Genetic Polymorphisms, Functional Genomics and the Host Inflammatory Response to Injury and Inflammation

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Introduction

The term ‘sepsis’ refers to the host’s systemic inflammatory response to an invasive microbial challenge; when the clinical constellation includes hypotension and/or concurrent end-organ injury, the condition is known as ‘severe sepsis’ or ‘septic shock’. There are more than 750,000 cases and 100,000 deaths each year in the US alone attributable to sepsis and septic shock [1, 2], translating into total costs of USD 16.7 billion annually [3]. Overall, septic patients have a 25–45% mortality [1, 3, 4]. The outcome of patients with sepsis and septic shock has improved minimally in the past 50 years, despite significant overall improvements in intensive care medicine. This systemic inflammatory response, or more explicitly, the interplay between the microbial pathogen and the characteristics of host response, determines the magnitude and diversity of the host response, and ultimately outcome. A growing body of evidence is mounting to suggest that much of the host response is a direct reflection of heritable traits, accounting for interpersonal differences, and allowing for genetic detection.
Systemic Inflammatory Diseases and Immunity

The role of the innate and the acquired immune response in chronic inflammatory diseases is regulated and sustained by the activation of an acquired immune response by antigen-presenting cells, such as macrophages and dendritic cells, with the subsequent release of inflammatory mediators. The inflammatory response is initiated and propagated by an element of immune recognition of self antigens [5], and is sustained by the overproduction of inflammatory mediators and T-lymphocyte-derived cytokines. Hence, both the reactive and chronic immune diseases occur as a result (at least at some level) of the dysregulation of the production of these immune mediators.

When these mediators are produced in excess, or when their counteracting receptor antagonists or antagonist-binding proteins are deficient, the protective role of the immune system devolves into a harmful one capable of systemic injury to the host [6].

Recent data have implicated the innate and acquired immune systems as being pivotal for the deleterious effects of acute inflammatory states, such as the systemic inflammatory response syndrome (SIRS). SIRS is defined by the physiological characteristics of the host’s response to any of a number of external or internal stimuli, infectious and noninfectious [7], and represents a nonspecific, systemic activation of the innate immune system [8]. SIRS clinically can result from ischemia-reperfusion injury, thermal injury, severe trauma, surgical injury, and microbial infection. Clinically, the SIRS response to an external stimulus is often followed by a compensatory anti-inflammatory response syndrome (CARS), during which there is a state of relative immunocompromise and a concomitant overexpression of genes encoding for anti-inflammatory cytokines (fig. 1) [9]. CARS has been observed for years in clinical practice as patients would develop potentially fatal secondary infections several days after the inciting traumatic injurious event.

The clinical presentation of sepsis and SIRS can be nearly identical, yet sepsis, unlike SIRS, requires the presence of a potentially identifiable microbial stimulus. It is now clear that the common response element for both of these syndromes is activation of the proinflammatory immune system, with a subsequent activation of the anti-inflammatory immune response. The scientific literature has recently become replete with attempts to characterize septic patients in terms of their immune status as a means to complement the clinical parameters (e.g., critical illness scoring systems) which more accurately measure the host’s physiological response to the injury and not the cause of the injury per se [10]. In an effort to develop new therapeutic approaches, specific biochemical mediators and cytokines involved in such processes, as well as their genetic control mechanisms have been targeted. Despite a number of successes in animal models, little gains have been
made with this approach in human studies. There likely are several reasons for the lack of success, including the improper timing of immune-altering measures, the complexity of triggering mechanisms, the redundancy of immune activation pathways, and the heterogeneity of the patient population being studied [11].

Clearly, the current practice of using physiological or immunological scoring systems to risk-stratify septic patients has scientific merit, but such an approach does not account for the underlying health status or predisposition of the individual. The genetic predisposition of patients to develop sepsis following traumatic or surgical injury, as well as their response to sepsis, has only begun to be explored experimentally. Practically speaking, hospital care of the septic patient comprises the bulk of the budget of any intensive care unit: each clinical case of sepsis costs approximately USD 22,100 [3]. Foreknowledge about a particular patient’s prognosis could decrease cost and improve the allocation of health care resources, thereby allowing clinicians to ‘apply preventative intensive care at lower cost with better outcomes’ [12]. If a relationship between the genetic constitution of a patient and the response to a major inflammatory stimulus could be established, better injury-scoring systems could be devised that more accurately predict the clinical trajectory of a patient.
The Triggering Event

The classic stimuli known to cause sepsis and SIRS in humans and animal models are microbial products, predominantly lipopolysaccharide (LPS), which is a component of the cell wall of gram-negative organisms. The innate immune system has been programmed to discriminate self from non-self and has developed elaborate recognition systems to detect the presence of minimal quantities of microbial products [13]. Secretory, acute phase protein products of the liver, including complement, play an integral role in this process. Probably, the best described innate immune system response occurs from the recognition of bacterial LPS. A key acute phase reactant protein involved in the recognition of bacterial LPS is LPS-binding protein (LBP), which is produced by hepatocytes and other cell types, and circulates in the plasma [14]. When bound to LPS, LBP forms a complex with either the soluble or membrane-bound receptor, CD14. This LPS:LBP:CD14 complex, acting via the TLR4 receptor, sets in motion a cascade of reactions which ultimately leads to the recruitment of neutrophils and the release of other inflammatory mediators [15].

This LPS-induced pro-inflammatory cascade is mediated largely by the production of proinflammatory cytokines, including tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1). In animal models, blocking TNFα or IL-1 signaling via its receptors prior to administration of *Escherichia coli* greatly reduces mortality [16, 17]. There is, however, a lack of success at reducing morbidity and mortality in human trials: in over 30 randomized studies with inhibitors of TNFα or IL-1 with more than 12,000 patients enrolled, there has been no proven clinical benefit [18]. ‘Merely blocking a single component may be insufficient to arrest the inflammatory process’ [18].

The presence of ‘non-self’ can explain, at least in part, microbe-induced SIRS, but clinical evidence has shown that nonmicrobial stimulation (e.g., mesenteric ischemia-reperfusion, pancreatitis, hemorrhagic shock, thermal injury) will often produce similar clinical outcomes. To reconcile this, Matzinger [19] has proposed that the immune system functions to recognize internal and external ‘danger signals’ as opposed to the more classical notion that the immune system functions to distinguish ‘self’ from ‘non-self’. In this ‘danger signal’ theory, the immune system can distinguish not only exogenous microbial products, but also internal endogenous signals such as heat-shock proteins (HSP) or necrotic (but not apoptotic) cells or tissue. In support of this theory, HSP-60 has been shown to be a known ligand for toll-like receptor-4 (TLR4) [20], capable of inducing the same inflammatory cascade as LPS.

The Biological Response

Regardless of the inciting event(s), the key initiators of inflammatory responses are cytokines. As listed by Oberholzer et al. [8], these proximal
proinflammatory cytokines function in three primary roles: (1) to induce the production of acute phase proteins by the liver; (2) to cause an elevation in body temperature, and (3) to induce the sequelae of local inflammation, namely an increase in vascular permeability and the chemotraction of other inflammatory molecules. Most cytokines are not stored within the cell as preformed entities, but rather are synthesized de novo from newly transcribed mRNA. Philosophically, the role of innate immunity and the acute inflammatory response is to prevent the systemic dissemination of the infection while the acquired immune response is being expressed.

The magnitude of the pro- and anti-inflammatory cytokine response is proportional to the severity of tissue trauma [8], and the risk of developing systemic complications. However, it should be noted that the production of these inflammatory mediators may not be uniform throughout the body. In fact, one of the initial responses of the acute inflammation mediated by the innate immune system (and specifically mostly by TNFα) is to induce a procoagulant state that serves to thwart the hematogenous spread of the organism or inciting molecule throughout the body, thereby allowing a focused or sequestered inflammatory response to the inciting event. In addition to the circulatory control of the regulation of cytokine distribution, many cytokines are expressed in a ‘cell-restricted manner’ [21].

There are three principle cytokines that mediate the early acute inflammatory response: IL-1, IL-6, and TNFα. IL-1 primarily serves to mount the febrile response, induces an acute phase reaction, stimulates the proliferation of early hematopoietic progenitor cells, triggers prostanoid release, and regulates ‘sickness behavior’; IL-6 stimulates hepatocytes to produce acute phase reactants and causes B-cell proliferation [22]; TNFα is a procoagulant, overlaps with IL-1 in its effects on the hepatic acute phase response, fever and prostaglandin production, and stimulates neutrophil recruitment and activation [22, 23]. TNFα and IL-1 stimulate the production of a variety of secondary proinflammatory and anti-inflammatory cytokines, including IL-6, chemokines (IL-8 superfamily), IL-10, IL-12, and IL-18. Increased levels of TNFα are seen in healthy volunteers after injection of endotoxin [24] as well as in patients with either gram-negative or gram-positive septic shock [25, 26]. The peak TNFα production generally occurs within the first 2 h after endotoxin challenge [24, 27, 28]. In a model of SIRS without microbial infection, elevated levels of TNFα and IL-6 were seen in the serum of patients after thoracoabdominal aneurysm repair, which necessarily includes a period of intraoperative mesenteric ischemia with subsequent reperfusion [29]. Increased frequency and magnitude of multiorgan dysfunction were seen in patients with elevated TNF and IL-6 levels [29].

While most patients survive the initial SIRS response, the proinflammatory mediators that caused the acute physiologic response then contribute to the immune-suppressed state (CARS), which is characterized by T-cell hyporesponsiveness, anergy, a defect in antigen presentation, and increased
T- and B-cell apoptosis [9]. Wood et al. [30] showed that T-cell lymphocytes taken from patients several days after severe injury do not produce IL-2, and furthermore, the degree of suppression of IL-2 production correlated with the likelihood of developing sepsis [30]. Kelly et al. [31] showed that severe thermal injury to mice depressed Th1 cell function, which was manifested by lower levels of IL-2, IFN-γ, and IL-10. Although this relationship was not causative, it gives clear insight into the direct control of the immune system onto clinical outcomes.

**Genetic Polymorphisms**

The Prophit survey, investigating the concordance of tuberculosis among monozygotic and dizygotic twins, first established the link between genetics and host response to disease states [32]. Links between the presence of genetic markers and the occurrence of common systemic diseases such as coronary artery disease have also been established [33, 34]. Given the converging biological pathways of inflammation, it is unlikely that a single gene will be identified that determines all of the clinical sequelae of sepsis. While a majority of studies have focused on the contribution of single genes or gene products, more recent data have focused on genome wide changes. The Human Genome Project is a large-scale, collaborative effort to sequence the entire human genome. While much of the genome is conserved in all members of the species, approximately 1% is repeatedly and reproducibly variable at a frequency much greater than that which would be expected from random mutations. As opposed to mutations that can occur and likely will be repaired by the cell’s defense mechanisms, these areas of variability, called polymorphisms, are stable within the genome. Strictly defined, a polymorphism is the existence of two or more sequence variants occurring at significant frequencies within a population. Common examples of polymorphisms are single nucleotide polymorphisms (SNPs) and variable number of tandem repeats (VNTRs). SNPs are single nucleotides which are substituted for another nucleotide within a sequence of the allele. A VNTR is a duplication of a short run of non-coding DNA arranged in tandem. While research efforts are investigating polymorphisms as genetic markers for disease processes, they are not necessarily causative of that disease, and therefore, afford no immediate therapeutic benefit [4].

**Tumor Necrosis Factor**

With respect to the host’s response to acute inflammation, several of the more well-studied polymorphisms occur within the gene cluster that encodes TNFα and TNFβ on chromosome 6. The TNFα gene is highly conserved
throughout evolution [35] and, therefore, has relatively few known polymorphisms. SNPs are known to occur, however, and some have been identified at positions −850, −376, −308, and −238 (fig. 2) [36]. At position −308 (308 basepairs upstream from the start codon), there is a guanine (G) nucleotide in 80% of the population; this is replaced by an adenosine (A) in the remaining 20% of the population. The sequence with a G is referred to as the TNF1 allele while that with the A is the TNF2 allele. Since each person has two copies of the gene, the relative frequencies of G/G, G/A, and A/A are 65–80%, 15–25%, and 2–5%, respectively [4]. While the TNF2 gene has been associated with the increased production of TNFα, the clinical relevance of this allele has been debated. Mira et al. [37] found that 39% of patients with septic shock had the TNF2 allele as opposed to only 18% of controls, and the frequency of TNF2 was higher among patients who died than those who lived (52 vs. 24%, respectively). Furthermore, stratifying the data according to the severity of clinical illness, there was higher mortality in patients who had at least one copy of the TNF2 allele (71.4 vs. 42.6%) [37]. Overall, there was a 3.7-fold increase in risk of death with at least one copy of TNF2 [37].

The genes for TNFα and TNFβ (LTα and LTβ) are juxtaposed on chromosome 6, within the HLA class-III locus. They are believed to have evolved from a common ancestral gene through duplication [38]. The protein products of these genes have similar functions, although TNFα is produced primarily by macrophages while TNFβ is produced predominantly by lymphocytes. Majetschak et al. [39] examined the frequency of a polymorphism within the first intron of the TNFβ gene in patients who developed post-traumatic sepsis. At position +1069 within the TNFβ gene, 70% of the population have an adenosine (TNFB2 allele) while 30% have a guanine (TNFB1 allele). The odds ratio for developing posttraumatic sepsis in patients with homozygous TNFB2 versus heterozygotes was 5.22 [39]. 

Fig. 2. Locations of the single nucleotide polymorphisms (SNPs) within the tumor necrosis factor-α (TNFα) promoter.
these results, Fang et al. [40] found that among patients with septic shock, those who were homozygous for the TNFB2 allele had an 81% mortality as opposed to patients who were heterozygous (TNFB2/TNFB1) or homozygous for the TNFB1 allele, who had 42 and 19% mortality, respectively. Stüber et al. [36] reported that patients with severe sepsis who were homozygous for TNFB2 had an increased mortality, a higher circulating concentration of TNFα, and on average, higher multiorgan failure scores than patients who were TNFB2/ TNFB1 heterozygous. These clinical data confirm earlier work that the TNFB2 allele is associated with higher TNFα release from LPS-stimulated monocytes ex vivo [41].

Interleukin-1

The gene for IL-1 is a complex sequence that encodes for 3 distinct protein products, IL-1α, IL-1β, and IL-1ra (a receptor antagonist which acts as a competitive inhibitor of IL-1α and IL-1β) [42]. When activated, the intracellular domain of the IL-1 type-I receptor provides the docking platform for the recruitment of several proteins, including MyD88 and IRAK, whose phosphorylation leads to the activation and translocation of nuclear factor κB (NFκB), which subsequently induces the transcription of IL-1, IL-6, IL-8, as well as other chemokine genes [43].

IL-1ra

IL-1ra is believed to offer some protective role against severe sepsis. In a study comparing 78 patients with severe sepsis, Arnalich et al. [44] found that the plasma concentration of IL-1ra was lower in patients who died compared to those who lived. Although overexpression of IL-1ra has protective effects in murine models of sepsis [45] and administration of IL-1ra protects baboons from bacteremic shock [46], no clinical benefit has been seen with human trials of the administration of recombinant IL-1ra [47].

Within intron 2 of the gene for IL-1ra is an 86 basepair VNTR with 5 known alleles, varying in number of tandem copies from 2 to 6. Seventy percent of the population has 4 copies (the IL-1ra A1 allele), 25% have 2 copies (IL-1ra A2), while the remaining 3 alleles (with 3, 5, and 6 copies of IL-1ra) are seen in less than 5% of the population (fig. 3). The risk of developing immune-mediated diseases, such as ulcerative colitis, alopecia areata, systemic lupus erythematosus, Graves disease, and lichen sclerosis, is higher in patients with the IL-1ra A2 allele [26, 48–52], and this allele has been associated with exaggerated IL-1β secretion [41]. More recently, Fang et al. [40] reported a 2.14-fold greater relative risk of developing severe sepsis for homozygous IL-1ra A2 patients and a 1.73-fold risk for IL-1ra A2 heterozygotes, although the occurrence of the allele did not translate into survival predictability.
This increased incidence of IL-1ra A2 among septic patients was confirmed by Ma et al. [53], further implying a potential susceptibility to sepsis in homozygotes of this allele. Arnalich et al. [54] found that even after adjustment for APACHE II score, homozygosity for the A2 allele was associated with a 6.47-fold increased risk of dying from severe sepsis. Ex vivo studies of peripheral blood monocytes from these same septic patients showed that at least one copy of the IL-1ra A2 allele was associated with elevated production of IL-1ra regardless of the presence or absence of ex vivo stimulation of these cells [54].
**IL-1α and IL-1β**

Within intron 6 of the gene encoding IL-1α, there is a 46-bp VNTR for which there are 7 known alleles (fig. 4). Ma et al. [53] noted that septic patients homozygous for IL-1α A2 had higher mortality rates than noncarriers or heterozygotes. At position −511 within the gene for IL-1β, there is a polymorphism commonly detected by the restriction enzyme AvaI. Previous work has shown that carriers of IL-1β B2 exhibited exaggerated levels of endotoxin-induced IL-1β [55]. In the same group of septic patients described above by Ma et al. [53], septic patients homozygous for IL-1β B2 had higher mortality than heterozygotes or noncarriers. A second polymorphism of IL-1β exists at position +3953, which likewise has been associated with elevated concentrations of IL-1β (fig. 4) [55].

**Interleukin-10**

IL-10 has classically been defined as an anti-inflammatory cytokine and serves to downregulate the expression of IL-1, TNFα, and other proinflammatory cytokines. In a murine and primate model, IL-10 has been shown to protect the animals from endotoxin-induced lethality and hypotension [56]. There are three known polymorphisms of the IL-10 gene, all SNPs, which are located within the promoter: at positions −1,082, −819, and −592. Unfortunately, it appears that the three SNPs are in linkage disequilibrium, suggesting that their frequency of occurrence is a nonrandom event. Nevertheless, in case-controlled study, Koch et al. [57] found no difference in either the frequency of the alleles or combinations of alleles among patients who had coronary artery disease, a history of a prior myocardial infarction, and normal controls. The allelic frequency of IL-10 polymorphisms in sepsis has not been well described, with the exception of some preliminary reports on meningococcal disease [58].

**Interleukin-6**

IL-6 has been shown to have both pro- and anti-inflammatory properties; it exerts its biologic effects by binding to its receptor on cell surfaces and then interacting with the gp130 signal-transducing complex. Clinical variation in the level of IL-6 has been noted: high IL-6 production has been shown to adversely affect the survival of human solid organ allografts [59]. Data from the 1997–1998 United Network of Organ Sharing Scientific Registry show that the rate of organ failure for the African-American population is significantly higher than that of the Caucasian population. Ethnicity studies have shown that 98% of African-Americans were 'high IL-6' producers while only 84% of
Caucasians were similarly classified [60]. While this has not been demonstrated to be a causative relationship, it does raise suspicion that genetic variances account for the differences in levels of the production of proinflammatory cytokines, and may help to identify those patients at increased risk of the untoward clinical sequelae of graft failure.

Systemic levels of IL-6 are believed to be regulated at the level of genetic expression [61]. Several studies have shown a relation between the level of circulating IL-6 and outcome of sepsis [62, 63]. A known polymorphism occurs within the promoter at position −174 at which either a guanine (G) or cytosine (C) nucleotide resides. In a study of 50 septic patients, Schlüter et al. [64] found that among the 25 patients who did not survive, only 2 were homozygous for the G allele (G/G), while 11 of the 25 survivors were G/G. Furthermore, there was good correlation of circulating IL-6 concentration and clinical outcome of these same study patients [64]. The authors concluded that there must be some survival benefit in homozygosity of the G allele. However, the G allele is known to cause spontaneous and inducible genetic expression of the IL-6 gene, and has been associated with elevated levels of circulating IL-6 in healthy adults, as compared to healthy adults without a G allele [65]. Schlüter et al. [64] attempted to explain this apparent incongruity by noting that most of the studies previously performed examining IL-6 production as a function of allelic frequency were done with ex vivo stimulation.

**Nuclear Factor κB**

NFκB is a DNA-binding protein that incites high-level transcription of several proinflammatory genes including TNFα, IL-1β, IL-2, IL-6, IL-8, IL-12, IFNγ, and others. It is activated by any of a number of inflammatory stimuli or mediators: ischemia-reperfusion, hypoxia, volutrauma, barotrauma, IL-1β, or TNF. The activation of NFκB by TNFα or IL-1β is mediated through a series of kinases, the end result of which is translocation of the heterodimer to the nucleus with subsequent binding to the 6B promoter region. In a study by Bohrer et al. [66], the level of monocyte production of NFκB from patients with sepsis correlated with end organ failure and predicted survival.

**CD14**

As described above, the CD14 receptor binds to LPS on the surface of monocytes, macrophages, and other peripheral blood mononuclear cells, and thus moderates signals of the innate immune system. The administration of monoclonal antibodies against CD14 has been shown to attenuate organ injury and mortality in rabbits that have been given LPS, even if administered
subsequent to the LPS dose [67]. Furthermore, soluble CD14 (sCD14) plays a role in the response of endothelial cells (which lack the CD14 receptor) to microbial stimulation. Within the promoter region for the gene encoding CD14 is a polymorphism at the −159 position. Prior ex vivo studies have shown higher levels of sCD14 (and, incidentally, higher TNFα) from cells of patients who had the T (thymine) nucleotide as opposed to the C (cytosine) allele at this position [68]. Clinically, elevated sCD14 has been associated with higher mortality in patients with septic shock [69, 70]. Gibot et al. [71] examined the frequency of the CD14 polymorphisms in patients with septic shock. They found that the T allele was more common in patients with septic shock as opposed to normal healthy control volunteers (55 vs. 43%). Furthermore, among septic patients, those who were homozygous for the T allele had a higher mortality than those who were either heterozygous or homozygous for the C allele (77% mortality for T/T vs. 26% for C/C). This translated into an odds ratio of 5.3 for death among patients with septic shock in T/T versus C/C [71].

**Toll-Like Receptor 4**

TLR4 is the major receptor for LPS and gram-negative bacteria, as outlined above. In the mouse strain C3H/HeJ, in which there is an amino acid substitution at position +712 of the TLR4 gene, stimulation with LPS induces an attenuated inflammatory state compared to that which is induced by the stimulation of mice with normal (wild-type) TLR4 [72]. In addition, TLR4−/− mice do not respond to LPS stimulation [73, 74], thereby proving the requirement of this cell surface protein for the induction of the inflammatory state. One known mutation of the TLR4 gene at amino acid position 299 may predispose to septic shock: Lorenz et al. [75] found that 5 of 91 patients with septic shock had this mutation, as opposed to 0 of 73 controls, a difference which reached statistical significance.

**Genome-Wide Measurements**

If the discovery of markers for systemic disease states represents one level of genetic pathophysiology, the natural sequel is the expression products of the genome. Patients do not die from sepsis because of polymorphisms in their genomic DNA. Presumably, the differential outcome is secondary to altered expression or activity of the protein translated from the expressed gene. The advancement of the Human Genome Project has enabled the development of new wide-scale technologies, not only for screening the human genome, but evaluating the gene expression products. This latter process has
been defined as ‘functional genomics’. Formerly, it was possible only to measure the level of certain known mediators of systemic inflammation using reductive approaches that focused on single or small numbers of mRNA or proteins, such as Northern or Western analyses, RT-PCR or ELISAs. Now, using oligonucleotide or DNA microarrays, scientists can simultaneously measure the level of expression of thousands of genes from a variety of tissue types in response to various stimuli. This has resulted in the need to challenge how we look at the host response to inflammation. As Lander [76] stated, ‘The traditional gene-by-gene approach will not suffice to meet the sheer magnitude of the problem. It will be necessary to take “global views” of biological processes: simultaneous read-outs of all components’. Not only does microarray technology enable simultaneous measurement of thousands of genes, but it also eliminates the need for a priori knowledge of the function of the gene being studied. In this way, the response of a known gene to a given stimulus can be qualified; but furthermore, the function of an unknown gene or expressed sequence tag can be inferred from its expression behavior, relative to thousands of other genes.

The field of oncology is among the first to bring gene expression microarrays into clinical utility. Pathologists are now able to differentiate tumor types based on the RNA expression profile. Golub et al. [77] described the use of gene expression to predict the class of leukemia, even without prior biological knowledge of the cancer. Thus, any class of cell type can be identified not solely by physiologic and phenotypic characteristics, but additionally by its gene expression pattern, the ultimate ‘blueprint’.

An overview of the technology and biostatistics associated with this emerging field of functional genomics is complex and beyond the scope of this review. However, Chung et al. [78] provide a succinct summary of the use of microarrays in sepsis and the intensive care setting. They summarize that ‘for intensivists, the goal of functional genomics is to better understand [the] integrated molecular response to injury’.

Several groups have begun to investigate the gene expression patterns of antigen-presenting cells of the innate immune system (dendritic cells, DCs) in response to external stimuli [79]. Immature DCs, confined to tissues most suited for immunosurveillance, are capable of detecting pathogens or other immune-activating stressors; once these cells detect such a stimulus, they lose phagocytic capabilities and exhibit enhanced production of cytokines [79]. Granucci et al. [79] compared the gene expression pattern ‘transcriptome’ of immature and LPS-stimulated and TNFα-stimulated matured DCs from murine bone marrow using a commercial microarray containing oligonucleotide segments representing more than 6,500 murine genes. Previous work has shown that DCs will mature when stimulated with either LPS or TNFα for 18 h; their maturity determined by functional traits such as antigen presentation or cytoskeletal characteristics [80, 81]. Despite the phenotypic similarities of DCs matured by either method, microarray analysis revealed
diverse genotypes. In fact, only LPS-treated cells, and not TNFα-treated, showed a gene expression pattern consistent with growth arrest and immune activation [79]. This serves to highlight, even at the level of single cell analysis, the increased detection capacity of genome expression analysis over phenotypic analysis.

Similarly, genome-wide analysis on a rat model of sepsis in which the level of expression of over 8,000 genes was quantified in a cecal ligation and puncture model showed that the genetic responses of inflammation are distinct for each organ [82].

Current research efforts are examining the host response to external and internal immunostimulatory triggers at the level of the genome. Such broad-scaled characterization offers a completely novel approach to the problem of systemic inflammation. A recent Large Scale Collaborative Research Program funded by the National Institute of General Medical Sciences seeks to characterize early gene response to major trauma or burns, highlighting the broad interest in this field.

Conclusion

The ability to detect and characterize the immunological status of patients to diseases has illustrated that the host response to the disease affects outcome greatly. In some instances, disease occurs only when there is dysregulation of this response, often at the level of DNA transcription. Several known genetic variants or polymorphisms have been described in an effort to relate genetic constitution and clinical trajectory. Genetic variants may be used to stratify patient response to disease, characterize level of illness, and possibly predict clinical response. Furthermore, continued efforts to characterize genome-wide responses to stimuli will serve to identify heretofore unsuspected genes and potentially novel therapies.

References


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Genetic Exploration in Injury


**Discussion**

*Dr. Baracos:* One of the things that really blows my mind in trying to appreciate all this comes from a couple of presentations where the investigators presented changes in gene expression using microarray approaches after an acute stimulus over time. The first question that I saw related to that has to do with a simple experimental model in which muscles were stimulated to contract and the pattern of gene expression at 1, 2 and 6 h following stimulation has genes that go up and up and up, and then go back down again and stay down or go down and come back up again on a time frame which leads you to believe that a snap shot might even be taken of these arbitrarily chosen gene expression changes. This would lead you to have a huge amount of traffic that escapes your perception. How do you work around that?

*Dr. Moldawer:* Your point is exactly correct. These sophisticated procedures to assess mRNA levels have really become very simple analytical tools, and we routinely...
have surgical residents and graduate students perform the analyses. There is a required learning curve, and the techniques require someone with analytical sophistication, but the techniques are relatively straightforward. The problem is that the cost to perform these studies is enormous. The Affymetrix instrument costs about USD 250,000 to purchase and USD 30,000 a year to maintain. Each chip, even with a University discount like ours, is approximately USD 400 per sample. The costs associated with sample preparation are about USD 350. So we estimate an analytical cost of approximately USD 750 for each data point. In a 13-patient study in which we have performed approximately 40 chips, the estimated cost for materials alone is about USD 35,000. So when designing these studies, we are limited by the overall number of time points and replicates available to examine changes in the pattern of expression over time. Single interval snapshots tend to be misleading, but the reality is that it is difficult to design the optimal study with all of the required time intervals. So as investigators, we are faced with a conundrum which is that you generate a great deal of incomplete data which really can either lead you astray or towards enlightenment. The challenge is how do you handle the data and how do you use the data to generate a hypothesis, and that is not an easy challenge.

Dr. Baracos: I guess I should additionally ask if that is an argument for having a presentation venue for that information, or can someone sit down and look over it on a Sunday afternoon. I mean what if you get a phone call from somebody who is reading the results of microarray analysis and asks you what you think about x protein? This gene expression has increased, and I don't see how we can have a venue for discussion even for the volume of results which might be generated by such an approach. What is not obvious to you on inspection of that list of expressed genes might be plain and evident to someone else who has a larger understanding of what those different proteins might be doing.

Dr. Moldawer: You are exactly correct. For example, we generated a list of 37 genes which seem to predict the outcome in this patient population. Several of these genes were for cytokines or inflammatory proteins that I am unfamiliar with, so I am looking at the expression profile of genes which I essentially have no knowledge of their function. I have to go to GenMapp and GenBank even to figure out their identity and function. Even with these limitations, however, there is immense power with these techniques. The power first of all is I can now take that list of genes and I can ask a specific question and test it prospectively. This is a supervised statistical method to extract data retrospectively. We do not need to know the function of these genes to use their expression for class prediction. Now the first question we need to ask is whether the genes whose expression predicted outcome, can be confirmed prospectively. So we are now going back and prospectively testing whether those 37 genes are actually predictive in the next 30 patients that we are studying. We are validating the expression by other techniques as well, because microarrays are not as good qualitatively as RT- or real-time PCR, so you need to go back and validate the expression levels with the polymerase chain reaction. Once you have validated the results, then the power of bioinformatics can be directed to start querying those pathways and networks that are invoked. In this manner, we can start to query which pathways or networks are involved in the sepsis response. Can I learn something about pathways? Is there a preferential hypothetical pathway which seems to be involved? Based on these results, you can formulate the questions to ask. And these studies can be performed quickly. The challenge is in the bioinformatics and dissecting the information from literally 800,000 data points.

Dr. Déchelotte: You said that there is a lot of convincing evidence that gene expression may be altered in various ways in these patients, but with the final products, the protein itself, do you think we can draw anything from this gene approach without having the proteomics approach together?
**Dr. Moldawer:** There are groups who have looked at high throughput proteomics and tried to correlate them with changes in functional genomics, and have generally shown a rather poor correlation between proteomics and genomic analyses. Now that does not negate the value of the genomics as a diagnostic tool, because it still reveals that changes in expression may not be cause and effect, but they may be prognostic. But you are indeed correct, and the problem from an analytical point of view is that the proteomics field is probably about 5 years behind the genomics field. We do have high throughput mass spectrometry techniques that can separate between 50 and 100 thousand peptide fragments from human blood, but the ability to annotate the human data base for the protein fragments is much more limited and much cruder than we have for the genomics.

**Dr. Martindale:** Do you feel that some day we will be doing a preoperative genetic analysis prior to a patient undergoing major surgery to predict and tell them that the risk of developing sepsis from the same insult is much higher than others?

**Dr. Moldawer:** Yes, I think this is inevitable. We will be doing two things. We will be doing genetic profiling in terms of single nucleotide polymorphisms (SNPs). The cost of a single SNP measurement can be reduced down to about 10 cents. Now if you have 100,000 SNPs, that is still approximately USD 10,000 per patient to profile, but these costs will undoubtedly decline, and genetic analyses will become routine. It is my belief that in the future we will not only examining SNPs in our patients and generating databases for our patients, but we will also be routinely doing transcriptome profiling, gene expression profiling, in the same patients. The technology is improving, and the costs are coming down; my belief is that it will be some time in the foreseeable future.

**Dr. Martindale:** Then if we think about the cost of giving Xigris™, the drug you mentioned, the activated protein C is USD 7,000 per patient and we have to give it to 13 patients to see 1 responder. If we are looking at USD 7,000 dollars for the delivery of a drug, it is not really that unreasonable to think that perhaps we should look preoperatively or in the immediate to postoperative phase, saying that this person is perhaps a responder versus a nonresponder, and then treat the responders.

**Dr. McClain:** You are assuming that polymorphisms are playing a major role in outcome. Could it also be that the body responds in only a limited number of ways to a variety of different insults? In my particular area of expertise, the liver for example, if you have a hepatic infection, the hepatocytes undergo apoptosis with subsequent release of cytokines such as IL-8 and IL-18. These cytokines then play a role in killing the invading bacteria in a beneficial response. However, if you have hepatocyte apoptosis due to toxins such as alcohol, then the cytokine release does not kill invading bacteria but instead just amplifies a toxin-induced liver injury in a maladaptive response. Thus my question is whether polymorphisms are the major factor determining outcome. Could it also not be the particular insult, and possibly a maladaptive response to that insult, which plays a role in outcome?

**Dr. Moldawer:** My statement was an oversimplification. I think what we do know is that the polymorphisms will alter the level of expression of certain genes and that will contribute to outcome. How that contributes to outcome will be determined not only by the polymorphism of that gene, but polymorphism in other genes as well as the nature of the inflammatory stimulus. The host has what we call a common response element, in the liver. The acute phase response is a classic example of a common response element to a variety of different inducing stimuli. But what I think the microarray data have told us and what we are beginning to learn when we look at genome-wide and proteosome-wide scans, is that this common response element is probably greater in diversity than we had anticipated. If we look genome-wide, the diversity of the response is either determined by the genetic predisposition of the patient or the inflammatory stimulus is much broader than we had anticipated.
Dr. Cynober: I understand that there is a poor correlation between research coming from genomics and proteomics with regard to this factor. I would like to know if, in that case, it may be correct to feel that a key control point could be transcription factors, such as IP1 or NFκB, which could explain this?

Dr. Moldawer: I think there are several places where expression is regulated post-transcriptionally, and cannot be detected with these microarrays. Expression can be regulated at the level of message stability but it is more likely to be at the level of protein processing. Functional genomics has no way of dealing with that issue. The activation state of kinases for IκB as you mentioned, has nothing to do with the message expression level, it has to do with post-transcriptional processes. So the supposition that genomics will give us the entire answer is really naïve and over simplistic. What genomics gives us is another set of questions.

Dr. Cynober: You did not comment on gene polymorphism as a gene encoding for nitric oxide synthesis. Can it be of importance?

Dr. Moldawer: I did not comment a lot about gene polymorphism because it is not my strength relative to the other aspects of the presentation. My expertise on this subject is probably no better than many people present in this room. What I have seen is that there has been compelling evidence suggesting a role for these polymorphisms on outcome. The problem has been that the background frequency for the polymorphism is always unique to the population studied and the ability to translate these results to other populations is frequently difficult. For example, I know the tumor necrosis factor (TNF) gene better than nitric oxide gene, and the ability to translate the −308 polymorphism from the Finnish population to the American population has been difficult. Stuber’s staff with the TNF-β locus is another example where there seem to be correlations between outcome from sepsis in some subpopulations, but not with others. Clearly, we have evaluated just the tip of the iceberg, from which we have picked out just enough information to feel comfortable that there is some importance in understanding the polymorphisms, but we haven’t been able to dissect those factors which lead to the importance in outcome.

Dr. Kudsk: In the 1970s prostaglandins were the main topic and in the 1990s cytokines were the main topic and created a certain amount of enthusiasm. For example, you used samples of serum or blood cells. How do you know that this is the correct window as opposed to liver or muscle?

Dr. Moldawer: Excellent question. Dr. Baracos mentioned the studies in which they studied 12 tissues and did not identify the correct one. So we don't know the appropriate tissue compartment to sample. First of all, in hospitalized patients, we assume that it is easy to obtain peripheral blood leukocytes. It is really the only one immune cell population that you can sample reproducibly and easily. Dr. David Herndon in Texas has the ability to sample a number of other tissues from burn patients, but we don't have that capability. We sample leukocytes as a marker of the reiculoendothelial and lymphoid systems, and we admit that in fact it may not be optimal because we know that the gut and the liver are the primary organs which are involved in the immune response to endotoxemia. The issue of compartmentalization of response is a fundamental question we have not fully addressed.

Dr. Berger: We are clearly not equal when facing sepsis. In the intensive care unit we have much fewer females coming in with sepsis and they generally do better. Many reasons have been given for that. Have genomics brought any new insights to this aspect?

Dr. Moldawer: It is such a new field. I think there have probably been only 25–30 reports in the field that have applied microarrays to sepsis in critical illness. So I think it is too early to answer your question. You know the seven stages of an experiment where the first one is euphoria and the last one is recrimination, we are still in the euphoria stage with microarrays. Anti-cytokine therapies for sepsis are definitively in
the recrimination stage. With microarrays, we see only the potential, we don’t see the limitations yet.

**Dr. Moore:** From our standard epidemiology assessment, we have developed prediction models using host factors, injury severity and indices of shock that fairly accurately predict the clinical trajectory of trauma patients within 6 to 12 hours of injury. I am struggling with how you would put genomics in such a data base and I am not quite sure how a patient who has sepsis goes on to get multiple organ failure. Exactly what causes patients to go into multiple organ failure? What do you think all those proteins are doing?

**Dr. Moldawer:** That is a good question without a ready answer. In fact the reason we did the study is because we don’t know why so many of these patients go on to develop single-organ or multiple organ failure. You look at the comorbidities preoperatively, you look at the surgical events, and there are some patients who have a rough surgical course, they lose blood and you know they are going to have a rough post-operative course. But in the majority of patients who undergo this surgical procedure, you don’t know what the outcome is going to be. In fact the general phenomenon is that on the first post-operative day these patients do pretty well, and then around days 2–4 you start seeing problems. We don’t know why, we only know that if we have long ischemia times they have a worse prognosis. So the idea was to go in early, look at the leukocytes and see if the gene expression pattern would give us something that might tell us which patients are going to crash and burn, and for what reason. You have to do a lot of statistics to get 37 genes and it is a supervised statistical approach. In this regard, you have to tell the analysis who got sick and who didn’t to draw out those genes. So I don’t want to say that is going to be the answer, it has to be tested prospectively. But I guess the reason we took this direction, very much like some of the things we are doing with sepsis and trauma, is to try to find techniques which tell us, a little better than now, what track the patients are going to take.

**Dr. Nitenberg:** Your presentation is very fascinating. I don’t know if you remember the Monty Python movie, The Life of Brian. When the people were coming in, those on the right were told that they were to be crucified, and those on the left that they were to be saved. It is almost the same and I don’t like this idea. I prefer to keep our work in searching for what we can do in terms of therapeutic goals. Do you think that by identifying patients at risk we can modify the treatment according to genetics?

**Dr. Moldawer:** I am not a clinician so I can stand up here and pontificate, and not have any clinical consequences to what I say. Whereas, there are those of you who deal with these patients daily and have to live with the consequences. The only point I can make is that there was never an attempt to say we are going to withhold therapy on individual patients because their genetic background is such that their likely outcome is worse or better. What these tools really gives us is an opportunity to relatively comfortably say, look, here is a group of patients who, as a group, are likely to have a better or worse clinical trajectory. The way I envision their use, is we have patients whose genetic polymorphism or the microarrays, for whatever reason, suggest that they are at high risk. As a physician, you will have that additional information, and say perhaps we need to watch this patient a little more aggressively.

**Dr. Déchelotte:** We discussed that between genes and proteins there is a great gap and perhaps we should come back to nutrients in that field because we all know that there is a lot of evidence now that nutrients, even in the weeks before injury, may condition the expression of several genes and final products. So in your study, where we have seen these profiles predicting bad or good issues in these patients, were you able to correlate these genotypes also with some basic nutritional parameters such as body mass index or fat stores or antioxidant vitamin stores?
Dr. Moldawer: I started my talk with the apology that I haven’t done nutrition research for about 10 or 15 years: I was headed out the door when one of my post-doctorates gave me an article showing the importance of TNF polymorphism in response to fish oil diets. Unfortunately I didn’t have the opportunity to review the results for this group, but here is an example of where we are going. It was a study showing that the ability of fish oil to suppress ex vivo TNF production was dependent upon the polymorphism for the −308 TNF locus. I purpose we let our fish oil lover, Dr. Calder, make the final comments.

Dr. Calder: Yes, what we found was that the −308 polymorphism made people more sensitive to fish oil but so does the polymorphism in the TNF-β gene, −252 I think it is, but we don’t really understand the interplay between the TNF-β gene and TNF-α production.

Dr. Moldawer: The TNF gene is located right in the middle of the major histocompatibility (MHC) class 3 complex and the linkage equilibrium between the TNF gene and MHC is not fully resolved. So the ability to dissect the contribution of the TNF polymorphism is very difficult. I apologize if I did not include your paper in the presentation.

Dr. Allison: Ljungqvist gives a simple carbohydrate drink 2 h before surgery, and this profoundly affects the metabolic and clinical changes after surgery. Is this linked through this kind of system, and if we are going to drive our car should we be sure to have a good breakfast?

Dr. Moldawer: I think technology will demand we move in this direction.