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Translational systems biology of inflammation: potential applications to personalized medicine

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Abstract

A central goal of industrialized nations is to provide personalized, preemptive and predictive medicine, while maintaining healthcare costs at a minimum. To do so, we must confront and gain an understanding of inflammation, a complex, nonlinear process central to many diseases that affect both industrialized and developing nations. Herein, we describe the work aimed at creating a rational, engineering-oriented and evidence-based synthesis of inflammation geared towards rapid clinical application. This comprehensive approach, which we call ‘Translational Systems Biology’, to date has been utilized for *in silico* studies of sepsis, trauma/hemorrhage/traumatic brain injury, acute liver failure and wound healing. This framework has now allowed us to suggest how to modulate acute inflammation in a rational and individually optimized fashion using

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engineering principles applied to a biohybrid device. We suggest that we are on the cusp of fulfilling the promise of *in silico* modeling for personalized medicine for inflammatory disease.

Keywords

inflammation; modeling; systems biology

Inflammation is communication: towards a personalized view of complexity

Inflammatory diseases include infection/sepsis, trauma, inflammatory bowel diseases, rheumatologic disorders and asthma; as well as many other diseases, such as cancer, diabetes, atherosclerosis, Alzheimer's and obesity are also associated with dysregulated inflammation. The US NIH Roadmap Initiative has emphasized the need to apply systems biology methods to the study of inflammation [101]. The acute inflammatory response to biological stress (e.g., infection or tissue injury/damage) involves a highly conserved cascade of events mediated by a large array of cells (e.g., mast cells, macrophages, neutrophils, natural killer cells, dendritic cells, and T and B lymphocytes) and molecules (inflammatory cytokines, free radicals and damage-associated molecular pattern molecules [DAMP's]) that locate invading pathogens or stressed/damaged tissue, alert and recruit other cells and molecules, eliminate the offending agents and finally restore the body back to equilibrium [1,2]. Inflammation is a well-coordinated communication network operating at an intermediate time scale between neural and longer-term endocrine processes [3,4], and may also be driven by slower degenerative processes that share many common mediators with acute proinflammatory insults [5]. Inflammation itself can cause secondary or ancillary damage to tissues, which in turn, leads to the production of molecules that amplify inflammatory response and, in extreme cases, promote organ dysfunction and death [6]. However, inflammation is a critical and necessary process that allows organisms to deal with a rapidly changing and often hostile environment, and is not intrinsically detrimental. For example, in the setting of trauma/hemorrhage, in which inflammation has previously been considered purely detrimental [7,8], recent studies suggest that morbidity and mortality ensue in the absence of an adequate early inflammatory response [9]. This requirement for robust early inflammation may represent a means of communicating the extent of injury, setting in motion both early processes that act to prepare the organism for a possible secondary infection following trauma or wounding, while also driving reparative and regenerative processes [4]. The beneficial effects of inflammation may explain why this robust biological process is evolutionarily conserved [2,10]. Paradoxically, it is likely that the detrimental effects of inflammation, and the difficulty in countering or reversing inflammation in a way that will avoid detrimental side effects, are due to the same evolutionary conservation and robustness.

With the plethora of cellular and molecular components, and their context-dependent interactions involved in inflammation, it is not surprising that at the level of the individual inflammation manifests in diverse ways that reflect aspects of computational modeling of complex systems. Each injury or infection has specific qualitative and quantitative initial conditions [11]. Individual parameter values may reflect genetic predisposition to produce specific inflammatory mediators at either pathological or physiological levels in response to an inflammatory stimulus of a given magnitude [12–14], or the effects of demographic factors such as age [15–18] or socioeconomic status [19]. An evolving literature points to the propagation of inflammation by various positive feedback loops such as the one consisting of DAMP molecules that reinduce the release of proinflammatory cytokines [20,21]. In parallel, various negative feedback mechanisms are set in motion to suppress, counter, or reverse inflammatory processes [22]. Under physiological conditions, these

features endow inflammation with robustness to a wide range of perturbations, but with simultaneous fragility at points of control whose failure, not surprisingly, is associated with disease [3,23]. These robust, yet fragile, mechanisms help communicate the qualitative, and perhaps, even quantitative details of the myriad perturbations of homeostasis that impact the individual, including both acute and chronic events [5]. The acute inflammatory response therefore exhibits many essential characteristics of a complex system – such as sensitivity to initial conditions, positive and negative feedback loops, combined robustness and fragility, and emergence of nonintuitive behaviors [4,6,24–29] – that complicate the ability to predict individual clinical outcomes in inflammatory diseases. Acceptance of these tenets regarding inflammation leads inexorably to the need to adapt complex systems study approaches to the study of inflammation.

Complexity of inflammation: basic & translational approaches based on modeling cells, experimental animals, simulated populations & individuals

Systems biology has been defined in many ways [30–33], but in essence is a discipline that attempts to use computational approaches often combined with high-dimensional datasets to span the multiple scales of organization that characterize biological systems. In particular, mathematical modeling and simulation technologies have been applied to the study of sub-cellular and cellular processes, and have led to a paradigm shift in biology that has yielded numerous basic insights [25,30]. In the nascent field of inflammation modeling, the impetus to learn more about the inflammatory response in sepsis, trauma/hemorrhage and wound healing has been characterized by studies attempting to span the cell→animal→human population→individual patient. These studies have utilized both mechanistic and data-driven modeling to improve translational research. Ultimately, our goal is to go beyond describing, modeling and even predicting the inflammatory responses of individuals. Rather, we wish to reprogram inflammation rationally and in an individualized fashion. This task is daunting given current technology focusing on drug-based [34–43] and emerging cell-based [44] anti-inflammatory strategies. Thus, we have suggested that lessons learned from the field of dynamic control systems could be used to design an extracorporeal device that would reprogram the inflammatory response as optimally as possible, based on insights from data-driven and computational simulations (see later).

In a sense, this is the ultimate distillation of disciplines to serve the goal of translational research, which aims to apply basic scientific discoveries into clinical practice in order to improve healthcare [45]. Accordingly, we have put forth the concept of ‘Translational systems biology’ [4,6,46,47]. Translational systems biology calls for the creation of computational models that are generated with an *a priori* focus on rapid translational application in areas such as *in silico* clinical trials, patient diagnostics, rational drug design and long-term rehabilitative care [4,6,46–48]. Below, we describe a cornerstone of translational systems biology, namely the use of mechanistic modeling to gain insights into the pathophysiology of individuals (i.e., patients) and populations (i.e., patient cohorts) in the context of inflammation, in a manner that incorporates insights from studies at the cellular and molecular level and that ultimately allows for rational modulation of inflammation at the individual level.

A large body of work in translational systems biology has made use of ordinary differential equations (ODE) and related analysis methods. For instance, stability and bifurcation analyses of mechanistic ODE-based models have been used widely to understand, explain and illustrate the dynamic behaviors of biological systems [49]. Also, detailed models of cellular signal transduction cascades may help identify the side effects of a drug and provide system-level insights into mechanism-based drug discovery [50]. Various systems biology approaches have been applied in the study of inflammation and immunity [51,52]. For

example, a set of ODE representing the time evolution of different inflammatory mediators or cells has been used to model the biochemistry reaction network of immune-receptor signaling [53], as well as basal and preconditioned inflammatory responses to Gram-negative bacterial lipo-polysaccharide (LPS) [54]. Larger ODE-based models were used to yield insights into the acute inflammatory response in diverse shock states [55–60], as well as the responses to anthrax infection in the presence or absence of vaccination [61]. A related multicompartiment ODE model was used to describe features of necrotizing enterocolitis (NEC), an inflammatory disease that affects many premature newborns; this model was also used to elucidate novel aspects of probiotic therapy for NEC [62]. Importantly, the same ODE model that was capable of describing acute inflammation in mice subjected to clinically relevant experimental paradigms of shock was also used to gain insight into the *in vivo* inflammatory consequences for inflammation of the deletion of a single key gene (*CD14*, a component of the receptor for LPS) [63]. Variants of these ODE models have been used to simulate clinical trials in sepsis [64], paving the way for a nascent biosimulation industry that has been helping pharmaceutical companies to streamline clinical trials [102].

In addition to ODE, the reaction-diffusion type of partial differential equations that can account for spatial effects have been used to simulate the formation of immunological synapse between a T cell and an antigen-presenting cell [65] as well as NEC [Tronzo M, Horvat C, Barber J *et al.*, Unpublished Data] [66]. Furthermore, rules-based approaches have also been utilized to model inflammation-related processes. For example, both rules-based [67] and agent-based [68] models helped explain features of LPS preconditioning (and could be compared with equation-based models of the same process [6]). A related agent-based model was calibrated with transcriptomic data from human leukocytes and led to insights into the inflammatory response to LPS of human volunteers [69]. Agent-based computation modeling has also been used to model sepsis *in vivo*, including carrying out *in silico* clinical trials [70–72,103]. Other agent-based models simulated multiscale and multiorgan interactions in inflammation [73]. This modeling method has also been used to simulate the inflammation and healing in the setting of diabetic foot ulcers, encompassing both existing and hypothetical therapies [74]. A similar agent-based modeling approach was used to elucidate features of surgical injury to the vocal folds in experimental animals [75], as well as to both reproduce and predict the inflammatory responses of individual human subjects experiencing vocal fold phonotrauma [76]. This last study is the first in which a generic computational model was calibrated for data in individuals and was not used only to predict the responses of these individuals at time points beyond the time course of available data, but also to predict responses to diverse treatment regimens [76].

Translational systems biology work has been adopted as a cornerstone of the work of the Society of Complexity in Acute Illness (PA, USA) [104] and the Center for Inflammation and Regenerative Modeling (PA, USA) [105], as a means of traversing the current ‘fragmented continuum’ of healthcare delivery, in which the domains of preclinical studies, clinical trials, in-hospital care and eventual long-term care are separate [48]. In the present article, we discuss progress to date in the nascent field of translational systems biology, and focus in particular on applications of this framework for personalized medicine. This work was spurred by our extensive success in modeling inflammation at the molecular, cellular, tissue/organ and whole-animal levels [4,6,10,28,29,47,77]. Multiple modeling approaches, namely data-driven, equation-based, and agent-based modeling [48,78,79] (all methods covered in detail in other reviews) have been utilized in our translational systems biology work [4,6,10,28,29,47,77]. Later, we describe how data-driven modeling can integrate with the type of aforesaid mechanistic modeling, in order to help gain translational insights for individuals.

Integrating data-driven & mechanistic modeling to understand inflammation in individuals

Animal models may simulate the human inflammatory response to various degrees [80,81]. However, like many biological processes in humans, inflammation and its manifestations in disease are significantly more multidimensional and complex than that observed in animal studies, and the translational systems biology framework is strongly focused on understanding the human condition through modeling-simulation studies on human and preclinical data [4]. Prior studies in cells and animals have utilized both data-driven and mechanistic models to examine the characteristics of patient subgroups as well as the inflammatory responses of individual humans.

The work described herein has utilized both literature data and prospectively gathered data, coupled with data-driven modeling techniques, to gain insights into the inflammatory responses of individuals. For example, multiplexed cytokine data derived from serial plasma samples was analyzed with principal component analysis (PCA), and subsequently subjected to clustering analysis, in order to gain insights into the inflammatory responses of trauma patients. The utility of this combined PCA/clustering technique is summarized in Figure 1. Traditional statistical analysis (Figure 1A) is generally based on defining patient subgroups *a priori* (e.g., based on clinical outcomes), and then determining the features (e.g., cytokine levels) associated with these patient subgroups. In a sense, this statistical analysis is a variant of the so-called ‘inverse problem’, in which one assumes that causal agents can be inferred from the outcomes they are presumed to cause. In the setting of inflammation, this inverse problem can be roughly stated as ‘different outcomes following an inflammatory challenge are driven by, and therefore associated with, defined cytokines’ (Figure 1A). A significant limitation of this statistical approach is that cytokine measurements in the setting of inflammatory diseases are often quite variable, requiring large observational studies in order to obtain statistically significant differences in inflammation biomarkers among different outcome groups [82–85]. An alternative approach, summarized in Figure 1B, is based on the realization that while numerous cytokine patterns may exist in a given patient population over time, only a few defined clinical outcomes are observed. In the setting of trauma/hemorrhage, for example, inflammation is associated with death, multiple organ dysfunction and secondary infection [82–85]. The resultant hypothesis is that much of the variability in patterns of inflammation biomarkers can be reduced to a few key drivers of inflammation in individuals. A corollary to this hypothesis is that these groups of individuals will naturally segregate based on these biomarker patterns (Figure 1B).

An example of this approach can be seen in Figure 2. In this study, a large degree of inflammatory and outcome variability could be observed in a cohort of 25 blunt trauma patients who were all survivors, leading to an inability to define naturally occurring groups (Figure 2A). Nonetheless, defined patterns in the early (within 24 h postinjury) time course of inflammation biomarkers could be identified via PCA focusing on the principal cytokines present (those cytokines that contribute to 95% of the variance in the patient-specific time course data). These patterns segregated the 25 patients into defined subgroups exhibiting distinct levels of organ dysfunction (Figure 2b) [10], and suggests that these patient subgroups, defined within the first 24 h postinjury, persist with different levels of organ damage for several days (data not shown) [Ghuma A, Ziraldo C, Namas R *et al.*, Unpublished Data].

Similar methodology was used in the setting of pediatric acute liver failure (PALF), a disease in which a dysregulated or disordered response to various challenges to the liver can disrupt hepatic homeostasis [86]. Based on the observation that a large number of PALF patients have an indeterminate outcome similar to that of the trauma patients described

earlier, we obtained inflammation biomarker data from PALF patients and analyzed these data using PCA. The results suggested that defined patterns of cytokines are associated with patients who go on to live or die, and further that these cytokine patterns may delineate patients that live following liver transplantation versus those that go on to die following liver transplantation [10].

In parallel to and intersecting with the data-driven approaches, mechanistic modeling techniques have been applied to study the inflammatory responses of individuals. Initial work in the area focused on acute vocal fold injury, a process that engenders inflammation and subsequent wound healing responses within laryngeal mucosal tissue [75,79,87]. A possible factor in the poor prognosis of patients following vocal fold injuries (e.g., due to phonotrauma or surgical trauma) is that vocal fold lesions display wide individual variability, hampering efforts to predict disease progression and treatment response across patients [87]. The first step in the modeling project involved developing an agent-based model (ABM) of inflammation and tissue healing, derived from literature on wounded skin as well as the vocal folds, to simulate the process of healing in responses to the biomechanical stresses associated with phonotrauma [76]. The model reproduced the basic behavior of inflammation and wound healing expected in acute phonotrauma. Individual-specific variants of the model were created based on initial conditions in human subjects experiencing phonotrauma, alone or in combination with further spontaneous speech or specialized voice modulation exercises. These modeling studies predicted that the long-term outcomes of wound healing would be substantially different in individuals exhibiting relatively small differences in the initial inflammatory marker profiles that were associated with the various treatment groups [76]. These results were consistent with empirical observation suggesting that large-amplitude and low-impact vocal fold tissue mobilization exercise may help to attenuate inflammation and promote healing following acute phonotrauma [87]. The simulation results also suggested that an immediate 'parainflammatory' response [5] could ensue without necessary overt tissue injury as in the case of acute phonotrauma. Compared to phonotrauma, a surgical trauma event often results in a higher magnitude of vocal fold mucosal damage clinically. By increasing the initial magnitude of tissue injury in the ABM, the model was able to simulate a full-scale of inflammation and healing response as characterized in surgical vocal fold trauma [75]. Following this initial foray into the construction of patient-specific variants of inflammation models, we have applied similar methods in diverse settings, as discussed later.

Equation-based modeling methods were also used to create patient-specific models calibrated with circulating levels of inflammation biomarkers obtained serially in the setting of polytrauma, leading to a nonintuitive ability to predict outcomes that standard clinical scoring systems could not [10]. Similarly to polytrauma, inflammation induced by traumatic brain injury (TBI) can lead to both morbidity and mortality. Data-driven models were developed in order to predict outcomes post-TBI, and did so using a novel approach we call 'dynamic profiling' [10]. As applied to TBI, initial clustering was based on the Glasgow Coma Scale, and subsequent clustering was performed based on demographic information and then further, sequential clustering was based on the levels of individual inflammation biomarkers over time. These clusters assess the risk of death of a new patient after each cytokine reading, based on the existing information in the previous data in the cluster to which the new patient belongs at the time [10]. The same data on TBI patients was used for mechanistic modeling using ordinary differential equations, following an initial step consisting of PCA to select among a subset of inflammation biomarkers likely to be central drivers of inflammation in these patients. Utilizing this method, 27 patient-specific models were fit to these data [10]. Ongoing work is aimed at comparing model predictions to various clinical indicators. This work, in concert with the purely data-driven analysis described earlier, points to the diagnostic potential of these computational approaches using

relatively simple measurements obtained at multiple time points in easily accessible biofluids.

Rational inflammation reprogramming: systems-based modulation of inflammation in individuals

The central lesson from the aforementioned translational systems biology studies has been that the therapeutic goal for most, if not all, inflammatory diseases should not be to abolish inflammation but instead to reprogram it in a rational fashion in individuals. Thus, our goal is to attenuate the positive feedback cycle of inflammation→damage→inflammation [48], by allowing the body to re-equilibrate its inflammatory response through a repeated, incremental reduction of proinflammatory influences. Towards this end a self-regulating device for individualized control of inflammation was conceived and prototyped [10]. The basic concept of the proposed device is to create negative feedback proportional to the exact degree of inflammatory stimulus by invoking the production of these antagonists. The fact that inflammation is primarily driven and regulated by cytokines [3] and regulated in part by the production of endogenous cytokine antagonists [88] led to the hypothesis that in response to a given inflammatory cytokine such rational therapy might stimulate the release of a defined amount of a given cytokine's endogenous inhibitor or neutralizing protein. Examples include: TNF and its endogenous inhibitor, sTNFR, as well as IL-1 β and IL-1Ra [3]. To carry out initial proof-of-concept studies [Mikheev M, Vodovotz Y, Unpublished Data], vectors were constructed coding for the mouse sTNFR under control of the mouse NF- κ B-responsive enhancer coupled to a reduced thymidine kinase promoter. A lentiviral vector was used to generate stably transfected variants of the HepG2 human hepatocyte cell line, coding for the NF- κ B/sTNFR. Gene-modified human HepG2 cells were grown either in standard 2D culture or in a prototype four-compartment, hollow-fiber culture bioreactor. Mouse sTNFR production was elevated 3.5-fold in standard 2D culture, and this was recapitulated in initial experiments using the bioreactor. Cell lines, which were stably transfected with mouse NF- κ B/sTNFR, exhibited a stimulation of 2.5- to seven-fold over baseline [Mikheev M, Vodovotz Y, Unpublished Data]. Studies in experimental animals subjected to acute inflammatory stress are now being initiated. These encouraging initial results may lead to a class of devices, designed using computational modeling to modulate inflammation in a given disease, but that are adaptable to an individual patient's own inflammatory characteristics. Moreover, such devices could be designed to both diagnose the levels of inflammation biomarkers (perhaps indirectly, through the concomitant expression of fluorescent proteins that could be detected in some fashion), as well as modulating the very same inflammatory processes.

Future perspective

To date, a great deal of the emphasis in the development of personalized medicine has focused on identifying genetic signatures of patient populations in order to categorize disease type, stratify disease risk, predict disease trajectory or optimize response to treatment. While it is clear that genetics plays a vital role in defined subpopulations, there is a growing recognition that the phenotype of inflammatory disease arises from a multiplicity of factors and processes insufficiently characterized by genetic signatures [89,90]. In addition, genetic profiling is a purely correlative process, and does not provide a means by which to evaluate the type or success of any potential intervention. Therefore, it is clear that if the promise of personalized medicine is to be achieved (and here, we define medicine as diagnosis and therapy) then the ability to mechanistically characterize the dynamics of a disease process is an inevitable step.

We believe that the translational systems biology approach described earlier provides the initial steps towards the goal of personalized medicine. We further recognize that the practical process of mechanism-based dynamic modeling requires the adoption of a standard of sufficiency (as opposed to being comprehensive). Specifically, the level of resolution of the models need only be detailed enough to sufficiently describe and predict the behavior of the clinical scenario being studied and the frequency of data being gathered. The examples described earlier all manifest characteristics of this approach: the PCA models help identify candidate markers, the personalized trauma and TBI models are based on relatively sparse time courses of inflammatory mediators, and the vocal fold ABMs focus on the primary cellular and molecular processes involved in mechanical trauma. In fact, the emphasis on characterizing inflammation at multiple levels of resolution underscores the translational systems biology approach. Therefore, we posit that the clinical implementation of personalized medicine will require the development of multiple frames of representation of a particular disease, creating a multidimensional disease space into which individual patients can be placed [91]. These levels of model representation will span the range from correlative genetic profiling, to identification of epigenetic mechanisms of phenogenesis [89,90], to multiscale representation of hierarchical control systems [73], to complex pattern analysis of physiological output [92]. The ubiquity of inflammation in both health and disease makes inflammation especially appropriate as a target for the translational systems biology approach. We believe that the ongoing work, focused on the characterization of inflammation as it manifests in various clinical scenarios, will have significant impact on the evolution of personalized medicine.

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▪ of interest

▪▪ of considerable interest

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Figure 1. Approaches to data-driven modeling

(A) Traditional statistical analysis requires defining patient subgroups *a priori* (e.g., based on clinical outcomes), and then determining the features (e.g., cytokine levels) associated with these patient subgroups. **(B)** An alternative approach involves using PCA or similar data reduction technique to define principal drivers of a given response (e.g., cytokines that drive inflammation) in a patient-specific manner. Subsequently, hierarchical clustering is performed to identify subgroups of patients with similar patterns of principal cytokines. In turn, these biologically-defined subgroups are queried as to the clinical outcomes that distinguish them.

PCA: Principal component analysis.

Figure 2. Data-driven patient segregation in the setting of trauma/hemorrhage

(A) Serial blood samples were obtained from 25 trauma patients (all survivors) in the first 24 h following admission. A total of 25 inflammatory cytokines in plasma were quantified using Luminex™. NO₂⁻/NO₃⁻ was measured using the nitrate reductase/Griess assay. Unsupervised hierarchical clustering based on correlation distance was carried out, showing little if any similarity among patients. (B) In the same samples described in (A), individualized principal component analysis was carried out in order to discern individual features of inflammation. Again, unsupervised hierarchical clustering based on correlation distances among biomarkers was carried out, and this analysis delineated groups that differed significantly in their Marshall score (a composite of organ damage/dysfunction in multiple organ systems) within the first 24 h postinjury. Thus, principal component analysis followed by hierarchical clustering appears to better delineate patient subgroups as compared with the raw values of inflammation biomarkers.