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Modeling Physiologic Variability in Human Endotoxemia

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Abstract

The control and management of inflammation is a key aspect of clinical care for critical illnesses such as sepsis. In an ideal reaction to injury, the inflammatory response provokes a strong enough response to heal the injury and then restores homeostasis. When inflammation becomes dysregulated, a persistent inflammatory state can lead to significant deleterious effects and clinical challenges. Thus, gaining a better biological understanding of the mechanisms driving the inflammatory response is of the utmost importance. In this review, we discuss our work with the late Stephen F. Lowry to investigate systemic inflammation through systems biology of human endotoxemia. We present our efforts in modeling the human endotoxemia response with a particular focus on physiologic variability. Through modeling, with a focus ultimately on translational applications, we obtain more fundamental understanding of relevant physiological processes. And by taking advantage of the information embedded in biological rhythms, ranging in time scale from high-frequency autonomic oscillations reflected in heart rate variability to circadian rhythms in inflammatory mediators, we gain insight into the underlying physiology.

Keywords

systems biology; inflammation; mathematical modeling

I. CLINICAL IMPORTANCE OF INFLAMMATION

Inflammation is a critical component of the physiological response of an organism to stressors. When the inflammatory response is successful, it leads to the healing of an injury or the clearance of infection, and then the inflammatory process self-regulates and returns to homeostasis. However, when anti-inflammatory mechanisms fail to adequately counterbalance proinflammatory activity, the body can reach a state of prolonged, unresolving systemic inflammation. This dysregulated inflammatory state can cause significant harm to the body, even in the absence of any exogenous stressor. Further complicating this issue is our general inability to effectively modulate persistent inflammatory states. Clinically, this represents a major challenge. For instance, therapies for

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Dedicated to Stephen F. Lowry

the management and control of inflammation in septic patients are limited,[1] and the only recently approved novel therapy (activated protein C) failed to show improved outcome in a repeat phase III clinical trial.[2] Sepsis is a syndrome resulting fundamentally from the activation of the systemic inflammatory response (SIRS) in the presence of a severe infection.[3,4] Sepsis has an immense cost, both in human life lost and in expenditure of health-care resources. Annually, there are more than three quarters of a million cases, having an in-hospital mortality rate of over 25% and an average cost per case of more than $20,000.[4] This leads to over 200,000 deaths annually in the United States, making sepsis one of the leading causes of death nationwide.[5] The manifestation of SIRS is the common clinical phenotype of stressed surgical patients and reflects the presence of significant systemic inflammation.[6]

In order to develop novel strategies to approach the clinical management of inflammation-linked diseases, we need to obtain a more fundamental understanding of the mechanisms driving inflammation. A useful experimental tool that has helped with progression toward these goals is the human endotoxemia model,[7] which consists of the injection of low doses of endotoxin (lipopolysaccharide, LPS) in healthy human volunteers. This evokes many signs and symptoms characteristic of systemic inflammation, making it a practical experimental model of systemic inflammation in humans. Furthermore, it is becoming increasingly apparent that issues of physiologic variability are tightly intertwined with inflammation. The existence of a homeostatic state does not imply that physiology is at a constant level during health; to the contrary, there is rich variability both at circadian and other time scales in many physiological processes, including those related to inflammation. This is particularly interesting in light of the hypothesis originally put forth by Godin and Buchman that the connectivity of a physiological system is related to the variability in its output, and that dysregulated interorgan communication in disease may be reflected in decreased variability in output signals.[8] These issues can also be studied in the context of human endotoxemia, since endotoxemia results in a significant loss of physiologic variability (often called “decomplexification”) at multiple scales.[9] Particularly relevant from a clinical perspective is interplay between endotoxemia, circadian rhythms,[10] and heart rate variability (HRV).[11] In this paper, we will discuss our work concerning the interactions between variable physiological control systems and the human endotoxemia response in the context of mathematical models. We believe that through iterative feedback between modeling and experimental work, we can characterize and investigate underlying mechanisms linking physiologic variability and the inflammatory response.

II. THE HUMAN ENDOTOXEMIA MODEL AND PHYSIOLOGIC VARIABILITY

Human endotoxemia is the response to the elective administration of endotoxin (lipopolysaccharide, LPS), a component of the outer membrane of Gram-negative bacteria that is recognized by the innate immune system and provokes an inflammatory response. Although acute, systemic inflammation is of course not reflective of all of the physiological changes occurring in complex disease such as sepsis, human endotoxemia does precipitate signs and symptoms characteristic of clinical sepsis,[7,12] acute respiratory distress syndrome (ARDS), [13] and trauma.[14] These physiological changes include transcriptional responses in immune cells, secretion of cytokines and hormones, and changes in autonomic activity.[6] All of these effects stem from responses generated by the binding of LPS to toll-like receptor 4 (TLR4), thus activating innate immune cells and stimulating pathways linked to the production of inflammatory genes such as the NF-κB, JAK-STAT, and MAPK signaling pathways.[15] This transcriptional activity leads to the production and release of both proinflammatory and anti-inflammatory cytokines, mediators of the inflammatory response and the return to homeostasis, respectively. This initial inflammatory response to LPS also propagates through afferent nervous signaling to the central nervous system (CNS), which

then forms a feedback loop to regulate the progression immune response through modulating sympathetic and parasympathetic activity, which may be reflected in increased heart rate (HR) and decreased heart rate variability (HRV).[11] In particular, central regulation plays a role in governing the inflammatory response both through autonomic activity itself[16] and though the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the subsequent release of anti-inflammatory hormones such as cortisol.[17]

Analysis of HRV, in particular, is an intriguing tool due to its broad availability through noninvasive measurement and its potential to give insight into the progression and recovery from diseases involving systemic infection and inflammation. Although it is difficult to generalize about how a change in HRV relates to underlying physiology,[18] in specific contexts, HRV has been shown to correlate with particular disease states and physiological processes. Recent studies have demonstrated potential clinical applications of predictive HRV analysis in trauma patients[19–22] and in sepsis[23,24] through an increasingly expanding library of HRV metrics.[25] Technology based on analysis of HRV signals is not constrained purely to scientific investigational purposes, as evidenced by a recent successful clinical trial that showed a reduction in mortality from neonatal sepsis in response to novel HRV monitoring techniques.[24]

Despite this, the mechanistic links between inflammation and HRV remain unclear. Endotoxemia experiments have begun to elucidate some of these details by assessing changes in HRV along with other biomarkers in response to LPS,[9,11,26–32] although significant work remains in deciphering the underlying mechanisms. For instance, a recent study showed a downregulation of sympathetic vasomotor tone in endotoxemia,[28] in contrast to what might have been expected due to increased HR and changes in low-frequency HRV oscillations that are conventionally believed to be indicative of sympathetic withdrawal. However, they did find profound decoupling between the autonomic nervous system and the heart in endotoxemia.[28] This phenomenon provides more evidence that changes in interorgan communication may be driving alterations in physiologic variability in illness.[6,33] It has been hypothesized that a reduction in HRV and cardiac vagal tone reflect increased isolation of the heart from other organs,[8] which is a concept with theoretical underpinnings provided by mathematical analysis of coupled oscillators.[34] If this is true, then reduced HRV may reflect systemic-level loss of interorgan communication.

Although HRV is a common metric of physiologic variability, in part due to the ease of noninvasive assessment of HRV, it is not the only physiological signal with rhythmic properties that are dysregulated in inflammation. For instance, circadian rhythms (~24 h patterns synchronized to a daily cycle) are altered in endotoxemia. Acute LPS treatment, given at different times throughout the day, suppresses and synchronizes the expression of clock genes in peripheral blood leukocytes.[10] This represents another level of decoupling between central control and peripheral effectors that is particularly interesting, given the observed relationships between circadian rhythms and disease,[35] and raises interesting issues related to the coupling between central and peripheral circadian controls.[36]

The relationships between biological rhythms, interorgan communication, and disease suggest both that reduction in complexity may have diagnostic value and that the restoration of complexity may improve clinical outcomes. The concept of variability being a positive clinical endpoint is, in some respects, contrary to current standard practices that generally aim at achieving a set point in the patient's state within the normal homeostatic range, but without much regard for variability.[6] Incessant lighting, continuous feeding and drug delivery, imposed ventilation rhythms, and altered sleep patterns all disrupt endogenous rhythmicity. If recovery from illness is indeed linked to the recovery of biological rhythms,

clinical care procedures could be assessed to optimize the restoration of physiologic variability.

III. TRANSLATIONAL SYSTEMS BIOLOGY IN INFLAMMATION

It has long been known tha the control of inflammatory balances plays a key role in the progression of a variety of inflammation-related disorders.[37] Yet even general classes of approaches that work in the context of one particular disease may not apply more broadly. For instance, novel therapies aimed at treating rheumatoid arthritis and inflammatory bowel disease with anticytokine therapies have shown promise in recent years, but similar strategies have not produced such promising results in treating sepsis.[38,39]

One of the primary challenges hampering the discovery of effective therapies for inflammation-driven diseases is that multiple interacting pathways regulate the inflammatory response, giving rise to complex dynamics and often unintuitive results.[40] Approaching this challenge requires a systems-level understanding of inflammation[41,42] through mathematical and computational models of inflammation.[43] Mathematical modeling offers the opportunity of studying the dynamics of the interacting elements of a complex system while it provides a systematic framework for integrating research work from many disciplines.[44] Systems-based modeling enables the systematic integration of massive amounts of relevant information shedding invaluable insight into the progression of the disease state and into possible therapeutic interventions.[45] Oftentimes, *in silico* models are viewed as the “digital analogs of transgenic animals” in which the activity of the immune system can be manipulated in controlled conditions.[46] Mathematical models represent quantitative, explicit hypotheses of system function at a wide range of scales and levels of detail. Simple models of inflammation can produce interesting and biologically relevant dynamic patterns and allow for more thorough mathematical analysis due to the relatively small number of variables in the models.[47–49] More complex models can account for spatial heterogeneity and stochasticity[50,51] as well as a larger number of components, working toward unraveling the redundant complexity of inherent in inflammation.

Modeling the inflammatory response has the potential to lead to innovations in translational medicine where scientific research is applied for improved clinical care, for instance, in rationalizing drug development, designing clinical trials, and optimizing patient care.[52,53] These tasks are all especially important in the context of inflammation-linked diseases such as sepsis where mortality rates are high yet novel clinical developments have been slow.[54] These kinds of translational applications require both experimental and computational work studying inflammation in humans. The following sections of this paper describe steps we have taken to study mathematical models of human endotoxemia.

First, in order to characterize the dynamic response to endotoxemia and the state space defining this response, we studied high-dimensional blood leukocyte microarray data[55] to identify key coordinated responses in the data representing the transcriptional dynamics of the response to endotoxemia and construct a mathematical model linking these dynamics to LPS recognition.[15] Then, taking advantage of pharmacokinetic/pharmacodynamic models of hormone activity[56] as well as experiments studying the relationship between immunomodulatory hormones and endotoxemia,[29,30,57,58] we extended our model to account for hormone-driven signaling cascades that regulate the inflammatory response[59] to assess the propagation of an external perturbation as the emergent response of a network of interacting components essential for the onset, resolution, and control of the host response. This provided a solid foundation for studying issues related to the endotoxemia response, such as through a more mechanistic model of the signaling pathways linking LPS to transcriptional effects to investigate phenomenon of endotoxin preconditioning[60] and

through the computational identification of transcriptional regulators in human endotoxemia, to decipher the time-dependent gene regulation that occurs in human endotoxemia.

To consider issues of physiologic variability, as discussed above, we modeled circadian rhythms as centrally imposed hormonal patterns to study their impact on the inflammatory response.[61] We also investigated a more mechanistic model of cortisol secretion by the HPA axis to study the effects of ultradian (~1 h period) rhythms in cortisol secretion, both in homeostasis[62] and in the acute stress response,[63] illustrating hypothetical roles for ultradian rhythmicity in setting homeostatic stress hormone levels while maintaining the ability to elicit a strong acute stress response. Exploring the relationship between endotoxemia and cardiac function led to models linking the endotoxemia response with HR (Ref. 64) and HRV.[65] A more mechanistic approach, i.e., considering autonomic modulation of the heart leading to discrete heartbeats, allowed for the generation of both HR and HRV signals from an integrated model.[66] To consider physiologic variability generated by stochastic interactions inherent in biological processes, we studied a novel agent-based modeling (ABM) framework as an alternative to continuous equation–based modeling that allows for more detailed investigation at the molecular level.[51]

These models are discussed below in more detail. Ultimately, we wish to demonstrate not only the potential implications of the proposed integrative approach toward the study of the inflammatory response, but also to advocate the possibility of the generalization of this framework to a wide range of disease progression models, thus enabling applications of systems biology in translational research.

IV. MATHEMATICAL MODELING OF HUMAN ENDOTOXEMIA

Characterizing the behavior of a dynamic system is an essential first step toward developing a model. Transcriptional profiling of human blood leukocytes over time[55] provides high-dimensional data that can be analyzed to discover how the leukocyte transcriptional state changes in endotoxemia. A computational framework was recently proposed that clusters high-dimensional time course gene expression data into an elementary set of responses.[67] Leveraging this methodology allowed us to evaluate the hypothesis that a defined network structure defines the emerging dynamics of the inflammatory response and that these core transcriptional responses can serve as surrogates for the host response to endotoxemia. We first applied a microclustering technique to assign each gene transcript profile to a motif. Then, we identified significant motifs by finding the subset of motifs that maximally deviated from the average response, thus generating a subset of critical transcriptional motifs that were considered to be characteristic endotoxemia. When this analysis was applied to data on healthy human volunteers exposed to endotoxin,[55] three critical expression motifs were identified and all three were comprised of relevant biological pathways. The three motifs were (i) upregulation of the early (2–4 h post-LPS) proinflammatory signaling, including genes in the TLR4 signaling pathway and members of the NFκB/RelA family; (ii) upregulation of the later (4–9 h post-LPS) anti-inflammatory response, including genes related to JAK-STAT and IL-10 signaling; and (iii) downregulation (2–9 h post-LPS) of genes largely related to cellular bioenergetic processes.[15]

The critical transcriptional responses were leveraged in an equation-based physicochemical[68] model, linking LPS recognition with its transcriptional effects through the NF-κB pathway. NFκB serves as the active signal produced by TLR4 signaling that leadas to the initial transcriptional response of proinflammatory signaling. Further propagation of these signals through the model activates other components. The anti-inflammatory response, for instance, responds to proinflammatory signaling by inhibiting the production rates of the proinflammation and energetic responses, leading to a self-

limited response. In total, this physicochemical model encompasses the normal self-limited endotoxemia response as well as, through regulatory feedback mechanisms becoming overwhelmed, an uncontrolled, sustained inflammatory state that is qualitatively similar to the clinical phenotype of critically ill patients. We also studied an expanded version of the LPS recognition and signaling module to study how the mechanisms of different LPS-responsive pathways lead to the generation of unintuitive responses to successive doses of LPS.[60]

Given the goal of applying systems biology models toward translational applications, it is important to consider the interactions between the inflammatory response and hormones, both endogenous and exogenous. These issues have been explored in human endotoxemia experiments in which hormone treatment is given either before or after LPS.[29,30,57,58] From a modeling perspective, we leveraged pharmacokinetic/pharmacodynamic models of hormone activity that have been developed to study the dynamic effects of glucocorticoids, a key class of anti-inflammatory hormones.[56] Such studies generally study the pharmacogenomic effect of glucocorticoids at the transcriptional level by modeling (i) the binding of the glucocorticoids to glucocorticoid receptors in the cytosol; (ii) the translocation of the glucocorticoid–receptor complex to the nucleus, which regulates transcription of glucocorticoid-responsive genes; and (iii) transcriptional responses leading to the autoregulation of glucocorticoid activity. Indirect response (IDR) models have been widely used in pharmacokinetic/pharmacodynamic models for these purposes due to the infeasibility of accurately modeling fully detailed signaling mechanisms.[69] By integrating an IDR model of cortisol (the endogenous glucocorticoid in humans) and epinephrine (another key immunomodulatory hormone), we were able to investigate how the interactions between the inflammatory response both exogenous and endogenous hormones play out in endotoxemia,[59] for instance, by showing how hormone-induced signaling can critically determine whether the system will undergo a healthy self-limited response and a return to homeostasis, or whether it will instead go to an unresolving chronic inflammatory state. This integrated model of both hormone- and LPS-driven mechanisms in human endotoxemia also served as a foundation into our research on biological rhythms and physiologic variability in endotoxemia.

V. RHYTHMIC INFLUENCES ON ENDOTOXEMIA

A growing body of evidence suggests that bidirectional cross talk between the circadian and immune systems plays a critical role in regulating the function of both systems.[70] To begin studying these issues within the context of endotoxemia, we modeled the relationship between endogenous circadian rhythms and their effects on inflammatory mediators.[61] Through incorporating pharmacokinetic/pharmacodynamic models of circadian production[71] for both cortisol and melatonin and modeling their downstream effects, we constructed a model that was able to reproduce homeostatic circadian patterns in inflammatory mediators and make predictions about responsiveness to endotoxemia in the presence of circadian rhythms.[61]

Twenty-four–hour circadian rhythms represent just one time scale of regular biological rhythmicity. Cortisol's roughly hourly secretion pattern, called an ultradian rhythm, represents a biological rhythm that has garnered much interest in recent years.[72] Fast dynamic associations between glucocorticoid receptor (GR) and DNA (Ref. 73) leads to "gene pulsing" in transcriptional activity, matching rhythmic glucocorticoid treatment.[74] This type of periodic activation of the glucocorticoid transcriptional machinery leads to broad transcriptional differences relative to a constant level of glucocorticoids.[75] We similarly investigated, in a mathematical model, how nonlinearity in ligand-receptor kinetics propagates from the HPA axis[76] through the glucocorticoid signaling pathway[77] to lead to

differential responses to rhythmic and constant cortisol exposure in homeostasis.[62] In a broader study,[63] we evaluated how dysregulation of feedback loops in the HPA axis can lead to altered patterns in homeostatic circadian rhythms, suggesting that analysis of ultradian rhythmicity can give insight into underlying physiological integrity. We also evaluated stress responsiveness of the HPA axis, quantified by the transcriptional activity of glucocorticoid-responsive genes. First, we generated a library of random parameter values for the HPA axis model that produced roughly the same mean homeostatic transcriptional levels, some of which had oscillatory patterns and some of which were flat. Then, we tested the acute stress response through acute CRH exposure, which propagates through the model to produce an acute increase in gene expression. We found stress responsiveness of the system to be proportional to the amplitude of ultradian rhythms. In other words, even when mean homeostatic transcriptional levels were the same, the presence of homeostatic ultradian rhythms served as a marker for the ability to elicit a larger peak stress response. This computational result is suggestive of how analysis of biological rhythms can reveal critical information about the state of a physiological system.

Here, we have largely discussed models of cytokine and hormone responses as can be measured in the blood. However, these circulating inflammatory mediators do not reveal the full inflammatory state of the host, and they are often difficult or expensive to assess clinically. Thus, we have explored modeling approaches to study how these physiological responses to endotoxemia are related to changes in HRV, which can be measured noninvasively and often correlates with disease severity.[78]

VI. SEMIMECHANISTIC MODELING OF DECOMPLEXIFICATION

It is well established that critically ill patients with heightened inflammatory states exhibit reduced HRV and that there may be clinical value in assessing HRV in these conditions. However, much of our clinically relevant understanding of HRV is phenomenological. A quantitative framework linking the underlying inflammatory processes with changes in HRV is lacking. Human endotoxemia represents one experimental avenue for studying the relationship between HRV and inflammation,[9,11,26–32] and one of our goals is to rationalize these experimental observations through semi-mechanistic modeling of cardiovascular effects of endotoxemia. We first attempted to link endotoxemia with changes in both HR (Ref. 64) and HRV (Ref. 65) through equation-based models based on the concept of indirect response modeling that we used to study cytokine and hormonal responses to endotoxemia as discussed above. While these models began to explore physiological changes in endotoxemia and their signal transduction to the heart, this modeling approach treats HR and HRV as distinct continuous processes that evolve through separate equations, when they are really quantities derived from a discrete time-varying signal, i.e., the series of heart beats. We worked toward a more mechanistic representation of this system by developing a continuous model of autonomic influence on the heart, designed to account for changes in both rate and variability, and combining this with a discrete model to output a series of heart beats to be analyzed to determine HR and HRV.[66]

Sympathetic and parasympathetic nerves converge at the sinoatrial (SA) node of the heart, imposing rhythmic patterns on action potentials that initiate the periodic contraction of cardiac tissue giving rise to heart beats. In homeostasis, short-term variability in autonomic activity is present largely in two frequency bands. High-frequency (HF) rhythms range from 0.15 to 0.4 Hz,[79] and are driven mainly by coupling between the breathing pattern and the heart via the vagus nerve.[80] Low-frequency (LF) rhythms that range from 0.04 to 0.15 Hz (Ref. 79) have a more uncertain physiological underpinning, related at least in part to baroreflex-mediated fluctuations in blood pressure and governed by both sympathetic and parasympathetic activity. At a much longer timescale, circadian rhythms also exert a clear

pattern on HRV. In endotoxemia, altered autonomic rhythmicity and decreased coupling between the autonomic nervous system and the heart[28] lead to diminished HRV.

By accounting for rhythmic autonomic patterns, as well as their disruption in endotoxemia, we developed a continuous model of autonomic modulation of the heart that is discretized by an integral pulse frequency modulation (IPFM) model to produce a series of discrete heartbeats as output.[66] This transformation from continuous oscillatory system output to a variable discrete signal is critical in more mechanistic modeling of HRV and also for interpreting model function in the context of clinical reality. HRV can be quantified by a wide range of time domain, frequency domain, and nonlinear metrics, all designed to either tease out some biological mechanism and/or generate signals that correlate with disease state.[25] Discrete modeling of heartbeats allowed for the application and evaluation of these diverse metrics, which interestingly showed different patterns in HRV metrics, with some reacting much more sensitively to LPS than others. This is particularly interesting given that, clinically, different HRV metrics have been found to perform best in different scenarios, but there is typically not a quantitative understanding of why that happens. Through increasingly mechanistic modeling of cardiac function in human endotoxemia, we will continue to work toward understanding the biological processes linking inflammation and decomplexification.

VII. CONCLUSIONS AND FUTURE PERSPECTIVES

In this review, we discussed our work on semi-mechanistic models of human endotoxemia and physiologic variability. While past studies have contributed toward understanding the physiological mechanisms underlying the human endotoxemia response and their relationships to biological rhythms, much work remains to fully elucidate these mechanisms and translate them to clinical practice. In particular, although models of HRV in endotoxemia can rationalize experimental data and put forth novel hypotheses, more fundamental experimental work is needed to test these hypotheses and evaluate both the mechanistic underpinnings of HRV changes as well as the specific clinical applications of HRV analysis. We hope to overcome these challenges through our combined experimental/computational studies of human endotoxemia.

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