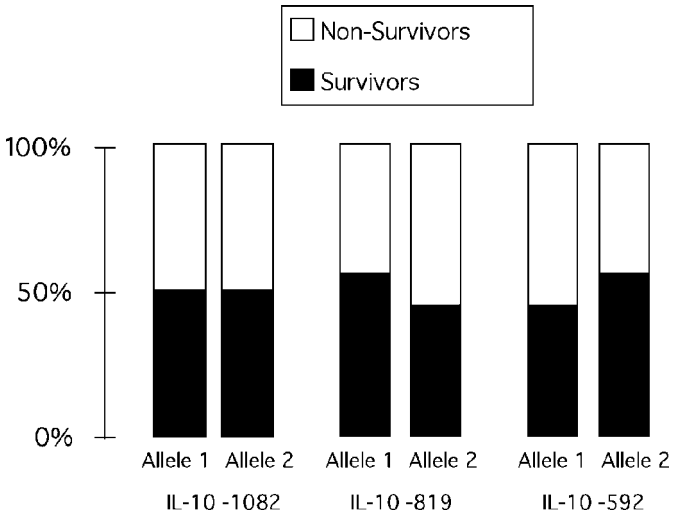

Fig. 1. Comparison of allele frequencies of IL-10 promoter polymorphisms in nonsurvivors (n = 67) and survivors (n = 63) with severe abdominal sepsis ($p > 0.05$).

Negative associations between IL-1ra polymorphisms and susceptibility to infection have been suggested for vaginal mycoplasma colonization, cytomegalovirus and Epstein–Barr virus infection as well as human immunodeficiency virus (HIV) reproductivity and hemorrhagic fever caused by a hantavirus (*see* reviews in refs. *46,47).*

3. FINDINGS IN OTHER CYTOKINE CANDIDATE GENES

Interleukin-10 shows well-defined haplotypic promoter variation. High IL-10 secretors presenting with fever of unknown origin display an increased mortality rate *(48)*, whereas susceptibility to severe meningococcal disease as well as poor outcome seem to be linked to inherited high IL-10 secretion and low TNF release in a family study testing ex vivo cytokine inducibility *(49)*. IL-10 promoter genotypes could not be associated with incidence or outcome of severe abdominal sepsis (Fig. 1).

In viral infections, IL10-1082 promoter polymorphism has been associated with susceptibility to chronic hepatitis C infection and resistance to antiviral therapy *(50)*. Another publication suggests that high IL-10 secretion indicated by the –1082 polymorphism or the promoter haplotype defined by single nucleotide polymorphisms at positions –1082, –819, and –592 protects against Epstein–Barr virus infection *(51,52)*.

Interestingly, another cytokine promoter polymorphism, interleukin-8-251A, has been associated with high IL-8 release and, possibly because of the IL-8 proinflammatory profile, also associated with the incidence of virus bronchiolitis in an excellent family-based study *(53)*.

Other results show that lipopolysaccharide (LPS)-binding protein may contribute to susceptibility to severe sepsis *(54)*, as suggested in a study also investigating the effects of gender. Genomic variability in chemokine genes have been demonstrated to influence the course of HIV infection *(55–57)*.

4. SIGNAL TRANSDUCTION PATHWAYS IN INFECTION INDUCING CYTOKINES

Transduction of the LPS signal into the cell has been an unknown mechanism until recently. An analogon of the so-called Toll-like receptor in *Drosophila* species that transduces signals for the elaboration of innate immune responses in flies directed against bacteria and fungi has been identified in mice and other species *(58)*. A single-basepair change resulting in an amino acid change of the

murine Toll-like receptor 4 (TLR4) renders the extensively studied mouse strain CH3/HeJ highly resistant to LPS challenge *(59)*. Ten Toll-like receptors (TLR1–10) *(60)* have been identified in mammals so far. TLR2 has been identified to transduce peptidoglycan stimulation by Gram-positive organisms *(61–63)*. In contrast, TLR4 seems to play a key role in the LPS-induced signaling pathway. TLR9 transduces inflammatory effects of bacterial DNA.

The presence of a functional TLR4 gene and gene product appears to be one of several determinants of outcome in Gram-negative infection. A first preliminary report suggests that a rare Arg753Gln mutation might render patients with sepsis susceptible to staphylococcal infection *(64)*. Another rare Arg677Trp variation of TLR2 has been linked to susceptibility to lepromatous leprosy *(65)*. Studies to test the association of the rare TLR4 variations with incidence and course of infectious disease are ongoing *(66,67)*.

5. CONCLUSION

Uncovering and understanding the genetic determination of the susceptibility to infection offers the chance of developing valuable diagnostic tools and new therapeutic approaches in severe sepsis. Evaluation of candidate genomic markers for risk stratification of individuals at high risk of developing infectious disease has just begun. Many candidate genes still have to be studied and clinical significance of genomic markers will be tested. In addition, this new approach may prove to be a valuable inclusion criterion for studies testing the prevention of infectious diseases in subpopulations known to be at high risk because of genomic predisposition. Most studies so far include rather small numbers of individuals and are in danger of being statistically underpowered and lack quality control of genotyping. New study designs will provide the scientific community with adequately powered studies, well-established concepts of genetic epidemiology, and quality control criteria of genotyping. These designs will include the determination of the genomic background variability in a given population to control for false-positive association (concept of genomic controls). Technical progress will allow researchers to step beyond candidate gene approaches and scan the genome to discover previously unnoticed loci of interest. Extension of single genomic marker analysis to haplotype analyses including functionally relevant alleles may reveal the highest informativity and diagnostic relevance even before the era of widely available genomic scans.

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