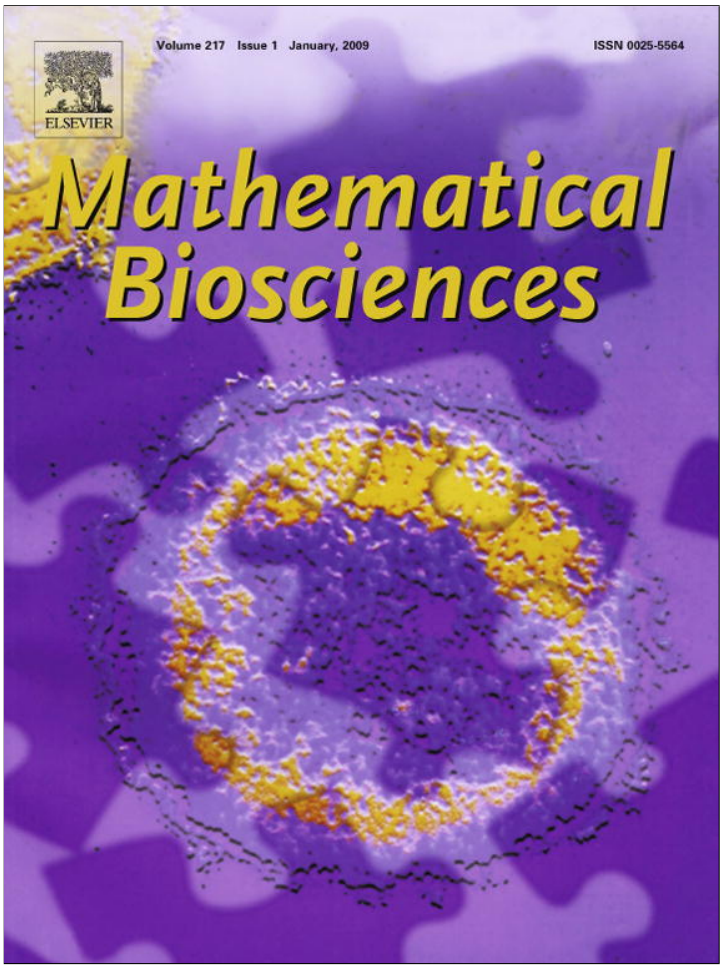

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Mechanistic simulations of inflammation: Current state and future prospects

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ABSTRACT

Inflammation is a normal, robust physiological process. It can also be viewed as a complex system that senses and attempts to resolve homeostatic perturbations initiated from within the body (for example, in autoimmune disease) or from the outside (for example, in infections). Virtually all acute and chronic diseases are either driven or modulated by inflammation. The complex interplay between beneficial and harmful arms of the inflammatory response may underlie the lack of fully effective therapies for many diseases. Mathematical modeling is emerging as a frontline tool for understanding the complexity of the inflammatory response. A series of articles in this issue highlights various modeling approaches to inflammation in the larger context of health and disease, from intracellular signaling to whole-animal physiology. Here we discuss the state of this emerging field. We note several common features of inflammation models, as well as challenges and prospects for future studies.

1. Introduction

Inflammation is the body's response to injury and danger. It is the central communication network and regulatory process that senses and controls threat, damage, containment, and healing, which are all critical aspects in the maintenance of an organism's integrity. There is a growing recognition that the role of inflammation in homeostasis is an integral component of many processes previously thought to be 'inevitable' during the course of life, such as aging [1–3], obesity [4,5], diabetes [6], and atherosclerosis [7]. Inflammation is constitutive and ubiquitous, and its role in a wide spectrum of diseases and responses to diseases is increasingly recognized [8].

Components of the inflammatory process are constantly involved in cycles of repair and remodeling after normal and pathologic challenges, and the high fidelity and robustness of these processes are clear characteristics of highly evolved complex biological systems. In addition to the complex course and regulation of normal inflammation, components of the inflammatory system also interact with non-inflammatory physiologic systems, making it difficult to reverse-engineer the architecture and control elements of the system. Though considerable progress has been made in elucidating many of the components of inflammation and their regulation, the inability to develop a coherent model of the dynamics of the entire complex system leaves physicians with inadequate treatment options for diseases in which inflammation is out of control, including cancer, AIDS, autoimmunity, sepsis, transplant rejection, obesity, diabetes, atherosclerosis, Alzheimer's disease

Abbreviations: ABM, agent-based model; LPS, Gram-negative bacterial lipopolysaccharide; ODE, ordinary differential equation; PDE, partial differential equation.

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and even aging. Progress in treating these processes requires a greater understanding of inflammation in its homeostatic context, and it is to be expected that a systems-level view will lead to improved predictability of the potential unintended consequences of therapeutic interventions.

It is becoming increasingly clear that achieving greater control over inflammation requires the application of formal analytical and synthetic methods drawn from other domains dealing with complex systems, and especially the adaptation of mathematical tools [9–11]. Mathematical and computational methods offer several significant benefits for the effective characterization of a complex system such as inflammation, for the following reasons:

(1) Mathematics allows a reduced but precise formal representation of hypotheses. Biological experimentation alone has not yielded such a formal representation, because of the difficulty of controlling all variables at multiple levels of resolution in experimentation. Specifically, biologists are limited to subsets of the larger problems because of constraints dictated by experimental work. Medicine is even more constrained, often relying on careful observation (retrospective and prospective) rather than experimentation, for practical, ethical, and medico-legal reasons. Despite a move toward evidence-based medicine, the nature of observation and decision-making in the clinical setting implies some degree of subjectivity. Using mathematical and computational tools, observations, hypotheses, and conceptual models are framed in a formal syntax, which can rigorously identify the role of assumptions and the implications of a hypothesis, and allow comparisons across experimental models, using a common language.

(2) Mathematical analysis can lead to a deeper understanding of the system. If a system can be adequately characterized mathematically, and equations describing the system can be reduced in dimension to a sufficient level, then formal mathematical analysis can be applied, leading to the development of 'axioms' concerning the dynamics of the biological system being studied. If the dynamics of biological systems such as inflammation is too complex to permit purely analytical solutions, mathematics can still come to the rescue by guiding the construction and analysis of approximate models that are evaluated numerically with computational methods.

(3) Mathematics allows hypotheses to be expressed in a form that can be analyzed with rigorous algebraic methods or simulated per computer. The mathematical formulation is a virtual analogue of the biological system offering the advantage that it can be queried and tested in uncounted variations to mimic real or hypothesized situations, even if this system is large and complex. Dimensionally-reduced models of whole-organism inflammation have yielded very useful insights into inflammatory diseases [12–15], but there is clearly an enormous number of components and their interactions involved in inflammation that are not captured by such models. Thus, the use of computational solutions and simulations has become the tool of choice for the study of inflammation [10,11,16,17]. With the desire to model inflammation in ever greater detail, the need for increased computational power in inflammation models is growing, and the modeling efforts have been benefiting from state-of-the-art simulation methods such as ensemble modeling [18,19], agent-based modeling [20–24] and different stochastic approaches (see below and [25]).

2. Modeling inflammation: diverse perspectives and current accomplishments

2.1. Current approaches to simulating inflammation

Properly built mathematical models can provide insights into observed behaviors in the system of inflammation that cannot be gathered from study of its component parts in the lab. Because of the complexity of inflammation, these models are predominantly assessed computationally, through dynamic simulations [26] or 'executable biology' [27]. Consequently, the models and methods described below should be seen as means of generating dynamic simulations of inflammation that are subsequently compared to experimental reference systems for calibration and validation. For the most part, simulations are intended to provide a more fine-grained dynamic map of the hypothesized mechanisms of inflammation than would otherwise be available using traditional wet lab experimental measurements.

The first and most traditional method used to characterize a dynamical system are equation-based models (EBM). This approach begins with assigning variables to various quantities that evolve over time (such as populations of cells or levels of measured mediators) and writing functions or differential equations that describe how those variables change over time. In the prominent case of ordinary differential equations (ODEs), the equations are linked to capture the dynamics of the system. ODEs use time as the sole independent variable (assuming that spatial effects can be ignored), while partial differential equations (PDEs) incorporate spatial dependence of the variables as well. Thus far, the primary EBMs used in the inflammation-modeling community are ODE based [13–15,18,26,28–30]. More complicated than the definition of variables are the choice of functional forms within the equations and the proper assignment of parameter values, which are often the most difficult parts of model design and are addressed in more detail in a later section.

ODE models have proven extremely successful in a variety of fields, but they also have their shortcomings. First, they are completely deterministic with respect to their behavior, given a certain set of initial conditions. Increasing evidence of stochastic behavior in critical biological processes, such as gene regulation and cellular behavior, points to the possible need to account for stochasticity in mathematical models [31,32]. Second, ODEs require the assumption that spatial aspects can validly be ignored, which allows for mean field approximations and mass-action kinetics, rather than partial differential equations. There is growing concern that the crowded environment and spatial architecture within cells, and, at a higher level in tissues and organs, might violate these assumptions to a point where ODEs are no longer valid [33].

The limitation of ODEs of not being able to account for stochasticity has been addressed by a series of methods developed by Gillespie [25]. Targeting reaction kinetics in systems with relatively few molecules, the Gillespie algorithms use probabilities to model discrete reaction events that form stochastic trajectories that propagate through a biochemical pathway. Simulations using the Gillespie algorithm rely heavily on the specification of all reactions in a system and their associated propensities. Reactions involved in inflammation are not always kinetic but also include interactions among evolving quantities, such as levels of activated macrophages and endotoxin in the system, which are difficult to quantify experimentally. Based on the collection of all rates in a system, each simulation step proceeds by first determining the time when the next reaction will occur. Next, the simulation determines which reaction will take place at this identified time. Levels of all evolving quantities are held fixed until the reaction occurs, at which time the levels of the quantities involved in the reaction are updated. This reaction-event based approach achieves major gains in computational speed relative to the more naïve approach of taking regular time steps and checking whether or not each reaction occurs systematically on each step. However, as it has been applied to increasingly complex biochemical pathways, the requirement to specify all reactions in a system has led to fast increases in the computational and data resources needed. These increases have placed constraints on the application of the traditional Gillespie method to systems such as inflammation. As a result, other means of signal network characterization have been proposed that focus

on the rules underlying molecular interactions [34]. These rule-based approaches render it possible to reduce the computational overhead required from explicitly representing every possible compound to representing only those generated through the specified molecular interactions. Furthermore, there has been considerable interest in developing hybrid approaches combining deterministic and stochastic methods [35–37].

The limitation of ODEs of neglecting spatial structure can be addressed in three possible ways. The first is the use of PDEs, a method that allows quantities to vary over both space and time. These models have been suggested as a means to characterize molecular signaling events [38,39], but have not been widely used in characterizing inflammation.

A second alternative is the subdivision of the spatial domain into compartments. In this approach, each compartment is assumed to be well-mixed, and the quantities of elements present in each compartment are tracked using ODEs. The ODEs incorporate coupling between compartments representing, for example, the movement of substances between compartments. This formulation retains the analytical tractability and relative ease of simulation of ODEs at the level of individual compartments; however, the need for a system of ODEs for each compartment leads to a proliferation of equations and variables that can slow down simulations and render analysis difficult.

A third option for incorporating spatial structure is agent-based modeling (ABM). As opposed to differential equation methods, ABM is more grounded as a computational simulation technique that was proposed by the computer science community and can be traced back to von Neumann's pioneering work on cellular automata [40,41]. Agent-based modeling is an object-oriented, discrete-event, rule-based, stochastic modeling method. The ABM framework consists of viewing a system as an aggregation of components (agents), which can be classified into populations or agent classes based on similar intrinsic rules of behavior (agent-rules). While each particular population of agents is assigned the same rules for behavior, the behavior of the individual agents is heterogeneous since all agents implement their rules based on local conditions that may differ considerably. The behavior of the entire system results from the aggregate interactions within and among its populations [21,40,42]. The advantages of AB models are several. They map well onto biological phenomena (such as cells interacting within tissues and organs) and are therefore fairly intuitive. Furthermore, agent-rule systems are typically expressed as conditional statements ('if-then'), thereby greatly facilitating the translation from the results of basic science experiments into agent-rules. Additionally, ABM intrinsically accounts for spatial components, because it permits rules governing local interactions and environmental heterogeneity. The main limitation of AB models is that they are computationally intensive. Moreover, due to the fact that relationships between the agent-rules and the system behavior are not always easy to infer, AB models can be difficult to calibrate in a quantitative way [26]. Nonetheless, several inflammation modeling studies have successfully made use of ABM [20–24].

2.2. Models of preconditioning in inflammation

An example of the application of mathematics to understand an important feature of the inflammatory process, namely behavior after preconditioning, highlights the usefulness of this kind of modeling. The concept of preconditioning, or the effect of past history on a system's behavior, is a critical factor in understanding and characterizing the inflammatory response as a dynamical system. Inflammatory preconditioning highlights the non-linear nature of the inflammatory response: stimulation with two or more pro-inflammatory stimuli in succession can lead to responses that are equal to, greater than, or lesser than each stimulus in isolation. Preconditioning is also critical in practical terms: if timed correctly, a therapeutic preconditioning stimulus might be used to augment natural inflammatory protective responses to a subsequent severe insult, or to blunt an overly exuberant (and hence detrimental) inflammatory response. To analyze inflammatory preconditioning mathematically, ODEs are particularly useful, since it is not possible to obtain explicit formulations for the time evolution of all variables associated with the entire inflammatory response. ODE models of the inflammatory response often permit several relevant asymptotically stable (AS) steady states, such as health, sustained inflammation with ongoing presence of an inflammatory instigator (e.g., a pathogen), and sustained inflammation without a sustained trigger. An important mathematical consequence of these multiple AS equilibrium points is that they can only coexist if the system also contains unstable states. The most fundamental configuration is the coexistence of two AS equilibria, which in the case of inflammation can be equated with 'health' and 'death', and an unstable intermediate state in the form of a saddle point. Such a saddle point is associated with a set of initial conditions, forming a separatrix, such that solutions that start within this set will approach the saddle. This separatrix is infinitesimally small, just as a two-dimensional sheet has no volume in three-dimensional space. Yet, the separatrix is essential, because it separates all other initial conditions into two sets, such that solutions starting in each set all approach the same AS equilibrium point.

To illustrate the concept of inflammatory preconditioning, one might consider the canonical response to pathogen-derived immunostimulants. Gram-negative bacterial lipopolysaccharide (LPS) is a central and canonical acute inflammatory stimulus in sepsis and related diseases [43], and LPS is used experimentally to mimic septic inflammatory responses in cellular and animal experiments. Inflammatory cells exposed to LPS generate both pro- and anti-inflammatory cytokines (protein hormones that are the principal mediators of inflammation) [8]. Repeated treatment with LPS can lead to desensitization or enhancement of subsequent pro-inflammatory cytokine responses (i.e., preconditioning) [44,45]. Since administered LPS decays relatively fast in a biological host, all equilibrium points in a model relevant to preconditioning should feature zero endotoxin levels. Thus, the relevant separatrix structure in the model is anchored in the absence of endotoxin. The side of this separatrix on which a solution curve is located determines the outcome of a preconditioning experiment. The position of a solution curve relative to the separatrix after LPS has decayed is like a memory of the LPS doses given, in that the side on which the separatrix is attained represents the outcome of the inflammatory response evoked by these doses. Interestingly, the separatrix has its own shape with respect to all system variables, just as a two-dimensional sheet hanging from a laundry line can take on different shapes under various conditions, despite being pinned in place at one end. Thus, the outcome of a preconditioning experiment cannot be determined by examining only one component of the response it evokes, such as the level of a particular pro-inflammatory cytokine, but requires knowing the full vector of system variable values. There are advantages of thinking in terms of dynamic structures, such as separatrices, compared to direct simulations. The main advantage is that knowledge about dynamic structures theoretically allows the outcome of a preconditioning experiment to be predicted by measuring the immediate response to an LPS challenge. This can be done without tracking the subsequent evolution quantitatively, although there are some technical details to address (see [15]). Importantly, we see that the relative timing of preconditioning and challenge doses is crucial to outcome, as the distance of a solution from the separatrix, measured in the direction of whatever system variables are directly changed by LPS administration, will vary over time [15].

Since the structure of the solution space that determines the outcome of preconditioning experiments is so general, it makes sense to assume that preconditioning phenomena should be ubiquitous in biological systems. The mathematical tools for predicting with certainty whether tolerance (the system responds to the insult in a benign way because of the preconditioning history) or potentiation (inflammatory over-reaction) will occur in a given setting, directly from dosing information without subsequent simulation, are still somewhat limited. Beyond the idea of a separatrix discussed above, additional techniques using isoclines, or collections of variable values along with the rate of change of a particular variable is constant, have recently been introduced (J. Day, J. Rubin, C.C. Chow, unpublished). One basic observation is that a key feature for allowing tolerance is the presence of an inhibitory interaction in a system, such that an increased level of one variable can suppress the growth of another system element, just as anti-inflammatory agents suppress pro-inflammation processes.

The importance of preconditioning as a central phenomenon in inflammatory dynamics is evident in a series of papers presented in this issue of *Mathematical Bioscience*. These articles describe computational simulations of inflammation using ODE, ABM, and rule-based modeling, and include, as one feature or even as the main focus, the concept of preconditioning in response to LPS. The central signaling receptor for LPS is Toll-like receptor 4 (TLR4) [46,47], and all five articles in this issue address this signaling pathway in some form.

The first modeling approach to preconditioning was carried out by Rivière et al. [48]. The authors created a four-variable ODE model to address the role of TLR4 in both normal and desensitized responses to LPS. They found that various, often subtle features of LPS-driven preconditioning could occur at the cellular level, without invoking the specific actions of endogenous anti-inflammatory cytokines [15]. Importantly, this study was calibrated using recent data regarding LPS/TLR4 binding *in vitro* [49], with other parameter values obtained in relation to the binding studies. Thus, many features of this model, including the preconditioning behavior, depend on the choice of parameter values (as explored explicitly in below). With appropriate parameter choices, this model shows that preconditioning effects of LPS depend on the amount used in preconditioning and on the time interval between the successive LPS injections. The way it is structured, this model suggests that preconditioning is controlled by the degeneration rate of TLR4, the time interval between repeated LPS stimuli, and the magnitude of each LPS stimulus.

Foteinou et al. [50] propose a larger, eight-variable ODE model of LPS-induced human inflammation subsequent to bacterial infection, that integrates transcriptional profiling and indirect responses. This study is a significant milestone in the attempt to integrate 'omics' datasets and computational modeling, carried out initially by Lagoa et al. in the setting of trauma/hemorrhage-induced inflammation [30]. Foteinou et al. sought to couple extracellular signals to essential transcriptional responses, and found that their model was capable of simulating a healthy (resolving) response to infection, a persistent infectious response, and persistent aseptic inflammation. As such, this model is similar conceptually to that of Kumar et al. [13], An [22], and Clermont et al. [51], though it incorporates aspects of transcriptional responses not presented in those earlier inflammation modeling studies. The model introduced by Foteinou et al. reproduces both priming and desensitization, similar to that of Day et al. [15] and the article by Rivière et al. [48]. Like Rivière et al., the model of Foteinou et al. demonstrates preconditioning in large part through the values of parameters representing ligand-receptor binding, internalization, and signaling.

An [52] has taken a different approach toward a simplified representation of the intracellular modulation of TLR4 signaling. He developed a computational agent-based model that combines a highly abstracted 'particle-event' view of molecular interactions, with a model morphology that reproduces spatial and structural relationships between the steps in the TLR4 signaling cascade. This method is termed Spatially Configured Stochastic Reaction Chambers (SCSRC). It is able to simulate the essential behavioral characteristics of TLR4 signal transduction, including stochasticity, negative feedback, dose-dependent responses, and preconditioning/tolerance to repeat doses of LPS. In this article, preconditioning also arises as a function of parameter values, though the stochasticity of the ABM framework results in a range of effective parameter values.

An and Faeder [53] introduce the concept of detailed, yet qualitative modeling utilizing a graphically based modeling toolkit, BioNetGen [34,54] to produce a model of TLR4 signaling. This approach attacks the issue of biocomplexity of inflammation from a very different perspective. As opposed to striving for quantitative parameter estimation, the structural complexity of the signaling cascade, in terms of its components, is modeled with a relatively high degree of detail. The model includes 31 molecule types and 41 reaction rules, which generates a network of 76 possible molecular species and 202 reactions. A total of 97 parameters are used for the initial concentrations of species and the reaction rate constants. Given the challenge of high-dimensional parameter estimation (see below), it is easy to see why a qualitative scaling of parameters would be desirable in a model of this complexity. Fortuitously, there is a strong suggestion that this type of qualitative approximation is useful in capturing the essential dynamics of signal transduction cascades [55–57]. The utility of expressing this degree of mechanistic detail is immediately evident when the 'inner' dynamics of the signaling cascade is examined. The gross output of the model, represented in terms of pro-inflammatory cytokine production both to initial LPS stimulus and reproducing preconditioning behavior, matches the behaviors of the models noted above. However, the detail of the model allows finer grained examination of the mechanisms of signal attenuation and tolerance behavior, and suggests the necessity for multiple nested feedback loops both in terms of explaining the overall dynamics, and to account for the degree of control and modulation seen in real biological systems. In practical terms, if the long-term goal is the design of molecular-level interventions on inflammation, then representation and identification of the mechanisms to be targeted is essential. The study shows that this type of detailed yet qualitative model can serve as a useful means of dynamic knowledge representation [58,59] to facilitate the discovery, design, and translation of molecular-level interventions.

Finally, Voit [60] proposes a conceptual framework that replaces the notion of health and disease *states* with health and disease *simplexes*, which are manifestations of physiological constraints that determine well-being. This formulation directly accounts for interpersonal variability and suggests that individuals migrate throughout their lives along their personal health and disease trajectories, traversing different parts of the health simplex, and temporarily or permanently crossing a boundary of disease. This disease model lends itself to rigorous mathematical implementation and demonstrates how it is possible that two individuals may show the same symptoms or drivers of inflammation but nevertheless have different risk profiles and health outcomes, because of their personal predisposition and features of their past trajectories, which among many other influences are affected by differences in preconditioning.

These five manuscripts conceptualize the acute inflammatory response in highly diverse ways. Regardless of the scale used, central features of inflammation display a preconditioning behavior. As a group, these studies suggest experiments that should be carried out in order to validate specific biologic predictions emerging from the mathematical representations. On a translational level,

the identified parameters may some day be utilized for the design of anti-inflammatory therapeutic strategies. On a foundational level, discussed below, these computational simulations suggest that certain mathematical structures, applicable to complex biological systems, display intrinsic preconditioning behavior. These mathematical structures may then be the core for the application of more rigorous mathematical methods of analysis, with potentially real applications in the clinical arena. One such process, that of parameter estimation and fine-tuning, is discussed in a later section.

2.3. Acute inflammation: the body's response to danger and damage as a unifying principle

Preconditioning can occur not only in response to microbial products such as LPS, but also in response to endogenous 'danger signals' released during states of cellular stress or tissue trauma [61] (Fig. 1). As mentioned above, the original impetus to develop translational computational modeling of inflammation was driven by the clinical challenge of sepsis and critical illness. However, one hypothesis that stemmed from the initial models was that the underlying acute inflammatory processes involved in the pathogenesis of sepsis were ubiquitous, and represented a basic, highly evolutionarily conserved homeostatic control mechanism. Evidence for this hypothesis arose from the fact that during the process of translating basic mechanistic knowledge into computational models, certain stimuli and pathways were recurrently invoked, and the dynamic characteristics of the initial inflammatory response seemed to require an autocatalytic, feed-forward process in order to generate the appropriate dynamics. Furthermore, the heterogeneous nature of 'entry points' into the acute inflammatory response suggested the need for the integration of an intrinsic 'damage' feedback component, and this feed-forward control logic was present at the earliest stages of development of the systemic inflammation models [13,20,21] (Fig. 1). It should be noted that at the time of the development of these models, there was no explicitly defined mechanism or mediator for this self-propagating dynamic; rather, the proposed interactions 'emerged' from an interpretation of disparate mechanistic information viewed from a modeling standpoint.

Concurrent developments in the traditional research community provided clues as to the nature of the molecular actor(s) inferred by the models. The re-discovery of High Mobility Group Box 1 (HMGB1) as a late pro-inflammatory mediator of sepsis related to cellular stress and/or death provided an example of a nearly ubiquitous biomolecule that, in situations of cellular and nuclear disruption, stimulates pro-inflammatory pathways [62,63]. The recognition that common signaling pathways via Toll-like receptors were induced not only by bacterial products but also were modulated by endogenous compounds (such as HMGB1) further linked the 'protective' inflammatory pathways identified for fighting infection to 'survey and maintenance' mechanisms [64–67]. These ideas coalesced into the concept of 'danger signals' and a new recognition of the role of acute inflammation in a whole host of disease processes [68]. Importantly, it is now appreciated that prior exposure to HMGB1 can lead to preconditioning [66], as would be predicted from the computational simulations that invoke the positive feedback loop of inflammation → damage → inflammation [13,20,21].

Fortuitously, similar recognition from a computational modeling perspective allowed for these concepts to be integrated into the design of the inflammation models, highlighting the evolvability of the models and ability to incorporate new knowledge not captured in the original concepts of acute inflammation. For example, one prediction of the schema presented in Fig. 1 (and borne out experimentally) is that alarm/danger signals should play a relatively late role in sepsis-induced inflammation [62] but an earlier role in trauma, hemorrhage, and ischemic injury [64,69]. The linkage between inflammation and healing described above represents one area where this linkage has already occurred, and other areas of potential application include atherosclerosis, Alzheimer's disease, cancer biology, fibrotic diseases, chronic transplant rejection, asthma, obesity, and aging.

In this regard, data collected from these clinical scenarios that play out over long time horizons could also inform the sepsis model. Sepsis is an acute disease and leads to a characteristic hemodynamic picture of low peripheral resistance, with a compensatory hyperdynamic cardiac profile. Patients with acute liver failure develop a remarkably similar picture, and patients with end-stage liver disease develop the same hemodynamic picture albeit over a longer time, and many of the mediators of the septic hemodynamic state are likely causes of the hemodynamic state characteristic of liver disease. Thus, insights obtained from modeling the interrelated actions of inflammation and cell/tissue/organ damage in sepsis and trauma may lead to novel insights into other pathological states such as end-stage liver disease.

2.4. Parameter variation in mathematical models of inflammation: the road to personalized medicine?

Independent of the modeling framework chosen for assessing inflammation with mathematical means, the analytical and simulation results obtained a given model are highly dependent on its parameter values. Unfortunately, there are no generally efficacious and easy methods for the difficult task for the estimation of these parameter values. We contrast in this section two complementary approaches and indicate how the fine-tuned specification of

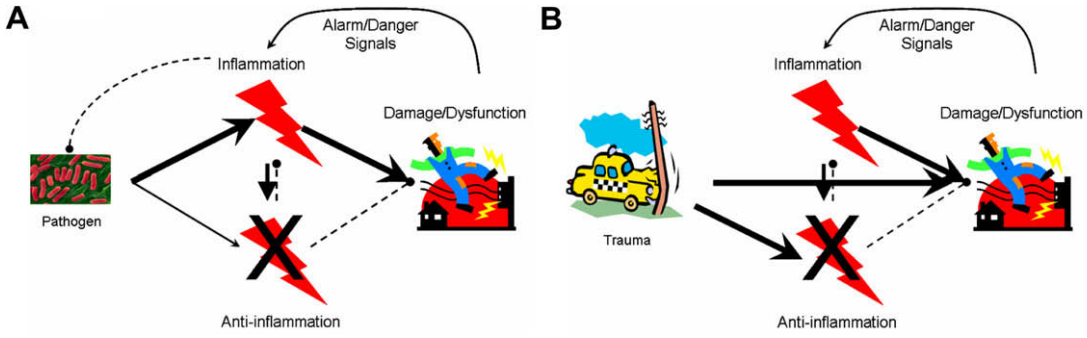


Fig. 1. The role of alarm/danger signals in inflammation, distilled for mathematical modeling purposes. Solid arrow: induction; dashed line: suppression. An initiating stimulus (e.g., pathogen (A) or trauma (B)) stimulates both pro- and anti-inflammatory pathways. In the setting of infection, pro-inflammatory agents (e.g., TNF) cause tissue damage/dysfunction, which in turn stimulates further inflammation (e.g., through the release of 'danger signals'). In the case of trauma, tissue damage occurs immediately and further simulates inflammation. Anti-inflammatory agents (e.g., TGF-β1) both suppress inflammation and stimulate healing.

parameter values can become a key ingredient in the personalization of biomedical models.

Regardless of the specific application area, the variables in the system interact with each other, and these interactions are numerically governed in AB or ODE models by functions containing parameters. Generically, such a function $v_{ij} = v_{ij}(X_i, X_j, \ldots, \mathbf{p}_{ij})$ relates two or more variables X_i and X_j and involves some or many of the components within and outside the system. The parameter vector $\mathbf{p}_{ij}$ contains elements that are to be estimated from biomedical information about the involved processes. Once all v_{ij} are mathematically defined, they are merged into an AB model or into an ODE systems model of the general form

$$\dot{X}_i = \sum_k \gamma_{ki} v_{ki} - \sum_j v_{ij}, \tag{1}$$

where γ_{ki} are stoichiometric coefficients that allow for the fact that the loss of one unit of a given source variable is needed to generate a certain number of units of a product variable.

Methods of parameter estimation depend on the types of available data, which fall into two distinct categories: 'Local' data, which are the basis for bottom-up model construction; and 'global' data, suggesting top-down modeling. In the former case, all processes in the system are modeled individually and subsequently merged into a comprehensive system model. The latter case relies on the availability of time series measurements that characterize the drivers of the system, and the task consists of the inference of parameter values from these data.

A typical example for bottom-up estimation is the construction of a traditional metabolic pathway model that contains of a number of biochemically related metabolites and is driven by the action of enzymes. If the pathway is involved in a chronic disease or associated with an acute imbalance like inflammation, the ultimate modeling goal could be a deeper understanding of the system or the development of counteracting measures. In order to represent the pathway dynamics mathematically, one consults with expert experimentalists and clinicians, and also queries the literature for the properties of all involved enzymes. The goal is to quantify properties of the enzyme and to obtain numerical values for their characteristics, such as kinetic constants, measures of affinity or the turn-over capacity with respect to specific substrates. The numerical characteristics, together with the listing of necessary cofactors and conditions required for enzyme activity, are then used to parameterize the mathematical representation of the functioning of each enzyme. Typical results of this procedure are Michaelis–Menten rate functions, more complex saturable functions accounting for activators and inhibitors [70], or power-law functions that derive generically from strategies established in the modeling framework of Biochemical Systems Theory [71–74].

In the case of global, top-down modeling, the equations may ultimately be exactly the same, but the parameter values in the vectors $\mathbf{p}_{ij}$ are estimated in a distinctly different fashion. Specifically, the data available for estimation consist of time series measurements $M_i(t_n)$ of the variables at N time points $t_1, \ldots, t_N$, which in the case of metabolites or proteins may for instance be obtained with methods of *in vivo* nuclear magnetic resonance or mass spectrometry. Instead of working from the bottom up, as before, the parameters are estimated with a non-linear regression method, a genetic algorithm, or some other computational technique that simultaneously attempts to determine all parameter values such that the solution of the system of differential equations in Eq. (1), which consists of all $X_i(t)$ over a desired time interval, matches the observed time series measurements $M_i(t_n)$ as closely as possible at all studied time points. In the ideal case, the resulting parameter values in $\mathbf{p}_{ij}$ should be the same as those found in the bottom-up strategy, but this does not always happen in reality, because of noise in the data, faulty assumptions and simplifications, and weaknesses in the estimation methods. Of course, if both types of information (local properties of processes and global time series data) are available, the two approaches can be employed concurrently.

When the numbers of equations and parameters are small (<10), it is often possible to estimate parameters from data as indicated above or by the use of Bayesian methods. The reliability of these estimations is subject to the relative magnitude of noise with respect to the signal. If the ratio is low, estimation can be quite good. Even in such favorable circumstances of successful parameter estimation, the subsequent prediction may not be satisfactory. Many causes are possible, but it is likely that failure is due to structural or regulatory flaws in the model equations (omission of certain key interactions, for instance) or to invalid assumptions and simplifications in the conceptualization of the system.

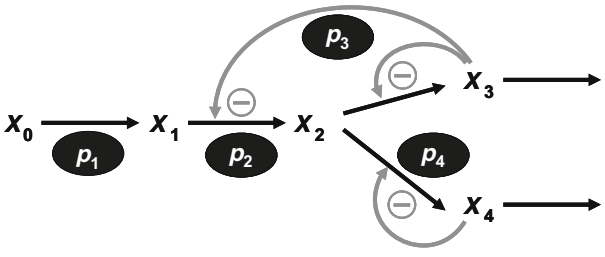
Issues of parameter estimation become tremendously more difficult for large systems with hundreds of interdependent parameters subject to estimation. As mentioned in Constantine et al. [75], current techniques are likely to fail and new methodologies of optimally surveying a high dimensional parameter space must be developed. However, before any such high-dimensional attack is undertaken, it helps to reduce the number of parameters at the modeling level. In addition to certain known rescaling methods and keeping parsimony in mind at all times, one may rewrite the non-linear model in piecewise linear form, which tends to reduce the dimension of the ensuing parameter space and permits methods of linear regression. If at all possible it also helps if issues of insufficient data and large noise-to-signal ratios can be overcome. Even if all is done to simplify the problem, parameter estimation remains to be very difficult for large systems, since experimental animals are limited as sources of biological data, especially if these data can only be acquired after death of the animal. The best that one can do with available data in such circumstances is to examine how the error in the data is related to the parameter sensitivity in the proposed model. Parameter sensitivity can be examined with analytical means or through extensive computer simulation, by mapping out basins of attraction for various parameter sets. Given the data, one may produce confidence regions around the observed data by using a data-based estimate of the error (we observed in Chow et al. [29] that the experimental error is often directly proportional to the signal). One then seeks parameter sets that yield model trajectories contained entirely within the confidence region. If the error is high, the difficulty is that essentially anywhere within the parameter space a region with some radius contains parameters that fit the data equally well, which complicated the isolation of meaningful parameter values. In such a case, additional data are needed to constrain the parameter estimation error sufficiently to guarantee meaningful modeling results. Our experience with large systems has been an observed erratic behavior, in the sense that in any ball of small radius we can find parameter values that yield qualitatively different model behavior. This phenomenon is independent of data or statistical technique of parameter estimation.

In almost all practical cases, the parameter values in the vectors $\mathbf{p}_{ij}$ are obtained from population averages. In the case of local estimation, information on the mechanisms of processes is typically obtained from biological experiments that are executed *in vitro* under standardized conditions and result in typical or average characteristics for a given organism or cell type. In the case of top-down estimation, the time series are mostly composites from many organisms, tissues, or cells. Thus, because the parameters are assigned average values within some population, the resulting model constitutes a representation of the biological system that is some sort of an average as well. This average may not necessarily be the arithmetic or geometric mean, but is somehow a reflection of what is considered 'normal' for this system.

Personalization of the model is based on the assumption that the 'normal' model is sufficiently robust. If so, the model accounts adequately for responses to moderate deviations in parameter values. These deviations can be interpreted as a reflection of the particular metabolic or physiological profile of an individual. Specifically, as far as measurements $\tilde{\mathbf{p}}_{ij}$ on an individual are known, they are substituted in the system model equation (1). If they have not been measured, it must be assumed by default that they are more or less equivalent to the 'normal' state $\mathbf{p}_{ij}$. The key point is that, by virtue of the substitution of $\tilde{\mathbf{p}}_{ij}$ for $\mathbf{p}_{ij}$, the average population model moves away from the normal state and assumes a personalized state that might be healthy or diseased.

It is difficult to predict how consequential the differences between $\tilde{\mathbf{p}}_{ij}$ and $\mathbf{p}_{ij}$ might be in a specific instance, but it is easy to compute them by integration of the ODE model, or, in the case of ABM, by executing the corresponding simulations. In particular, if it is known that certain profiles of metabolite concentrations, gene expression levels, or proteins are associated with disease, it is possible to define metrics that characterize how close an individual may be to a given disease state. In the same vein it is feasible to establish an individual risk profile of the following type: given the current state of $\tilde{\boldsymbol{p}}_{ij}$, the individual's physiological distance from a disease state of interest is $D(t)$, and this distance is a measure of the person's health risks. One may dissect this distance into dimensions for each metabolite X_i in the profile and assess which metabolite might exceed the disease threshold first. This analysis may be based on mathematical analysis of the effects of changes in $\tilde{\mathbf{p}}_{ij}$ on each X_i or on simulation studies that change components of $\tilde{\mathbf{p}}_{ij}$ individually or in combination and record the resulting metabolic profile associated with each change. A more detailed account of a theoretical formulation of health and disease is presented elsewhere in this volume [60].

As an illustration for assessing interpersonal variations, consider the branching process in Fig. 2, which could represent a metabolic pathway or signaling system or even a logical cause-and-effect diagram. For simplicity of discussion assume that the pathway is metabolic. It contains an initial substrate X_0, four intermediate metabolites $X_1,\ldots,X_4$, and three inhibitory signals. Corresponding equations are also shown in the figure, along with



$$\dot{X}_1 = p_1 X_0 - p_2 X_1^{0.5} X_3^{p_3}$$
$$\dot{X}_2 = p_2 X_1^{0.5} X_3^{p_3} - 1.5 X_2^{0.5} X_3^{-0.1} - p_4 X_2^{0.75} X_4^{-0.5}$$
$$\dot{X}_3 = 1.5 X_2^{0.5} X_3^{-0.1} - 0.5 X_3^{0.5}$$
$$\dot{X}_4 = p_4 X_2^{0.75} X_4^{-0.5} - 0.1 X_4^{0.8}$$

$$X_0 = 1,\ X_{10} = 0.829,\ X_{20} = 0.170,\ X_{30} = 1.43,\ X_{40} = 1.70$$

$$p_1 = 3,\ p_2 = 1,\ p_3 = -0.5,\ p_4 = 0.75$$

Fig. 2. Generic branched pathway used to illustrate the personalization of an average pathway model. Equations are formulated in the manner of Biochemical Systems Theory. The given initial values correspond to the stable steady state, which is obtained with parameter settings as indicated. To personalize the model, one or more of the parameter values are altered and the responses are checked.

initial values and parameters that are subject to numerical variation in this illustration. Suppose the main feature of significance in the system is the ratio X_3/X_4 which, when out of balance, is a sign of disease. With the settings given in Fig. 2, this ratio happens to be 0.84, which represents the 'normal,' healthy case. The question now becomes what happens if an individual exhibits variations in one or more of the parameters $p_1,\ldots,p_4$. The consequences of such variations are difficult to intuit, but easy to compute numerically. For instance, if p_1 is increased by 50%, the total amount of material in the system increases. X_1 changes the most; with a value that is increased approximately fivefold (Fig. 3). Nevertheless, the ratio X_3/X_4 is essentially unchanged. The change in input affects the metabolic profile quite considerably, but the resulting metabolic state would not be considered 'unhealthy,' even though variable X_1 is potentially 'outside the norm.' Expressed differently, neither p_1 nor X_1 is a good biomarker for diseases associated with an imbalance in X_3/X_4. Note that the transients are not of particular interest for this illustration, and are shown primarily to facilitate comparisons with the normal state at which each simulation starts. If p_2 is doubled, X_1 decreases considerably, but other variables, including the ratio X_3/X_4 are essentially unaffected. Parameter p_3 reflects the strength of inhibition of X_3 on the transformation of X_1 into X_2. If the inhibition is doubled in strength (from -0.5 to -1), X_1 increases, but the remaining system features are more or less unaffected. In contrast, if p_4, representing the turnover rate of the conversion of X_2 into X_4, is doubled, the ratio X_3/X_4 decreases from 0.84 to 0.46, which could be a characteristic of disease. In this case, p_4 could be considered a biomarker.

It is also easy to study combinations of deviations. For instance, if all perturbations are implemented simultaneously, all variables increase in value, but the ratio X_3/X_4 decreases from 0.84 to 0.59 (results not shown). Suppose this case occurred frequently, but that p_4 was never measured. The natural interpretation would be that $p_1,\ldots,p_3$ should be considered strong biomarkers of the disease, because their abnormal values would 'typically' be observed in subjects with a decreased X_3/X_4 ratio. Of course, we know from the above that this deduction would be wrong.

Monte Carlo simulations may also be executed, in which each parameter is drawn from a probability distribution that is to be defined over the range that has been observed within a population or is clinically reasonable. These simulations reveal the most likely as well as rare scenarios and discover all parameter combinations that lead to a profile of system variables that corresponds to a disease state.

Thus, the average model, which is established and parameterized entirely from population information, can be 'adjusted' to an individual patient and, given criteria of disease, it is feasible to study exhaustively all deviations in physiological parameters that are either harmless or lead to disease. Furthermore, if these parameters change over time, as it happens in chronic diseases like type-2 diabetes over a long time scale or in inflammatory processes over a much shorter time scale, the model not only classifies ultimate health and disease states but also characterizes trajectories toward a disease state. While the example above used metabolic pathways as a focus for illustration, the general concepts are independent of this particular aspect and any health and disease model can be personalized and analyzed in the same fashion.

3. Conclusions and future directions

The field of acute inflammation is inundated with literature that describes various aspects of the process but fails to link these important details into a comprehensive whole suitable to the goal of clinical translation. The recently presented concept of 'translational systems biology' aims to unify mechanisms described in

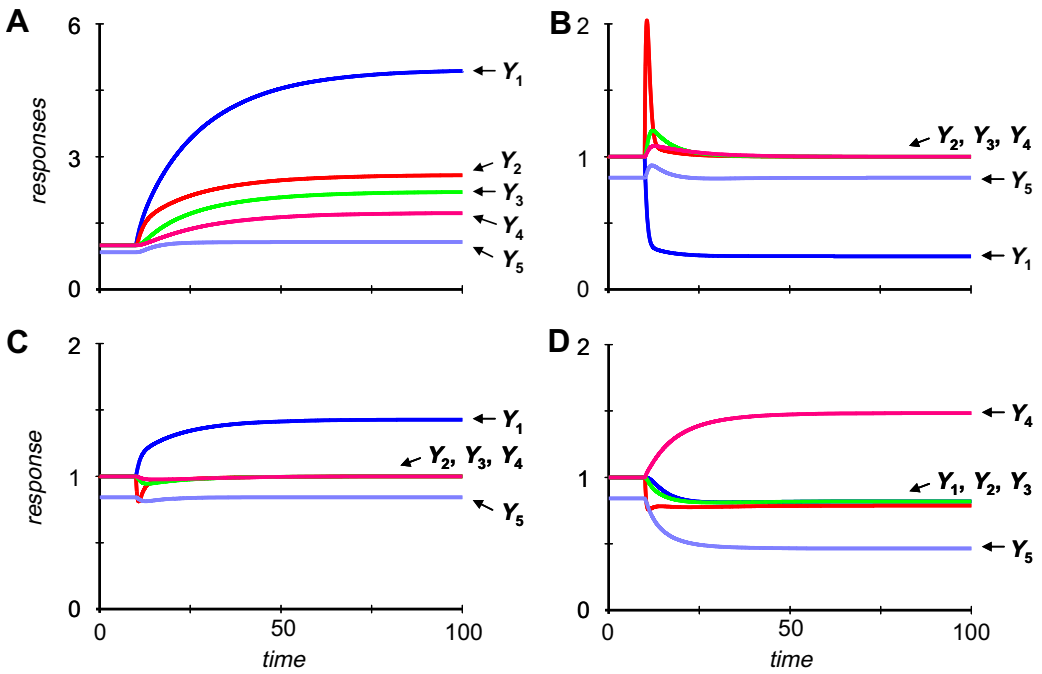


Fig. 3. Results of select simulations of the pathway model in Fig. 2. $Y_1,\ldots,Y_4$ denote $X_1,\ldots,X_4$ divided by their 'normal' steady-state initial values, respectively. Y_5 denotes the ratio X_3/X_4. (A) Increase of p_1 by 50%. (B) Doubling of p_2. (C) Increase in strength of inhibition (p_3) from −0.5 to −1. (D) Doubling of p_4.

the scientific literature using methods and tools developed by the computational and systems biology communities [10,11]. By doing so, we hope to uncover novel insights into the pathobiology of inflammation and the intertwined damage/healing response, and add a mechanistic, rational basis to the design and implementation of therapies [10,11]. Progress in this area is dependent not only on incorporating many mathematical tools, but examining data from many related clinical processes. The not-too-distant future includes rational, model-driven design and testing of novel therapies; clinical trials that are first run in computational simulations [22,51,76]; inpatient care in which diagnosis is aided by mathematical models [24]; and outpatient care plans prepared using model-driven decisions along the fragmented continuum of care which currently constrains modern medicine (Fig. 4).

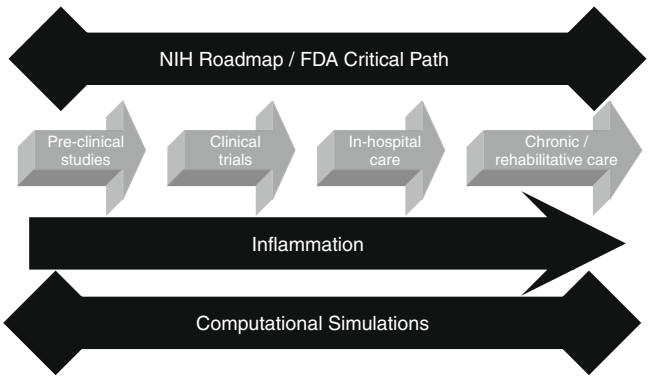


Fig. 4. The 'fragmented continuum' from pre-clinical studies to ultimate clinical utility. The current paradigm of healthcare delivery can be thought of as starting with *in vitro* and *in vivo* pre-clinical studies. Candidate therapies from these efforts are tested in clinical trials, yet the ultimate efficacy and safety of these therapies is revealed only via the process of in-hospital care over an often prolonged timeframe that may involve chronic and/or rehabilitative care. These domains are generally separate, and there is currently no framework under which these domains can be linked. Both the United States Food and Drug Administration, in its 'Critical Path' document [80] and the United States National Institutes of Health, in its 'Roadmap' statement [81] have called for the use of computational simulations to bridge this 'bench to bedside' gap. Translational systems biology focused on the inflammatory response may serve this role through the creation of computational simulations that span pre-clinical studies, *in silico* clinical trials, patient diagnostics and other aspects of in-hospital care, and ultimately long-term care and rehabilitation.

Successful achievement of these objectives will benefit from several advances. Models are currently initiated and modified through a painstaking and time-consuming process of manual extraction of relevant data from the scientific literature. Thus, translational systems biology will benefit from automated means of searching the literature and mining and extracting data in a form that will support continued updating of the core models [77,78]. Similarly, non-mathematically inclined clinician-investigators often struggle with converting even simple biological interactions into mathematical models using software optimized for mathematicians. Accordingly, translational systems biology would benefit from software designed to facilitate the translation of biological and clinical knowledge into mathematical models, especially software integrated with electronic medical records.

The ultimate therapeutic utility of these approaches is still in debate within the clinical community [79]. We in the translational systems biology community hope that the exciting developments outlined herein, and the many more on the way, will build bridges to the larger computational and systems biology communities to aid us in these translational efforts.

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