Mathematical models of the acute inflammatory response

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Purpose of review

Trauma and infection elicit an acute inflammatory response. In certain circumstances the degree of the acute inflammatory response may result in pathologic manifestations, namely, sepsis and multiple organ failure. Despite an extensive series of clinical trials designed to modulate inflammation in sepsis, only one compound, activated protein C, has emerged from more than 250 failed trials. There is a growing recognition that the complexity of the acute inflammatory response precludes the efficient development of therapies for sepsis and multiple organ failure until systems approaches are brought to bear on this problem.

Recent findings

Work carried out by the authors' groups suggests that mathematical modeling can provide a means by which *in vitro* and *in vivo* data can be synthesized into system-level analytic models of the acute inflammatory response. The authors have focused on agent-based modeling and modeling with ordinary differential equations. Some of the advantages and disadvantages of these modeling approaches are presented, and methods for calibration and validation of these models are discussed. Finally, the usefulness of mathematical models to evaluate the prospective therapeutic strategies in clinical trials of sepsis and trauma is examined.

Summary

Simulations using various methods can shed insight into the pathophysiology of the acute inflammatory response and may lead to better design of clinical trials in sepsis and trauma.

Keywords

sepsis, mathematical model, methodology, outcome prediction, randomized trials, intensive care

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Abbreviations

ABM	agent-based modeling
AIR	acute inflammatory response
IL	interleukin
MOF	multiple organ failure
ODE	ordinary differential equations
SIRS	systemic inflammatory response
TNT	tumor necrosis factor

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Introduction

The management of sepsis and its related conditions systemic inflammatory response (SIRS) and multiple organ failure (MOF) remains the greatest clinical challenge in critical care [1]. Similarly, the search for effective pharmacologic therapies for sepsis/SIRS/MOF remains the primary focus of the vast majority of basic science research in the critical care community. Much has been learned regarding the cellular and molecular mechanisms of the acute inflammatory response (AIR). However, except for recombinant human activated protein C (drotrecogin-α [activated]), this knowledge has not led to effective therapies for inflammation-induced shock [2–4]. Despite showing promise in animal and early-phase human studies, virtually all attempted anti-inflammatory strategies have failed to improve outcome in large, randomized clinical trials [5–8].

Clinical trials of mediator-directed therapies: what is missing?

We propose that one potential reason for this dearth of therapeutic options is the relatively recent recognition that the inflammatory system demonstrates complex, nonlinear behavior [9–13]. The complexity of the AIR calls into question the applicability of the traditional scientific paradigm of reductionism, which focuses on reducing a system into its constituent parts and assuming that the behavior of the full system can then be inferred from recombining these constituent parts. This approach is successful only for systems that behave linearly so that the results of various independent experiments can be linearly summed to obtain the behavior of the whole system.

Systems such as the AIR that have multiple feedback loops and saturating dose response kinetics are inherently nonlinear. Analyzing individual components in isolation may not illuminate how the entire system will

behave. These processes must be examined with techniques of nonlinear analysis. It is now recognized that such an approach is necessary to understand complex biologic processes [9–11,14,15].

The inherent nonlinearity and complexity of the AIR makes it difficult, if not impossible, to predict the effect of modulating a given pathway or mediator of inflammation given only the knowledge of that pathway or mediator in isolation. This fact is borne out by the general failure of therapies for SIRS/MOF that modulate inflammation [5–8]. Furthermore, redundancies in the immune system suggest the need for therapeutic strategies that target multiple pathways and mediators (*ie*, combination therapies) [16•]. However, such multimodal approaches require an understanding not only of how all the pieces fit together but also of how they behave together over time. This understanding is necessary to make informed decisions regarding the targets and timing of such prospective regimens [16•]. With these concerns in mind, we propose the use of mathematical modeling to improve the characterization of the AIR and its disease states of sepsis/SIRS/MOF.

Rationale for mathematical modeling of the acute inflammatory response

Scientists intuitively create mental models. In some disciplines, such as physics, these mental models can be formalized into mathematical equations. Chemistry, as well, can be characterized in terms of mathematical relations. These equations can then be solved analytically or numerically on computers. However, biology has for the most part resisted mathematical characterization. The aforementioned nonlinearities associated with biology in general and human physiology in particular have made that task daunting. Biologic systems display heterogeneity of behavior that resists reduction to simple quantifiable principles. Furthermore, the dense quantitative data necessary for mathematical modeling have been, until recently, mostly unavailable.

The advent of plentiful computing power and the availability of more quantitative data has made the prospect of mathematical modeling of biologic systems more feasible. Mathematical tools from the fields of nonlinear analysis, complexity theory, machine learning, Bayesian analysis, and fuzzy logic have recently been used in this endeavor [11,17]. Two standard approaches for understanding the dynamics of complex systems (not just biologic systems) are agent-based modeling (ABM) and modeling with ordinary differential equations (ODE). In recent years, these approaches have moved toward commercial applications as companies such as Entelos, Inc. (www.entelos.com), and Immunetrics, Inc. (Pittsburgh, PA, USA) (www.immunetrics.com) have begun to carry out simulated clinical trials such as those described below [18,19•]. Herein we describe some initial experience with using these methods to address the complexities of sepsis/SIRS/MOF.

Examples of mathematical modeling

Various approaches have been used to construct simulations of complex biologic processes. All these methods have distinct advantages and disadvantages. Below, we discuss two such approaches and present examples of models of the AIR.

Ordinary differential equations

The ODE type of modeling consists of establishing a series of differential equations that describe the sequential change in the states of the components of the system over time. The differential equations are derived from known and hypothesized kinetics of the components of the biologic system. This approach has been used for many years to describe chemical systems, for example Michaelis-Menton kinetics. The variables of the equations generally represent average concentrations of the various components. These equations rely on large numbers of individuals of these components. When the numbers become small, differential equation descriptions break down. If spatial dynamics can be ignored, the behavior of the system can be characterized with ODE. If simple enough, ODE can be solved analytically. If not, they can be easily solved computationally. Additionally, methods from nonlinear analysis can explore the properties of ODE without completely solving them. Because these equations are based on biologic interactions, these models can potentially predict outcomes beyond the range of available data. Furthermore, manipulation of a biologic mechanism can be entered into the model and an outcome derived.

Agent-based modeling

The ABM type of modeling focuses on the rules and mechanisms of behavior of the individual components of a system. The components of a system are classified into types of "agents" by virtue of shared mechanisms that have been identified experimentally. The mechanisms are expressed as a series of conditional ("if-then") statements, and computer programs are written to describe the rules of behavior. An example would be the sequence of receptor activation involved in neutrophil adhesion. The model defines a "virtual world" based on characteristics of the reference system and generates populations of the various types of agents. The agents interact based on responses (defined by their rule systems) to inputs and outputs from their environment. For example, simulated cells would respond to variables in their immediate neighborhood, representing the extent of a cell's interaction with its extracellular milieu. The agents run in a parallel fashion to simulate simultaneous behavior, and the dynamics of the system are allowed to emerge from the multiple interactions between the agents over time. This type of modeling is "bottom up,"

inasmuch as all measured parameters and outcomes from the model are generated by the actions of the agents. Agents should ideally have well-identified, confirmed, simple rules. Because ABMs are mechanistic models, any intervention that deals with a defined mechanism in the model can be simulated. Because they are based on rules, ABMs are often more intuitive to nonmathematicians.

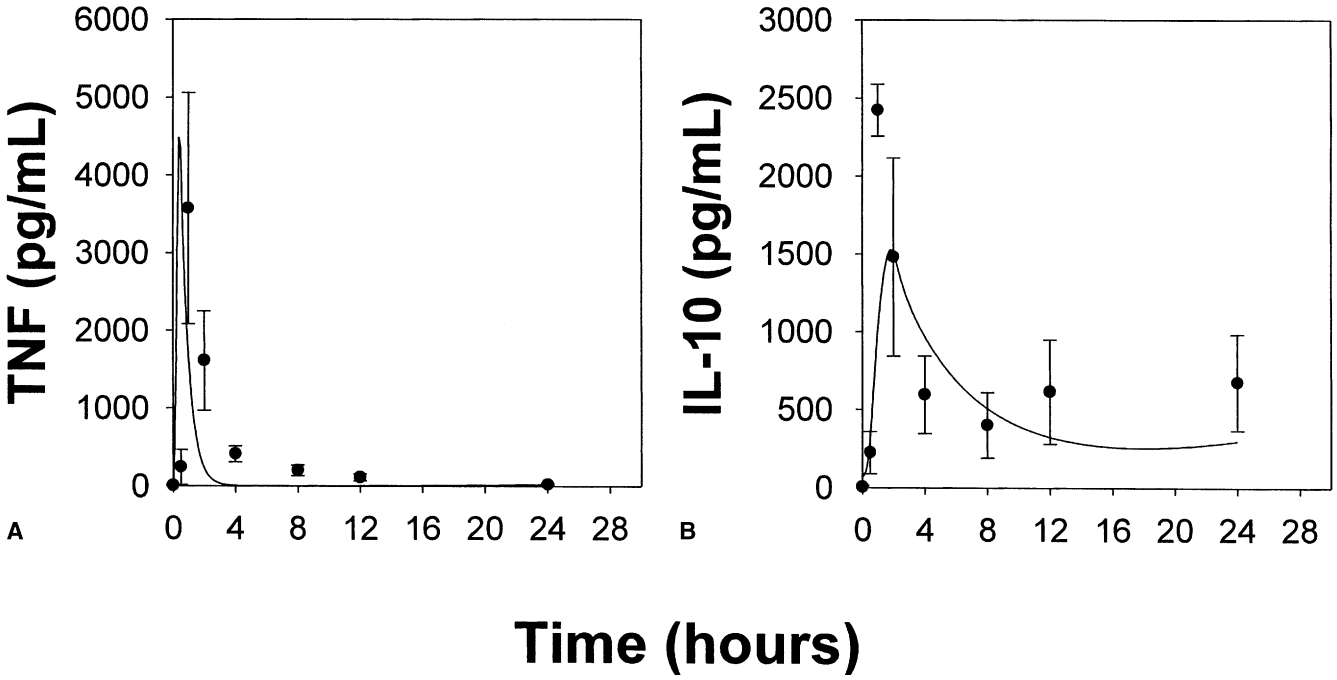
It needs to be emphasized that the methods of modeling described above are complementary, and ideally, both would be used to provide mathematical characterization of a complex dynamical system. Ordinary differential equation models focus on the collective behavior of a population of individual components (*eg*, concentrations). Agent-based models simulate the behavior of actual individuals and can easily encode complicated history-dependent internal states of cells that are not easily captured in ODE models. Additionally, the ABM approach provides a very intuitive means of translation of basic science data (for a nonmathematician) and allows flexibility in proposing interventions. The downside is that extensive computational power may be required to simulate large numbers representative of real systems, and thus ABM can be difficult to validate and calibrate with experimental data. The recognition that both approaches have their advantages and limitations has placed emphasis on cross-platform validation (see below). The following sections will describe specific models of the AIR using ODE and ABM, validation strategies for both, and a series of *in silico* ("in computer," *ie*, simulated) experiments and results that demonstrate the potential uses of these forms of analysis.

Ordinary differential equations model of acute inflammation

Clermont *et al.* [19•] and Vodovotz *et al.* [20•] have developed and calibrated an ODE model of acute inflammation based on the kinetics of well-accepted constituents of the AIR. The model was initially designed to simulate inflammation in the peritoneal cavity with spillover into the circulation; this mimics the situation both in many clinical scenarios and in well-accepted animal models. In this ODE model, neutrophils and macrophages are activated directly by bacterial endotoxin (lipopolysaccharide) or indirectly by various stimuli elicited systemically upon trauma and hemorrhage. These stimuli affect both the systemic circulation and injured or ischemic tissue [21,22].

Once activated, macrophages and neutrophils produce and secrete both proinflammatory and antiinflammatory cytokines that together serve to restore homeostasis after clearing the initial infection or withstanding the initial injury. This homeostasis leads either to a restoration of the conditions preceding the insult to the organism or; alternatively, to the establishment of a new, "healed" equilibrium. However, when overproduced, antiinflam-

Figure 1. Calibration of ordinary differential equations model of acute inflammation



Mice received 6 mg/kg lipopolysaccharide intraperitoneally. At various time points after this injection, the mice were killed and sera obtained. Serum tumor necrosis factor (**A**) and interleukin-10 (**B**) were measured by specific enzyme-linked immunoassay. Black symbols represent mean ± SD for 3 to 8 separate animals. Black line indicates prediction of mathematical model.

matory cytokines lead to detrimental immunosuppression [23–25]. Both proinflammatory and antiinflammatory cytokines in this model are elaborated with distinct temporal characteristics, with "waves" of cytokines occurring both early and later after the initial challenge.

Proinflammatory cytokines also induce macrophages and neutrophils to produce free radicals, such as superoxide NO, which are toxic to bacteria and indirectly to host tissue [26–28]. The induced damage can incite more inflammation by activating macrophages and neutrophils [29]. However, NO can also protect tissue from damage induced by shock, even though overproduction of this free radical causes hypotension [27,30–32]. Simultaneous numeric solution of the equations of this general model generates predictions of the time courses of these elements (Fig. 1).

Agent-based model of the acute inflammatory response

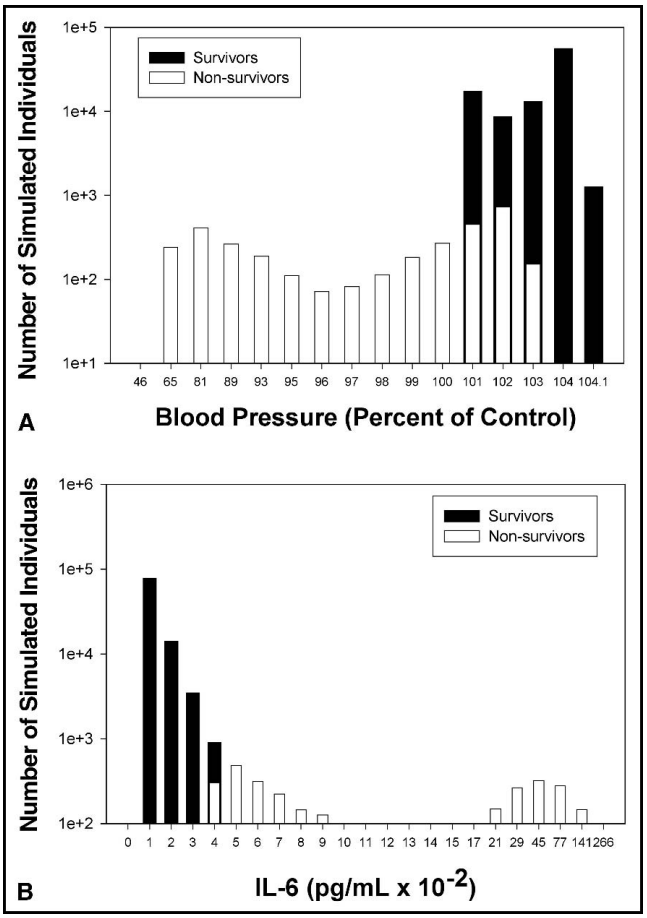
An [15,33•] has produced a very abstracted ABM of the AIR that is based at the cellular level. The entire model, as well as extensive documentation, is available online through the following portal: http://ccl.sesp.northwestern.edu/cm/models/community/. A brief summary of the model and its actions follows here.

The model focuses on the interactions that occur at the interface between endothelial cells and blood-borne inflammatory cells and mediators. Cells were selected as the agent level because cells can readily be subgrouped based on common behavioral rules and the responses of single types of cells to various mediators are extensively characterized in the basic science literature. The endothelial-blood interface was chosen because endothelial injury and activation initiate nearly all inflammatory processes, and propagation of inflammation requires the circulating components of the AIR in the blood. The model is designed to respond to insults that simulate both infection and noninfectious tissue injury such as trauma; as in the ODE model described above, this premise is based on the idea that similar pathways and actions are responsible for the propagation of inflammation once the process has been initiated.

Agents that represent endothelial cells populate the background grid, and agents that represent neutrophils and circulating monocytes move over the surface of this grid. An injury pattern is induced on the endothelial surface, and as a result the injured endothelial cells express variables that simulate the activation of cell surface receptors and produce variables that simulate mediators that diffuse across the endothelial surface. Neutrophils or monocytes respond to these values by initiating their own activation rules. Activated neutrophils undergo an adhesion and migration sequence, culminating in a simulated respiratory burst. These variables will eliminate infectious vectors in their vicinity but will also cause some degree of damage to surrounding endothelial cells. The monocytes produce further activating and potentiating mediators that augment neutrophil function, and they also execute an abstracted "heal" function that simulates phagocytosis and repair of damaged endothelial cells. The result is the creation of a "virtual organism" that mimics the basic processes of the AIR. Obviously, this is a very abstract approximation of a real organism; it is actually more akin to a giant endothelial cell culture model because there is no tissue differentiation or organ function. However, it is believed that the model captures the essence of the processes and behavior of the AIR.

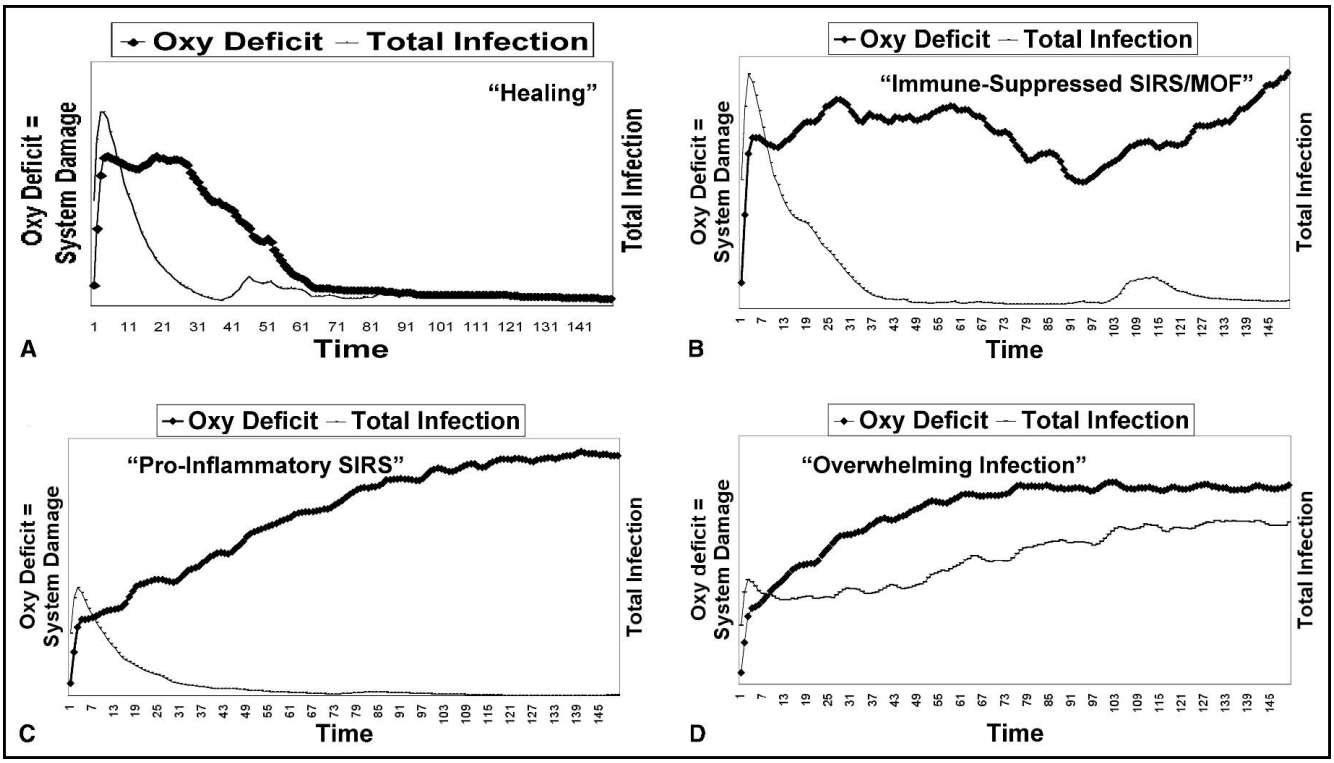
As we describe below, both models, which were derived independently and focus on slightly different mechanisms occurring in different anatomic regions, often predict similar results both in individuals and in simulated

Figure 2. An *in silico* observational trial of cardiopulmonary bypass–induced inflammation and mortality



The ordinary differential equations model of inflammation was used to generate a simulated clinical trial, as described in the text. Elevated interleukin-6 levels (**A**) and hypotension (**B**) 6 hours after cardiopulmonary bypass are associated with mortality at 48 hours.

Figure 3. Individual runs with progressing initial levels of infection, demonstrating various outcomes corresponding to clinical scenarios



(**A**) Healing, clearing infection, and correcting system damage system. (**B**) Immune-suppressed systemic inflammatory response/multiple organ failure, clearing infectious vectors by approximately Time 40 but inability to reduce system damage sufficiently until a "trivial" second hit at approximately Time 100 increased system damage. (**C**) Pro-inflammatory systemic inflammatory response, clearing infection but with the forward feedback on the pro-inflammatory actions leading to progressively increasing system damage. (**D**) Overwhelming infection, with no clearing of infection or reduction of system damage. Reprinted by permission from [33].

clinical trials. This "virtual organism" can now be used to run *in silico* experiments to integrate hypotheses based on the results of basic science studies.

Validation strategies

The key for any model, be it cell culture, animal, clinical, or mathematical, is the relation between the model and the real-world process that is being modeled. Hence, a systematic means of validation of the models is necessary. Validation of a mathematical model is focused at two basic levels: the assumptions that go into the construction of the model, and the subsequent behavior of the model.

The validation of the assumptions of the model can be addressed through transparency: making explicit the process by which the architecture and rules of the model are chosen and implemented, because all models represent some degree of abstraction, and the degree is the choice of the modeler. In the models described above and in the publications cited, the assumptions and interactions are stated, and the models themselves are made available for review. As new mechanisms of inflammation become established, and their role demonstrated reproducibly, these mechanisms can be incorporated into both the ABM and the ODE models. For example, the contributions of the autonomic nervous system to the production of cytokines such as interleukin (IL)-10 are currently being integrated into the ODE model, and the effects of newer mediators such as HMGB-1 are being added into the ABM.

Validation of the behavior of the model can be approached through a series of strategies, some of them specific to the type of model being examined. All these strategies, to some degree, consist of comparing the behavior of the model with some real-world data set of expected behavior. When the behavior of the model matches the real-world data set, then the model is deemed valid for that particular test. If the model does not match, then the model is reexamined, and either the basic structure of the model is reconfigured, or specific variables and relations are fine-tuned through a calibration process. One immediately apparent problem involves determining when a lack of fit is due to calibration or to an error in the basic structure of the model, and this

step often involves extensive literature searches to obtain additional mechanistic insight as well as trial and error (or more systematic, as described below) calibration strategies.

The ODE model and parameters were validated and calibrated in three stages. In the preliminary stage, the model was constructed so it could reproduce qualitatively several different scenarios described in the literature. The resulting qualitative model was then calibrated to experimental data in mice, rats, or humans (note that separate mathematical models were generated for each species). In the second stage, the model was matched to experimental data by adjusting parameters for which exact or approximate values were unknown, using literature information regarding specific biologic mechanisms together with the dynamics of the model. In the third stage, the parameters were optimized using a stochastic gradient descent algorithm that was implemented in proprietary software of Immunetrics, Inc. A statistical analysis of the model's ability to account for the data was performed, showing that model fit was not significantly different from the most optimal regression fit to each data set. Figure 1 gives a representative example of the ability of our model to describe the production of tumor necrosis factor (TNF) and IL-10 in response to 6 mg/kg endotoxin in C57Bl/6 mice; similar data at 3 and 12 mg/kg lipopolysaccharide, as well as data on IL-6 and NO_2^-/NO_3^-, are not shown.

This model has demonstrated its utility in simulating acute inflammation induced in mice by endotoxin, surgical trauma, and surgery/hemorrhage. Its predictive ability was tested in trauma (sham surgery/surgical instrumentation) followed or not by hemorrhagic shock + lipopolysaccharide given 0.5, 3, or 27 hours after the beginning of surgical instrumentation (Lagoa *et al.*, unpublished data). Alhough the model was able to predict to a large extent the levels of TNF, IL-10, IL-6, and NO reaction products (NO_2^-/NO_3^-) in these animals, we note that in some combinations of insults and at some time points, the model prediction did not agree with experimental results. We believe that these discrepancies will help us improve the model by pointing out incorrect simulations of mechanisms or dynamics.

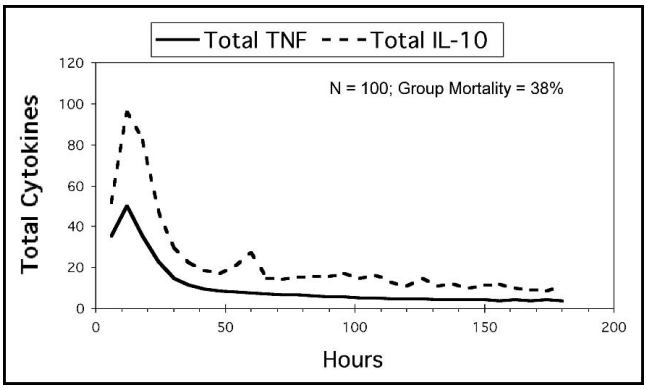
As an example of the behavior of this model in the setting of population dynamics, we generated an *in silico* trial of 100,000 "patients" subjected to randomized levels of surgical trauma (cardiopulmonary bypass) and hemorrhage (Vodovotz *et al.*, unpublished data). The "patients" showed the random variations in the production of proinflammatory and antiinflammatory cytokines as well as NO in response to trauma, and they were "injured" at various random levels. We carried out the simulation for 48 hours. Death was defined as damage/dysfunction equal to or greater than 0.15 arbitrary units, because that was the cutoff point for damage/dysfunction that was predicted to rise indefinitely. Under these conditions, 3262 "patients" (~3%) fit this criterion for death, in agreement with published estimates of mortality after cardiopulmonary bypass [34]. We examined the characteristics of the "patients" who died and found that, in agreement with published studies in both rodents and humans, death tended to occur in those cases in which IL-6 (examined at 6 hours) and early hypotension were evident (Fig. 2) [35,36].

Furthermore, the ODE model was used to simulate an anti-TNF trial, demonstrating a lack of efficacy of therapy administered randomly to sepsis patients with 30 to 40% mortality in the control population. Interestingly, this therapy might have demonstrated efficacy if the ODE model had been used to establish inclusion and exclusion criteria for administration. For example, "patients" helped by anti-TNF treatment in this simulation had higher peak levels of TNF and IL-6 and more severe infections, whereas those hurt by this intervention tended to have infections of moderate severity but were low TNF responders and high antiinflammatory responders [37].

In the ABM, validation can be accomplished at three levels: the level of individual response (individual dynamic), the level of the behavior of a population with respect to intrinsic variables (population dynamic), and finally the behavior of the population with respect to an intervention (population response).

In evaluating the individual dynamic, a single run of the ABM is considered the equivalent of the behavior of a single patient, with the focus on the pattern of system damage and clearance of infection. The ABM is able to

Figure 4. Patterns of mean cytokine levels (tumor necrosis factor and interleukin-10) over 7 days in populations of runs with mortality rate of 38%



Model is qualitative only, and therefore the actual numeric values of the cytokine levels are dimensionless. Reprinted by permission from [33].

Table 1. ABM *in silico* experiments of existing clinical trials: simulated clinical trials using the ABM

Model mode	Infectious mortality (IIL = 1200) (%)	Sterile mortality (IIL = 1700) (%)
Control	38	37
3 days anti-TNF	40	34
3 days hrIL-1ra	36	36
7 days GCSF	35	35

N = 100 for all populations. Mortality rate is determined after 28 days of simulated time (FDA standard for efficacy of intervention). The simulated control group was selected for initial injury level (IIL) that produced a mortality rate approximating that seen in clinical sepsis trials. This IIL was not subsequently varied for the simulated trials. Simulated interventions were modeled using published pharmacokinetics of the study drugs, and all results were generated prospectively. No intervention resulted in a statistically significant difference in mortality rate, qualitatively matching the results of those trials.
anti-TNF, anti–tumor necrosis factor; hrIL-1ra, interleukin-1 receptor antagonist; GCSF, filgrastim.
Data from Reinhart *et al.* [38], Opal *et al.* [39], and Root *et al.* [40•].

reproduce the basic responses and behaviors seen in the clinical setting: "healing," "proinflammatory predominant SIRS," "immune-suppressed SIRS/MOF," and "overwhelming infection." The graphs demonstrating these dynamics can be seen in Figure 3.

With respect to the population dynamic, the ABM is able to reproduce the patterns of cytokine expression seen in a matched clinical group. Figure 4 demonstrates the patterns of values of variables that represent TNF and IL-10 for a simulated population with a mortality rate of 38%.

Evaluation of the population response consisted of carrying out *in silico* experiments of therapeutic interventions. In the validation arm, the ABM was used to reproduce the results of existing clinical mediator-directed therapies, namely, anti-TNF, anti-IL-1, and GCSF supplementation [38,39,40•]. The reported pharmacologic action of each of these drugs was taken into account, their effects were simulated in the base model, and none of them resulted in a positive treatment effect (Table 1), qualitatively matching the results of those clinical trials. Unfortunately, the lack of coagulation in the ABM precludes the modeling of the single positive mediator directed therapy, namely, activated protein C. The ODE model does include the coagulation cascade, but this pathway still requires calibration. Ongoing work in both models is being directed at simulating the actions of activated protein C. Reproduction of a positive intervention would strengthen the population response level of validation of both models.

Conclusions and future directions

In summary, it is our contention that the state of medicine is at a point at which the traditional research paradigm of linear reductionism has reached its limits. In system-level disease processes, like sepsis/SIRS/MOF, a synthetic framework is necessary, and mathematical modeling can provide that [9–13]. There are many approaches to mathematical modeling, and here we present two approaches, ABM and ODE, that have different focuses but produce similar results. It is hoped that this paper will stimulate interest in these and other techniques.

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