Mathematical Simulations of Sepsis and Trauma

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Disclosure: *This author is a co-founder of and consultant to Immunetrics, Inc., which has licensed from the University of Pittsburgh the mathematical model of acute inflammation described in this manuscript*

Abbreviations

ELISA, enzyme-linked immunosorbent assay; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, bacterial lipopolysaccharide (endotoxin); NO, nitric oxide; TNF, tumor necrosis factor-α

Acknowledgments

The authors would like to acknowledge the contributions to this work of several excellent technicians (Derek Barclay, David Gallo, and Binnie Betten). This work was supported by National Institutes of Health grants R01-GM-67240-02 (CCC, GC, YV), and P50-GM-53789-08 (TRB, MPF, YV, GC, JR) and a grant from the Pittsburgh Lifesciences Greenhouse (YV, JB).

Summary

Sepsis and trauma elicit an acute, complex inflammatory response, leading to organ dysfunction and death. We constructed mathematical models of increasing complexity, using differential equations that encompass the dynamics of relevant cells and cytokines as well as the resulting global tissue dysfunction, in order to begin to unravel these inflammatory interactions. The simplest model, consisting solely of a pathogen, a single population of inflammatory cells, and a measure of global tissue damage/dysfunction could describe both recoverable infection and septic shock. A more complex model was used to create simulated populations of septic patients and to simulate population responses to anthrax in the presence or absence of vaccination. The most

complex model was calibrated in various inflammatory scenarios in mice and was able to predict responses to combinations of insults on which it was not trained. Mathematical modeling provides insights into the complex dynamics of acute inflammation and organ dysfunction in sepsis and trauma, and may help in the development of novel therapies and diagnostic strategies.

Introduction

The initial response of the body to 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 clinical signs 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 additional 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] It is therefore not surprising that therapies that modulate inflammation in sepsis and trauma have largely failed clinically.[2]

Interdisciplinary Approach to Modeling Inflammation

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 biologic pathways,[3] Given the complexity described above and the need to examine distinct pathways as part of a whole, we and others have initiated systems approaches to this problem[4-6] As such, we have concentrated our efforts on forming multidisciplinary teams consisting of clinicians, biologists, mathematicians, and computer scientists to approach and solve the seemingly intractable dilemma of sepsis and trauma.[7] Our team is carrying out an iterative program of model generation, verification and calibration in both mice and humans, and subsequent hypothesis generation and testing. While An has previously developed an agent-based model of inflammation and organ dysfunction in sepsis,[6, 8, 9] we chose to create our model using a set of ordinary differential equations, an approach that allows for the possibility of mathematical analysis of the behavior of a complex system. We sought to create a series of mathematical models of various degrees of realism (detail), in order to address both funda-

mental, global issues relating to the pathology of sepsis and trauma, as well as directly practical issues of streamlining experiments, deriving therapies, and simulating clinical trials.[6, 10, 11] In so doing, we have also considered general questions relating to the use of statistics to examine goodness-of-fit of large mathematical models such as ours.[10]

Methods and Results

Small Models, Big Questions

We first set out to create a reduced model of inflammation that would be amenable to formal mathematical analysis.[12] In this initial foray into modeling inflammation, we considered a simplified picture of the acute inflammatory response, as follows. An infectious agent/microorganism triggers early pro-inflammatory responders that attempt to kill the pathogen. The early inflammatory mediators then activate later inflammatory mediators, which can further excite the early mediators. Thus, this model consists of three variables: (1) a pathogen (p), which is an instigator of the innate immune response; (2) an early pro-inflammatory mediator (m), which can be thought of as representing the combined effects of immune cells such as macrophages and neutrophils together with early pro-inflammatory mediators such as TNF and IL-1; (3) a late pro-inflammatory mediator (l) that represents a combined effect of cytokines such as IL-6 and HMGB1 together with the stimulatory effects of tissue damage/dysfunction. This model is capable of simulating 1) a healthy response to infection, in which the pathogen is then completely cleared and the inflammatory response subsides; 2) persistent non-infectious inflammation, a state in which even though the pathogen is cleared, the inflammatory response does not abate; 3) persistent infectious inflammation, in which the inflammatory response is high but the pathogen still cannot be cleared (i.e., severe septic shock). Under some settings, we could simulate recurrent infection. This latter scenario could be likened to infection with tuberculosis, yeast infections or low-grade bacterial infections in which low levels or concealed pathogens fail to trigger an immune response, are not eradicated, and reemerge after a time of apparent quiescence. Finally, the model could also simulate a state of immunosuppression or immunodeficiency, in which the pathogen grows to saturation and does not elicit any response from the body, as would be seen in patients that have already been immunocompromised by previous infection or trauma. Using this very simple model, we suggest that very different therapeutic strategies are called for to deal with the diverse negative outcomes. For example, any therapy for persistent noninfectious or post-infectious inflammation should target the late-phase pro-inflammatory mediators (e.g., IL-6, HMGB1).

Alternatively, if the patient is suffering from persistent infectious inflammation secondary to infection, then therapies must be aimed at both reducing the pathogen load and the late pro-inflammatory response. Conversely, immunostimulatory therapy might be effective in situations of recurring or persistent, low-level infections. These predictions are in line with expectations expressed in the literature.[13] A slightly larger version of this model, consisting of LPS, an early inflammatory cell population (with its attendant cytokines), a late anti-inflammatory cytokine (e.g., TGF-β1), and late damage/dysfunction (e.g., HMGB1[14, 15]) was able to simulate both priming and desensitization phenomena when we simulated repeated LPS administration (depending on the exact timing of the LPS doses; J. Day *et al*, manuscript in preparation). Using a similar approach, we have constructed a model that describes epithelial barrier disruption and ensuing bacterial translocation, such as happens in necrotizing enterocolitis[16] or possibly following hemorrhagic shock.[17] Using this model, we can obtain situations of return to health or persistent inflammation. (J. Rubin, B. Ermentrout, B. Riviere, I. Yotov, G. Clermont, and Y. Vodovotz, manuscript in preparation; Fig. 1). Future

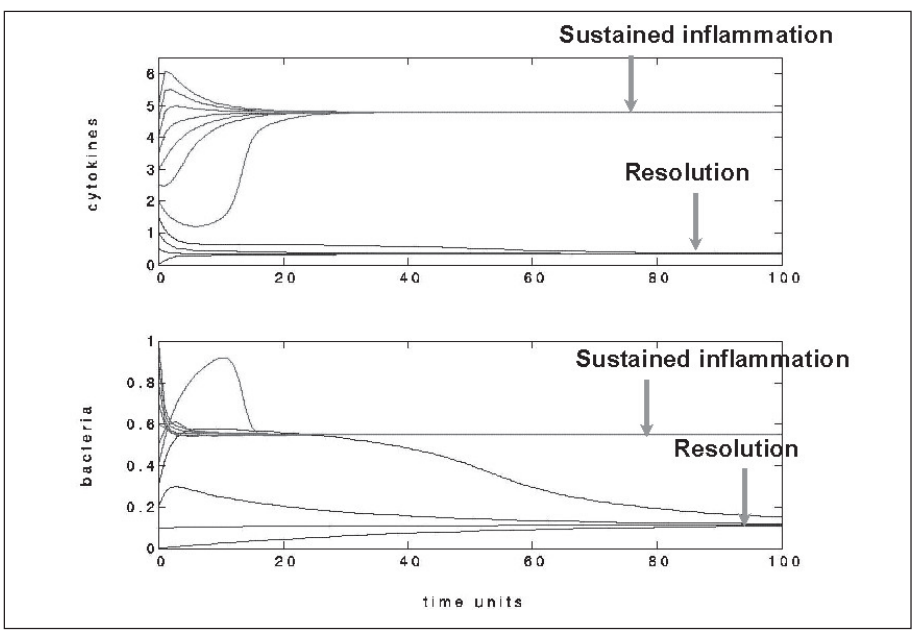

Fig. 1. Output from a simulation of inflammation and gut dysfunction following bacterial translocation. The model consists of bacteria, cytokines, and an epithelial barrier. The various curves represent different times courses (in abstract time units) generated by different starting conditions for the model, and show a low (healthy) and a high (inflamed) fixed point.

work will use this mathematical model to obtain insights into pathophysiological mechanisms and possible therapeutic strategies.

Simulating Clinical Trials and Population Studies Using a Qualitative Model of Inflammation

Seeking to increase the utility of our modeling approach beyond basic insights into sepsis, we created a larger model. The differential equations that make up our mathematical model represent the kinetics of well-accepted constituents of the inflammatory response, including macrophages, neutrophils, early and late pro- and anti-inflammatory cytokines, and effectors such as nitric oxide and superoxide radical anion. Importantly, we incorporated elements of coagulation and the concept of global tissue damage/dysfunction as in our three-variable model; this variable is both a marker of the adverse effects of inflammation as well as a driver of further inflammation. Thus, damage/dysfunction in our model approximates the release of "danger signals" such as HMGB1.[14, 15] Nonetheless, elements are still either totally lacking in the model, for example dendritic cells and T cells. Moreover, not all of the components are calibrated to actual data; these deficiencies are being addressed in our most comprehensive model (see below). Nonetheless, this qualitative model has proven useful for demonstrating the same basic states of sepsis found in our three-variable model and for simulating clinical trials in sepsis.[11] Using a random number-generating algorithm to modify a limited number of parameters in the model (e.g., capacity to produce TNF, NO, IL-6, etc. or other mediators in response to infection, thereby essentially allowing for genetic variability such as that derived from single nucleotide polymorphisms) as well as bacterial burden, we created several identical populations of 1,000 virtual patients. These virtual patients were further randomized to receive placebo or one of several doses of anti-TNF antibodies for various durations of treatment. We chose to simulate this therapy because it exhibited an excellent pre-clinical, Phase I, and Phase II profile, yet essentially failed to demonstrate efficacy in Phase III trials.[18] In our simulations, we equated persistent damage/dysfunction with death, while damage/dysfunction that returned to baseline was a surrogate for survival. Our simulations suggested that some patients receiving anti-TNF would benefit from the therapy, while some would actually be hurt; many would be neither helped nor hurt by the therapy.

We modified the properties of the invading bacteria in the model to those specific to *Bacillus anthracis* a potential bioterror organism that induces a sepsis-like inflammatory process that can lead to death following inhalational exposure.[19] In our model, we included the anthrax products lethal factor (LF), edema factor (EF), and protective antigen (PA), which dimerize in various combinations (LF + PA = Lethal Toxin;

EF + PA = edema toxin), and then simulated the host response to anthrax infection. We also simulated treatment strategies against anthrax, including 1) antibiotic treatment initiated at various time points; 2) vaccination, which induces anti-PA antibodies; and 3) a combination of antibiotics and vaccine. In agreement with studies in mice, our simulations showed that antibiotics only improve survival if administered early in the course of anthrax infection. Vaccination is anti-inflammatory and beneficial in averting shock and improving survival. However, antibodies to protective antigen alone cannot universally protect from anthrax infection; rather, an optimal strategy would require both vaccination and antibiotic administration (Kumar *et al*, submitted).

The Devil is in the Details: Towards a Large, Unified Model of Acute Inflammation

We next sought to add elements of adaptive immunity to our model, as well as calibrating it to actual data. Accordingly, we augmented the model described in the previous section to include additional cytokines, dendritic cells, and T_H1 and T_H2 cells.[6, 10, 20] Simultaneous numerical solution of the equations of this general model generates predictions of the time courses of these elements. 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, and life spans of cells. 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. *However, unlike regression fitting, we used a single set of equations and values for the parameters in those equations, changing only the starting conditions, to account for all inflammatory scenarios.*

In order to test the ability of our mathematical model to predict inflammation in settings on which it had not been trained explicitly, we simulated the response to hemorrhagic shock (2.5 h at a mean arterial pressure of 25-30 mmHg, followed by 3 mg/kg LPS administered at 3 h from the initiation of the experiment). We also examined the response to repeated administration of 3 mg/kg LPS (doses separated by 24 h, a desensitization paradigm). Like the smaller mathematical model described above, this larger model was also capable of simulating this scenario; moreover, it predicted the levels of cytokines and NO reaction products fairly accurately (Fig. 2; Lagoa *et al*, manuscript in preparation). We

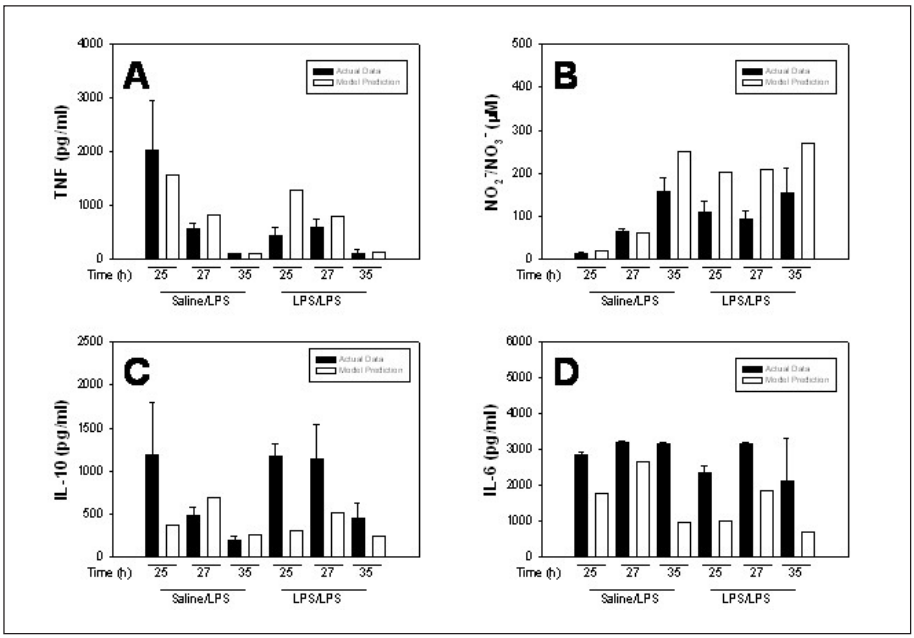

Fig. 2. Prediction of the inflammatory response to repeated administration of LPS in mice. Mice were subjected to 3 mg/kg LPS at 0h and at 24h, vs. saline at 0h and LPS at 24h. Cytokines were measured using specific ELISA's (R&D Systems, Minneapolis, MN). NO_2^-/NO_3^- was measured using the nitrate reductase method (Cayman Chemical, Ann Arbor, MI). Black bars represent mean ± SD for 3-4 separate animals per time point. White bars indicate prediction of mathematical model.

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 representations of certain mechanisms or interactions.

We have adapted the above model to simulate acute inflammation in rats (Lagoa *et al*, manuscript in preparation), swine (Y. Vodovotz, J. Wei, J. Bartels, A. Baratt, and G. Clermont, unpublished observation), and humans (using data obtained from Dr. Anthony Suffredini, Critical Care Branch, National Institutes of Health, Bethesda, MD).[20] We emphasize that these models in species other than mouse are still preliminary and require substantial additional calibration and validation studies. However, we have noted more similarities than differences among species with respect to the overall dynamics of evolution of inflammation and the scatter obtained in the data when comparing responses to a similar stimulus (e.g. LPS).

Conclusions

In conclusion, we have created a set of rules, written in the language of differential equations, to encompass these interactions among key components of the early inflammatory response. This is the first model that unifies mechanisms of the inflammatory and physiologic responses to infection, trauma, and hemorrhage and that has been validated and calibrated in both rodents and humans. Though 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 an iterative process of model creation, calibration, and validation. 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.

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