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• PATHOPHYSIOLOGY OF POST-INJURY INFECTION AND ORGAN FAILURE

FUNDING

National Institute of General Medical Sciences

Severe traumatic injury results in biochemical and physiological changes that often lead to the development of nosocomial infection (pneumonia, wound infections, etc) and remote organ (lung, kidney, liver) failure. Excluding those patients who succumb to their injuries and die in the immediate (≤ 1 hour) or early (≤ 24 hours) post-injury period, infection and organ failure (MODS; multiple organ dysfunction syndrome) are leading causes of death. Furthermore, infection and organ failure contribute to prolonged and resource intensive hospital stays. However, if these complications are not lethal, they do not appear to result in major long-term disabilities.

Despite considerable progress in the understanding of the pathophysiology of post-injury infection and organ failure, it has been difficult to translate the observations made in well-designed animal experimentation into effective therapeutics in humans. Two possibilities exist that are, in part, responsible for this inability to clearly influence the course of post-injury infection and organ failure. First, it is likely that our understanding of the problem is incomplete, not from

genetic influences on the risk for and outcome from injury-related nosocomial infection and organ failure and to better characterize the nature of the inflammatory response to tissue injury. In this report, we will demonstrate our findings regarding (1) the effect of genetic variations in the form of single nucleotide polymorphisms (SNPs) on cytokine production by whole blood leukocytes exposed to bacterial endotoxin, (2) relationships between SNPs and the severity of acute appendicitis and the associated cytokine production and, (3) the role of SNPs as markers for the development of severe sepsis and septic shock after trauma.

Polymorphisms in the Interleukin-6 (IL-6) Gene Promoter Affect Cytokine Production

Interleukin-6 (IL-6) plays a key role in the acute-phase response to infection and injury. A single nucleotide polymorphism guanine to cytosine substitution at -174 in the promoter region of the IL-6 gene has been associated with a lower circulating IL-6 concentration in healthy humans who carried the C-allele. However, there is controversy whether this polymorphism

We have identified associations between gene polymorphisms and severe sepsis and septic shock after trauma.

an informational perspective, but rather a conceptual oversimplification in an attempt to force a simple linear “cause - effect” model on a condition that represents a complex biological system with numerous inputs and multiple possible outputs or phenotypic expressions. Second, failure to consider individual variability, in the form of gene polymorphisms, may have reduced our ability to detect beneficial effects of novel therapies.

We are interested in both of these related phenomena and our research program aims to characterize

is associated with the IL-6 response to inflammatory stimuli. Thus, we sought to determine whether carriage of the less common C-allele was associated with a reduced production of IL-6 after stimulus using an *ex vivo* model.

In this series of experiments, diluted whole blood from healthy volunteers was stimulated with bacterial endotoxin (lipopolysaccharide; LPS) for 24 hours and IL-6 production was measured in the supernatant fluid. Individuals were genotyped for 3 polymorphisms in the promoter region of the IL-6 gene; each of which is

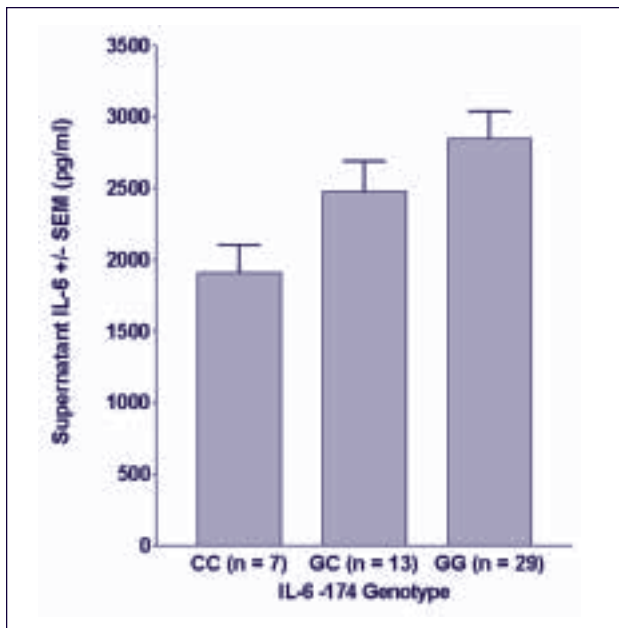


FIGURE 1

considered to possibly influence IL-6 gene transcription. We observed that the polymorphism at the -174 position was associated with IL-6 cytokine production. The number of C-alleles that were carried was associated with a progressive decrease in the IL-6 response to LPS (results shown in figure 1).

While the *ex vivo* model that we use is meant to reflect the complex *in vivo* situation and avoid difficulties attendant with leukocyte isolation, it is not clear how these observations might translate into various, complex clinical circumstances. For example, a reduced capacity to produce IL-6 (or other cytokines) may be detrimental under some circumstances and beneficial in others. We therefore recognize that all our studies and observations made here must be re-examined in a range of clinical scenarios.

Evaluation of Single Nucleotide Polymorphisms in Five Innate Immunity Genes and the Severity of Acute Appendicitis

Innate immunity is the body's first line system for recognizing and destroying invading microbes. In the study summarized here, we hypothesized that polymorphisms in genes involved in these defenses would be associated with clinical outcomes in local infections caused by the body's commensal microbial flora. We tested this hypothesis by studying patients with acute infection-inflammation of the vermiform appendix, a localized infection that requires prompt surgical extirpation of the appendix to avoid complications.

We looked for associations between the severity of acute appendicitis and allelic polymorphisms located within genes involved in recognizing bacterial molecules [CD14 (-159 C(T); TLR4 (896 A(G))] and in mounting the inflammatory response [IL-6 (-174 G(C), TNF- α , (-308 G(A), IL-1 β (-31 C (T))].

We studied 134 patients with acute appendicitis. A total of 91 patients had uncomplicated disease and 43 had complicated appendicitis; which refers to the presence of microscopic evidence of gangrene, necrosis or perforation of the appendix. Polymorphisms in the IL-6 and TNF- α promoters were associated with a greater risk for complicated appendicitis; polymorphisms in the other genes were not. The results of our multivariate analysis are shown in the table below.

Logistic regression analysis of the association between single nucleotide polymorphisms and complicated appendicitis.

Interestingly, a "high-risk" genotype, defined by the presence of at least one A-allele at TNF- α (-308 and GG-homozygosity at the IL-6 -174 position was associated with a 50% risk for complicated appendicitis. In contrast, a "low-risk" genotype, defined by the absence of the TNF- α (-308 A-allele and at least one C-allele at IL-6 -174 position was associated with a 12% risk of complicated appendicitis. So, it seems that the severity of a common surgical disease, may in part be determined by genetic differences in at least two cytokine genes.

RISK FACTOR	ODDS RATIO	95% CONFIDENCE INTERVAL	P-VALUE
Symptom duration	1.02	1.01-1.03	0.03
IL-6 -174 GG-homozygotes	5.2	1.5-18.5	0.01
TNF- α -308 A-allele	2.7	0.9-7.9	0.07

TABLE 1: Results of Multivariate Analysis

An additional advantage of appendicitis as a model of human inflammation is that it allows sampling of regional (peritoneal) and systemic (blood) compartments for cytokine measurements. We observed that both plasma and peritoneal fluid IL-6 concentrations were higher in the GG-homozygotes than the C-allele carriers (see data shown in figures 2 and 3). These observations are consistent with the findings in our *ex vivo* model of LPS stimulation of leukocytes.

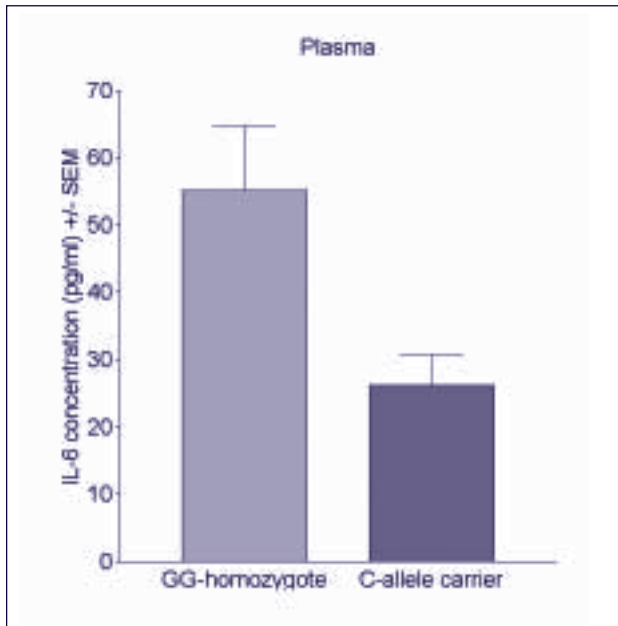


FIGURE 2: Plasma Concentrations

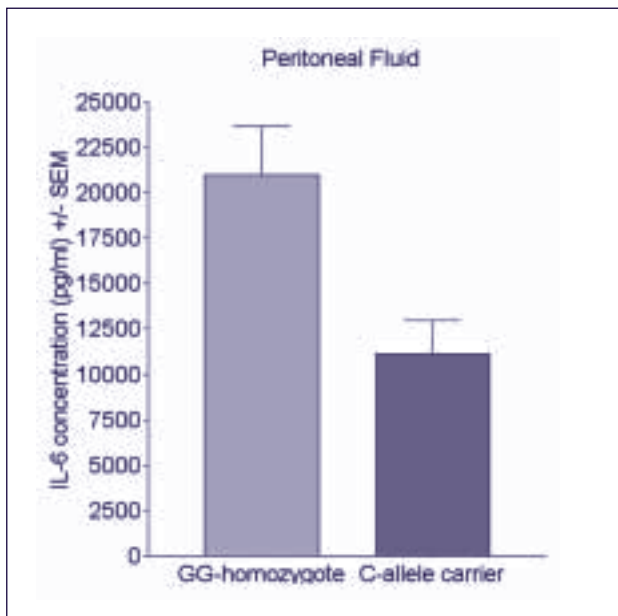


FIGURE 3: Peritoneal Concentrations

The G→A Single Nucleotide Polymorphism at the -308 Position in the TNF-α Promoter Increases the Risk for Severe Sepsis after Trauma

We have also conducted similar genetic evaluation in patients with severe injury, who are at risk for sepsis complicated by organ failure (severe sepsis) and septic shock — which are referred to here as “complicated sepsis”. We have initially focused our efforts on the TNF-α promoter, in which a number of SNPs may affect transcriptional regulation of TNF-α production.

A SNP at the -308 position (G→A substitution) was shown to alter TNF-α gene transcription in a transfection model. Carriage of the less common A-allele has been associated with increased risk of acquiring several infectious and inflammatory diseases and with adverse clinical outcomes in a number of clinical settings.

For example, increased risk for renal transplant rejection, death from meningococcal sepsis, and mortality from septic shock has been reported in A-allele carriers. However, others have found that the A-allele does not increase transcription rates in vitro and that carriage of this allele is not associated with increased risk for severe sepsis. Although less extensively studied than the -308 polymorphism, other TNF-α promoter SNPs may also be important in transcriptional regulation of TNF-α production. In the case of the -376 G→A transition, Knight and colleagues determined that carriage of the uncommon A-allele was associated with a higher incidence of cerebral malaria and that basal gene expression was significantly greater in monocytes transfected with the A-allele than those transfected with the G-allele.

In the study summarized here, we asked whether these naturally occurring genetic differences in the TNF-α promoter are markers for the development of complicated sepsis in severely injured patients. We hypothesized that carriage of the uncommon (A) allele at the -238, -308 or -376 position in the TNF-α promoter is associated with an increased risk for complicated sepsis.

Of the three SNPs in the TNF-α promoter that we studied, only the G(A transition at the -308 position was more frequent in patients with severe sepsis. The single patient who was homozygous for the A-allele developed severe sepsis, as did 15 of the 34 (44%)

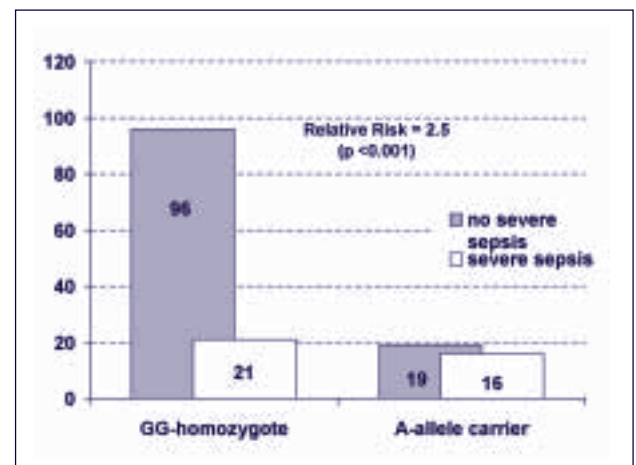


FIGURE 4: Sepsis Risk Factor

heterozygotes. Therefore, carriage of the A-allele was associated with a 46% risk (16/35) of severe sepsis (See Figure 4). This is in contrast to the 18% risk (21/117) in patients homozygous for the G-allele (wild type).

The unadjusted relative risk for complicated sepsis associated with the A-allele was 2.5 (95% CI = 1.5–4.3). Carriage of the A-allele at either the -238 or -376 position was uncommon and was not associated with complicated sepsis. What could be considered as traditional clinical risk factors for complicated sepsis (age < 55 years, early post-injury shock as indicated by an arterial base deficit of < 6 meq/L from 6–24 hours after injury) were present to a similar extent in the -308 A-allele carriers and GG-homozygotes. After adjusting for these risk factors, carriage of the TNF- α -308 A-allele was associated with a 4.6-fold increased risk for severe sepsis or septic shock.

Summary

We have identified associations between gene polymorphisms and severe sepsis and septic shock after trauma. It will, however, be necessary to generate DNA

databanks, linked to reliable detailed clinical data, in considerably larger cohorts of injury victims; this is one aim of our ongoing work. Appendicitis represents an interesting and potentially useful clinical model of inflammation. We will continue to study these patients in detail. Finally, ex vivo experimentation may provide data to identify important SNPs that can then be evaluated in more complex human models.

Our congruent observations regarding IL-6 production in patients with acute appendicitis and in healthy control subjects, suggest that this polymorphism does affect IL-6 production and potentially also the severity of local inflammation. Our research program will continue to address multiple SNPs for their effect on cytokine release and for the severity of acute inflammation. We will examine which SNPs are related to the most severe manifestations of sepsis — sepsis with organ failure and septic shock.

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