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Toward Computational Identification of Multiscale "Tipping Points" in Acute Inflammation and Multiple Organ Failure

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Abstract—Sepsis accounts annually for nearly 10% of total U.S. deaths, costing nearly $17 billion/year. Sepsis is a manifestation of disordered systemic inflammation. Properly regulated inflammation allows for timely recognition and effective reaction to injury or infection, but inadequate or overly robust inflammation can lead to Multiple Organ Dysfunction Syndrome (MODS). There is an incongruity between the systemic nature of disordered inflammation (as the target of inflammation-modulating therapies), and the regional manifestation of organ-specific failure (as the subject of organ support), that presents a therapeutic dilemma: systemic interventions can interfere with an individual organ system's appropriate response, yet organ-specific interventions may not help the overall system reorient itself. Based on a decade of systems and computational approaches to deciphering acute inflammation, along with translationally-motivated experimental studies in both small and large animals, we propose that MODS evolves due to the feed-forward cycle of inflammation → damage → inflammation. We hypothesize that inflammation proceeds at a given, "nested" level or scale until positive feedback exceeds a "tipping point." Below this tipping point, inflammation is contained and manageable; when this threshold is crossed, inflammation becomes disordered, and dysfunction propagates to a higher biological scale (e.g., progressing from cellular, to tissue/organ, to multiple organs, to the organism). Finally, we suggest that a combination of computational biology approaches involving data-driven and mechanistic mathematical modeling, in close association with studies in clinically relevant paradigms of sepsis/MODS, are necessary in order to define scale-specific "tipping points" and to suggest novel therapies for sepsis.

Keywords—Inflammation, Sepsis, Trauma, Mathematical model.

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ABBREVIATIONS

APRV	Airway Pressure Release Ventilation
ARDS	Acute Respiratory Distress Syndrome
AST	Asparagine aminotransferase
DAMP	Damage-associated molecular pattern molecule
DBN	Dynamic Bayesian Network
DyNA	Dynamic Network Analysis
FiO_2	Fraction of inspired O_2
IL	Interleukin
MIST	Minimally Invasive Suction and Treatment device
MODS	Multiple Organ Dysfunction Syndrome
PaO_2	Partial arterial O_2 pressure
PEEP	Positive end-expiratory pressure
SIRS	Systemic Inflammatory Response Syndrome
TNF-α	Tumor necrosis factor-α

INTRODUCTION

The systemic inflammatory response to injury, hemorrhage, or microbial pathogenic insult elicits an initially local inflammatory response that, while attempting to defend the body and prevent further damage, paradoxically induces systemic inflammation and attendant dysfunction at the cellular, tissue, and organ levels and can eventually kill the host. The ultimate outcome for a given patient spans the gamut from a healthy resolution of the initial insult, to a prolonged, but recoverable stay in the intensive care unit to support the so-called Multiple Organ Dysfunction Syndrome (MODS), and in many cases to

death. These diverse outcomes are determined both by pathogen virulence and the host's inflammatory response, which in turn is based on the patient's genetics as well as prior history of co-morbidities.[7,25,40] Sepsis alone is responsible for more than 215,000 deaths in the US per year and an annual healthcare cost of over $16 billion,[10] while trauma/hemorrhage is the most common cause of death for young people in the US, costing over $400 billion annually.[61,72,98]

The responses to severe infection and trauma/hemorrhage both involve a generalized activation and systemic expression of the host's inflammatory pathways—the so-called Systemic Inflammatory Response Syndrome (SIRS). Inflammatory stimulation of host immune effector cells leads to release of potent inflammatory mediators, including chemokines, cytokines, and reactive oxygen/nitrogen species. Most cytokines function properly when they are restricted to specific tissues, wherein local injury or infection induces a well-regulated inflammatory response. Inflammation becomes harmful, however, once the levels of these cytokines rise sufficiently so that they begin to appear in the bloodstream. Sustained systemic elevations of both pro- and anti-inflammatory cytokines, and their subsequent complex interactions with inflammatory, endothelial, and parenchymal cells, identify those sepsis patients who develop MODS, shock, and death.[1]

Effectively modulating the inflammatory response in sepsis and trauma, without adverse effects, has proven daunting. There is currently not a single drug approved by the U.S. Food and Drug Administration (FDA) for the treatment of MODS. The one drug that had previously been approved for sepsis, recombinant human activated protein C, was found on an FDA-mandated repeat Phase III clinical trial to offer no benefit over standard of care; this drug was subsequently removed from the market.[9,58] We suggest that the following combination of factors is responsible for this state of affairs. Inflammation and associated cellular, tissue, and organ dysfunction form an interconnected complex biological system whose very architecture is both robust and fragile[90,92,95]; identifying the critical control points in such systems is extremely challenging. In addition, the animal models that have formed the primary preclinical experimental platforms have often failed to replicate the full spectrum of human responses to infection or injury.[54,71,93] Together, these factors are likely to blame for the failure of the current reductionist paradigm for discovery of novel therapeutics for these diseases (see below). We assert that the development of novel treatment strategies for disordered acute inflammation[15,16,67,85,94] must be driven by a combination of translationally-motivated computational modeling[96] and clinically relevant animal models of trauma/hemorrhage, sepsis, and MODS.[50,51,77,82,83] We further suggest the following stepwise process in order to extract translationally meaningful knowledge from the "data deluge" caused by the advent of "omics" methodology[38]: (1) acquisition of high-dimensional data, (2) data-driven modeling in order to derive quasi-mechanistic insights, (3) mechanistic modeling, and ultimately (4) experimental validation.[7,8,57,64,92] Herein, we discuss recent progress in applying this combined approach to glean insights into the pathophysiology of sepsis and trauma/hemorrhage.

ACUTE INFLAMMATION AND ORGAN DYSFUNCTION: A COMPLEX, INTERCONNECTED SYSTEM

The current paradigm for targeting acute inflammation in sepsis and trauma, much as in many other complex diseases, centers on the identification of a putatively important biological pathway, followed by interference with that pathway (using genetic methods, antibodies, pharmacologic inhibitors, or scavengers), and evaluation of the results on some biologic endpoint. The advent of high-dimensional genomics, proteomics, metabolomics, physiomics, and related approaches has resulted in both a deluge of data[38] with the hope that a key mediator of sepsis and trauma/hemorrhage that could be identified and targeted in the reductionist fashion described above.[20,21] However, inflammation is a prototypical complex system, exhibiting nonlinear behavior resulting from the multi-feedback nature of the interactions between its components, robustness to perturbation coupled with the potential for severe failure in key nodes, and emergent properties that are difficult to intuit from a reductionist analysis of central mediators in isolation.[23,92] While the reductionist paradigm has been extremely useful in elucidating many of the physiological mechanisms that contribute to the pathogenesis of shock and sepsis,[19,73] this conceptual framework has also perpetuated the "single mediator" approach to the development of novel therapeutic agents. Unfortunately, while a significant number of "single mediator" therapeutic approaches to inflammation have worked well in highly constrained animal models, these interventions have shown little benefit when applied to the complex disease state of human hemorrhagic shock and sepsis.[16,19,33,47,66,68,85]

In addition, the clinical approach to MODS is also based on a reductionist view (though to a great degree driven by necessity), focused on attempting to provide supportive care, often without a clear concept of "ripple effect" of possible injury caused by such supportive care (e.g., provision of fluids and drugs to

alleviate hypotension, placement on a ventilator to support failing lungs, etc.).[22,30,78] Indeed, the possibility that inflammation and physiology are tightly coupled such that altered physiology in sepsis is a signal to the inflammatory response and *vice versa*,[7,8,64,91,92] is a relationship that has been poorly explored.

We have suggested a computational modeling approach to the dilemma of MODS that represents a conceptual departure from the current view of acute inflammation and MODS, and offers a new paradigm of MODS pathogenesis based on multifaceted, multicompartment, and multiscale inflammatory processes.[8] This approach is based on the following premises:

- The recognition—both driven and supported by computational modeling—that the inflammatory response, much like physiology, is both compartmentalized and connected.
- The critical importance of computational modeling as a bridging methodology as a means of linking *in vivo* models and clinical scenarios across different experimental platforms and conditions, thereby serving a critical translational role.
- The hypothesized forward feedback loop of inflammation → damage/dysfunction → inflammation, which is predicated on the interconnected nature of inflammation and physiology.
- The corollary to the above, namely the hypothesis that this interconnected structure and behavior leads to compartment-specific "tipping point" that drive systemic "all-or-none" behavior.
- The need to target therapies based on this structure and dynamic behavior.

Below, we discuss recent progress in understanding the inflammatory pathophysiology of trauma/hemorrhage and sepsis as an illustration of these concepts.

COMPUTATIONAL AND EXPERIMENTAL STUDIES ON COMPARTMENTALIZED ACUTE INFLAMMATION

The inflammatory response is compartmentalized both structurally and across multiple scales of organization.[14,17,59,79] Moreover, inflammation is both regulated by, and coupled to, physiological processes *via* neural pathways.[1,87] Using our experience with both theoretical computational models and a multiplicity of laboratory experimental platforms, we have sought to refine the dynamic relationships between inflammatory compartments in order to develop a rational roadmap toward the translational engineering of therapeutic interventions. As with all engineering/development tasks, the first step is establishing a sufficiently expressive and robust hypothesis upon which further development can proceed. To achieve this characterization, we employ a traditional, progressive use of *in vivo* experiments and platforms, but augment this process through the use of computational models to explore, explain, and hopefully bridge the fundamental aspects of multi-compartment inflammation.

In order to utilize laboratory methods across a continuum of experimental platforms, all developed with an explicit translational goal in mind, we have worked in rodent models of sepsis in which mortality more closely reflects the clinical reality (25–30%),[28] and have developed an experimental paradigm of peritoneal sepsis combined with ischemia/reperfusion injury in swine (PS + I/R) that replicates much of the complex pathogenesis of sepsis/MODS, including the identical clinical time frame, as seen in human sepsis patients[50,51,73,76,82,83] (Namas *et al.*, submitted). In our large-animal studies, we have observed hints of compartment-specific "tipping point," all-or-none behavior at which the animal is no longer responsive to fluids, to increased FiO_2, to pressors, or to increased PEEP.[50,51,77,82,83]

Suppressing the initial inflammatory response in animal models of sepsis results in immunosuppression and elevated morbidity and mortality, due to overwhelming infection.[65,75] Moreover, even in nominally sterile trauma/hemorrhage in both humans and swine, the inability to produce an adequately robust systemic TNF-α response is likewise associated with elevated morbidity and mortality.[62] In contrast, in a rat model of sepsis, we have found that hemoadsorption (HA, the non-specific removal of middle molecular weight inflammatory mediators *via* adsorption to beads in an extracorporeal column), a therapy that reprograms inflammation in a fashion that preserves appropriate compartmentalization, optimizes both the reduction of bacterial burden, and reduces dysregulated systemic inflammation (Namas *et al.*, submitted). Specifically, we found that plasma cytokine levels at baseline were the same in animals subjected to HA and sham treatment, but that plasma TNF-α, IL-6, CXCL-1, CCL-2, and AST were significantly reduced in HA vs. sham animals. We next utilized Principal Component Analysis, a dimensionality reduction tool that has been used in *in vitro* systems biology studies[45,46] and in recent *in vivo* mouse trauma/hemorrhage work from our group,[56] in an attempt to identify the subsets of circulating mediators that are most strongly indicative of membership in the HA or sham treatment group, and that thereby might be considered principal drivers of each response. This data-driven analysis suggested that the circulating inflammatory response in the sham

group was primarily driven by IL-6 and TNF-α, whereas the response to HA was primarily driven by TNF-α, CXCL-1, IL-10, and CCL2. This analysis suggests that in this animal model—and in the time range studied—TNF-α and IL-6 are principal drivers of sepsis, and that HA modifies this process *via* CXCL-1, IL-10, and CCL-2 (and with a concomitantly reduced role for IL-6). Peritoneal bacterial counts were significantly lower in HA vs. sham. Liver damage, assessed by plasma AST was reduced in the HA group. These results suggest that HA may reduce, reprogram, and re-localize inflammation, while improving bacterial clearance and reducing organ damage in experimental sepsis. Thus, we hypothesize that inflammation is beneficial as long as it remains compartmentalized, while systemic spillover is both harmful in and of itself (inasmuch as systemic inflammation drives organ damage) as well as in that dysregulated inflammation inhibits the effective clearance of bacteria. Finally, this study points to data-driven analyses such as PCA as useful quasi-mechanistic tools for studying inflammation *in vivo* (Namas *et al*., submitted).

Despite the many useful features of the rat sepsis model, it shares the same limitations inherent in all rodent models (as mentioned above), with the consequence that it will not be as clinically realistic as large animal models of sepsis, especially with regard to probing the interactions between inflammation and organ pathophysiology and the ability to apply clinically relevant interventions. Based on studies in a clinically realistic porcine paradigm of sepsis, we and others have shown that disordered inflammation in the gut/peritoneal compartment occurs early and plays a critical role in the pathogenesis of MODS.[36,37,50] The gut is a prime candidate for the source of the second "hit" that perpetuates and amplifies systemic inflammation leading to MODS and the Acute Respiratory Distress Syndrome (ARDS).[8,37,48,50] Injury to the intestine occurs early and often in ICU patients,[89] disrupting the gut microcirculation,[42–44,49,88] inducing I/R injury,[34,99–101] and leading to the accumulation of toxic ascites that perpetuates systemic inflammation.[29,53,55] Intestinal edema,[35] lymph,[12,26,27,80] and ascites[50] contain inflammatory mediators that can exacerbate the primary pathology and perpetuate SIRS. Thus, damage to the gut becomes a continual source of inflammation, propagating systemic inflammation and ultimately causing MODS and ARDS.[31,50,60,84] We have shown that removing peritoneal ascites with abdominal negative pressure therapy, in a peritoneal sepsis + ischemia/reperfusion model of MODS, decreased the inflammatory milieu in both the ascites and plasma, which correlated with a reduction in histopathology of the lung, liver, kidney, and intestines.[50] These data suggest that by reducing the inflammatory milieu in the peritoneal compartment below the "tipping point" threshold we can prevent dysfunction from propagating to a higher biologic scale. Our hypothesis does not include altered gut *epithelial permeability* causing bacterial translocation, an old disproven mechanism,[81] but rather implicates increased *endothelial permeability* as the mechanism perpetuating systemic inflammation and ARDS.[34,41]

We have augmented our laboratory explorations with computational models that can integrate, suggest, explain and potentially predict biological knowledge and data. One of our earlier computationally-based efforts at identifying inflammatory "tipping points" centered on a multi-scale, multi-tissue and multi-organ agent-based model (ABM) of the gut–lung axis of systemic inflammation.[3] Agent-based modeling is a computational modeling method that creates system-level behavior through the *in silico* interactions of populations of computational "agents" that are used to represent subtypes of real-world objects in the reference system.[6] This class of computational models is increasingly common in biomedical research, since there is a natural mapping between cellular subtypes and classes of computational agents, and as such ABMs are well-suited to translating rule-based mechanistic knowledge generated from basic science research. In our particular gut–lung inflammation ABM, both organs are represented by spatially distinct, aggregated populations of epithelial and endothelial cells that nonetheless share populations of circulating inflammatory cells and mediators. Initially intended as a means of integrating molecular biological pathway knowledge into a multi-scale context, *in silico* experiments involving the effect of gut ischemia on the development of pulmonary edema demonstrated a clear gut ischemia threshold, or "tipping point," beyond which there was a consistent propagation first to ARDS, then to subsequent systemic hypoxia (including worsening gut ischemia) and finally system death (Fig. 1).[3] The addition of an abstracted form of ventilatory support allowed the system to tolerate more severe gut ischemia, but could not eliminate the "tipping point." While this ABM was quite abstract, it did provide early evidence of the role of compartmental inflammation on the generation of inflammatory tipping points, and suggested that at least at some level compartment directed interventions could affect the dynamics of the overall system. These findings provided some theoretical substantiation for the tipping point hypothesis as laboratory experiments progress.

In a similar vein, in order to more closely integrate the iterative loop between computational modeling and laboratory experiments, we created a two-compartment mathematical model of porcine endotoxemia,[69] based on an existing mathematical model of

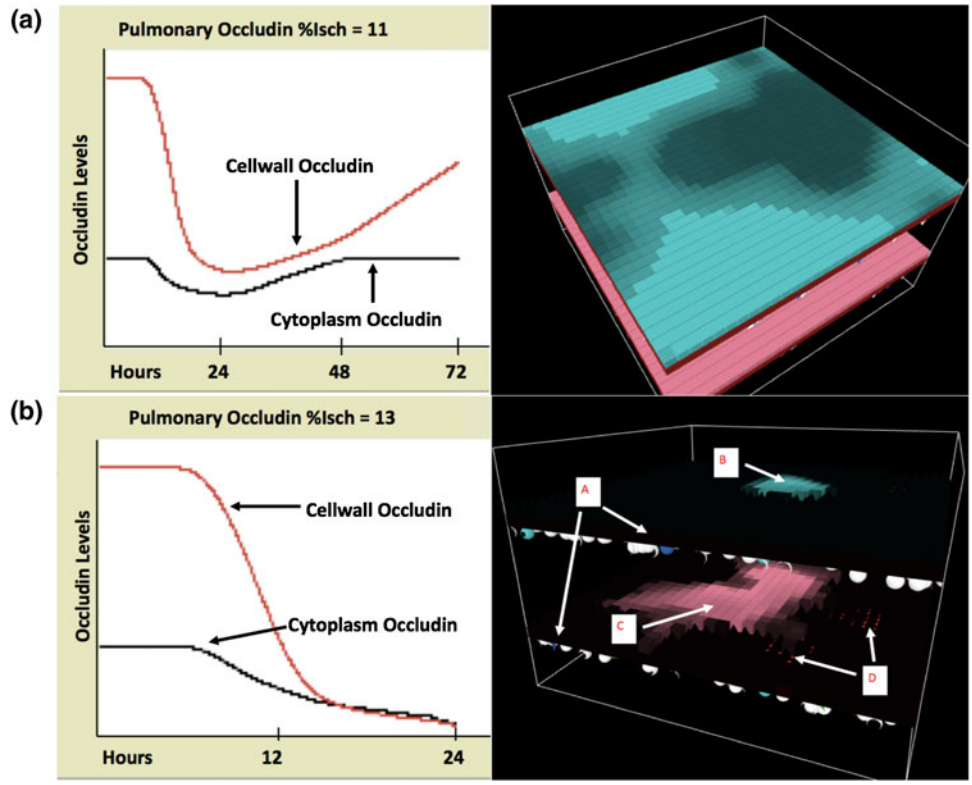

FIGURE 1. Computationally-defined inflammatory "tipping points" using agent-based modeling of inflammation. Output from the gut-lung ABM (An[3]) in simulations of the effect of gut ischemia on the development of pulmonary edema (manifest as loss of pulmonary tight junction integrity). *Panel A* demonstrates a degree of gut ischemia (11% in arbitrary units) below a compartmental "tipping point", such that pulmonary tight junctions can recover (24–48 h), with a screenshot showing spatial distribution of loss of pulmonary barrier function. *Panel B* demonstrates a degree of gut ischemia (13% in arbitrary units) above the compartmental "tipping point", at which the propagation of inflammation leads to severe enough pulmonary dysfunction so that the system cannot recover (at 24 h). In the corresponding screenshot, dysfunctional lung and gut epithelial cells are noted by red letters A and D, whereas remaining intact epithelial cells are labeled red letters B and C. Note that these simulations did not include organ support, such as ventilation for the failing lung component in *Panel B*. Figure reprinted from An[3] under the Creative Commons license.

mouse endotoxemia.[18,52,74,86] This previous single-compartment mathematical model of inflammation was capable of making qualitative and quantitative predictions with regard to endotoxin-induced inflammation and a single measure of physiology (mean arterial pressure) in genetically identical mice.[18,52,74,86] Our goal in generating the multi-compartment model was to set the stage for addressing the "tipping point" hypothesis described above, in a mathematical model that combines both inflammation and physiology and calibrated with data obtained in genetically diverse swine. The model was also extended to support clinical interventions administered such as a fluid resuscitation and mechanical ventilation, in order to increase its clinical realism. Circulating inflammatory mediators (HMGB1, IL-1β, IL-6, IL-8, IL-10, and TNF-α) exhibited statistically significant changes at some point in the 6-h time course of this experiment. Principal Component Analysis was carried out in order to define principal inflammatory drivers (as in the examples described above). This analysis suggested that the contributors to the systemic inflammatory response, in order of effect from greatest to least, were IL-1β > IL-10 > IL-8 > TNF-α > IL-6 > NO_2^-/NO_3^-.[1,3,6–10,12,14–17,19–23,26–31,33–38,41–51,53–62,64–69,71–73,75–85,87–96,98–101] This finding was surprising, since the pro-inflammatory cytokine TNF-α was elevated sooner than IL-1β, and since many prior studies (including those involving mathematical modeling[18,24,74]) suggested that TNF-α should be the primary driver of inflammation in the setting of endotoxemia.

Based on PCA, we constructed a key module of our mathematical model, in which HMGB1 led to the production of IL-1β, with the eventual production of IL-6; interestingly, both IL-1β and IL-6 exhibited "tipping point" behavior, with a rapid elevation that plateaued but did not decrease.[69] This mathematical model describes these interactions and depicts these behaviors, along with other pro- and anti-inflammatory cytokines, blood pressure, lung functional parameters (e.g., PaO_2/FiO_2 ratio), and a damage variable that recapitulates the health of the animal.

Importantly, this mathematical model could be fit to both inflammatory and physiologic data in the individual swine. Moreover, the predicted time course of damage could be matched to the Oxygen Index (OI) in three of the four swine utilized in the study.[69]

More recently, we augmented the two-compartment mathematical model structure to include a third compartment (''tissue''), which nominally represents all tissues in the body other than the lung. This three-compartment model was initially calibrated with data from individual surviving trauma patients [Injury Severity Score (ISS) of approximately 25, representing moderate to severe injury]. Knowledge of variability among these patients was leveraged to produce 10,000 ''virtual patients''. Each ''patient'' was subjected to three insults of trauma: low ISS (5–20), moderate ISS (20–35), and high ISS (35–50). Parameter sensitivity analysis was employed to understand the relative role of inflammatory mediators on predicted ICU length of stay and organ dysfunction. This analysis suggested that in patients with a low ISS, IL-1β was the predominant driver, while IL-6 was the main driver in ''patients'' with moderate or severe ISS. These findings were in agreement with PCA and dynamic network analysis performed on plasma samples from mouse trauma/hemorrhage.[56] Principal Component Analysis of the circulating inflammatory mediators from the original 33 patients suggested that IL-1β was the principal driver of inflammation in these actual patients, in line with the results from the virtual patients. This study raises the possibility of determining novel basic mechanisms in trauma, of individualized outcome prediction for trauma patients, and of virtual clinical trials based on a small number of actual patients.

Based on this combined *in silico–in vivo* approach, we have suggested the need to identify, predict, and modulate the combination of mediators that cause the phase transition from a lower (e.g., cellular) to a higher (e.g., organ) biological level, and, ultimately, MODS.[8] More specifically, we have observed a ''tipping point'' approximately 24 h following the initial injury (peritoneal sepsis and gut ischemia/reperfusion), at which point the lung was no longer able to respond to increases in FiO_2 and PEEP with improved oxygenation.[50,51,77,82,83]

We suggest that our evolving mathematical models will help identify and predict potential bioactive interventions, and validate those predictions in further experiments in swine and, ultimately, in clinical trials based on our *in silico* studies. The multi-compartment/multi-scale computational models could be used to test interventions targeted at effector organs, such as ventilation strategies aimed at manipulating the lung. Importantly, mechanistic mathematical models can allow us to bridge the gap from rodent studies to large-animal studies and eventually to clinical studies.[4,93] Trans-species and trans-compartment mechanistic and data-driven modeling will also allow us to integrate those inflammatory networks that help drive ''tipping point'' behavior.

To define these inflammatory networks, we have begun to use our recently-published Dynamic Network Analysis (DyNA) algorithm,[56] along with a more recently developed Dynamic Bayesian Network (DBN) algorithm (adapted from Grzegorczyk and Husmeier[39]). Given time-series data, DBNs provide a way of inferring causal relationships based on probabilistic measures. While our DyNA method infers the networks present at each time interval,[56] DBN's assume a static network structure across all time points. This approximation, however, helps to suggest the overall network structure (including central nodes that exhibit positive feedback). As one example of the utility of the DBN methodology for linking to mechanistic mathematical models and for deriving novel predictions therefrom, we subjected C57Bl/6 mice to trauma/hemorrhage, with hemorrhagic shock lasting from 0 to 4 h. Cytokines/chemokines were assayed in the plasma by Luminex™, and the data were subjected to DBN analysis. This analysis suggested a network that contains a so-called incoherent type I feed-forward loop,[2] driving the pulse-like behavior of the cytokine IL-1α (Azhar *et al.*, unpublished). An ordinary differential equation-based mathematical model of the core network predicted pulsatile behavior for IL-1α, a behavior that was indeed observed in mice subjected to trauma/hemorrhage (Azhar *et al.*, unpublished).

We also utilized this DBN method to examine the connectivity of inflammation in multiple organs in endotoxemic mice. We subjected C57Bl/6 mice to endotoxemia (3 mg/kg) for various periods of time ranging from 0 to 24 h. At each time point, mice were sacrificed, and their liver (Fig. 2a), lung (Fig. 2b), intestine, heart, kidney, spleen, and plasma (all data not shown) cytokines and chemokines were assessed by Luminex™. These data were subjected to the DBN, which suggested a high degree of connectivity as well as feed-forward behavior for the chemokines CXCL10, CXCL1, and CXCL9. Importantly, this network structure suggests that the cytokine IL-6, which is a biomarker of aberrant inflammation,[1,61] is produced systemically as a consequence of the activation of these chemokine networks. Based on these studies, we suggest that the compartment-specific response to inflammatory stimuli initially remains within a given compartment, helping to coordinate responses appropriate to a given stimulus. However, when the magnitude or duration of an inflammatory stimulus exceeds certain (likely genetically encoded) thresholds, the response spills over into other compartments. This process probably occurs at least in part *via* the systemic

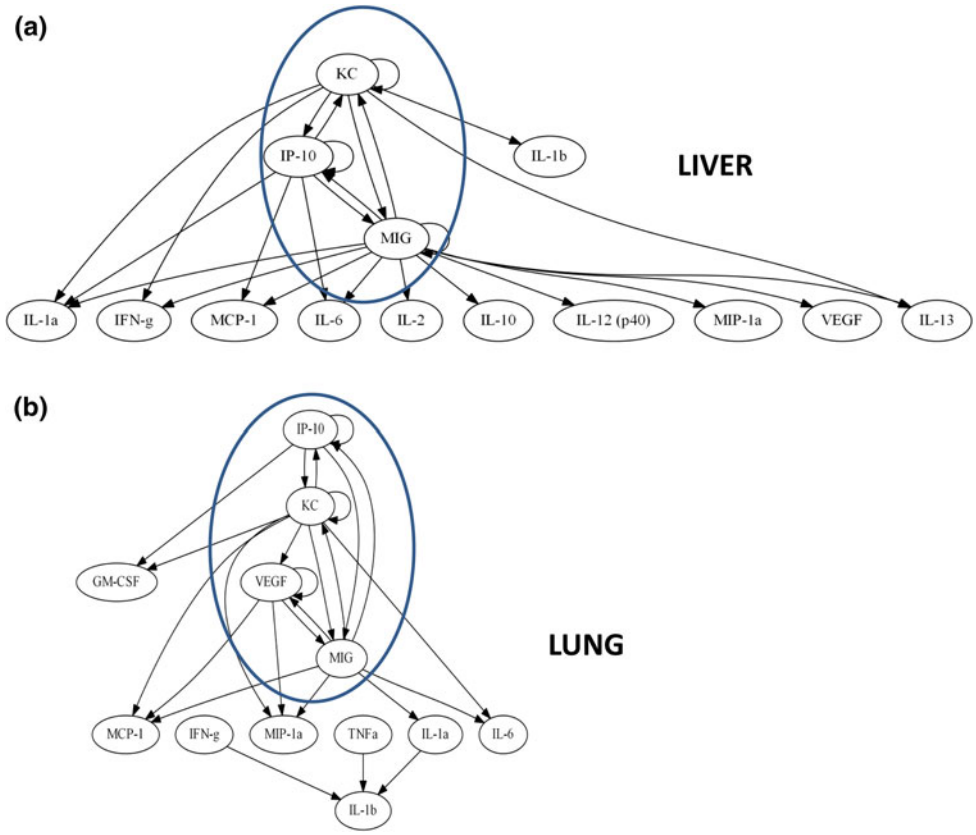

FIGURE 2. Dynamic Bayesian Network analysis suggests a core, multi-organ inflammatory network in murine endotoxemia. C57Bl/6 mice were subjected to endotoxemia, and cytokines/chemokines were assayed in their liver (*Panel A*) and lung (*Panel B*). Circles indicate core networks, suggesting a high degree of connectivity as well as feed-forward behavior.

circulation, and also likely *via* the lymph (as detailed above), leading to progressive organ dysfunction. This dysfunction, in turn, further aggravates inflammation.

COMPUTATIONAL INSIGHTS FOR RATIONAL INFLAMMATION REPROGRAMMING

Based on the inflammatory "tipping point" hypothesis, we suggest that the acute inflammatory response, which forms the core of the detrimental effects of inflammation, may simply evolve too rapidly to be modulated appropriately given the time necessary for proper diagnosis and administration of therapy. We further suggest that the current approach to anti-inflammatory therapeutics—extinguishing inflammation to the greatest degree possible coupled with the support of failing organs—is misguided. The therapeutic goal should not be to abolish inflammation, but rather to reduce damage or dysfunction (i.e., promote healing) by attenuating the positive feedback cycle of inflammation → damage → inflammation.[4] Likewise, rather than simply supporting organs, attempts should be made to modulate organ function in order to avoid "tipping points" and thereby also help avoid this vicious inflammatory cycle.[8]

We have initiated work on multimodal therapies such as HA (see above; Namas *et al*., submitted), in order to help re-compartmentalize and thereby reprogram the inflammatory response. Due to the forward feedback interactions between inflammation and organ pathophysiology, we hypothesize that modulating inflammation should reduce organ dysfunction and *vice versa*. We have shown that modulating peritoneal inflammation may provide control of an early, vital "tipping point," and, reciprocally, that altering ventilatory pattern may prevent inflammation and ARDS. Peritoneal negative pressure therapy[50] is an example of the former approach (targeting inflammation to impact organ dysfunction). Furthermore, we have designed a dual modality device [the *Minimally Invasive Suction and Treatment* device (*MIST*)] to simultaneously remove ascites with suction and continually treat the gut with dialysis fluid. Although the mere removal of ascites is likely to have a therapeutic effect,[50] we would ultimately want to modulate "tipping points" more precisely through the use of bioactive agents in the peritoneal dialysate, to modulate the inflammatory

response for specific biological circumstances as predicted by our computational models.

Airway Pressure Release Ventilation (APRV)[50] is an example of the latter approach (treating organ function to affect inflammation). Our study demonstrated that early application of APRV prevented the pulmonary edema, increased vascular permeability, and surfactant dysfunction, all of which are hallmarks of ARDS pathophysiology; avoiding these pathologic changes blocked progression to the "tipping point" and prevented the development of ARDS.[50] Thus, by understanding that the progression of disease in not linear but rather an all-or-none event we may be able to change the clinical paradigm from *treating* to *preventing* ARDS.

We have also recently created a biohybrid device for the control of systemic inflammation,[63] i.e., once "tipping points" have been exceeded leading to systemic spillover of inflammatory mediators. This is a novel class of devices, in which gene-modified cells are seeded in a bioreactor that is connected to the systemic circulation. The current version of this device acts to suppress TNF-α *via* its endogenous inhibitor, soluble TNF-α receptor [sTNFR]. *In vivo*, this biohybrid device resulted in elevated circulating sTNFR, reduced the levels of TNF-α and other key inflammatory mediators as well as alleviating hypotension and reducing circulating markers of organ damage in endotoxemic rats. It should be noted that this was achieved in rats by human cells producing mouse proteins. Moreover, we were able to maintain the bioreactor solely using the rat's circulation, and re-use the bioreactor multiple times, with transport between laboratories, over a period of a month.[63] We suggest that these diverse strategies could be modified based on computational modeling, and deployed in a combined fashion based on our improving characterization of inflammatory "tipping points."

COMPUTATIONAL BIOLOGY AND THE FUTURE OF SEPSIS AND TRAUMA RESEARCH

The acute inflammatory response to bacterial infection or trauma is mediated by cells and molecules that locate invading pathogens and damaged tissue and act to restore the body to equilibrium.[13] However, the feed-forward loop of inflammation → tissue damage/dysfunction → inflammation can lead to organ dysfunction and death.[8,90,96] And yet, acute inflammation is not in and of itself detrimental. It is in most cases a well-coordinated communication network that helps maintain homeostasis.[8,90,96] A central question, then, is: how do we harness the beneficial effects of inflammation and allow proper lines of communication, while simultaneously not allowing inflammation to exceed "tipping point" thresholds that drive organ dysfunction? We suggest that the solution to this conundrum lies in recognizing that the acute inflammatory response is a complex system, both in structure and behavior.[8,70] Based on insights from combined *in silico*/*in vivo* studies, we suggest that manipulating the acute inflammatory response will require an extension beyond the traditional scientific paradigm of analysis *via* sequential reductionist experimentation.[90,96] Mathematical modeling of complex systems has emerged as an approach by which to tame the seemingly unpredictable behavior of such biological systems, including the rational design of synthetic biology strategies.[4,11,97] We and others have shown that the complexity of inflammation can be addressed rationally through computational simulations[32,90,96] in the context of interdisciplinary teams.[5,8] In this "Translational Systems Biology"[90,96] approach, we have focused on *in silico* (simulated) clinical trials, personalized diagnostics, and rational drug/device design driven by computational models.[4,96] Herein, we have discussed key insights derived from this combined approach, which have led us to hypothesize a novel concept by which inflammation propagates and drives organ dysfunction in sepsis and trauma. Future studies will help us refine and test this hypothesis, *via* a combination of clinically-relevant animal models, data-driven modeling, and mechanistic computational simulations.

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