Challenges for modeling and interpreting the complex biology of severe injury and inflammation

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Abstract: Human injury is associated with inflammatory responses that are modulated by the acute and chronic activity of endogenous factors and exogenous interventions. A characteristic feature of chronic, severe inflammatory states is the diminished signal output variability of many organ systems, including innate immune responsiveness and endogenous neural and endocrine-mediated functions. The attenuation of signal/response variability and integration of feedback capacity may contribute to systemic and tissue-specific deterioration of function. Some well-intentioned therapies directed toward support of systemic and tissue functions may actually promote the loss of system(s) adaptability and contribute to adverse outcomes in severely stressed patients. In vivo and in silico models of stress, injury, and infection have yet to fully define the influences of ongoing stressful stimuli as well as genetic variation and epigenetic factors in the context of an evolving inflammatory state. Experimental and human models incorporating variable, antecedent stress(es) and altered neuroendocrine rhythms might approximate the altered adaptability in immune and organ function responses. Such models may also provide insights into the salient mechanisms of risk and outcome more precisely than do the constrained study conditions of current animal or human models of systemic inflammation. J. Leukoc. Biol. 83: 553–557; 2008.

Key Words: systems biology · endotoxin · heart rate variability

“Errors are not in the art but in the artificers.”

INTRODUCTION

The late Roger Bone [1] cited the above from Newton’s Princípia as a challenge to basic and clinical injury biologists to “revise our models as new information becomes available, to admit when our explanations are incomplete, and to consider new explanations for the same data”. Mechanisms of disease prevention, evolution, and reversal must ultimately be put to the test of prospective, clinical investigation. Despite supportive preclinical studies, many hypotheses regarding the management and treatment of severe human inflammation have failed during confirmatory clinical testing [2–18]. These unexpected results occurred frequently when the more constrained, single variable assumptions of the original model(s) were extended to clinical scenarios, where prolonged stress and inflammation prevailed [11]. Clermont et al. [19] have recently noted, “the difficulty of predicting the impact of modifying single components of the highly complex, non-linear, and redundant inflammatory response” and that “prediction of the behavior of such systems derived from local insights ... may be impossible”. With relatively few simplifying assumptions, he and his colleagues [19] have shown that in silico modeling may replicate clinical trial results for some anti-inflammatory agents (i.e., anti-TNF-α) and provide unique insights regarding target populations and treatment strategies. Recent reviews have discussed these modeling approaches and the alternative statistical approaches being used [20–25] for such investigations. Such models do, however, rely on data (or assumptions) derived from experimental and clinical models in immune “competent” populations and may not be applicable where the nonlinear relationships among interacting systems are not established [26].

THE OBVIOUS CONFOUNDERS

It is now widely appreciated that genetic variation and epigenetic factors influence the course and outcome of severe clinical inflammation. The role of genetic variation has been widely considered and discussed in the context of injury and critical illness [27–30]. Most studies have used a candidate gene approach to assess the influence of single nucleotide polymorphisms for inflammatory ligand/receptor/signal pathway responses to a prototypic stimulus, such as endotoxin, or have sought risk and outcome correlations of genetic variation among diverse, critically ill populations.

Studies using stress or other perturbations that alter the antecedent state of host physiologic regulatory systems before infectious or traumatic challenge will likely be essential for our understanding of complicated injury/inflammation biology. One such study has demonstrated that even a seemingly modest, stressful event influenced the subsequent gene expression pattern significantly in liver following severe hemorrhage [31]. Examples of alteration of gene and protein expression from other disciplines, where “sterile” stress is also invoked, such as exercise biology [32–35], might assist future investigations in this area.

How many other epigenetic and environmental factors have been overlooked in our current stochastic or nonlinear models...
of inflammatory risk? Some, such as age and gender [36–42], as well as ethnicity [43, 44], are receiving attention, and other interacting variables, such as heritable or acquired disease comorbidities and therapies, require greater emphasis. One example is the level of physical fitness of patients subjected to inflammatory conditions. It is suggested that such subjects exhibit a more “anti-inflammatory” phenotype than do humans of lesser fitness and/or greater body mass indices or those with metabolic syndrome [45–47]. In an age where physical fitness varies widely across the population, this highly individualized variable merits further attention. The interactions of “physical fitness” with age or gender covariates have received virtually no attention in the setting of severe inflammation.

SOME LESS-OBVIOUS CONFOUNDERS

The concept of “fitness” may also be applied to “adaptability” in a larger sense. As discussed previously [26, 48], the nonlinear dynamics of normal homeostasis reveals considerable variability in the signaling substructure(s) of biologic systems. It has been suggested [48, 49] that complex biologic oscillators underlie the integration (connectivity) of tissue and organ systems and that such variability reflects, at least in part, the normal function and adaptability arising from this integration. As originally espoused by Godin and Buchman [48], developmentally directed coupling of variable strength and frequency (oscillatory) signals promotes adaptability to stressors. Examples of such oscillatory signals are evident throughout biologic systems, from intermittent cellular uptake and processing of substrate to episodic hormone secretion and respiratory variability. Indeed, the beneficial coupling of variable oscillatory systems, as, for example, of cardiomypathic function, is demonstrable in disparate conditions, ranging from mechanical ventilator weaning [50] to biofeedback for asthma [51].

Signal variability may not be revealed by limited or intermittent sampling of tissue (e.g., for gene expression) or blood-borne signals, and inferential statistical methods normally do not detect such variation. Illness, be it organ-specific or systemic, may result in a detectable loss of organ or systemic signal variability that is associated with reduced adaptability (decomplexification).

The loss of variability may be manifest across short (e.g., heart rate) and long (e.g., circadian) time and/or oscillatory frequency spectra. For example, an acute endotoxin challenge in healthy humans induces a diminution of short-term heart-rate variability (HRV) [48, 52, 53] and diminished autonomic activity (Fig. 1). Reduced HRV is also observed in higher risk, severely injured patients [49, 52, 54–61] and as autonomic imbalance in severely infected [62] and organ-dysfunction patients [63].

An evolved state of diminished system(s) variability and autonomic imbalance has many implications for homeostatic feedback loops regulating blood pressure and cardiac performance as well as metabolic processes. This loss of autonomic activity extends to the potential influence of tissue and systemic, proinflammatory regulation that results from vagal parasympathetic signal disruption during sterile [64] and infectious [65] conditions. A diminution of neural acetylcholine signaling through the α7-nicotinic receptor may promote excessive, proinflammatory mediator (TNF-α) activity [66, 67], and pharmacological stimulation of this receptor via cutaneous nicotine application attenuates endotoxin-induced manifestations of inflammation in humans [68]. The extent to which agonist therapies directed toward tissue macrophage α7-nicotinic receptors also influence sympathetic autonomic activity remains uncertain. Recent studies suggest that attenuation of proinflammatory activity (TNF-α) via β-adrenergic stimulation does not alter endotoxin-induced changes in autonomic balance [69].

The normal pattern of circadian hormone secretion is also altered during severe or prolonged inflammatory stress [26, 70, 71]. Recent studies have confirmed the circadian expression of “clock” genes, not only in relevant central nervous and solid organ tissues but also within circulating immune cells [72, 73]. The increased expression of these environmentally influenced genes is superimposable on the enhanced circadian variability of heart rate in normal humans [74]. This diurnal pattern contrasts diametrically with potential immunoprotective signals emanating from the hypothalamic-pituitary-adrenal axis [70] and raises interesting questions as to the teleologic benefit of this phase-shifting pattern of immunomodulatory signals. Metabolic as well as innate and adaptive immune activities are influenced by circadian rhythms, and the diurnal incidence of some adverse events may be related to stress-induced alterations in the normal circadian pattern [75, 76].

The existence of age- and gender-specific circadian patterns of HRV [74] raises intriguing possibilities for the disruption of neuroendocrine signaling as but one manifestation of systemic decomplexification.

As a potential surrogate marker for system decomplexification, diminished HRV and autonomic dysregulation as revealed by very low, low, and high-frequency spectra will, doubtless, receive increasing attention in the mathematical modeling of critical illness. Although these parameters have been captured after the onset of unstable conditions, such as septic shock [77] and established organ dysfunction [63], several recent studies have ascertained prospectively the relative incidence of altered HRV during the early postadmission phase and before overt evidence of systems deterioration [57, 58, 61, 78]. There is a striking relationship of very early diminution in parameters of HRV to the later adverse outcome of patients that appears to be independent of subsequent multiple organ dysfunctions. It is unclear whether such alter-
ations result from the subclinical presence of inflammatory ligands such as endotoxin or other proinflammatory agonists. Our recent studies suggest, however, that the endogenous neuroendocrine stress response may contribute, in part, to this deterioration of HRV indices [53].

A recent study implies that disordered neuroendocrine functions are also associated with diminished HRV in stressed patients. Morris and colleagues [79] have demonstrated that reduced HRV is associated more frequently with relative adrenal insufficiency in trauma patients. Further, this interesting study suggests that treatment of the underlying adrenal condition results in enhanced HRV over the subsequent several days and is associated with improved outcome [79].

The disruption of short- and longer-term oscillatory rhythms during inflammatory illness may also have implications for common support therapies. As discussed by Buchman [26], most such interventions are designed around monotonous programs (inputs) that do not replicate healthy physiologic variability. He speculates further that redesigning many such interventions to emulate physiologic variability might “accelerate recovery from critical illness.” Indeed, our own preliminary results suggest that a commonly applied support intervention, continuous enteral or parenteral feeding, leads to diminished HRV in normal subjects as compared with subjects on a normal, episodic feeding schedule [80]. It is tempting to speculate that this widely used intervention may, like others that promote limited variability, contribute to the acquired endocrine dysfunction of severe illness [70]. Further, the implications that (relatively) invariant energy and substrate provision may have for stressed organ and cellular functions have yet to be addressed in humans [81–85]. The continuous nutrition support-related loss of physiologic variability that may place subjects at risk for noninfectious organ-specific and systemic events also has implications for autonomic regulation of subsequent inflammatory insult [86, 87].

**SYSTEMIC AND COMPARTMENTALIZED RESPONSES DURING EVOLUTION OF THE STRESS RESPONSE**

Some mathematical models of inflammation assume a dominante of proinflammatory activity that is seldom demonstrable beyond the early phase of injury or infectious challenge. Munford and Pugin [88] have noted that the propensity of systemic inflammatory responses to injury/infection is toward sustaining a net, anti-inflammatory state, and this can be modeled to some extent [22]. By contrast, existing evidence suggests that the organ-specific balance of pro- and anti-inflammatory activities is highly dependent on the experimental model(s) and is more consistent with a “reprogramming” of systemic and resident immune cells [89]. Indeed, patients with end-organ injury exhibit a net reduction of anti-inflammatory activity [90], but this seldom translates into an measurable, proinflammatory phenotype. The assumed state of immune “hyporesponsiveness” that frequently accompanies an evolving stress state has been derived almost exclusively from the study of blood cells [91]. It is now clear that any interpretation of the prevailing immune-responsive state and inflammatory balance must consider antecedent conditions as well as the nature of secondary inflammatory stimuli.

**IS THE EMPHASIS ON INNATE IMMUNITY MISPLACED DURING CHRONIC STRESS?**

Based on emerging data [92, 93], it is reasonable to question whether the implied mechanisms of system(s) deterioration during evolving stress conditions differ fundamentally from our present assumptions derived from acute immune responses in previously healthy subjects. There are many well-intentioned technologies with the potential to diminish systems variability and integration that are applied to patients. It may well be that our assumptions about the primacy of innate immune activation are less robust under such circumstances. Are stably expressed “patterns” [94–97] more representative of conditionally influenced states of decomplexification than corridors to mechanistic insights or targets of opportunity?

**SUMMARY**

Severe injury, inflammation, and infection may eventuate in systemic and organ systems deterioration that are associated with increased morbidity and mortality. The evolution of these life-threatening conditions occurs under the guise of ongoing neuroendocrine stress and a failure of tissue and cellular responsiveness to homeostatic signals. This so-called “decomplexification” of systemic and inter-organ feedback may result from inciting inflammatory signals as well as commonly applied therapeutic and support technologies. The increasing evidence that attenuation of physiologic variability increases outcome risks will require a rethinking of current treatment strategies and the development of models that account for such alterations in a clinically relevant manner.

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**REFERENCES**
