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Deciphering the Complexity of Acute Inflammation Using Mathematical Models

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Abstract

Various stresses elicit an acute, complex inflammatory response, leading to healing but sometimes also to organ dysfunction and death. We constructed both equation-based models (EBM) and agent-based models (ABM) of various degrees of granularity—which encompass the dynamics of relevant cells, cytokines, and the resulting global tissue dysfunction—in order to begin to unravel these inflammatory interactions. The EBMs describe and predict various features of septic shock and trauma/hemorrhage (including the response to anthrax, preconditioning phenomena, and irreversible hemorrhage) and were used to simulate anti-inflammatory strategies in clinical trials. The ABMs that describe the interrelationship between inflammation and wound healing yielded insights into intestinal healing in necrotizing enterocolitis, vocal fold healing during phonotrauma, and skin healing in the setting of diabetic foot ulcers. Modeling may help in understanding the complex interactions among the components of inflammation and response to stress, and therefore aid in the development of novel therapies and diagnostics.

Key Words

Inflammation
Mathematical model
Sepsis
Trauma
Wound healing

In Silico Approaches to Inflammation in the Era of Interdisciplinary, Translational Research

An important component of the body's initial response to stress such as bacterial infection or tissue trauma is the *acute inflammatory response.* This response is characterized on a systemic level by clinical signs such as fever, which contribute to optimizing the various defense mechanisms involved. While the normal inflammatory response is critically important in maintaining and restoring health under external stress, dysregulated inflammation can be destructive to healthy tissue. This tissue damage can result in a feed-forward

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cycle that stimulates further inflammation *(1)*. Resolution of the inflammatory response is necessary for proper tissue healing *(2)*.

Systems biology approaches *(3)* may offer a solution to understanding these interactions. For addressing complex biological processes such as the acute inflammatory response in sepsis and trauma *(4)*, both the NIH in its Roadmap Initiative (http://nihroadmap.nih.gov/) and the FDA in its "critical path" document *(5)* have called for the use of *in silico* (computer) models to augment preclinical animal studies in order to develop novel therapies. We *(4)* and others *(6)* have initiated multidisciplinary modeling teams to study inflammation in the settings of sepsis, trauma, hemorrhage, and wound healing, and have helped found the Society for Complexity in Acute Illness (www.scai-med.org). In our studies, we used both equation-based models (EBM) and agent-based models (ABM). Below, we describe various aspects of this multifaceted approach.

Modeling Inflammation and Organ Dysfunction in Sepsis

We first set out to create a series of reduced EBM of inflammation that would be amenable to formal mathematical analysis, in order to gain insight into the basic process of inflammation. In these models, an infectious agent triggers early pro-inflammatory responses in order to kill the pathogen, followed by later inflammatory mediators that further exacerbate inflammation *(7)*. These models are capable of simulating various states known to occur in septic patients; analysis of these models suggested different therapeutic approaches for these diverse scenarios (e.g., inhibiting late pro-inflammatory mediators such as HMGB1 for persistent, non-infectious inflammation while conversely suggesting pro-inflammatory therapies in the case of infections with slow-growing pathogens) *(7)*.

We next examined the anti-inflammatory response in acute inflammation *(8)*. While anti-inflammation inhibits the subsequent build-up of pro-inflammation and the damage to tissue that may be caused by pro-inflammation, this response also mitigates the subsequent production of anti-inflammatory mediators. An augmentation of our previous EBM to include anti-inflammatory influences illustrates the health advantage conferred by a dynamic anti-inflammatory response and suggests that the rates of this response might be modified therapeutically to yield optimal outcomes following pathogenic infection *(8)*.

Experimental observations demonstrate that the inflammatory response induced by bacterial lipopolysaccharide (LPS; endotoxin) can be either blunted (tolerance) or augmented (potentiation) with repeated administration of endotoxin. Both of these preconditioning phenomena are of clinical relevance. We demonstrated that a related four-dimensional EBM of this response *(9)* reproduces many scenarios involving repeated endotoxin administration, including both tolerance and potentiation, from a single parameter set under different administration protocols. The key determinants of the outcome of our simulations are the relative timescales of model components *(9)*.

In parallel to the work described above, we sought to obtain quantitative predictions by creating a more realistic EBM and calibrating it to experimental data in mice (endotoxemia, surgical trauma, and surgical trauma followed by hemorrhage). The model was able to predict doses at which mice would die, despite being calibrated on data from sublethal insults *(10)*. The model was further validated by testing quantitative predictions of cytokine and NO_2^-/NO_3^- levels in preconditioning regimens in mice (endotoxin tolerance as well as hemorrhagic shock followed by endotoxin),

similar to that described above *(9)* albeit with quantitative predictions as to circulating cytokine levels at various time points (Lagoa et al., manuscript in preparation). We also calibrated this model to simulate acute inflammation in rats (Lagoa et al., manuscript in preparation), swine *(11),* and humans (using data on experimental endotoxemia in human volunteers obtained from Dr. Anthony Suffredini, Critical Care Branch, National Institutes of Health, Bethesda, MD) *(12).*

We have augmented these models in various aspects. Matrix metalloproteinases (MMPs) are a family of proteases participating in extracellular matrix (ECM) degradation and remodeling during wound healing, angiogenesis, tumor metastasis, and many inflammation-associated diseases, including sepsis. We created an EBM that describes the interrelationships among MMPs and the tissue inhibitors of metalloproteases (TIMPs), and incorporated these data into our mathematical model of inflammation *(11).* We incorporated literature data regarding the production of TNF and IL-6 in swine undergoing treatment with LPS. Figure 1 depicts the output of our mathematical model of inflammation in swine, including the interrelated effects of cytokines (TNF and IL-6) and MMP-2 *(11).*

Clinical trial simulations represent another useful application of inflammation simulations, with the potential to greatly streamline the design and execution of randomized, placebo-controlled clinical trials, ultimately leading to personalized medicine *(4,13,14).* Using a different EBM, we carried out simulated clinical trials of a prototypical failed anti-sepsis therapy that had a promising preclinical profile, namely, anti-TNF neutralizing antibodies *(15).* We initially created a population of simulated individuals that differed in initial pathogen load, antibiotics, time of "admission" of the virtual patient, and genetic variability (i.e., relevant cytokine gene polymorphisms, implemented as ranges of values of relevant parameters in our model). This virtual patient population was "cloned" and subjected to simulated anti-TNF treatment at various doses initiated at various times and lasting for various time periods. In addition to reproducing the basic outcome of these trials, a key finding was that

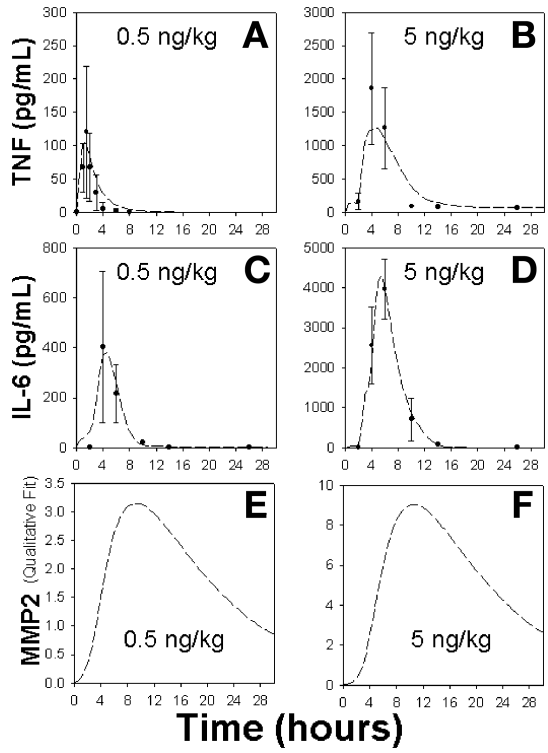

Fig. 1. Mathematical model of porcine endotoxemia. The mathematical simulation of acute inflammation was calibrated to literature data relevant to endotoxemia in swine. Simulations were matched to data for pigs challenged with 0.5 or 5 ng/kg LPS iv. Note that this simulation includes the effects of surgical trauma, because this is how the experiment was performed. Also shown are qualitative simulations of the dynamics of MMP2, based on literature data regarding the influences of inflammatory mediators present in the simulation (although experimental data at these doses were not available). Symbols represent digitized data from published studies. Dashed lines indicate model output.

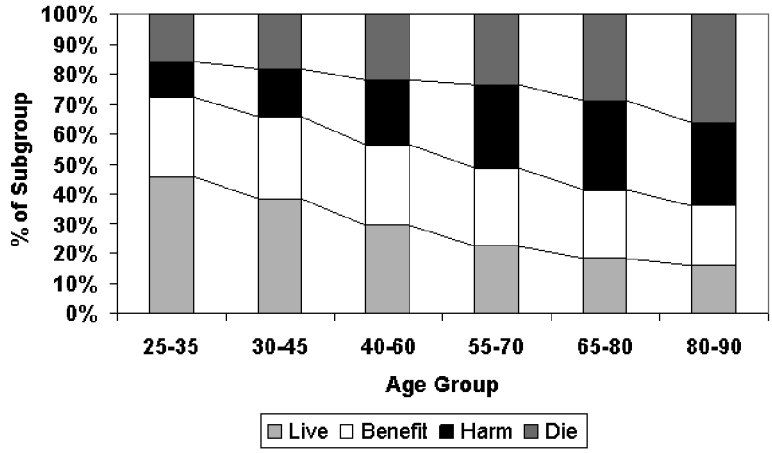

Fig. 2. Simulation of an interventional trial in sepsis, incorporating the effects of aging. A simulation of anti-TNF therapy in sepsis *(13)* was augmented to include the following effects of aging *(16):* (1) killing capacity of macrophages and neutrophils decrease with age and respiratory burst also decreases; (2) the death rates of the resting and active macrophages increase with age to reflect decrease in the macrophage precursors and impaired macrophage chemotactic response; (3) production of reactive nitrogen intermediates decreases with age; (4) superoxide production by neutrophils and macrophages decreases with age; and (5) TNF and IL-6 production by activated macrophages decreases with age. For simplicity, these changes were assumed to increase in magnitude linearly with age. Simulated patient cohorts consisted of 10,000 individuals each, essentially as described *(13).* Indicated are those virtual patients predicted to live or die regardless of therapy, as well as those predicted to either benefit or be harmed by the therapy (as compared to the placebo).

anti-TNF treatment helps a significant percentage of individuals, but also harms many *(13).* We augmented this simulation of anti-TNF therapy in sepsis to include some of the known effects of aging on innate immunity *(16),* including alterations in various aspects of macrophages and neutrophils. Although none of the aging-associated changes listed above could by itself be considered to have intrinsically beneficial or detrimental effects in sepsis, as a whole the simulation concurred with the literature *(17)* by showing that elderly patients would have a greater placebo mortality rate (Fig. 2). Moreover, anti-TNF was found to cause more harm as the age of the simulated patients increased, and thus the overall efficacy of this therapy was lower with increasing age *(18).* We incorporated clinical data, examining realistic age distributions by utilizing de-identified demographic data from three hospitals within the University of Pittsburgh Medical Center Health System. Despite similar mean age and simulated placebo mortality, only one hospital was predicted to show a statistically significant survival advantage for anti-TNF vs placebo (Hospital B, 6.1%; Table 1). The survival advantage in this simulation is identical to that reported for the only FDA-approved immunomodulatory therapy for sepsis, recombinant activated protein C (Xigris™, Eli Lilly and Company) *(19).* This simulation suggests that the mean age is not the critical component, but rather it is the age distribution about the mean that determines this difference in survival advantage; modeling approaches such as this one may suggest other non-intuitive hypotheses.

Table 1. Simulations of Age-Related Effects on Survival in an anti-TNF Sepsis Clinical Trial

Hospital	Mean age (yr)	Simulated placebo morality (%)	Simulated treated morality (%)	Treatment delta (Treated – Placebo)(%)
A	61.5	40.2	36.5	3.6
B	58.9	40.2	34	6.1
C	56.9	39.1	33.7	5.4

A simulated clinical trial of anti-TNF in sepsis was carried out essentially as described *(13),* except that cohort size was 10,000 per treatment arm and that aging was incorporated into the simulation. Aging effects were modeled by altering the effectiveness of macrophages and neutrophils with respect to killing capacity; production of superoxide, TNF, and IL-6; and macrophage and neutrophil half-lives. Hospitals A, B, and C are actual hospitals in the University of Pittsburgh Medical Center System, whose de-identified patient data were analyzed for mean age as well as actual age distribution and used for prediction of therapeutic efficacy of anti-TNF.

Modeling Inflammation and Organ Dysfunction in Trauma/Hemorrhage

Our large inflammation EBM *(10)* predicted fairly similar degrees of tissue damage/dysfunction in surgical cannulation (sham) trauma alone vs cannulation followed by hemorrhagic shock in mice, suggesting that similar pathways of different magnitude were operant as the degree of total body damage increased. We sought to validate this hypothesis by examining the global response of the liver, a central organ in the pathophysiology of acute inflammation in general and in trauma/hemorrhage in particular, by interrogating the hepatic transcriptome of mice subjected to these stresses using DNA microarrays. In agreement with the model prediction, our microarray analysis demonstrated that cannulation alone resulted in a substantial proportion of the observed hepatic gene/pathways changes when compared to control animals. The addition of hemorrhage further increased the magnitude of gene expression, but relatively few additional genes were recruited, suggesting that insults of different magnitude seem to evoke similar genetic and molecular pathways by the host *(20)*.

We next sought to utilize this model to address controversies *(3)* in acute inflammation. Although some studies support this notion, both animal and clinical studies have failed to implicate LPS or bacterial translocation in trauma/hemorrhage-induced inflammation. We brought to bear on this controversy a combination of *in silico* and in vivo approaches. We first recalibrated our mathematical model using inflammatory analytes data obtained in mice that are genetically different in their response to LPS from their wild-type counterparts ($CD14^{-/-}$ mice). Rather than generating a single recalibrated model parameter set, we created automatically an ensemble of models that fit data from $CD14^{-/-}$ mice; this practice is used extensively in the field of weather forecasting to increase the accuracy of forecasts *(21)*. Our $CD14^{-/-}$-specific ensemble of models was able to both fit the data obtained in these mice and predict that damage/dysfunction would be no different in $CD14^{-/-}$ mice as compared to wild-type mice subjected to trauma/hemorrhage. These predictions were borne out in vivo, suggesting that LPS does not mediate inflammation via the classical CD14–TLR4 pathway in trauma/hemorrhage *(22)*.

We hypothesized that mathematical modeling could contribute to understanding the relative importance of not only inflammation but also inadequate or delayed restoration of

homeostasis, i.e., resuscitation in the setting of severe hemorrhagic shock. In addition to the severity of the blood loss, the length of the interval between injury and restoration of homeostasis through fluid resuscitation may contribute to the progression to irreversible hemorrhagic shock. We therefore examined the characteristics of reversible and irreversible hemorrhagic shock using a yet larger EBM that includes elements of the adaptive immune response (Lagoa et al., manuscript in preparation; Torres et al., manuscript in preparation), with the goal of defining early predictors of late organ dysfunction and death. Qualitatively, our model demonstrated that within 1 mmHg of simulated hemorrhage, the organism would either live following this procedure, or die at approx 3–5 d, thereby defining the reversibility and irreversibility of shock, respectively. Our simulations suggested that circulating inflammatory analytes would not be statistically predictive of later mortality, when assessed at the time point at which the simulated hemorrhagic shock was predicted to be irreversible. Thus, our mathematical model appears to exhibit emergent properties (reversible vs irreversible hemorrhage), and may be of use in exploring the features of irreversible hemorrhagic shock, as well as guiding diagnosis or therapy (Torres et al., manuscript in preparation). We are currently also modeling the effects of the cardiovascular system and its compensatory control on the syndrome of severe hemorrhage-induced circulatory collapse. Eventually, we aim to decipher possible links among cardiovascular decompensation, early inflammation, and mortality (Zenker et al., manuscript in preparation).

Modeling Inflammation and Wound Healing

We have created an ABM to address the interrelationship between inflammation and healing, and have calibrated it in different instantiations to address healing in several contexts. In the first case, we simulated the dynamics of the vocal fold inflammatory and wound healing in responses to the biomechanical stresses associated with phonotrauma (Li et al., submitted). Our model, calibrated with human and animal data, reproduced the basic behavior of the inflammatory and wound healing responses expected in phonotrauma. Moreover, the model predicted that the long-term outcomes of wound healing outcomes would be substantially different given small changes in the initial magnitude of damage to the tissue. The model's results were consistent with recent in vitro and human data suggesting that large-amplitude/low-impact vocal fold tissue mobilization may actually reduce inflammation following acute phonotrauma.

Inflammation and wound healing are deranged in the setting of chronic, non-healing diabetic foot ulcers (DFU). An ideal therapy for DFU should suppress excessive inflammation while enhancing the production of cytokines that enhance healing. We calibrated our inflammation/healing ABM with literature data on skin wound healing, including changes in cytokines such as TNF and transforming growth factor-β1 (TGF-β1). We utilized this model to examine the genesis of DFU, given that diabetes and DFU have been associated both with increased TNF and with decreased TGF-β1 (Qi et al., manuscript in preparation).

We have also created an ABM and two different EBM in order to examine inflammation and healing in the setting of necrotizing enterocolitis (NEC), a severe inflammatory disease of the newborn. Damage to the inflamed intestine leads to a disruption in the normally impermeant enterocyte monolayer. This disruption leaves the host susceptible to the translocation of pathogenic microbes from the intestinal lumen into the systemic circulation,

often leading to systemic inflammation and organ dysfunction *(23).* We have modeled both the localized inflammation/healing process and the systemic inflammation components of NEC. Healing of the inflamed intestine—and reversal of the pro-inflammatory cascade—occurs through the process of *intestinal restitution,* which involves the migration of healthy enterocytes to sites of mucosal disruption *(23).* Using live-cell, high-resolution video microscopy, we have shown that the process of enterocyte migration into the inflamed/injured intestine is thwarted after exposure to high levels of LPS (as occurs during NEC) *(24,25).* We have created an ABM of the intestinal restitution process that simulates this effect of LPS, and have correlated this process with measures of cell–matrix adhesiveness (Mi et al., manuscript in preparation). We have also begun to collect serial systemic inflammation biomarker data from a well-established neonatal rat hypoxia/formula-feeding model of NEC in order to calibrate our existing inflammation EBM (Upperman et al., manuscript in preparation).

We have also developed an EBM for the migration and proliferation of enterocytes, based on a novel assumption of elastic deformation of the cell layer and incorporating (i) a motility-promoting force due to lamellipod formation, (ii) a motility-impeding friction due to the adhesion to the cell matrix, and (iii) enterocyte proliferation. Our model successfully reproduces the behavior observed for enterocyte migration on glass coverslips, namely the dependence of migration speed on the distance from the wound edge and the finite propagation distance in the absence of proliferation, which results in an occasional failure to close the wound (Mi et al., manuscript in preparation).

We have extended the above models to include spatial effects, such as diffusion of inflammatory agents, chemotaxis, and cell migration in NEC (Sullivan et al., submitted) This EBM is comprised of four compartments—lumen, epithelial layer, organ tissue, and blood. The model accounts for the different material characteristics by assigning specific diffusion parameters to the four compartments. The ability of lumen pathogen to infiltrate the organ tissue is affected by the integrity of the epithelial wall. The latter depends on factors such as cell migration and strength of tight junctions, which in turn are affected by the inflammatory process. Our results show that even normally harmless bacteria in the lumen can lead to serious infection, even sepsis, if the epithelial wall is damaged due to stress or other factors. The ability of a damaged wall to heal depends on the level of initial infection through the amount of LPS present in the system. The ability of the pathogen to diffuse through the organ tissue may also affect the outcome of the inflammatory process (Sullivan et al., submitted).

Conclusions and Future Prospects

In conclusion, we have created a set of models to encompass key interactions of acute inflammation. We continue to explore the utility of this *in silico* approach. For example, we have embarked on studies to improve an existing hemoadsorption device using a combination of mathematical models of the device design and our inflammation model to guide an iterative improvement process (Kellum et al., unpublished observations). We have utilized the same ensemble modeling methodology described above for $CD14^{-/-}$ mice to examine the changes that accompany inflammation in aging mice, as well as to examine the mechanism of action of novel anti-inflammatory agents (Vodovotz et al., unpublished observations). We have demonstrated several distinct uses and benefits of

our combined *in silico*/in vivo approach, which should continue to yield insights into the biology of inflammation.

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