Chapter 8
Integrating Data-Driven and Mechanistic Models of the Inflammatory Response in Sepsis and Trauma

Nabil Azhar, Qi Mi, Cordelia Ziraldo, Marius Buliga, Gregory M. Constantine, and Yoram Vodovotz

Introduction

Inflammation is an essential process in maintaining health and responding to disease. Acute inflammation is driven largely by the innate immune system, which not only serves as the first line of defense against invading pathogens but also functions to resolve tissue damage and restore homeostasis upon a variety of inflammatory conditions including sepsis, trauma, wound healing, and many more. However, when inflammation is either insufficient to address the original disruption of homeostasis, or becomes dysregulated and systemic, it can contribute substantially to morbidity and mortality in these conditions. Dysregulated systemic inflammation also

N. Azhar, B.S.
Department of Computational and Systems Biology, University of Pittsburgh, 3088 BST 3, 3501 Fifth Ave, Pittsburgh, PA 15260, USA

Q. Mi, Ph.D.
Sports Medicine and Nutrition, University of Pittsburgh, 6072 Forbes Tower, 3600 Forbes Ave., Pittsburgh, PA 15218, USA

C. Ziraldo, Ph.D.
Computational and Systems Biology, University of Pittsburgh, 3501 Fifth Ave, BST3 Suite 3076, Pittsburgh, PA 15260, USA
e-mail: cordelia.ziraldo@gmail.com

M. Buliga, Ph.D.
University of Pittsburgh at Bradford, 300 Campus Drive, 103 D Fisher Hall, Bradford, PA 16701, USA

G.M. Constantine, Ph.D.
Department of Mathematics, University of Pittsburgh, Thackeray Hall, Pittsburgh, PA 15260, USA

Y. Vodovotz, Ph.D. (✉)
Department of Surgery, University of Pittsburgh, W944 Starzl Biomedical Science Tower, 200 Lothrop St, Pittsburgh, PA 15213, USA
e-mail: vodovotzy@upmc.edu

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plays a significant role in the pathophysiology of diseases that are not primarily attributed to innate immunity such as cancer and diabetes. Although the list of diseases is broad and the processes important to each setting may differ in certain respects, the core architecture of the inflammatory response to biological stress is highly conserved [1].

The systemic inflammatory response syndrome (SIRS) is a major driver of morbidity and mortality in the settings of sepsis and trauma/hemorrhagic shock. Sepsis is one of the leading causes of death in the USA and is responsible for nearly $17 billion in health care costs annually [2]. Trauma/hemorrhage is the most common cause of death for young people in the USA, costing over $400 billion every year [3]. In both sepsis and trauma, the acute inflammatory response is concomitant with physiologic manifestations including changes in heart rate and body temperature, responses that act in a concerted fashion in order to help optimize host defense while minimizing tissue damage. Indeed, although a well-regulated inflammatory response is crucial for effective healing and host defense, an excessively vigorous response can become self-perpetuating and lead to organ dysfunction and death [4, 5]. Both sepsis and trauma patients are particularly susceptible to multiple organ dysfunction syndrome (MODS), a poorly understood syndrome that may be partly attributed to excessive and dysregulated inflammation [5]. These vastly different outcomes can be explained by the overall framework of the immune response, which includes a positive feedback loop from inflammation → damage/dysfunction → inflammation that can drive pathophysiology in inflammatory diseases [6–8].

The adverse effects of self-sustaining inflammation are likely responsible for the general perception of inflammation as an intrinsically harmful process [9, 10]. However, in addition to the aforementioned beneficial roles of inflammation in the resolution of tissue injury, recent studies suggest that morbidity and mortality are worse in animals with low levels of early proinflammatory signals [11]. The emerging view of inflammation is indeed more nuanced, casting inflammation as a highly coordinated communication network that allows the body to sense and respond to challenges and subsequently restore homeostasis [6, 12]. One may consider the complexity resulting from this coordination to be an indicator of a well regulated and properly orchestrated response, and consequently a less complex response would be indicative of a pathological dys- or mis-connectivity of the network. Guided by insights from studies on the dysregulated physiology characteristic of sepsis and trauma/hemorrhage, which have reported that a decrease in variability/complexity of heart rate can presage increased morbidity and mortality, we have suggested that well-organized dynamic networks of mediators are crucial to an appropriate inflammatory response [2, 13]. Indeed, such networks are induced early in the response to experimental surgical trauma in mice, and these networks become disorganized and less complex with the addition of hemorrhagic shock to this minor trauma [13].

The current paradigm for acute inflammation, based in large part on studies in response to trauma, hemorrhage, or infection, involves a dynamic cascade of cellular and molecular events. Innate immune cells, such as mast cells, neutrophils, and macrophages, are activated directly by bacterial endotoxin or indirectly by various stimuli elicited systemically upon trauma and hemorrhage [14–17], including the

release of damage-associated molecular pattern molecules (DAMPs) [7, 18, 19]. Both DAMPs and proinflammatory cytokines—primary among them tumor necrosis factor-α (TNF-α) [20–26]—further activate both parenchymal and immune/inflammatory cells and can affect tissue/organ physiology adversely. These stressed tissues/organs feed back positively to promote further production of inflammatory mediators. We have hypothesized that this behavior could lead to multicompartment and multiscale inflammatory "tipping points" [27–29].

A Systems Approach to Inflammation

The complexity and nonlinearity of the acute inflammatory response as described above has largely stymied the development of novel therapies for trauma/hemorrhage and sepsis. Systems biology is an emerging paradigm for tackling complex biological systems in a holistic fashion [30]. Approaches in systems biology span a broad range of techniques and can be categorized roughly into correlative or causative methods, with focus on either learning basic principles of system organization and function [31–33] or building predictive computational models [31, 34]. Although there is overlap between these areas, most efforts at elucidating biological mechanisms from high-dimensional data have traditionally focused on particular points along this spectrum of computational approaches. We suggest that gleaning translationally relevant insights into the inflammatory response and its interconnected (patho)physiology will require integration of methods from across this spectrum [13–17, 35–38], in order to progress from data to models to actionable knowledge and prediction (ideally in an in vivo or clinical context) [18, 27].

Data-Driven (Correlative) Approaches to Dynamic Inflammation Data

Statistically based approaches, with which most biologists and clinicians are generally familiar, include regression techniques that build models predictive within the conditions of the data on which the models were trained [39]. Although these methods cannot provide detailed mechanistic insights, they can be used to understand abstract features of the response such as the presence of nonlinearities or the identification of factor interactions that affect the response. The main drawback of this class of models is the fact that they often are devoid of mechanistic insight, and their linearity in the parameters can overfit to the data on which they were trained. Associative methods, such as hierarchical clustering, may be used to highlight the natural variability, as well as any overlap, across experimental or clinical conditions. Hierarchical clustering is a simple and unbiased clustering method, which aims to build a hierarchy of clusters. The limitation is the cluster must be built pairwise; since it is purely based on the similarity between the data, the cluster may lack

biological relevance [20]. Hierarchical clustering is used extensively in the genomics field and was used to discern patterns and coregulated clusters of gene expression associated with sepsis and trauma/hemorrhage in both animals [40–43] and humans [44–48].

A less-utilized data-driven method is principal component analysis (PCA), which reduces a high-dimensional dataset into a few principal components that account for much of the observed variance in the data. When applied to time-series data, PCA may identify the subsets of the variables under study (genes/proteins/etc.) that are most strongly representative of the response. Thus, these principal components may be interpreted as the principal drivers of the observed response and can give some mechanistic insights into the underlying process [13, 49]. In the setting of inflammation, correlative approaches, such as PCA, could facilitate the development of therapeutics by yielding insights into the mechanisms by which these therapeutic modalities may function [50]. Similarly, PCA may aid the development of diagnostics by analyzing the cytokine milieu in the blood resulting from inflammatory spillover in order to identify the health state of individuals and possibly inform patient-specific interventions [51].

Nonetheless, principal components, being linear combinations of the original mediator variables, often do not lend themselves to clear biological interpretations [32]. Principal components do, however, greatly ease dimensionality issues and provide a compact and efficient explanation of the data in terms of meaningful groups of mediator variables. Successful implementation of PCA within this context requires some adjustments. Mediators are measured on widely different scales which need to be appropriately adjusted for meaningful comparisons. This may be done in several ways, taking into account known biological effects. Two mediators may show significant variation within their possibly very different ranges, in which case we can rescale them appropriately. However, this should not be done, for example, if one of the two hypothetical mediators has small variation simply because it is an inert factor. Rescaling inert factors would simply amplify the error in the data. Once this rescaling issue is settled, a PCA can be carried out. In our own studies, we augmented such analysis in two additional ways. We reevaluated the importance of a specific mediator as follows: deem k principal components as being significant (by explaining, as usual, a certain fraction of the total variance). Next, assess the importance of each mediator in view of these k principal components, by adding the absolute values of the weights associated to that mediator within the k principal components. The higher the sum, the more relevant the mediator. This allows us to rank the relative importance of the mediators. A word of caution: a mediator that is naturally very noisy may be ranked as important by the PCA method, but it need not necessarily be highly relevant to the phenomenon under study. The last point we make is that it is often more convenient to work with only biologically intuitive linear combinations of mediators rather than principal components. Such intuitive linear combinations are usually suggested by the principal components themselves from which we may delete certain mediators that appear nonintuitive. This still reduces the dimension, offers good biological interpretation, but the analysis that results is more complicated, since these linear combinations become correlated [18, 32].

One clinically important area in which we have carried out data-driven modeling is traumatic brain injury (TBI). Inflammation induced by TBI can lead to both morbidity and mortality [3, 52]. We obtained both clinical data and data on the dynamic changes in multiple inflammatory mediators in the cerebrospinal fluid of TBI patients. The clinical data on each patient consisted of one-dimensional variables such as age, gender, presence of infection, bleeding, decompression, presence of subarachnoid hemorrhage, and Glasgow Coma Scale (GCS), which quantifies the nature of the initial brain injury on a numerical scale. The Glasgow Outcome Score (GOS) is the outcome variable; we view it as the response variable to study and predict as a function of the other input variables. The GOS quantifies the state of health of the subject when hospital treatment ceases. Our initial approach involved extracting orthogonal polynomial trends from each cytokine's time series, up to a specific degree d. The degree d was constant across both cytokines and subjects. The trends, by merely encapsulating linear, quadratic or cubic growth, have the distinct advantage of not being dependent on the actual length of the time series (which generally have widely different lengths). We then used these polynomial trends, quantified as one-dimensional variables, as predictors for the GOS and explored multinomial logistic as well as probit models. The models emerged upon fitting to data, and subsequent selection of the statistically significant clinical predictors as well as the orthogonal polynomial time trends of cytokines. Upon extracting polynomial trends, we carried out a study of the residuals. The model was obtained by using 80 % of the available data and was tested on the remaining 20 %. Ultimately, a logistic model was found as an optimal predictive tool (unpublished observations).

We next hypothesized that changes in the probability of survival vs. nonsurvival are related to the dynamics of the inflammatory response, the factors intrinsic to the patient (i.e., key demographic indicators) as well as to metrics related to the injury itself. To test this hypothesis, we developed a method which we call "Dynamic Profiling," as a means of assessing the dynamic course of a TBI patient within the hospital environment (Fig. 8.1). In the TBI application of Dynamic Profiling, a cluster is a subset of TBI patients that share similar characteristics. The set of clusters, recalculated after each set of cytokine readings, forms a partition of the TBI patients. To a given cluster, we associate three statistics based on the GOS score: the number of GOS scores equal to 1 in the cluster (this is the number of patients that died, to which we refer as "red flags"), the average GOS score of the subjects in the cluster, and the standard deviation of the GOS scores in that cluster. The vector of these three statistics is called the "weight" of the cluster. A cluster has a favorable weight if it has a small number of deaths, a high GOS average and a low GOS standard deviation. A useful statistic for the cluster is the probability of death of a patient belonging to that cluster (a "red flag"); it is derived as the ratio of "red flags" to the total number of subjects in the cluster. During the hospital stay, the aim is to diagnose, and ideally, reduce the probability of death (as we pass from stage i clusters to stage $i+1$). The data on which clustering is based consists of vectors in Euclidean space, with the most natural metric to use being the usual Euclidean distance. Hartigan's k-means routine is particularly well suited to clustering such

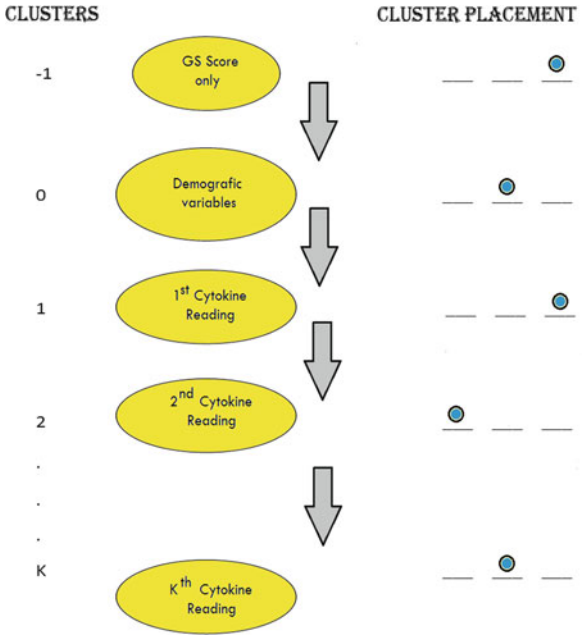

Fig. 8.1 Patient data is used to generate clusters. The initial cluster is based solely on the GS initial injury. A cluster adding in demographic data follows. Upon the reading of cytokine data, successive clusters are produced. The stage K cluster yields the probability of survival at that stage of the hospital stay

high-dimensional Euclidean data. We used the following variables to obtain the clusters: GCS, the subset of statistically significant demographic and clinical variables, the statistically significant polynomial trends in the time series of inflammatory mediator readings up to stage $i-1$ clustering (inclusive), and the inflammatory mediator readings during the current time interval. We note that the number of variables used to cluster on does not increase as we move to higher stage clustering. Indeed, we only use polynomial trends of degree at most d, irrespective of the length of the time series, or, equivalently, irrespective of the stage of clustering. This yields robustness to the clustering process while simultaneously bounding the dimension in which clustering takes place. The clusters' weights offer the opportunity of identifying patterns in the inflammatory mediators that yield favorable GOS scores. At each clustering stage, the fraction of "red flags" (deaths) in the cluster, in which the new patient falls, estimates the probability of death of the patient. The procedure lends itself easily to a Bayesian approach by placing a prior distribution (of probability of death) on existing clusters based on known medical expertise not pertaining to the data at hand. This is then updated by the observed data through the Dynamic Profiling method described above. The resulting posterior distribution encapsulates both the medical expertise as well as the observed probabilities of death within the data. Using this method, we were achieved a 72 % success rate in prediction of outcome post-TBI, a rate considerably higher than that of 50 % obtained by assigning the outcome to Low or High randomly (unpublished observations).

Like most biological processes, inflammation proceeds as a series of interacting cascades of signaling events that are often reflected in the production and secretion of

inflammatory mediators that likely form well-coordinated networks [13, 47, 53–59]. In order to better discern organizational aspects of interacting networks of inflammatory mediators, such as coregulation or autoinduction, a variety of methods have been developed. Hierarchical clustering and Bayesian methods use high-throughput genomic or proteomic data of several time points and/or conditions to correlate gene expression patterns with function and infer regulatory networks of correlated genes [60, 61]. Several developments in these methods over the last 15 years have yielded more informative networks that can be more easily translated into mechanistic models [62, 63]. A key point is that any network analysis method must reflect, and yield insights into, the dynamics of a given inflammatory response. For example, we have utilized a relatively simple network analysis method employed over discrete intervals of data to analyze the commonality and differences between experimental surgical cannulation trauma + hemorrhage in mice vs. the sham procedure (surgical cannulation only). This analysis suggested that the circulating mediators produced in response to the sham procedure were characterized by a high degree of interconnection/complexity at all time points, while the response to trauma/hemorrhage consisted of different central nodes, and exhibited zero network density over the first 2 h with lesser connectivity vs. sham at all time points [13].

Among network methods, dynamic Bayesian networks (DBNs) are particularly suited for inferring directed (causative) networks of interactions based on the probabilistic measure of how well the network can explain observed data. DBNs provide a good platform for incorporating biological knowledge alongside data in order to increase our knowledge of connectivity in biological processes and may be supplemented by additional experimental evidence and expert knowledge to hypothesize mechanistic models. As an example of the application of this methodology to the acute inflammatory disease, we have begun to examine the systemic inflammatory responses of pediatric acute liver failure (PALF) patients (unpublished observations). PALF is a complex, catastrophic, rapidly evolving clinical syndrome. The clinical trajectory of PALF is dynamic and the precise onset of disease rarely identified, with an exception being acute ingestions (e.g., mushrooms and acetaminophen). Patient outcome is reflected, in part, by the interaction among etiology, disease severity, supportive management, and treatment. Yet, outcomes vary among children with seemingly similar etiology, disease severity, and treatment; thus, additional factors are likely involved to explain these variations. Such factors likely include a complex interaction among the inflammatory milieu, end-organ damage, immune activation, potential for liver regeneration, and interventions [64, 65]. We hypothesized that dynamic networks of immune/inflammatory dysregulation drive outcomes in PALF, and that DBN analysis would shed insights into the structures of these networks. We assayed 26 inflammatory mediators on stored serum samples obtained from 49 children in the PALF study group (PALFSG; http://www.ccm.pitt.edu/research/projects/multi-center-group-study-acute-liver-failure-children) collected over 7 days after enrollment. Data were subjected to DBN analysis to suggest how inflammatory mediators are connected over time in spontaneous survivors, nonsurvivors, and PALF patients who received liver transplants (outcomes were assessed within 21 days of enrollment). Whereas raw inflammatory mediator levels assessed

over time did not distinguish among PALF outcomes, DBN analysis revealed distinct chemokine-related networks that distinguished spontaneous survivors from those who died. The DBN pattern identified in patients who underwent liver transplantation was more like that seen in spontaneous survivors than in those who died. Thus, we suggest that DBN may have general utility in other complex diseases with an inflammatory etiology.

Dynamic, Mechanistic Modeling of Inflammation

Mechanistic computational models are derived from more-detailed biological and physical descriptions of a system and have a rich set of tools for both analysis and simulation. These models, based on causative interactions, can be constructed as ordinary differential equations (ODEs), rules-based models (RBMs), and agent-based models (ABMs) among other methods (including hybrid methods) and have the advantage of potentially being predictive outside the range of conditions/time-points on which they were calibrated. Although it is often difficult to parameterize such models, they can unveil emergent phenomena not immediately obvious from the interactions that are encoded in the model. There are several analytic tools, for ODE models especially, that have been developed and used to decipher the organizational principles of networks (or subnetworks), the properties that explain the dynamics and robustness/sensitivity of a given complex system, and, perhaps most importantly, the critical points of control in the system [33]. These tools are particularly important in order to help define the complex interplay between the inflammatory mediators in the blood and other compartments both within the host (organs/tissue) and without (e.g., in the case of interactions with blood-feeding vectors). Tools from dynamical systems theory allow identification of the possible steady state(s) of a system as well as the dynamics of the system's time evolution. These tools have been used extensively to explain (or predict, depending on the context) diverse behaviors such as bistability, hysteresis, and oscillations in a variety of biological systems [66]. Bifurcation diagrams, in particular, can be used to map out the effects of a particular parameter on the possible steady-state behaviors of a system and to indicate the transition from a healthy steady state to a pathological one [16, 35, 67, 68]. The relative importance of parameters can also be quantified by calculating the change in the model output in response to changes in the parameter values using sensitivity analysis [33, 69]. These methods work in a complementary fashion to identify the key points that can be modulated to change the behavior of a system.

The analysis of ODE models of biological systems can be approached from a control theory perspective as well. Achieving robustness and efficiency are core principles of both evolution as well as engineering. Indeed, feedback, a pervasive biological phenomenon, is also a fundamental component of control strategies [70]. An ODE model is the equivalent of a state space representation of a control system. Thus, it is possible to decompose the biological system into a control structure and analyze the role of each component using control theoretic tools that characterize

their robustness and identify the key mediators that modulate the performance of such a control system [71]. These analyses are especially relevant given that the "tipping point" phenomenon in the inflammatory response is likely the result of a failure of the body's control structure to handle stress.

While we wish to navigate through the process of data → data-driven model → mechanistic model → prediction and understanding of the innate immune response, we seek to put it in the perspective of translational applications with a focus on clinical and preclinical settings. Much of the work in systems biology has understandably been in simpler, well-studied model organisms, but even among studies focused on preclinical science, there has been an overall lack of translation to the clinical arena. *Translational Systems Biology* is a framework with a focus on translational insights for novel diagnostic or therapeutic purposes and predictive mathematical models that inform in silico clinical trials [6, 72, 73]. Initially formulated to deal with the clinical challenge of integrating acute inflammation and organ dysfunction in critical illness, this work expanded to include healing of acute and chronic wounds and infections in various diseases, and rational dynamic modulation of inflammation.

We and others have created mechanistic computational models of acute inflammation in sepsis [16, 37, 74–76], endotoxemia [14, 35, 36, 77–88], and trauma/hemorrhage [14, 15, 17, 36]. In large part, these models (both ODE and ABM) are based on the typical progression of the inflammatory pathway described in the preceding section. Some of these models are purely theoretical (e.g., [16, 35, 37, 74–76]), while others are based on data either at the protein [14, 15, 17, 36] or mRNA [78, 79, 84–86] level. Similar mechanistic models have focused on related diseases such as necrotizing enterocolitis [89, 90].

Inflammation is an inherently multiscale process that manifests at the molecular, cellular, tissue/organ, whole organism, and population levels [28]. Early models of acute inflammation at the cellular level highlighted the nonlinear responses to multiple exposures to the same stimulus (Gram-negative bacterial lipopolysaccharide) [78, 80–82, 84, 88]. Some of these computational studies based on in vitro data suggested molecular control mechanisms that lead to the phenomena of nonlinear responses to repeated inflammatory stimulation at the cellular level [80–82, 84, 88]. One recent in vitro study involved mouse macrophages treated with extracellular β-nicotinamide adenine dinucleotide (NAD^+), a ubiquitous intracellular molecule that is anti-inflammatory when given extracellularly [91]. In that study, we hypothesized that extracellular NAD^+ would modulate the anti-inflammatory cytokine transforming growth factor (TGF)-β1. Indeed, NAD^+ led to increases in both active and latent cell-associated TGF-β1 in mouse macrophages. The time and dose effects of NAD^+ on TGF-β1 were complex and biphasic. A statistical model suggested that the effects of NAD^+ on TGF-β1 were nonlinear and this model was capable of predicting not only the levels of active and latent TGF-β1 but also the biphasic dose effect of NAD^+. Based on these data-driven modeling studies, we inferred that the effects of NAD^+ on TGF-β1 are nonlinear. Accordingly, we created a nonlinear ODE model of interactions we considered the most parsimonious and yet still capable of recapitulating the complex biological phenomena observed experimentally.

Model-predicted levels of TGF-β1 protein and mRNA were not only largely confirmed experimentally but also suggested the presence of other mechanisms of regulation of TGF-β1 by NAD^+ [92]. These studies highlight the utility of traditional biochemical/pharmacological studies coupled with computational modeling in defining novel biological mechanisms.

Combining Data-Driven and Mechanistic Modeling of Inflammation

We have utilized dynamic data, data-driven modeling, and dynamic mechanistic modeling in diverse contexts. We also utilized both correlative (transcriptomic analysis, PCA, and regression) and causative (ODE) models in our in vivo studies on the role of trauma in the murine response trauma/hemorrhagic shock. Initial studies using a literature-based, in vivo-calibrated mechanistic ODE model suggested that the underlying trauma is central in driving the inflammatory response to combined trauma/hemorrhage, both systemically and in the liver [15]. Transcriptomic data supported these model predictions as indicated by a large overlap between the genes and pathways induced in trauma alone vs. those induced in the setting of experimental trauma/hemorrhage [15]. This ODE model was extended to include details of experimental trauma/hemorrhage in mice (e.g., bleeding rate and target blood pressure) and further validated using a unique, computerized platform for automated hemorrhage that was constructed specifically to test the behavior of this mathematical model [17]. Later, multivariate regression, hierarchical clustering analysis, PCA, and dynamic network analysis all suggested that despite a large overlap at the level of unprocessed inflammatory mediator data (as shown by inconclusive hierarchical clustering of these data), there were major mechanistic differences between surgical trauma alone vs. trauma/hemorrhage [13].

In addition to the data-driven modeling work on TBI described above, we also carried out combined data-driven and mechanistic modeling in TBI using the same data on TBI patients described above (unpublished observations). Initially, we carried out PCA, which suggested that primary drivers of inflammation in this TBI cohort. Based on this analysis, we created patient-specific, mechanistic ODE models that were fit to each patient's data. These modeling-based studies raise the possibility of personalized modeling for TBI patients during their hospital stay.

In a similar fashion, we created a two-compartment mathematical model of porcine endotoxemia [83], based on an existing mathematical model of mouse endotoxemia [14, 15, 17, 36], in order to further test the hypothesis that a conserved inflammation framework could have radically individual manifestations. PCA of circulating inflammatory mediators suggested a central role for the cytokine IL-1β in this inflammatory response. Based on this analysis, we constructed a two-compartment ODE mathematical model that encompasses inflammation, lung (patho) physiology, and a damage variable that recapitulates the health of the animal [83]. This mathematical model could be fit to both inflammatory and physiologic

data in the individual swine, whose outcomes ranged from a self-resolving inflammatory response with fairly normal lung histopathology and function through various degrees of dysregulated inflammation and lung damage to death accompanied by severe lung injury [83]. More recently, we augmented this pig-specific two-compartment ODE model to include a third "tissue" compartment. This three-compartment mechanistic model was initially calibrated with data from individual surviving trauma patients, data that were used to produce 10,000 in silico patients subjected to virtual trauma/hemorrhage. This study raises the possibility of individualized outcome prediction for trauma patients as well as showing the potential for in silico clinical trials based on a small, but representative, cohort of actual patients.

Conclusions

We have increased our understanding of the inflammatory response beyond description of its symptoms and unveiled an ever-increasing complexity underlying this evolutionarily conserved internal communication mechanism [2, 7] that manifests at multiple biological scales [27, 28]. Clinically, translational systems approaches to inflammation have the potential for the identification of novel, rationally designed therapies and diagnostics—as well as for gaining new basic mechanistic insights—via combined data-driven and mechanistic modeling.

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