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Review Article

IN SILICO MODELS OF ACUTE INFLAMMATION IN ANIMALS

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ABSTRACT—Trauma and hemorrhagic shock elicit an acute inflammatory response, predisposing patients to sepsis, organ dysfunction, and death. Few approved therapies exist for these acute inflammatory states, mainly due to the complex interplay of interacting inflammatory and physiological elements working at multiple levels. Various animal models have been used to simulate these phenomena, but these models often do not replicate the clinical setting of multiple overlapping insults. Mathematical modeling of complex systems is an approach for understanding the interplay among biological interactions. We constructed a mathematical model using ordinary differential equations that encompass the dynamics of cells and cytokines of the acute inflammatory response, as well as global tissue dysfunction. The model was calibrated in C57Bl/6 mice subjected to (1) various doses of lipopolysaccharide (LPS) alone, (2) surgical trauma, and (3) surgery + hemorrhagic shock. We tested the model's predictive ability in scenarios on which it had not been trained, namely, (1) surgery ± hemorrhagic shock + LPS given at times after the beginning of surgical instrumentation, and (2) surgery + hemorrhagic shock + bilateral femoral fracture. Software was created that facilitated fitting of the mathematical model to experimental data, as well as for simulation of experiments with various inflammatory challenges and associated variations (gene knockouts, inhibition of specific cytokines, etc.). Using this software, the C57Bl/6-specific model was recalibrated for inflammatory analyte data in $CD14^{-/-}$ mice and was used to elucidate altered features of inflammation in these animals. In other experiments, rats were subjected to surgical trauma ± LPS or to bacterial infection via fibrin clots impregnated with various inocula of *Escherichia coli*. Mathematical modeling may provide insights into the complex dynamics of acute inflammation in a manner that can be tested *in vivo* using many fewer animals than has been possible previously.

KEYWORDS—Sepsis, trauma, mathematical model, inflammation, mouse, rat

The acute inflammatory response to infection and trauma

The initial response of the body to acute biological stress such as bacterial infection or tissue trauma is an acute inflammatory response. This response involves a cascade of events mediated by a large array of cells and molecules that locate invading pathogens or damaged tissue, alert and recruit other cells and effector molecules, eliminate the offending agents, and finally restore the body to equilibrium. This response is accompanied by physiological manifestations such as fever and elevated heart rate and redistribution of blood flow to tissues, which contribute to optimize the various defense mechanisms involved. In this process, the inflammatory response also can be destructive to healthy tissue, resulting in further tissue injury and further stimulating inflammation. In some instances, this can lead to a runaway effect in which a persistent dysregulated inflammatory response promotes organ dysfunction and death (1, 2).

Inflammation is necessary for the removal or reduction of challenges to the organism and subsequent restoration of homeostasis. In an attempt to reestablish homeostasis, the inflammatory response is pivotal in clearing invading organisms and offending agents, enhancing wound healing, and promoting tissue repair. The acute inflammatory response involves a coordinated mobilization of cellular and molecular elements of the innate and adaptive immune systems, along with the neurohormonal axis, and subsequently impacts all organ systems (3, 4). In conditions of normal homeostasis, the inflammatory response restores the body to healthy function after clearance of the offending agents and appropriate tissue repair. However, in cases of unchecked systemic inflammation, it remains persistently activated and compromises healthy tissue, thereby leading to the detrimental consequences described above (2, 5).

Given the complexity of inflammation and the difficulty in translating reductionist animal studies to clinical trials (6), it is not surprising that therapies that modulate inflammation in sepsis and trauma have yielded disappointing clinical results (7–11). Despite promising results in animal and early human trials, large-scale trials of therapies targeted at inhibiting specific inflammatory mediators have generally failed to improve survival (12). To address problems such as this one,

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[1]These authors are cofounders of and consultants to Ummunetrics, Inc., which was licensed from the University of Pittsbutgh the mathematical model of acute inflammation described in this abstract.

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as well as the rising cost of production and approval of new drug candidates for all diseases, the U.S. Food and Drug Administration recently stated that "A new product development tool kit—containing powerful new scientific and technical methods such as animal or computer-based predictive models, biomarkers for safety and effectiveness, and new clinical evaluation techniques—is urgently needed to improve predictability and efficiency along the critical path from laboratory concept to commercial product" (13). Here we describe a unique interface between mathematical models and animal studies, in which well-established paradigms of acute inflammation in animals were used to calibrate and validate a mathematical model of acute inflammation. In turn, the mathematical model has led to streamlined usage of animals as well as leading to new insights in the pathology of acute inflammatory states such as sepsis, trauma, and hemorrhage. We also show how the model can be translated to human inflammation and applied to simulations of randomized, placebo-controlled clinical trials.

Experimental models of infection and trauma

There is a long history of experimental investigation of acute inflammation, using various animal models (summarized recently in several articles in *Shock*, vol. 24, supplement 1) (14, 15). With various degrees of fidelity, these animal models attempt to reproduce features of human septic, traumatic, or hemorrhagic shock. Much of the work has focused on manipulations of single components and deducing their influence, but relatively few studies have followed the dynamics of several components simultaneously under controlled situations. Thus, for reasons of expense and time, as well as regulatory issues (15), complex, dynamic physiological and pathological processes become reduced to measurements at specific time points. Adding to the difficulty in interpreting these studies is the fact that they are carried out in different animals, with different stimulants or challenges. Because of their disparate nature, these studies are, by themselves, inadequate to validate global models of the acute inflammatory response (6). While animal models have been developed in rodents, dogs, swine, and primates, rodent models are particularly attractive because of the availability of genetically similar or identical individuals, relatively low cost, and ease of handling (14). These limitations, and especially the issue of increasing regulatory oversight on research involving animals subjected to severe inflammatory stress (15), have prompted us to initiate a project to create an *in silico* (computer-simulated) platform for acute inflammation. In the following sections, we describe the development, calibration, and validation of this platform. We note that this is a work in progress but suggest that our approach may help investigators achieve the elusive goals of reduction and replacement of animals long sought by the institutional animal care and use committee. Furthermore, we describe our initial efforts to link the simulation of acute inflammation across several species, including humans, to allow for the extrapolation from studies in animals to simulated human clinical trials of therapeutics for sepsis and trauma. Finally, we conclude with the challenges faced by investigators trying to adopt complex systems and simulation methodologies into their research.

A PLATFORM FOR *IN SILICO* INFLAMMATION EXPERIMENTS

Mathematical modeling of complex systems is emerging as an approach by which to tame the seemingly unpredictable behavior of such biological phenomena and account for the plethora of known and unknown interactions among biological pathways (16). A mathematical model that captures the dynamic interactions among several key components of the acute inflammatory response might provide new insights into the global consequences of (1) differing initial conditions on outcome, (2) manipulating individual components of inflammation, and (3) modulating several components of inflammation simultaneously or in sequence (5, 17–19). Achieving these goals would facilitate the use of *in silico* models of acute inflammation to streamline and improve current preclinical models of sepsis and trauma in various species. In attempts to deal with this complexity, others have created mechanistic models that describe some of the inflammatory characteristics of sepsis (19–23). However, the mathematical model we describe here is the first to have been validated with animal and human data and the first to unify diverse inflammatory insults such as trauma, hemorrhagic shock, and endotoxemia (24). Moreover, this model was capable of realistically simulating clinical trials in sepsis (25, 26) and is the first model used to discern the inflammatory phenotype of genetically modified mice (27) (Prince et al., Mol Med 2006, in press).

Our approach to modeling inflammation

Given the complexity described previously and the need to examine distinct pathways as part of a whole, we heeded calls in the literature (17, 28, 29) for the formation of a multidisciplinary team to tackle this problem. We assembled a team consisting of clinicians, bench scientists, mathematicians, and computer scientists to carry out an iterative program of model generation, verification, and calibration in both mice and humans and subsequent hypothesis generation and testing (Fig. 1). We created our model using a set of ordinary differential equations, which are most valid in the limit of large concentrations in a well-mixed volume. When these assumptions are invalid, agent-based models as pioneered

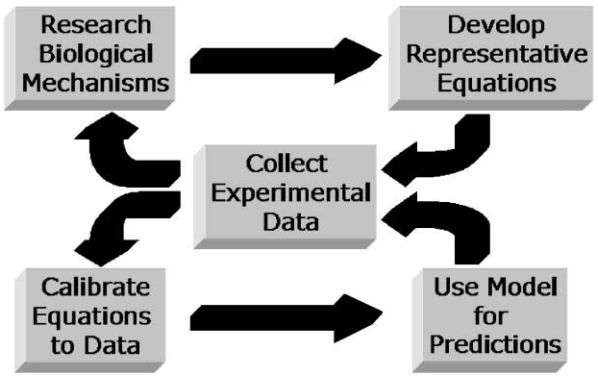

FIG. 1. **Iterative approach to modeling inflammation.** The approach taken by our team involves an iterative and repeating process of model creation based on existing literature, validation in relevant experimental paradigms, and hypothesis generation (testing of predictions).

by An (21, 22) may be more appropriate. The advantage of our approach is that it has a long history in biological modeling and amenable to mathematical analysis. Indeed, we have used differential equations in several reduced mathematical model of inflammation (30) (Reynolds et al., J Theoretical Biol 2006, in press; Day et al., J Theoretical Biol 2006, in press).

The differential equations that make up our mathematical model represent the kinetics of well-accepted constituents of the inflammatory response (Table 1). Nonetheless, elements are still either totally lacking or require calibration, as indicated in Table 1. Although it may be argued that no simulation of a biological process can ever be complete, the process of augmentation of our mathematical model of inflammation is continuous and follows the iterative process described in Figure 1. In our model, neutrophils and macrophages are activated directly by bacterial endotoxin (lipopolysaccharide [LPS]) or indirectly by various stimuli elicited systemically upon trauma and hemorrhage. Although not included explicitly in our model, early effects, such as mast cell degranulation and complement activation (5), are incorporated implicitly in the dynamics of our endotoxin and cytokine variables. These stimuli, including endotoxin, enter the systemic circulation quickly and activate circulating monocytes and neutrophils (14). Activated neutrophils also reach injured or ischemic tissue by migrating along a chemoattractant gradient (31).

Once activated, macrophages and neutrophils produce and secrete effector molecules that activate themselves and also other cells, such as endothelial cells. Proinflammatory cytokines—tumor necrosis factor (TNF), interleukin (IL) 6, and IL-12 in our mathematical model—promote immune cell activation and proinflammatory cytokine production (32). The concurrent production of anti-inflammatory cytokines counterbalances the actions of proinflammatory cytokines. In the ideal situation, these anti-inflammatory agents serve to restore homeostasis. However, when overproduced, they may lead to detrimental immunosuppression (33–35).

Our model includes a fast-acting anti-inflammatory cytokine, IL-10, and expressions for the slower-acting anti-inflammatory action of transforming growth factor $\beta 1$ (TGF-$\beta 1$), soluble receptors for proinflammatory cytokines, and cortisol. Proinflammatory cytokines also induce macrophages and neutrophils to produce free radicals. In our model, inducible nitric oxide synthase (iNOS)–derived NO is directly toxic to bacteria and indirectly to host tissue (36–38). Although the actions of superoxide (O_2^-) and lytic mechanisms (38) do not appear explicitly in the model, their activity is accounted for implicitly through proinflammatory agents. In the model, the actions of these products that can cause direct tissue dysfunction or damage are subsumed by the action of each cytokine directly. The induced damage, which we define in much the same way as inflammation-promoting "alarm/danger" signals derived from stressed or dysfunctional cells (39), can incite more inflammation by activating macrophages and neutrophils (40). However, NO can also protect tissue from damage induced by shock (41–43), although overproduction of this free radical causes hypotension (37). Proinflammatory cytokines also reduce the expression of

TABLE 1. **Elements of the mathematical model of acute inflammation**

Model component	Present in model	Requires further calibration
Initiator element		
Pathogen/endotoxin	Y	Y
Blood pressure	Y	N
Tissue trauma	Y	Y
Innate immune element		
Resting neutrophils	Y	Y
Activated neutrophils	Y	Y
Resting macrophage	Y	Y
Activated macrophages	Y	Y
NK cell	N	Y
NKT cell	N	Y
Mast cell	N	Y
Adaptive immune element		
Immature DC	Y	Y
DC1	Y	Y
DC2	Y	Y
T_H1 cell	Y	Y
Effector element		
Constitutive nitric oxide synthase (eNOS)	Y	N
iNOS	Y	N
NO_2^-/NO_3^-	Y	N
TNF		
S-nitrosothiols	N	Y
IL-10	Y	N
IL-6	Y	N
Generic anti-inflammatory activity	Y	Y
IL-12	Y	Y
IFN-γ	Y	Y
IL-2	Y	Y
IL-4	Y	Y
IL-18	N	Y
Coagulation elements		
Tissue factor	Y	Y
Prothrombin	Y	Y
Thrombin	Y	Y
Protein C	Y	Y
Activated protein C	Y	Y
Protein S	Y	Y
Factors VIIa, IX, IXa, X, and Xa	Y	Y
PAI-1	Y	Y
Physiological target		
Blood pressure/heart rate/circulating volume/ cardiac output	Y	N
Tissue damage/dysfunction	Y	Y

NK, natural killer; NKT, natural killer T cell; DC, dendritic cell; IFN-γ, interferon γ; PAI-1, plasminogen activator inhibitor 1.

endothelial nitric oxide synthase (eNOS), thereby increasing tissue dysfunction (44).

Simultaneous numerical solution of the equations of this general model generates predictions of the time courses of these elements. The differential equations describing these interactions were written and solved numerically using the XPPAUT freeware written by Dr. G.B. Ermentrout (University of Pittsburgh, Department of Mathematics; www.math.pitt.edu/phase) as well as proprietary software of Immunetrics, Inc. (www.immunetrics.com; Pittsburgh, Pa). The model and parameters were specified in three stages. In the preliminary stage, the model was constructed so it could reproduce qualitatively several different scenarios that exist in the literature, using as much information as could be gleaned from the literature as to cytokine half-lives, life spans of cells, and so on. The resulting qualitatively correct 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 our experimental data by adjusting those parameters for which exact or approximate values were unknown, using our knowledge of the biological mechanisms together with the dynamics of the model, to attain desired time course shapes. 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 with the S-Plus statistical and programming package (Statistical Sciences, Inc., Seattle, Wash), showing that model fit was not significantly different from the most optimal regression fit to each data set. However, unlike regression fitting, we used a single set of equations and values for the constants in those equations, changing only the starting conditions, to account for all inflammatory scenarios.

An In Silico model of mouse endotoxemia

We describe the response to endotoxin first (24), because this is generally regarded as a relatively simple *in vivo* paradigm of acute inflammation (14). In endotoxemia, the model assumes that LPS enters the bloodstream, incites a systemwide response (45), and is cleared in approximately 1 h (46, 47). Circulating neutrophils are activated directly and produce TNF (48) and IL-10 (49–51). The newly produced TNF combines with LPS to activate macrophages that then secrete TNF, IL-6, IL-12, and IL-10 (52). Activated neutrophils, macrophages, and endothelial cells produce NO through iNOS (53). The model assumes that locally produced NO is eventually detected as the measured serum end products NO_2^-/NO_3^-, and this process depends on the differential induction of iNOS in various organs over time (54, 55). Interleukin 10 suppresses TNF production profoundly (56, 57), causing circulating levels of this cytokine to rise and fall within a few hours. The model also includes other slow anti-inflammatory cytokines, including IL-6 (58, 59). We believe that this anti-inflammatory action could be mediated by inducing or activating TGF-β1 (60) on the surface of neutrophils and macrophages, as has been shown for cytokines such as interferon γ (61). Interleukin 10 is inhibited by IL-12 (57) and stimulated by TGF-β1 (62) that can come from various sources.

As an example, we show the ordinary differential equation (ODE) governing the level of LPS (referred to as "PE" for "pathogen-derived endotoxin") over time, including the way in which LPS is removed, in Eq. (1). The first term of the equation reflects LPS generation resulting from death of bacteria (killed by activated macrophages and, more importantly, neutrophils), action by superoxide, and (to a lesser degree) NO production. The second term encodes clearance from the circulation. The last term explicitly represents a therapy that promotes the decay of endotoxin. In that term, the step function square (ton,toff) equals 0 except during the time interval of therapeutic intervention, [ton,toff], when the function equals 1.

$$\mathrm{d}\frac{(\mathrm{pe})}{\mathrm{dt}} = \mathrm{k_{pep}}*\left(\mathrm{k_{pm}}*\mathrm{M_A} + \mathrm{k_{pNO}}*\mathrm{NO} + \mathrm{k_{pO2}}*\mathrm{O_2}\right) *\mathrm{P} - \mathrm{k_{pe}}*\mathrm{pe} - \mathrm{square(ton,toff)}*\mathrm{pe}$$

In a similar fashion, the relevant interactions of acute inflammation are represented mathematically by equations for the rates of change of the relevant variables (for each variable, this is essentially the combined effects of those factors that make the variable increase minus the effects that lead to a decrease), with experimental data used to constrain relevant parameter ranges whenever possible. As seen in Figure 2, we show a representative example of the ability of our model to describe the production of IL-6 and IL-10 in response to 3 mg/kg endotoxin in C57Bl/6 mice; similar data at 6 and 12 mg/kg LPS, as well as data on TNF and NO_2^-/NO_3^-, are not shown.

We next sought to assess the ability of the mathematical model to predict organ dysfunction and mortality in mouse endotoxemia. Our model includes an equation describing global tissue damage/dysfunction, which is promoted by inflammatory stimuli and which itself is proinflammatory. Thus, the mathematical model is calibrated to the levels of circulating cytokines in a manner that takes into consideration the role of global damage/dysfunction. However, it is important to determine the point at which this currently unitless quantity corresponds to death. Accordingly, we assessed survival in the mice given 3, 6, and 12 mg/kg LPS. These doses were well

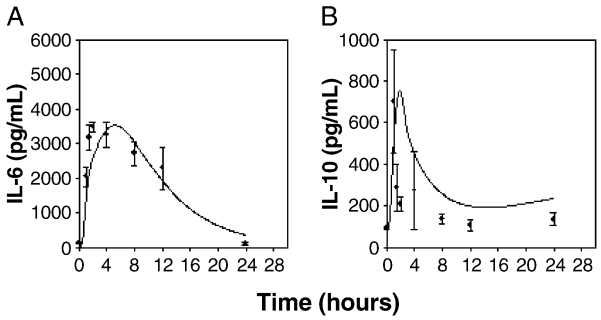

FIG. 2. **Simulation and calibration of the cytokine response to endotoxin in mice.** Mice received 3 mg/kg LPS i.p. at various time points after this injection, the mice were killed, and sera were obtained. Serum IL-6 (A) and IL-10 (B) were measured by specific enzyme-linked immunosorbent assay. Black symbols represent mean ± SD for three to eight separate animals. Black line indicates prediction of mathematical model.

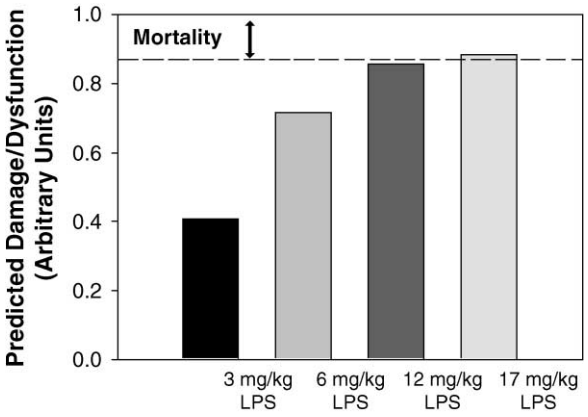

FIG. 3. **Comparison of damage/dysfunction versus mortality in mice.** Mice were subjected to 3, 6, 12, or 17 mg/kg i.p. LPS, and their mortality was assessed. Damage/dysfunction was predicted by the mathematical model of inflammation. Dashed line indicates the division between survival and mortality.

tolerated, and all animals survived. We next treated a separate cohort of mice with a lethal dose of LPS (17 mg/kg). In Figure 3, we show the predicted damage/dysfunction levels and note that a level of approximately 0.9 on this arbitrary scale had to be reached to observe mortality. When subjected to simulated endotoxin loads exceeding 9 mg/kg, the model predicts persistent high levels of IL-6 and high levels of damage. In fact, the model predicts that cumulative damage will grow rapidly and fail to resolve within the first 24 h with doses exceeding 12 mg/kg (24).

An In Silico model of mouse surgical trauma and combined surgery/hemorrhage

The response to trauma differs from endotoxemia, and our mathematical model can account for this fact (24). We know that the inflammatory response to hemorrhage is less pronounced than that of sepsis (63, 64). We also recognize that direct tissue injury is a more prominent initiating event than occurs with sepsis or endotoxemia. We incorporated the finding that localized trauma and hemorrhage cause a burst of catecholamine release (65, 66), as well as inducing platelets to release TGF-β1 (67), which then chemoattracts circulating neutrophils to the site of injury (68–70). Simultaneously, elements associated with the systemic response to injury and dysfunctional/locally damaged tissue (possibly HMGB1 (71, 72)) are released, activating neutrophils when they arrive. The trauma-induced products combine with TNF to activate local macrophages to produce IL-6 and IL-10. We assumed that the released TGF-β1 induces activated macrophages to produce IL-10 (73), causing a massive release of IL-10 in comparison to TNF (Fig. 4) and IL-6 (data not shown) when mice are subjected to surgical trauma combined with hemorrhage. Our data also showed an initial drop in circulating NO_2^-/NO_3^- values in response to surgery/hemorrhage (data not shown). We know that trauma causes a severe drop in eNOS expression and/or activity (74–78). We believe that this phenomenon may account for the dip in NO_2^-/NO_3^- seen in trauma patients (79, 80). Although trauma results in a rapid reduction in availability of L-arginine (81), this effect is generally seen at later time points than those observed in our studies. The model assumes that blood loss in hemorrhage causes some tissue damage as well as directly contributing to neutrophil and macrophage activation (63). This causes release of TNF, which in turn induces IL-10 and IL-6 release. Tissue dysfunction can be induced or exacerbated by a rapid lowering of blood pressure as well as by proinflammatory mediators. As seen in Figure 4, our mathematical model predicts the production of TNF and IL-10 induced by surgery for vessel cannulation followed by hemorrhagic shock. Not shown are the simulations for IL-6 and NO_2^-/NO_3^- discussed previously. Also not shown is the fact that the kinetics of elaboration of these analytes is essentially identical (although of lower magnitude) in mice subjected to cannulation only (so-called "sham" surgery, which we understand to be surgical trauma). Thus, we hypothesized that surgical or other trauma is the main driver of inflammation observed in survivable hemorrhagic shock, at least in rodents. As an illustration of the utility of a mathematical model for addressing this issue, we can simulate hemorrhage in the absence of any trauma whatsoever (a near impossibility *in vivo*). We have attempted to correlate this prediction with another global measure of the response to either surgical trauma alone or in combination with hemorrhagic shock/resuscitation, namely, DNA microarray analysis of the liver transcriptome (82) (Lagoa et al., submitted for publication).

In Silico models of combined surgery, hemorrhage, and endotoxemia

To test the ability of our mathematical model to predict inflammation in settings on which it had not been trained explicitly, we considered the following inflammatory paradigms: surgical trauma (cannulation) with or without hemorrhagic shock + LPS given at 0.5, 3, or 27 h after the beginning of surgical instrumentation (Lagoa et al., submitted for publication). To determine the magnitude of the inflammatory response, the animals were killed at either 27 h (for the animals in which LPS was administered at 30 min or 3 h after surgery ± hemorrhage) or 32 h (for the animals in which LPS was administered at 27 h after hemorrhage) from the beginning of experimentation. This was done since the mathematical model predicted that maximal IL-6 levels would be found in the circulation of mice subjected to the latter regimen. As seen in Figure 5, the model succeeded in

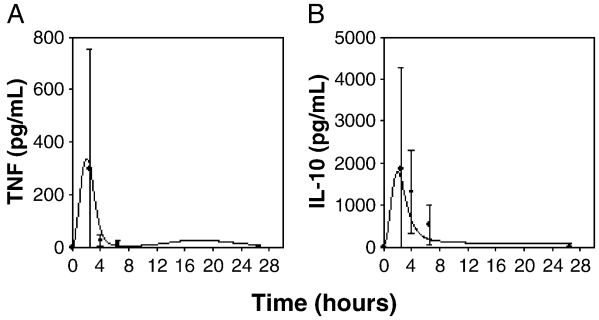

FIG. 4. **Experimental data and model predictions for surgery/hemorrhage-induced inflammation.** Mice were subjected to combined surgical trauma and hemorrhagic shock. All analytes were measured as described in Figure 2. Black symbols represent mean ± SD for three to six separate animals. Black line indicates prediction of mathematical model.

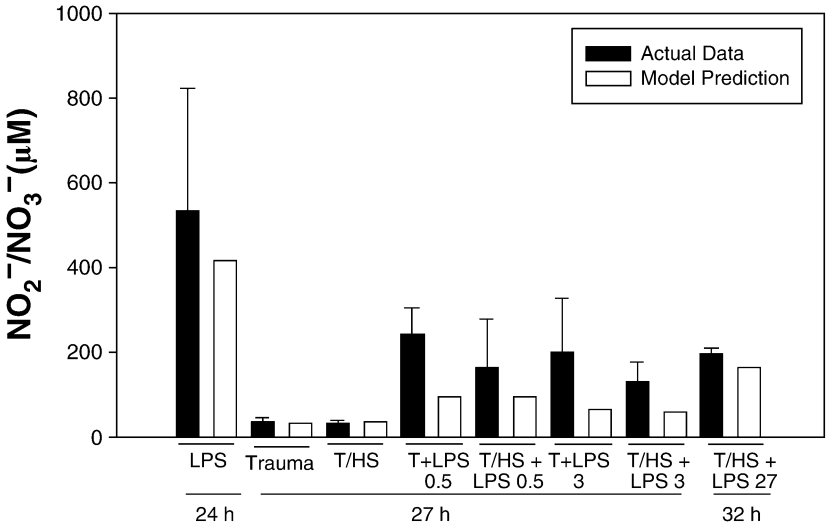

FIG. 5. **Prediction of the inflammatory response to a combination of surgery/hemorrhage followed by LPS in mice.** Mice were subjected to trauma or trauma/hemorrhage, as described in the text, and then received 3 mg/kg LPS i.p. at either 0.5, 3, or 27 h from the time of initiation of surgery ± hemorrhage. At 27 h after surgery ± hemorrhage, the mice were killed, and sera were obtained. Circulating NO_2^-/NO_3^- was measured as described in Figure 2. Black bars represent mean ± SD for three to eight separate animals. White bars indicate prediction of mathematical model.

simulating the elaboration of the NO reaction products, NO_2^-/NO_3^-, in single or combined insults. Not shown are similar data for TNF, IL-6, and IL-10. Moreover, the predicted damage/dysfunction was well below that associated with mortality in the setting of endotoxemia alone (Fig. 3), and indeed survival was 100% in the multiple-hit studies. 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 (Fig. 1).

In Silico model of combined surgery/hemorrhage with bilateral femur fracture

We next explored the ability of our mathematical model to guide the design of pilot experiments on the inflammatory effects of surgery + 1.5-h hemorrhage + bone fracture (Lagoa et al., manuscript in preparation). The simulation of combined surgery/hemorrhage/bone fracture suggested that (1) dysfunction induced by surgery + bone fracture followed by 1.5 h of hemorrhage would be approximately equivalent to the dysfunction induced by surgery only followed by 2.5 h of hemorrhage, and (2) that the predicted increase in dysfunction in animals subjected to surgery + bone fracture followed by 1.5 h of hemorrhage versus surgery only + 1.5 h of hemorrhage approximately matched the true organ dysfunction as assessed by serum alanine aminotransferase. Our model further predicted that combining 2.5-h hemorrhage with bone fracture would lead to the death of the animals (Lagoa et al., manuscript in preparation). As a demonstration of the practical utility of our mathematical model, the simulation described previously was used to establish this specific animal model in the Animal Models Core in the Department of Surgery at the University of Pittsburgh and to guide preliminary work for a major grant application, given the requirement to define a set of conditions that would lead to reproducible inflammation and dysfunction but not to death. To our knowledge, this is the first time a mathematical model was in fact used for this purpose, greatly reducing the number of animals and time required to establish a new animal model of this complexity.

Combined In Silico and In Vivo studies used to elucidate the complex inflammatory phenotype of CD14-deficient mice

Another novel application of our mathematical modeling approach is to help elucidate mechanisms and address controversies underlying the complex inflammatory phenotype of transgenic and gene knockout mice. One such issue is the role of LPS in hemorrhagic shock–induced inflammation. It is well known that the intestine is highly sensitive to ischemia-reperfusion injury and experiences a marked reduction in blood flow during circulatory shock due to a disproportionate constriction of the splanchnic circulation. Several studies have proposed that many of the inflammatory changes characteristic of trauma or hemorrhage are secondary to the release and recognition of gut-derived immunostimulants, such as LPS, or after bacterial translocation caused by increased intestinal permeability (83–93); nonetheless, both animal (94) and clinical (95–97) studies have failed to implicate LPS or bacterial translocation in this process. As mentioned previously, we found that our single mathematical model with different initiators, including the autonomic system, could describe the response to both hemorrhagic shock and LPS *in vivo* (24). This mathematical model of acute inflammation could account for hemorrhagic shock–induced inflammation without invoking endogenous release of LPS, a hypothesis that required *in vivo* validation.

We sought to use our mathematical model in a novel way to address this controversy. We hypothesized that inflammatory analytes data obtained in mice that are genetically different in their response to LPS from their wild-type counterparts could be used to modify the values of relevant constants in our mathematical model. Inflammatory analytes inform the "damage/dysfunction" variable in our model (which itself recursively induces further inflammation (24)), and this currently unitless variable can be compared with the overall

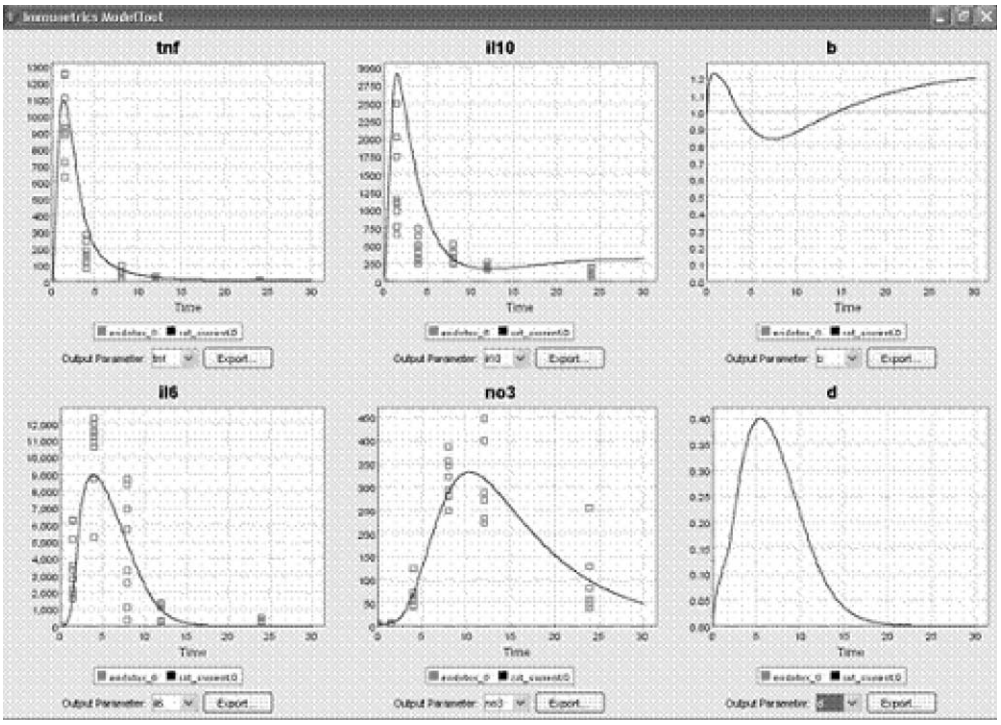

FIG. 6. **Screenshot of Immunetrics software for *in silico* experiments and visualization of the mathematical model of inflammation.** This software allows for rapid manipulation of the mathematical model, as well as visualization of actual data (symbols) and model fit (lines). Multiple models in various species and various scenarios (e.g., endotoxemia), as well as the kinetics of evolution of multiple analytes (shown are TNF, IL-6, IL-10, and NO_2^-/NO_3^- ["no3"], predicted blood pressure ["b"], and predicted global damage/dysfunction ["d"]), may be viewed simultaneously. Not shown is the ability of this software to generate *in silico* experiments combining multiple insults, "deleting" genes, or "inhibiting" specific cytokines, and so on.

pathological response of animals. Mice lacking in CD14 ($CD14^{-/-}$), a molecule crucial to the recognition of LPS and subsequent cellular activation (98), have been reported to be highly LPS-insensitive (99); however, a detailed time course of the response of $CD14^{-/-}$ mice to LPS demonstrated that their production of TNF, IL-6, IL-10, and NO_2^-/NO_3^- could not be explained solely by lack of sensitivity to LPS. For example, although TNF and NO_2^-/NO_3^- were 10-fold lower in $CD14^{-/-}$ as compared with wild-type controls, IL-6 levels were only partially changed, and IL-10 levels remained essentially identical (Prince et al., Mol Med 2006, in press). Moreover, although C57Bl/6 mice subjected to hemorrhagic shock expressed elevated levels of hepatic CD14 protein, $CD14^{-/-}$ mice did not differ from their wild-type controls with regard to circulating levels of IL-6 and IL-10 after either surgical trauma or surgery + hemorrhage (Prince et al., Mol

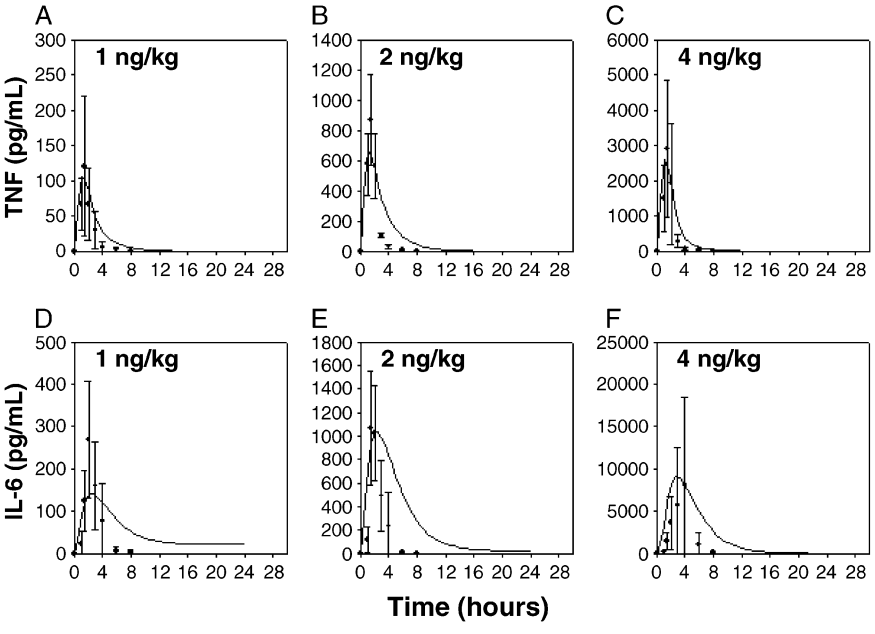

FIG. 7. **Mathematical model of human endotoxemia.** Healthy human volunteers received 1, 2, or 4 ng endotoxin/kg body weight. Serum TNF and IL-6 were measured serially over 24 h. Note the similar time courses among the groups and the similar scatter in data between genetically identical mice (Fig. 2) and genetically diverse humans.

Med 2006, in press). These latter data supported (but did not prove) our hypothesis that LPS translocation is not necessary for hemorrhage-induced inflammation.

We sought to use our mathematical modeling approach in a novel way to address this issue, reasoning that if we could recalibrate our C57Bl/6-specific model to circulating analyte data in $CD14^{-/-}$ mice, we could gain insight into some of the underlying biological changes characteristic of these mice since our model is based on the mechanistic interrelationship among various elements of the inflammatory cascade (as detailed previously). Rather than generating a single recalibrated model parameter set as we had in the past, we followed a practice developed recently in the field of weather forecasting, known as "ensemble modeling" (in which 5–100 different models of the same process are, in aggregate, capable of more accurate forecasts than any one given model) (100). Accordingly, we created an ensemble of possible models, to improve prediction accuracy. The changes made in the models by our algorithm to account for inflammation in $CD14^{-/-}$ mice included both ones that would have been chosen intuitively (e.g., decreased responsiveness of leukocytes to LPS) and others that were not intuitively obvious (e.g., altered IL-6, IL-10, and NO production; Prince et al., Mol Med 2006, in press). We note that these models in aggregate predicted changes in IL-6, IL-10, and NO that were separate from the decreased responsiveness to LPS that would, by itself, be expected to reduce production of these mediators. Moreover, we note that the $CD14^{-/-}$-specific model, like the C57Bl/6 base model on which it was based, did not invoke LPS release in trauma- or hemorrhage-induced inflammation. Indeed, baseline production and turnover of IL-6 by macrophage-type cells are suggested to be significantly enhanced; eNOS production and iNOS responsiveness are significantly down-regulated in $CD14^{-/-}$ mice (27). We suggest that this methodology could be used to describe and predict the actions of novel anti-inflammatory drugs in a given species and to use a model calibrated in one species to yield a model calibrated in another, closely related species. If so, this approach should greatly streamline animal use in preclinical studies of sepsis and trauma, as well as increase the overall predictive ability of these models.

Ongoing studies and future direction

We have created a preliminary model of acute inflammation for rats subjected to surgical trauma ± LPS (Lagoa et al., manuscript in preparation). We note that, in rats, unlike mice, we implanted cannulae and were able to assess the production of cytokines and NO_2^-/NO_3^- in serial measurements from single animals. In Figure 6, we show the results for combined surgical trauma and LPS in the context of software designed by Immunetrics, Inc., to allow for rapid manipulation of the model and for the design of *in silico* experiments. Using data obtained from Dr. Anthony Suffredini (Critical Care Branch, National Institutes of Health, Bethesda, Md), we also created an initial human endotoxemia model (Fig. 7), which we are currently augmenting with additional human clinical data in sepsis and trauma. We emphasize that these models in species other than mouse are still preliminary and require substantial additional calibration and validation studies. However, we point out both the similarities among species with respect to the overall dynamics of evolution of inflammation and the scatter obtained in the data, as well as the differences in sensitivities to various stimuli (e.g., LPS) that our mathematical model will hope to address through methodology similar to that described previously for $CD14^{-/-}$ mice.

We are currently carrying out extensive studies in rats subjected to implantation of fibrin clots containing various inocula of *Escherichia coli* (101), to establish a mathematical model of bacterial sepsis stimulated by known levels of bacteria. We are also in the process of fleshing out our model of inflammation in swine, baboons, and humans and carrying out simulated clinical trials in the sepsis (25) and trauma (19).

CONCLUSIONS

Detailed cellular and molecular analyses explored in isolation have provided valuable but limited information in the setting of trauma and sepsis (17, 18). Despite the sophistication of our current schematics of the pathophysiology of critical illness, they are no more than the sum of their individual parts, and they fail in three broad areas: (1) to represent dynamic changes (i.e., changes over time in response to multiple influences) appropriately, (2) to weigh the relative importance of separate components, and (3) to capture properties of an organ system that emerge from the integrated behavior of multiple separate components.

Accordingly, we created a set of rules, written in the language of differential equations, to encompass these interactions. This is the first model that unifies elements of the inflammatory and physiological response to infection, trauma, and hemorrhage and that has been validated and calibrated in both rodents and humans. Although the model does and will incorporate many known mediators and pathways, there are undoubtedly mediators and pathways that are unknown that likely influence the outcome from shock or interact with known variables in the model. The model accounts for some of these unknowns, and many may be incorporated in the future once their interactions are known. Our modeling platform is also capable of incorporating treatments such as antibiotics and immunomodulatory agents (e.g., neutralizing anti-TNF antibodies) (25). This process will yield a model that will serve to generate hypotheses regarding the propagation of inflammation, streamlining animal work and possibly yielding insights into novel therapies through simulated clinical trials to test sepsis/trauma therapeutics (19, 25).

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