Yoram Vodovotz
Gary An *Editors*

Complex Systems and Computational Biology Approaches to Acute Inflammation

A Framework for Model-based Precision Medicine

Second Edition

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Preface

We are now standing at the cusp of systems-based understanding of the processes that underlie, drive, and control the acute inflammatory response. Based on decades of painstaking progress, mostly driven by the reductionist methods that have served science well for hundreds of years, this new systems approach is poised to redefine how we approach diagnosis and therapy for sepsis/infection, trauma, and wound healing. The development of systems and computational biology over the past decade has brought a new understanding, new methods, and new terminology to the forefront of the biomedical enterprise.

These new tools and new understanding have begun to yield insights into basic biological mechanisms. What has lagged has been a clear understanding of how systems and computational biology could be applied systematically to change clinical practice. Indeed, we have not yet fully leveraged the power of computational and systems biology. There is currently no fully rational, computationally driven, pipeline for drug discovery, clinical trials, "smart" diagnostics, and patient-specific therapy driven by computational modeling (i.e., Translational Systems Biology). However, we are at, or near, an inflection point in this transition from reductionism to systems approaches applied clinically. Translational Systems Biology as a concept was formulated in an attempt to give initial definitions and directions to the biomedical community. A decade of Translational Systems Biology has resulted in increasingly realistic computational models that can recapitulate inflammation at the cellular, small animal, large animal, and human levels. As such, Translational Systems Biology is at an inflection point between early studies subjected to intense scrutiny and, to a degree, resistance from the research community, vs. widespread adoption.

This book presents a snapshot of this inflection point. In this book, we present the current state of Translational Systems Biology, including overviews of the inflammatory response in trauma, sepsis, infectious disease (focusing on malaria), and wound healing. We also present overviews of relevant computational methods, and

how these methods have been and are being used to gain novel—and hopefully clinically useful—insights into these diseases. This book will hopefully leave the reader with a clear idea of the background and motivation for these systems approaches, and also for what must yet be done in order to fully realize the vision of Translational Systems Biology.

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About the Editors

Gary An is a Professor of Surgery and Vice-Chairman for Surgical Research in the Department of Surgery at the University of Vermont Larner College of Medicine. He is a founding member of the Society for Complex Acute Illness and past president of the Swarm Development Group, one of the original organizations promoting the use of agent-based modeling for scientific investigation. In addition to being an active trauma/critical care surgeon, he has worked on the application of complex systems analysis to sepsis and inflammation since 1999 and consists of development of mechanism-based computer simulations and integration of machine learning and artificial intelligence with multiscale simulation models for discovery of therapeutic control modalities.

Yoram Vodovotz is currently a Professor of Surgery, Immunology, Computational and Systems Biology, Bioengineering, Clinical and Translational Science, and Communication Science and Disorders at the University of Pittsburgh School of Medicine. His research focus is the systems biology of inflammation. He is the Director of the Center for Inflammation and Regeneration Modeling at the McGowan Institute for Regenerative Medicine, a cofounder and past President of the Society for Complex Acute Illness, and a cofounder of Immunetrics, Inc., a Pittsburgh-based company that is commercializing this mathematical modeling work. He has published over 300 manuscripts, including three books.

Part I
Overview

Chapter 1
An Overview of the Translational Dilemma and the Need for Model-Based Precision Medicine

Yoram Vodovotz and Gary An

Introduction

The greatest challenge for the biomedical research community is the effective translation of basic mechanistic knowledge into clinically effective therapeutics, most apparent in attempts to understand and modulate "systems" processes/disorders, such as sepsis, cancer, and wound healing. Over 15 years ago, a United States Food and Drug Administration report titled "Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products" (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html) clearly delineated the increasing expenditure on research and development concurrent with a progressive decrease in delivery of medical products to market. This was, and remains, the *Translational Dilemma* that faces biomedical research and necessitates a re-assessment of the scientific process as an initial step toward identifying where and how the process can be augmented by technology [1, 2].

This book is focused on systems approaches to understanding and potentially controlling the acute inflammatory response. It is now beyond doubt that inflammation, with its manifold manifestations at the molecular, cellular, tissue, organ, and whole-organism levels, drives outcomes following injury and infection, and can lead to diverse manifestations of chronic diseases such as rheumatoid arthritis, neurodegenerative diseases, the metabolic syndrome, and cancer [3]. Though properly regulated inflammation allows for timely recognition and effective reaction to injury

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or infection, acute inflammatory derangements such as those that accompany trauma/hemorrhage, sepsis, the wound healing response, and key aspects of host–pathogen interactions are manifestations of insufficient and/or disordered inflammation that in turn impairs physiological functions. It is critical to note that inflammation is not in and of itself detrimental. Well-regulated, self-resolving inflammation is necessary for the appropriate communication and resolution of infection and trauma, and for maintenance of proper physiology and homeostasis [3]. This paradox of a robust, evolutionarily conserved network of inflammation whose very structure may lead to disease [4–6] has resulted in its near ubiquitous involvement in those diseases that most dramatically manifest the Translational Dilemma [2]. Indeed, most evidence suggests that either insufficient [7] or self-sustaining [8–10] inflammation drives the pathobiology of trauma/hemorrhage, sepsis, inadequate or exaggerated wound healing, and a breakdown of appropriate host–pathogen responses. Over a decade ago, there was recognition of the complex interplay between inflammation and physiology in critical illness and of the need to apply complex systems approaches such as computational modeling to unravel this complexity [11–13]. The advent of "omics" methodologies, with the theoretical capability of interrogating the complete responses of cells and tissues, spurred the application of these methodologies to critical illness following injury or infection [14–21], wound healing [22, 23], and host–pathogen interactions [24–28].

It is now increasingly recognized that merely suppressing inflammation is an ineffective therapeutic strategy, and that controlling and reprogramming inflammation in order to reap the benefits of this evolutionarily conserved process is the future therapeutic paradigm. However, there is currently no rational, reductionism-based approach by which to accomplish this goal. In addition to the multiscale complexity inherent in its organizational structure, inflammation manifests very differently based on personalized factors. These factors include individual features of the initial inflammatory perturbation, the individual's demographic and disease histories (including genetic predispositions and setpoints/thresholds for inflammatory processes), and the impact of environment and clinical care. Recent studies suggest a key role for epigenetic reprogramming as a central mechanism underlying these individual predispositions to mount differential inflammatory responses [29]. We assert that mathematical modeling and computational biology can help decipher this multidimensional puzzle, and that, when geared toward practical applications, these methods hold the potential to transform the entire process of healthcare delivery from pre-clinical studies, through clinical trial design and implementation, to personalized diagnosis and therapy, and ultimately to long-term care.

Progress in Translational Systems Biology of Inflammation

We and others have suggested a rational, systems engineering-oriented, computationally based framework, Translational Systems Biology, for integrating data derived from basic biology experiments as well as pre-clinical and clinical studies,

and ultimately leading to rational inflammation reprogramming [30–34]. Translational Systems Biology involves using dynamic mathematical modeling based on mechanistic information generated in early stage and pre-clinical research to simulate higher-level behaviors at the organ and organism level, thus facilitating the translation of experimental data to the level of clinically relevant phenomena [2]. This book will introduce and demonstrate the Translational Systems Biology approach. Furthermore, we suggest that therapy for complex inflammatory diseases could be thought of as a model-based dynamic control problem, involving dynamic reprogramming of inflammation and associated processes. Together, these are the central features of Model-based Precision Medicine [34, 35], which includes our proposed Axioms of Precision Medicine:

Axiom 1:
Patient A is not the same as Patient B (Personalization).

Axiom 2:
Patient A at Time X is not the same as Patient A at Time Y (Precision).

Axiom 3:
The goal of medicine is to treat; prognosis is not enough (Treatment).

Axiom 4:
Precision medicine should find effective therapies for every patient and not only identify groups of patients who respond to medicine (Inclusiveness).

These axioms were developed to address the insufficiency of current approaches to "precision medicine." Axiom 1 highlights the importance of being able to individualize therapy, as opposed to the current approach of "precision medicine" that identifies subgroups of populations' responses to specific therapies. Axiom 2 recognizes that as a specific patient progresses through the course of a disease, they may require differential treatment at one phase versus another, and thus emphasizes the fact that diseases are *dynamic* processes. Axiom 3 points out that while diagnosis and prognosis are critical (see Axiom 2), the ultimate goal of medicine is to take a bad outcome and turn it into a good one (i.e., altering a disease trajectory to one of health and recovery). Axiom 4 corrects the tendency the current focus of "Precision Medicine" on identifying groups of responders but provides no clear path or recourse for patients for whom no effective therapy can be identified. We assert that any precision medicine project must incorporate at pathway that facilitates the discovery of new therapies that have not been tested or developed previously, thus providing hope for those left out in the current paradigm.

This book brings together leaders from the interdisciplinary field of inflammation modeling as well as thought leaders in in the fields of trauma/hemorrhage, sepsis, wound healing, and host–pathogen responses. This book is divided into five sections, covering recent progress in Translational Systems Biology as applied to disease states involving acute inflammation. This chapter comprises Part I and is intended as an overview of Translational Systems Biology and Model-based Precision Medicine. In Part II (*Computational Modeling Methods and Biomedical*

Applications), the relevant methods for computational modeling of inflammatory diseases are discussed. In Part III (*Translational Modeling of Sepsis and Trauma*), the relevant clinical and experimental features and challenges of systemic inflammation in trauma/hemorrhage and sepsis are detailed, along with recent progress in computational modeling of these diseases. Similarly, in Part IV (*Translational Modeling of Organ/Tissue Specific Inflammatory Disease Processes*), the relevant clinical and experimental features and challenges of specific inflammatory diseases are discussed, along with computational modeling studies in their respective fields. Finally, in Part V (*Future Perspectives: Translation to Implementation*), we discuss the challenges that remain in order to fully implement the vision of Translational Systems Biology of Inflammation and Model-based Precision Medicine.

As summarized in this book, in silico modeling has yielded both basic insights and translational applications in critical illness [4, 5, 31, 33, 36–40]. Indeed, key translational applications such as in silico clinical trials were pioneered in the arena of critical illness [41, 42]. Recent studies show the potential to predict the individual inflammatory and pathophysiologic outcomes of human subjects [43] and large, outbred animals [44] subjected to acute inflammatory stress. Such studies highlight the maturity of computational modeling in the clinical arena and suggest the possibility of predicting the outcomes of—and possibly tailoring therapy for—individual critically ill patients [33, 38, 45]. This work is detailed in Chaps. 2–4, 8, 12, and 15.

Early studies utilizing complex systems approaches in critical illness suggested the concept of "coupled oscillators" that become uncoupled as inflammation becomes dysregulated and organ dysfunction progresses [11], which has led to the explosion of studies on the use of physiological waveforms (e.g., those derived from heart rate or breathing pattern) to diagnose outcomes in sepsis and trauma/hemorrhage. This work will be reviewed in Chap. 7. More recent in silico modeling work has posed specific hypotheses with regard to the mechanisms by which inflammation is coupled nonlinearly to physiological (dys)function at multiple scales [4, 31, 37–42, 44, 46–56] (summarized in Chaps. 2–4, 7, and 8).

Challenges and Future Perspectives

As we detail in the final section of this book, many challenges remain for the field of Translational Systems Biology and especially as this field transitions toward Model-based Precision Medicine [34, 35]. As useful as mechanistic computational modeling has been in integrating known interactions gleaned from the literature, this approach is inherently biased, given the tremendous volume of information that could, in theory, be incorporated into models and that is deemed irrelevant or unnecessary for the degree of abstraction chosen by the modeler. In recent years, there has been an attempt to couple the less-biased data-driven approach with mechanistic mathematical modeling of the acute inflammatory response [5, 32, 33, 37–40]. In these studies, mechanistic computational simulations were created based on biology abstracted from "omics" data [55–60] or inferred from data-driven analysis of

principal drivers [44]. This type of combined data-driven and mechanistic modeling reflects the maturity of computational modeling in the context of complex diseases, and is likely to be the area of study with most growth in coming years due to the inherent appeal of unifying—and gaining testable mechanistic insights from—the growing repository of "omics" data.

At the most practical level, in silico modelers must also prove the translational benefit of this technology through prospective clinical studies and ultimately through the development of computationally based diagnostics or therapeutics. The central challenge in this field is to integrate the multiscale, multisystem nature of acute inflammation. Translational Systems Biology must therefore rise to the challenge of integrating inflammatory, neuro-endocrine, and physiologic processes in order to unravel the multidimensional, multicompartment, and highly dynamic landscape of trauma/hemorrhage, sepsis, wound healing, and host–pathogen interactions. As we describe in Chaps. 10 and 12, this is now beginning to occur (e.g., a recent Defense Advanced Research Projects Agency (DARPA) project aimed at creating a "smart bandage" that is being designed with the help of an agent-based model of wound healing). These are exciting developments, which, when coupled with the increasing emphasis on in silico clinical trials by international organizations such as the Society for Complex Acute Illness (www.scai-med.org), the Avicenna Alliance (https://avicenna-alliance.com/), and the Virtual Physiologic Human Institute (https://www.vph-institute.org/) give us much hope for the future of Translational Systems Biology and Model-based Precision Medicine.

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Part II
Computational Modeling Methods and Biomedical Applications

Chapter 2
Translational Equation-Based Modeling

Gilles Clermont

Biological systems are complex and evolving. Because of the intense network of interaction present, intuition often fails to predict the system-level impact of altering one of a few components of this system. At a preclinical level, gene knock-out mice often result in phenotypes that are more complex than a mouse with the inability to express a given gene. Rather, knock outs are more typically highly adapted survivors of this gene deletion, where other mechanisms have compensated for what is otherwise an important biological function. Yet, biological knowledge of isolated mechanisms, such as ligand–receptor dynamics, transcription-factor binding, second-messenger cascades, and myriad other cell, tissue, and organ-level interactions, has expanded immensely in the last few decades. Basic knowledge of causal links has improved, but tools to interpret and predict the integrated effect of the combined dynamics are limited in number.

A pragmatic approach, therefore, is to create a simplified representation of the system, a model, and to define rules that describe the presence, nature, and intensity of interactions present between components of this model. We focus on the use of differential equations as the mathematical implementation of this set of rules and describe the time evolution of the components included in the model. At a given point in time, the state of the system is described by the actual values of all components included in the model, a vector of real numbers. Solving the equations yields a description of the time evolution of the system. In other words, the solution of this system of equation describes the trajectory of the system as its state evolves in time. Once a computational model has been developed, it will be used to generate predictions and evaluate the potential effect of perturbing the system.

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Equation-Based Models of Biological Systems

Historical Perspective

Equation models of biological systems have been used for several decades, initially applied in ecology, infectious disease, and organ physiology. More recently, considerable efforts have been devoted to computational models of the immune response. Yet, the discipline of computational systems biology and the extensive use of models as a discovery tool in modern biology is less than two decades old [1]. Although computational models have been used for several decades in pharmacokinetics, their use as tools to enhance drug discovery and clinical trial simulation is less than a decade old [2, 3]. Therefore, computational models is just emerging as a potential tool in translational science [4–6], and in particular in personalizing treatments [7, 8]. The vast majority of these models have been implemented using differential equations as mathematical formalism, partly for historical and familiarity reasons, and also because methods to enhance the scalability and computational efficiency of alternative modeling methods are still very much in the process of being developed.

Types of Equation-Based Models

The simplest mathematical expression of is in the form of a difference equation $X(t+1) = f(X(t))$, where $X(t+1) = \{x_1(t+1), x_2(t+1), \ldots, x_n(t+1)\}$ and $X(t) = \{x_1(t), x_2(t), \ldots, x_n(t)\}$ are vectors representing the state of the system, that is counts of each of n components $\{x_1, x_2, \ldots, x_n\}$ at consecutive times t and $t+1$, and the function f embodies the underlying biology of interactions dictating the evolution of the system. Such models describe the evolution of the system in discrete time steps and are common in mathematical biology and chemical reactions. For example, in such systems, the state of the system is simply a series of numbers representing the number of individuals of different species in an ecosystem, or the number of molecules of each single chemical species in a network of chemical reactions. An important ecological problem involves modeling the population size of a given species, for example dividing cells or bacteria. The change in population size during the interval between these times is given by the following growth equation, also known as the logistic map $x(t+1) = rx(t)(1 - x(t))$, where $x(0)$ represents the initial population at time 0, r is a positive number corresponding to an overall growth rate, and the last negative term represents increased competition as the population grows. Similar models have been used to describe the dynamics of an epidemic, where, for a standard Susceptible-Infected-Recovered (SIR) model, the system state is the number of individuals in the susceptible, infected, and recovered pools at a given point in time. All large-scale simulation of epidemic disease are sophisticated implementations of this construct [9], with geospatial information and constraints also included in more realistic, large-scale models. Discrete models are typically

computationally intensive to simulate, but they have a clear advantage in the limit of small numbers, when spatial information is so detailed that alternative forms of spatial models, such as partial differential equations, are impractical.

Ordinary differential equations (ODE), the most popular mathematical implementation of biological models, express the rate of change of each component of a model $dX/dt = f(X(t), \Delta, t)$ as a function of other components in the model, a vector of parameter Δ and time t. Also, implicit in this formulation is that the state of the system at time t is typically dependent on the initial state of the model $X(t = 0)$. So, the simplified mathematical representation of the biological system includes the components of the model one chooses to include, their initial values of each component, and the form and strength of the interaction one chooses to represent.

Yet, if the system evolves for a long enough time, it may evolve toward a basin of attraction, which may be a single state (fixed point), an orbit of states (limit cycle or limit tori), a set of states within a defined region of state space with no particular structure (strange attractor), a feature of chaotic systems. A feature of ODE is that the evolution of states is deterministic. In other words, for given initial conditions, the trajectory will always be the same. Although this feature can be perceived as a weakness of this formalism and inconsistent with real-life behavior of biological systems where randomness and uncertainty often plays a key role in the dynamics, techniques have been developed which incorporate elements of randomness in the formalism of ODEs. Therefore, ODEs are currently the main platform used by most for modeling the acute inflammatory response in a variety of contexts, cellular networks, and models linking inflammation to macroscopic observables such as blood pressure or heart rate variability [10–14].

Equation-based models can also describe processes where physical compartments or the spatial characteristics of the biological system are important. Examples of such system include wound progression and healing [15], propagation of infection, inflammation, or gas exchange [16]. Generally however, biological processes are compartment specific, and communication between compartments is often limited and regulated. Preserving compartmentalization is often, but not always, required for a reasonable computational simulation of the underlying biology. ODEs are particularly well suited to compartmental modeling, while partial differential equations, which explicitly include continuous spatial evolution, are more appropriate for certain processes such as wound healing, tumor growth, and other systems where there is not a clear concept of compartment. More recently, alternative modeling formulations, such as rule-based or agent-based simulations, have grown in popularity and are more appropriate for the simulation of systems where it cannot be assumed that individuals of a species (e.g., molecules of a chemical species, people, etc.) are distributed homogeneously within a compartment, and where there is a sufficient number of individuals to express interactions as reaction rates [17].

Advantages of Equation-Based Models

Differential equations, as a modeling approach, have enormous appeal. They (1) provide an intuitive implementation of causal mechanisms into a mathematical framework, (2) can be analyzed using a large body of existing techniques, (3) can be numerically simulated easily and inexpensively on a variety of computing platforms including portable devices, (4) provide *both* qualitative and quantitative predictions, and (5) allow for the systematic incorporation of higher levels of complexity and uncertainty. Furthermore, this modeling framework integrates existing knowledge embodied within the structure of the model, yet admits the flexibility of being data driven and stochastic. Therefore, knowledge gaps are readily identified, unlike alternative modeling approaches. Further, the speed of existing numerical solvers for differential equation-based models allows for massive experimentation with parameters that may not be determined experimentally, leading to the development of hypotheses on the roles of individual parameters, reflecting the presence and relative importance of biological processes or interactions.

Disadvantages of Equation-Based Models

An equation-based representation of a moderately complex biological system usually depends on a large number of parameters that quantify biological interactions, and identifying these parameters can be a challenging and often impossible task. Although some of these parameters might have been determined experimentally a priori, this is rarely the case and several parameters are therefore poorly understood and constrained. Methods of inferring parameters must integrate the fact that limited data will be reflected in limited knowledge of model parameters. This is however a general issue with models, irrespective of their mathematical implementation.

As mentioned above, equation-based representations violate basic assumptions of their underlying mathematical theory when the number of instances of model components is small, of when components cannot be assumed to be well mixed within explicitly modeled compartments [18]. For partial differential equations, this must also hold approximately true within the limits of the spatial resolution of the model because of numerical solving schemes, and boundary effects and phenomena must be considered with care. When these assumptions are significantly violated, alternative modeling frameworks should be used.

Models Big and Small

Small models are highly simplified representations of a system and are comprised of a very limited number of components [19, 20]. These components will typically not represent measurable biological entities but rather lumped biological actions or principles. Several small models of the inflammatory response aggregate a complex network of cytokines and immune active cells under the general categories of pro-inflammatory and anti-inflammatory mediators (e.g., [20, 21]). Because the mathematical theory of differential equations is mature, small models allow formal mathematical analysis and the derivation of broad conclusions such as long-term stability and exhaustive enumeration of all possible types of evolution of such models. Empirical evidence will often restrict this range of possible behaviors, which in turn may limit the range of possible interaction between components (model parameter values), or impose restrictions on how interactions are coupled. Small models are not meant to be calibrated to experimental data, but to ensure that, from the outset, the proposed model structure is biologically supported.

If more insight is sought into biological details and in particular, if experimental data is explicitly available, then a larger model must be synthesized such that specific quantitatively verifiable predictions are formulated. The larger model must preserve empirically observed or plausible time evolutions. It is generally useful to initially construct the simplest model representation of a system such that expected behavior is indeed possible in this simplified representation. Models, much like pieces of a puzzle, are enhanced by the inclusions of modules that offer a more biologically realistic description of aspects of the biological system the modeler wishes to focus on. A cellular pathway for which empirical data are available or which is the potential target of a drug intervention would constitute a good candidate for more detailed representation. As general rule, a model should be as detailed as the data it wishes to describe or explain but no more. Simple models can provide insight as to broad therapeutic strategies such as structured interruption of antiretroviral therapy in HIV patients [22]. Yet, it is imperative that realistic models are used to offer quantitative predictions which can be validated experimentally, or offer specific insight on potential therapeutic targets, or on specific timing and dosage of a potential therapeutic agent.

Validating Equation-Based Models

When applied to statistical models, the concept of model validation is intuitive and well characterized: are the predictions of a statistical model developed from experimental data verified in a different set of observations gathered when the experiment is repeated? For example, the experiment could be an observational cohort of patients exposed to a disease or treatment. The model provides predictions on to a cohort of patients different from the cohort used to develop the model. A valid

model will yield predictions within statistical error of observed predictions. The validity of the prediction is typically judged on the entire cohort, on not on any single individual.

On the other hand, validation of a dynamical system is uncharted territory. The burden of proof is unclear, since the claims as to what computational models are attempting to accomplish are admittedly more extensive than for statistical models [23]. However, a roadmap could be constructed and applied to equation based, and other simulation platforms for complex systems (Fig. 2.1). A first set of criteria could be constructed as follows. First, because computational models are knowledge rich and meant to relate components of a system causally, it should include biologically verified or plausible interactions. Second, simulation of the computational model should result in biologically plausible behaviors under a wide range of initial conditions and perturbations representing realistic experiments. Third, when a model generates time courses for experimentally accessible data, deviations between model predictions and observed data should be statistically insignificant. And fourth, parameter ranges should fall within biologically verified or plausible values. A second set of criteria which are more directly related to model predictive ability and external validity are then considered. First, given limited data (e.g., the first few hours), can the model offer accurate predict the future evolution of the system, at least within a biologically or clinically relevant time horizon. A similar criterion exists for statistically based longitudinal models and is key to validating weather forecasting models [24]. Second, and a unique expectation from mechanistic models, is the ability of the models to predict the results of an experiment not used for estimating parameters. For example, what is the expected impact of exposing the system to a drug at a specific concentration and time duration on model components or ultimate behavior of the system? Will drug X decrease mortality? Such predictions are not easily formulated with standard statistical models and represent a unique challenge to mechanistic models. At the very least, attempts at validation should include an effort at externally validating the model in a different

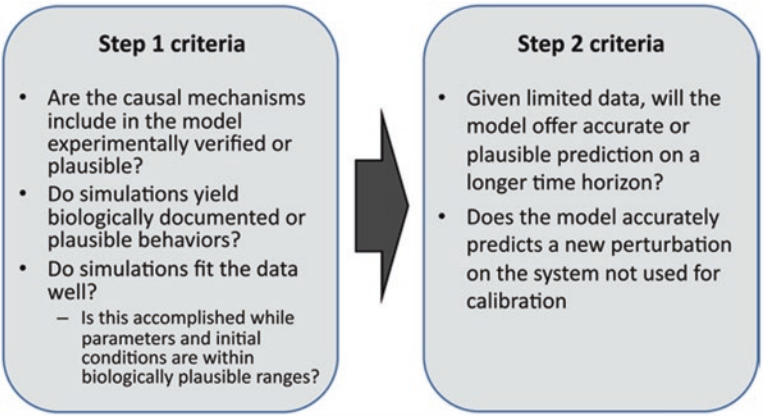

Fig. 2.1 Roadmap for the validation of equation-based models of biological systems

experimental system. It is apparent that model validation may be an iterative exercise, where failure of external validation leads to a reformulation of the basic mechanisms included in the model, while preserving desirable behavior and predictions. Further expansion of this roadmap would be a useful addition to the current modeling literature.

Using Equation-Based Models as a Prediction Tool

Parameter Estimation and the Inverse Problem

In its simplest expression, the inverse problem consists of reconstructing model parameters given observed data. In statistical linear regression model, there is only one optimal solution to the inverse problem, which is the set of parameters minimizing the sum of square residuals. In such situations, the inverse problem is said to be well posed. Equations-based models, especially larger, more complex models include a number of parameters, which are poorly known. Formal identifiability analysis will confirm that many such models are not structurally identifiable [25]. In other words, even if provided with perfect and infinitely rich data, not all parameters of the models can be identified. It behooves the modeler to develop a priori sound models where such issues are minimized. A second obstacle lies in the sparsity of data available to estimate model parameters and in the experimental uncertainty associated with experimental measurements. This type of problem contributes to the practical identifiability of the model. A third obstacle is that nonlinear systems may admit a large number of good solutions, and thus potentially a large number of parametrizations are compatible with observed data. The lack of a unique solution to the inverse problem is referred to as ill-posedness [26]. Therefore, in complex models, it is rarely realistic to identify a set of parameters that describes a system uniquely, while preserving the ability of such a parametrized model to offer robust predictions. Indeed, although this single parameter set may create a good fit to the data at hand (e.g., maximum likelihood in the immediate vicinity of this parameter set) or display all the require biological behaviors, it will in all likelihood not hold to extended validation (see above).

Approaches to Solving the Ill-Posed Inverse Problem

In an equation-based framework, a satisfactory solution to an ill-posed inverse problem is tantamount to identify a set of model structures (the equations themselves), and for each structure, as set of parameters and initial conditions that will provide a good enough explanation of the data available on the biological system being studied. In the simpler case when only one model structure is under consideration, that

is, the set of mechanisms represented by the equations is believed to be well understood, solving the inverse problem is limited to the identification of parameters and initial conditions that will produce a good solution, while maintaining biological fidelity of simulations under a variety of input to the model, as described above in the discussion on model validation. A good solution can be defined using a variety of metrics, or cost functions, expressing how close predicted trajectories are from observed data. A simple cost function would be, for example, the sum of squared residuals.

Ideally, all system variables are observed at a level of time granularity sufficient to provide a good description of the longitudinal dynamics, up to restoration of homeostasis or stabilization to a different state. In the absence of fine-grained data obtained over a wide range of experimental conditions, the next step is to complement the dataset with a set of heuristic rules that will define a priori the plausible ranges of observations and limiting behavior. Heuristic rules are based on prior knowledge and expert opinion of system behavior. These rules are of particular importance to system variables for which data are sparse or missing. For example, there almost always exists literature or other empirical evidence where some variables of the system were measured under a different set of circumstances, or in a somewhat different biological system (e.g., endotoxemia in human vs. sepsis in humans). Yet, data from human endotoxemia may still be of use in a computational model of human sepsis in constraining the potential range of unmeasured variables in this model. More generally, such data can be used "qualitatively" to create rules that restrict the range of plausible behaviors of these variables. These rules are used in the calibration of computational models as (1) models and parameter combinations for which the system violating a rule are excluded a priori or (2) a number expressing the severity of the deviation from a rule is added to the cost function (the function that quantifies numerically the difference between experimental data/heuristics and predicted behavior), decreasing the likelihood of this model which is minimized by the optimization process.

In the discussion that follows, we use the notion of a generalized parameter $\mathbf{p}$ vector of a model as encompassing both standard parameters. Δ and initial conditions $X(0)$, $\mathbf{p} = \{\Delta, X(0)\}$. It is apparent from the discussion above that standard algorithms searching for local minima are not well suited for addressing ill-posed inverse problems. The ill-posedness may open the possibility to a large, possibly infinite, number of local minima. A practical approach is to initiate the calibration process from a large number of initial points in parameter space, selected in such a way as to offer reasonable coverage of parameter space such as through Latin hyper-square sampling. This approach is referred to as multistart optimization and yields a set of local minima, many of which may fit data relatively well and display suitable heuristic behaviors [27]. Yet, many regions of the cost function landscape may be very flat and consequently such algorithms may not converge to realistic solutions. This is fact to be the case for larger models when data constrains the plausible parameter distributions to a submanifold of lower dimension than the number of parameters including the model, demonstrating the practical nonidentifiability of the model. It does not necessarily follow that such models are useless and they may in fact offer good,

albeit probabilistic, predictions if one considers the entire set of parameters. Indeed, each parameter set leads to a deterministic prediction, but the ensemble of parameter vectors produces a variety of plausible predictions. Although some problems, such as the tertiary structure of a macromolecule, plausibly admit a single global minimum of the cost function, which in this case is the conformational energy for a given local microenvironment, there is neither assurance nor intuition that this is generally true of out of equilibrium complex dynamical systems constantly adapting to changing environments and under varying energetic constraints.

It would seem unwise therefore to think of calibration of a complex model as the identification of an optimal parameter set, but rather of an ensemble of parameters.

There is a growing tendency to conduct stochastic searches of parameter space as a solution to ill-posed problems [28–30], typically following a Bayesian estimation procedure. In such a scheme, the calibration process also involves the construction of an ensemble $E(\mathbf{p})$ of a large number of good solutions, each characterized by a generalized parameter vectors $\mathbf{p} = \{\Delta, X(0)\}$ as described above. For a given data-set $Y = \{y_i(t_j)\}$ or i variables collected at j time points and model $\mathrm{d}X/\mathrm{d}t = \mathbf{f}(X, \mathbf{p}, t)$, the distribution $\rho(\mathbf{p})$ will be computed using $w(\mathbf{p}) = \prod_{ij} P_{ij}\left(x_i\left(t_j;\mathbf{p}\right)\right)$, where it is understood that $w(\mathbf{p}) \equiv w(\mathbf{p} \mid Y)$. If prior knowledge on parameters $P(\mathbf{p})$ is available, a Bayesian scheme is adopted where the posterior distribution $w(\mathbf{p}) = \prod_{ij} P_{ij}\left(x_i\left(t_j;\mathbf{p}\right)\right) P(\mathbf{p}) / P(Y)$ is known within a normalization fraction, $P(Y)$. Yet, this normalization factor is generally not required for practical computations as one is typically interested in ratios of probabilities in the process of selecting suitable parameter sets. The probability functions P_{ij} are determined by the data accuracy. If the data point $y_i(t_j)$ has Gaussian uncertainty σ_{ij}, then $P_{ij}\left(x_i\left(t_j;\mathbf{p}\right)\right) = Q\exp\left(-\left(x_i\left(t_j;\mathbf{p}\right) - y_i\left(t_j\right)\right)^2 / 2\sigma_{ij}^2\right)$. By normalizing the weight function, we obtain a probability density $\rho(\mathbf{p}) = w(\mathbf{p})/\int w(\mathbf{p})\mathrm{d}\mathbf{p}$ over the space of parameters. Efficient sampling of parameter space, although alleviated by dimensional reduction methods, remains computationally challenging and will be approached using stochastic sampling methods such as parallel tempering [30, 31]. A posteriori analysis of the distribution is conducted to determine the width of the distribution, modality (number of local maxima), separation of local maxima, approximate dimension, etc. Simulation of the ensemble model produces not only a single trajectory but also an ensemble of trajectories parameterized by $\mathbf{p}$ with weights proportional to $\rho(\mathbf{p})$. These trajectories will provide probabilistic prediction of the outcome of the model as a time-dependent distribution of values of system variables $\phi(\mathbf{x}, t) = \int \delta(\mathbf{x} - \mathbf{x}(t; \mathbf{p}))\rho(\mathbf{p})\mathrm{d}\mathbf{p}$. This distribution can be used to compute the average trajectory $\mathbf{x}(t) = \int \mathbf{x}\phi(\mathbf{x}, t)\mathrm{d}\mathbf{x}$, the time-dependent variance of average trajectory $\sigma^2(t) = \int (\mathbf{x} - \mathbf{x}(t))^2\phi(\mathbf{x}, t)\mathrm{d}\mathbf{x}$, the probability $P\left(x_i(t) < x_j^0\right)$ that a value of a given variable drops below a prescribed threshold at time t, and various other quantities of interest.

Hybrid Models

A growing number of computational models simulate phenomena observed at different scales, for example, intracellular pathways and cellular phenotypes, intrahost models of viral infection and population epidemic models. Scales are typically physical and be best approaches using different simulation platforms. Hybrid models consist of computational models comprising two or more simulation platforms.

Such models may be of particular interest in translational applications, where the largest scale of the simulation is at the individual or population levels, and the lowest scale determined by the type on perturbation we wish to impose on the system. It could be, for example, an antiviral medication with a pharmacodynamics profile dependent on the age or preimmune status on an individual. Simulations using coarse distributional assumptions "average" behavior of individuals may lead to result very significantly different from more-detailed simulations of drug action within an individual. Hybrid models raise considerable computational challenges. Expanding on the example above, representing each individual in a population using an equation-based model of intrahost infection (e.g., [32]) is not feasible within the framework of a large population scale agent-based simulation. Creative solutions exists however to mitigate the additional computational cost associated by multiscale hybrid simulations [33]. Use can be made of large differences in temporal dynamics that exist at different scales of the simulation. A method our group has implemented in the context of epidemic simulation is to create, from intrahost models of Influenza A infection, algebraic response surfaces using preexisting simulations of the equation-based model to generate an algebraic input–output map [34]. Consequently, given a patient's age, preexisting immune status and initial viral load, a daily profile of infectivity and symptomaticity is generated by applying a regression equation rather than embedded simulation of an equation-based model. The incremental computational cost was negligible and such an approach is imminently scalable. A class of applications of particular interest in inflammatory diseases relate organ function to specific anatomic intricacies as the playground for molecular or cellular inflammatory effectors [16, 35–38]. Such models may not be explicitly hybrid in nature but typically present very similar challenges in that efficient computation require creative solution to bridging anatomical scales.

Translational Applications

The Interdisciplinary Perspective

Computational models are practical instantiations of the state of current knowledge and a representation tool for competing hypothesis of processes driving biological systems and therefore constitute a framework for hypothesis generation and efficient experimental design for testing these [1, 7, 39]. The truly impactful concept is

that this approach allows a model-centered discussion between clinicians, biologists, and modelers [7]. Hypotheses and thought experiments can be pushed computationally to their logical outcome as well as regions that may offer unexpected clinical benefit. This is extremely difficult to achieve from discussions only, without an existing quantitative model of disease. We believe that computational models will become a powerful and standard tool that promotes effective interdisciplinary research and scientific epistemology.

Enhancing Current Trial Design

The ultimate purpose of basic mechanistic research is to improve human health. The mantra of pharmacological intervention remains that the right drug at the right dose must be administered to the right patient at the right time. In addition to basic lack of efficacy of biologics tried for sepsis, several have raised the issue that the design of clinical trials itself was to blame [40], namely that patients were enrolled too late given the biological rationale of the intervention, that phenotype-based enrolment may not be ideal, or that dosing might have been inappropriate. For example, patients presenting with community-acquired sepsis already have elevated circulatory biomarker levels upon enrolment [41, 42]. These circulatory biomarkers generally peak between 3 and 36 h postadmission and diminish over the subsequent 72 h [41]. The vast majority of immunomodulatory trials have enrolled patients well after key biomarkers have peaked [43].

The US food and Drug administration has published a report on the use of adaptive clinical trial design to maximize information extracted for clinical trials and minimize sample size required to detect real treatment benefit [44]. Recent methodological developments in adaptive clinical trials design, such as sample size reestimation as proposed in the recent failed confirmatory trial of drotrecogin alfa [45], are for the most part only tangentially applicable to sepsis trials [44, 46–49]. However, computational models of human sepsis could contribute to an adaptive design in two distinct ways. Every large trial has prespecified interim analyses. If at this point in trial execution, a computational model calibrated from data accrued up to the interim analysis was more sensitive than standard data analysis to identify treatment effect as we suspect if would be, of lack of a difference in biological activity as suggested by extensive overlap in extensive overlap of model ensembles, one could envision (1) consolidating trial arms that show no difference in biological activity and thus improve power to detect differences between residual arms of the trial or (2) declare futility with greater confidence. A second potential application of computational models to augment adaptive design resides in their ability to identify cohorts of patients with a better probability to respond to the proposed intervention. Adaptive patient enrichment design allows the modification of enrolment criteria as the enrolment accrues as it becomes clear that some types of patients clearly do not benefit from the intervention [50]. Both types of contribution are potentially promising for future trials of immunomodulatory intervention in acute and chronic inflammatory diseases.

In Silico Clinical Trials

Clinical trials of immunomodulation in acute inflammatory disorders have a generally dismal track record. This is particularly true for sepsis, a clinical syndrome arising from the systemic host response to infection with clinical manifestations that span a broad set of inflammation-related signs and symptoms [51]. Although the host's response to sepsis strives to contain infection and promote repair, the intensity of the inflammatory response often leads to compromised tissue function, uncontrolledinflammation and/or profound immune suppression, organ failure, and death [52, 53]. Severe sepsis accounts for between 2 and 11% of all admissions to hospitals or ICUs, approximately 750,000 cases a year, with an associated mortality of 35%, most often from progressive organ failure in an ICU [54]. An intense effort by the critical care community to raise sepsis awareness and provide evidence-based recommendations is ongoing. The Surviving Sepsis Campaign (http://www.surviving-sepsis.org) published guidelines in 2004, 2008, and 2013 [55–57]. These recent guidelines support the implementation of "care bundles," sets of care decisions that constitute generally accepted competent critical care, but all recommendations regarding immunomodulation have been withdrawn. Care environments, which have implemented sepsis bundles, have seen a modest improvement in outcome paralleling that of general ICU care [54, 58–62]; yet, recent spectacular failures of confirmatory trials of immunosuppressive agents [63, 64] have further contributed to the profound consternation, skepticism, and soul searching permeating the critical care community regarding breakthrough treatments for sepsis [65, 66]. Presumably, major reasons for this dismal record are the failure to integrate the complexity of sepsis pathophysiology towards mechanistically sound therapeutic rationales and the failure to translate knowledge acquired from in vitro and preclinical experiments to clinically and genetically diverse human beings [67]. Computational simulations of immunomodulatory agents in sepsis will hopefully contribute to bridge the gap between a reasonably well-known pathophysiology, generally favorable preclinical data, and clinical trial results. In addition, such simulation may contribute to proper patient selection, dosage selection, and duration of intervention. A computational simulation of an acute inflammatory disease, such as sepsis, would provide recommendations on the basis of point-of-care measurements of biomarkers, a platform which is currently not commercially available, presumably because there is currently no market for such methods.

There have been few prior attempts at computational simulations of clinical trials [2, 68, 69]. A simulation of an anti-TNF intervention pointed out such of the potential advantages underlines above. A potential for harm was identified in patients who were predominantly immunosuppressed at the time of initiation of therapy, in patients offered high doses for longer than 48 h, and in those with particularly aggressive bacterial infection [2]. These predictions are teleologically plausible, and the computational model identified, early in the course of treatment, responders from nonresponders with high probability. Although such conclusions are speculative until verified in the clinic, animal data supported many of the predictions of this

computational model [70]. Opportunities for computational models to gain legitimacy in the clinical realm are ongoing, extend well beyond acute inflammatory disease to cancer and cardiovascular interventions, and are encouraged by registration entities such as the Food and Drug Administration in the USA [44].

Parameter Ensembles vs. Data: Different Worldviews

One typically expresses differences in terms of statistically significant levels of biomarkers, incidence of organ failure, or mortality. The computational model-based alternative is to express differences in terms of differences in the distributions of model parameters that characterize important phenotypes such as survival or death. For example, in a recent publication, statistical tools were compared the parameter ensembles of a computational models fit to animals that survived a septic challenge to the distribution obtained from a model fit to data of animals that died [31]. Analyzing parameter distributions rather than data provides insight on mechanisms explaining the difference in outcome, not merely a description of what is different. Although both the "data world" and the "parameter world" can be used to produce predictive models, only a parameter-based description can offer insight on mechanistically motivated therapeutic strategies that may alter outcome.

Novel Approaches to Personalized Therapies for the Critically Ill

Personalized, or precision medicine is often confused with genomic medicine and an inference is often made that gene-level understanding of biological processes is sine qua non to the development of personalized therapies [7]. Standard approaches have had some, yet limited success in linking gene expression profiles to local and circulatory protein levels, to clinical disease severity and outcome [70–72]. Care givers, and acute care physicians have been offering effective, individually titrated care to their patients based on a different, yet complementary premise of recognizing patterns of organ function and injury, framed in a conceptual framework of mechanistic pathophysiology, and establishing individualized therapeutic targets to mitigate these mechanisms. Computational approaches offer the possibility of reconciling these concepts of translating differences in data, which does not easily lead to hypothesis generation, into differences in mechanisms, which typically have a more direct interpretation and may suggest individualized, time-dependent, therapeutic approaches.

Two features of equation-based computational models may contribute to the development of personalized approaches to treatment. Computational models, and equation-based models in particular, map data to mechanism and thus offer a way to identify mechanistically based phenotypes. As more data becomes available and model parameters reestimated, mechanistically based phenotype becomes better

defined. Interventions in acute care, pharmacologic or physical organ support, interact directly with mechanisms. Indeed, practitioners constantly select therapies based on perceived mechanisms. Therefore, a mechanistically based decision support system suggesting which mechanisms are particularly important in a given individual at a given time might enhance data interpretation, diagnostic ability, and treatment selection [26]. The recent rise of control-based approaches in cancer pharmacotherapy of glucose control in type I diabetes offers a second application for equation-based models applied to personalized care. The underlying concept is that, if a biological process is well modeled computationally, then using tools of control engineering facilitates achieving a preset therapeutic objective, such as a specific drug concentration, or glucose zone target much as an artificial endocrine pancreas would achieve [73, 74]. Such approaches have already been ushered in clinical practice.

Conclusion

Equation-based models of translational relevance are recent. Their acceptance as useful knowledge discovery and decision support tools, although unquestioned in the basic sciences, has met with considerable criticisms in the translational arena. The immediate task of modelers and clinicians alike is to build and disseminate success stories. There are more likely to emerge from cancer research, diabetes, or immunomodulation of chronic or acute inflammatory disorders.

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Chapter 3
Agent-Based Modeling in Translational Systems Biology

Gary An

The Translational Dilemma and the Need for Dynamic Knowledge Representation

As noted else where in this book, the Translational Dilemma, the inability to translate the successes at obtaining basic mechanistic knowledge about biological processes into clinically effective therapeutics, is the greatest challenge facing the biomedical community [1]. The Translational Dilemma consists of two primary barriers that need to be breeched: (1) the need to accelerate the scope of hypothesis testing necessary to deal with the multiplicity of possible explanations of high-resolution data (the experimental throughput problem) and (2) the ability to adequately evaluate the consequences of highly complex, multicomponent, multihierarchical integrative hypotheses (the multiscale problem). Both issues are related directly to the requirement to move forward; biomedical researchers must greatly increase their ability to evaluate the *plausibility* of mechanistic hypotheses and their manifestation at the systemic level. Meeting this requirement will almost certainly involve harnessing the power of advanced computational modeling and computer hardware for the dynamic knowledge representation of biological systems in such a way that hypotheses can be instantiated and evaluated in silico. The ability to execute in silico experiments offers potentially the only viable path to substantially accelerate and enhance the Scientific Cycle by providing a plausibility filter for putative hypotheses. This will substantially reduce the set of possible mechanistic explanations for a particular observation and will help direct and focus the design of traditional laboratory experiments to further refine the set of possible hypotheses. This chapter will discuss the use of agent-based modeling (also known as

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individual-based modeling), for dynamic knowledge representation with an explicit translational goal in the area of acute inflammation.

Dynamic Knowledge Representation with Agent-Based Modeling

Agent-based modeling is an object-oriented, discrete event, rule-based computational modeling method [2–6]. Agent-based models (ABMs) consist of virtual environments populated with objects (agents) that execute behaviors based on programmed rules that govern their interactions with the local environment and other agents. An ABM represents a system as populations of components ("agents") where the simulation agent level of the ABM corresponds to the primary component level of the system being studied: for instance, a cell-level ABM uses agents that primarily represent biological cells. An ABM *agent-class* is defined by a specification of the properties, characteristics, and rules of an agent type that govern its identity and behavior. As an ABM is executed, it creates a population of individual computational instances (an agent) of each agent-class, where each individual agent possesses the behavioral rule sets and defined properties of its agent class, but once created can have diverging behavioral trajectories based on the different inputs it receives within a heterogeneous simulation environment. ABM rules are often expressed as conditional statements ("if-then" statements), making ABMs suited to expressing the hypotheses generated from basic science research, though it should be noted that the general conditional nature of simulation agent rules does not preclude the encapsulation of other types of mathematical or computational models (i.e., differential equation, stochastic or network) as rule systems [7–9]. A standard conditional agent rule for a cell agent interacting with its environment might have the following format:

if Compound A (in the environment) is present, then bind to and activate Cell-Surface Receptor B (in the cell-agent).
if Cell-Surface Receptor B is activated, then increase Signal Transduction Enzyme C (in the cell-agent) by x.
if Signal Transduction Enzyme C is increased beyond threshold y, then activate Transcription Factor D.
if Transcription Factor D is activated, then express Gene E and so on…

As noted above, the rule sets for an agent can be of any formal type, such as a series of logical statements or a differential equation. Regardless of the specific ABM rules, ABMs allow a close mapping between the natural language expression of hypotheses present in publications (the current means by which this knowledge is communicated within the community) and the rule structure of ABM [10, 11]. Results can be readily used for dynamic knowledge representation, particularly for researchers not expressly trained in either computational or mathematical modeling

by allowing them to more easily translate their biological knowledge into a computational form.

ABMs also intrinsically cross multiple scales of biological organization by necessarily involving at least three levels of system organization. Scale #1 is the lowest level of system process represented, and this is accomplished by the agent's behavioral rules. Scale #2 is the "middle" level corresponding to the primary component level chosen, and processes at this level are represented by the behavior of an individual agent. Scale #3 is the "system" level consisting of the global phenotype under investigation and is generated by the aggregate behavior of populations of agents. To use an example of a cell-as-agent ABM, Scale #1 then represents molecular events associated with signaling and protein synthesis, Scale #2 represents the behavior of an individual cell as it changes state, secretes something or moves, and Scale #3 represents tissue behavior arising from the interactions between populations of cellular agents. Furthermore, these levels can theoretically be nested, to provide a comprehensive depiction of a multiscale biological system (see Fig. 3.1), making ABMs well suited for creating modular models [6, 7, 12–14].

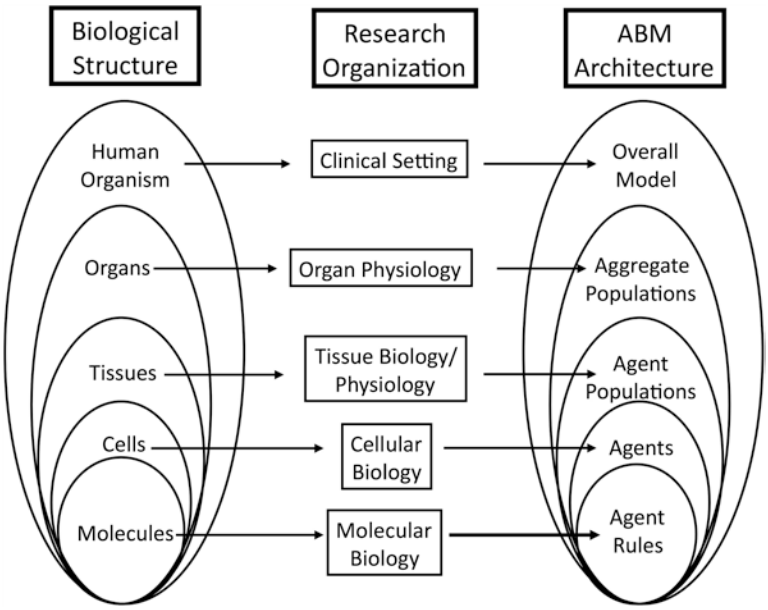

Fig. 3.1 The mapping between scales of biological organization, research community structure and agent-based models. This diagram maps the similar structure of organizational scales present in biological systems, the research communities studying them and the architecture of an ABM. Note that scales of organization are nested in the biological system and the ABM, reflecting the trans-scale coupling seen in both systems. Alternatively, the research community structure is disparate and compartmentalized, arising from both social and pragmatic logistical factors. (Reprinted with permission from Ref. [11])

Related Modeling Methods

Given the description above, it is clear that agent-based modeling is actually a very general means of system representation, and as such is viewed as quite similar to many other modeling methods. In fact, many of these types of modeling methods can be considered as sub-types of ABMs, leading to a great deal of variability in the use of the term "ABM." As such, it is useful to clarify the distinctions between certain other commonly used modeling methods and agent-based modeling as the term is used in Translational Systems Biology. One of the most closely related modeling methods is cellular automata (CA), particularly two-dimensional CAs. Cellular automata involve a discretely divided space into a series of "cells," such that the state of each particular cell is defined by a set of rules dependent upon the states of some defined neighborhood of cells. Classical examples of two-dimensional CAs are Conway's Game of Life [15] and Kaufman's N-K System [16]. These systems can be seen as ABMs where there is a single agent class (the basic unit "cell"), which does not move, and a set of agent rules that govern an agent's state transitions. Another closely related modeling method is the Cellular Potts Model (CPM), developed by Glazier and Graner, where the states of points on a lattice are determined using probabilistic rules, and membership in a particular group of points is used to define superstructures representing cells or aspects of tissue [17]. Each of these methods has its own benefits and uses, most often governed by a combination of the resulting model's use and the data available to construct the model. For instance, while "movement" can be simulated using a CA, it is often less intuitive for a biologist to think of a cell's movement as a progression of cellular variables across a grid as opposed to a specific computational object that changes its position. As another example, while a CPM can allow cells to change their size and shape (where a "cell" is defined by a group of lattice points), the means by which a lattice point's membership in a particular cell, often expressed as a Hamiltonian representing an effective energy function, does not readily map to a biologist's knowledge set (as evidenced by the relative incomprehensibility of the prior terms!). At one level (i.e., in terms of the actual execution of the binary code) the distinction between these methods and agent-based modeling may be a distinction without a difference; however, in terms of facilitating knowledge representation the component-centric emphasis of agent-based modeling is more consistent with how most biological systems are conceptualized (i.e., "things doing things").

Agent-Based Models Versus Multiagent Systems

In addition to closely related modeling methods, there is also ambiguity in the use of the term "agent." The distinction between an "agent-based model" and a "multiagent system" is just such a situation. Both terms are widely used in the computer science and modeling and simulation community and are often used to mean the

same thing: a computer program that utilizes multiple computational agents. However, in terms of the types of systems they usually describe, these two methods actually represent very different types of computational tools. Therefore, for purposes of comparison, we make a distinct difference between these two entities (noting that that the following distinction is not intended to be a definitive description of the distinction, but rather is intended to clarify the differential usage of the term "agent" in the context of Translational Systems Biology).

We consider "agent-based modeling" as a simulation method, where the model constructed is intended to mimic or represent some other reference system, which is the subject of investigation. The computational agents making up the ABM are intended to represent specific types of components in the real world where selected characteristics of the real-world object are reflected in the nature of the rules incorporated into the simulation agent. Since a main benefit of agent-based modeling is the ability to represent populations of real-world objects at the individual level with simulation agents, in many circumstances ABMs consist of a large number of individual instances of simulation agents derived from a single agent class.

Alternatively, an "agent-directed" or "multi-agent system" is generally used to describe a computer system design solution, where computational agents perform tasks related to the implementation of a particular computing goal. These computational agents generally have some decision-making capacity, which may be augmented using artificial intelligence approaches, that allows them to manage the information flow within a particular software implementation. In multiagent computer systems, the computational agents generally do not have a specific real-world reference object for a computational agent; rather, there is a set of recognized tasks in information flow management that can be expressed as a set of algorithms and packaged for execution by a computational agent.

Properties of Agent-Based Models

As noted above, ABMs are related to other spatially discrete modeling methods, most notably cellular automata, though the mobile capability of ABM agents and their ability to represent a wider range of model topologies could lead to consideration of cellular automata as a special type of ABM. However, in practice, many ABMs have several characteristics of agent-based modeling that set it apart from other object-oriented, rule-based modeling systems (such as Petri nets, Boolean or Bayesian Networks), even though at its purest definition, they could all be potentially viewed as ABMs.

Representation of Spatial Relationships

Agent-based models (ABMs) readily incorporate *spatial relationships*, be they manifest in an actual spatial topology, or a topological interaction neighborhood linking individual agents. In an ABM agent behavior is driven by interactions determined by agent neighborhoods defining the communication and interaction network for each agent. An agent neighborhood can be represented as a two-dimensional square grid (very common), a 3-dimensional cubic space [7, 12], 2- or 3-dimensional hexagonical space [18, 19] or as a network topology, as a neighborhood does not necessarily mean physical proximity but rather the configuration of some set of other agents with whom an agent can interact. This definition of an agent neighborhood is consistent with the bounded nature of the sense-and-respond and message passing capabilities of biological objects. This may also be used to represent physical interactions and forces between agents that affect their subsequent behavior.

Representation of Parallelism and Concurrency

ABMs utilize *parallelism*. In general, each ABM agent class has multiple computational instantiations that form a population of agents, each capable of having different behavioral trajectories. These heterogeneous behaviors produce population dynamics that are the observable, system-level output of the ABM. A classic example of this phenomenon is the behavior of flocks of birds, in which simulations utilizing relatively simple interaction rules among birds can lead to sophisticated flocking patterns without an overall controller [20]. This property is well suited to the tendency in biology toward classification: the grouping of similar biological entities that share some set of properties and behaviors. Biological systems are then readily characterized as being composed of some types and numbers of these entities. This type of conceptual representation exactly suits the architecture of an ABM.

Incorporation of Stochasticity and Randomness

ABMs readily incorporate *stochasticity*. Many biological systems have behaviors that appear to be random [21, 22]. Whether these behaviors are truly random, or just merely appear to be due to a lack of finer grained knowledge is, from an operational standpoint, often irrelevant as long as the probabilities of a particular behavior can be determined for the population as a whole experimentally. These probabilities are then used to generate a probability function for the behavior of a single agent that is then incorporated into the agent's rules. As a population of agents executes their rules during the course of a simulation, each agent follows a particular behavioral trajectory as its behavior rules' probabilities are resolved as the simulation progresses. A set of behavioral outputs is thusly generated from a single ABM, producing system behavioral state spaces representing the set of population-level biological observations.

Modular Architecture

ABMs are *modular*. Agents represent a distinct and circumscribed modular level into which new information can be added through either the introduction of new agent types or the modification of existing agent rules without having to re-engineer the entire simulation. Agent classes representing generic cell types can be subdivided and expanded to include a finer degree of detail with respect to sub-categories of cells while the remainder of the ABM remains essentially intact. New mediators can be similarly added by creating new cellular state or environmental variables and rules. Multiple ABMs can be aggregated, providing that their points of contact and interaction are consistent across the incorporated ABMs [12, 19].

Generation of Nonintuitive System-Level Phenomenon

A central hallmark of ABM is that they generate system-level behaviors that could not have been reasonably inferred from, and often may be counterintuitive to, examination of the rules of the agents alone. This is our definition of *emergent* behavior. ABMs are capable of generating this type of behavior due to the local/neighborhood knowledge constrained and stochastic nature of agent rules, and the population effects of their aggregated interactions. For example, in the bird flock an initial observation would suggest an overall leader, thereby requiring a means of determining rules for flock-wide command and control communication. This, however, is not the actual case; birds function on a series of locally constrained, neighborhood-defined interaction rules, and the flocking behavior emerges from the aggregate of these interactions [20]. The capacity to generate nonintuitive behavior is a vital advantage of using ABM for conceptual model verification, as often the translation of generative mechanisms to system-level behavior produces paradoxical and unanticipated results that break a conceptual model.

Readily Facilitates Useful and Detailed Abstraction

ABMs provide for high-fidelity component abstraction of system structure. ABMs can be readily constructed using incomplete and abstracted knowledge yet produce surprisingly highly "realistic" system-level behavior. Because of this property it is advantageous in the initial steps of developing an ABM to keep the rules as simple and verifiable as possible, even at the expense of some detail. As such, meta-analyses of existing basic research often guide the development of an ABM [23]. ABMs constructed with admittedly incomplete and uncertain mechanisms representing statements of hypotheses can provide qualitative verification of those hypotheses [24]. As with all computational models, the greater fidelity of mapping between the ABM and its biological counterparts enhances the correlation between simulation results and the real-world behaviors. An iterative process of refinement of an ABM

will lead to increased detail, possibly a stronger correlation to real-world data and a greater confidence in the ability of the ABM to describe observable phenomena.

Agent-based modeling is an integrative modeling framework that can readily be used for communicable dynamic knowledge representation [10–12, 25] (see Fig. 3.1). Agent-based modeling, because of its emphasis on "things doing things," is generally more intuitive for nonmathematicians/computer scientists than more formal mathematical modeling methods such as ordinary differential equations, partial differential equations, and their stochastic variants. Agent-based modeling presents a lower threshold barrier for researchers to "bring to life" their conceptual models and integrate in silico methods with traditional in vitro and in vivo experiments [2].

Since ABMs are knowledge-based models, constructed by instantiating bottom-up mechanisms (as opposed to inductive models, where mechanisms are inferred with the goal of explaining data), agent-based modeling addresses different modeling questions than equation-based inductive models. For instance, ABMs are not readily developed directly from a mass of raw data; they require that the modeler has a mechanistic hypothesis that, when instantiated in an ABM, can be used to generate simulated data, which can then be compared to the real-world data set. One can envision an iterative process by which inductive models are applied to large datasets, wet lab experiments are carried out to investigate the mechanisms inferred from the inductive model, and the experimentally confirmed mechanisms are used as a basis of an ABM which would close the discovery loop by recapitulating the original dataset.

Agent-based modeling was pioneered in the areas of ecology, social science, and economics, but since 2000 it has increasingly been used in the biomedical arena. Notable disease processes include cancer (see [26] for an excellent review), sepsis [11, 12, 27–30] cellular trafficking [31–35], wound healing [36–38], and intracellular processes and signaling [8, 25, 39–44]. The majority of biomedical ABMs utilize cells as the primary simulation agent level, though there are several exceptions of modeling intracellular processes from Refs [8, 25, 39–44]), and we consider the use of agent-based modeling in epidemiology, with its extremely rich background [45], as a separate discipline. From the standpoint of addressing the Translational Dilemma, cells form a ready level of "encapsulated complexity" that is both highly studied as a unit (i.e., cellular biology) and can be addressed with relatively straightforward input-output rules [6]. As noted above, while ABM agent rules are often logical or algebraic statements, rules can be a mathematical model in itself. There are multiple examples of embedding complex mathematical models within a cell-level ABM agent [6–9, 14, 38, 46]. These examples emphasize the potential unifying role of agent-based modeling as a means of "wrapping" different simulation methodologies. This suggests that the meta-structure of an ABM can be used as a template into which structured biomedical knowledge can be integrated to facilitate the instantiation of multiple mechanistic hypotheses [47].

Tools for Agent-Based Modeling

Agent-based modeling environments require addressing certain software issues beyond the basic capabilities of more traditional object-oriented programming tools. These issues include emulating parallel processing to represent the actions of multiple agents within populations, dealing with associated execution concurrency issues within those populations, establishing means of defining model topology (i.e., agent interaction neighborhood), and the development of task schedulers to account for the multiple iterations that constitute an ABM run. As a result of these issues, along with the case that many researchers who utilize ABMs are not trained computer scientists or programmers, many biomedical ABMs are created using existing ABM development software packages. These agent-based modeling environments attempt to strike a balance between representational capacity, computational efficiency, and user-friendliness. A noncomprehensive list of such ABM toolkits can be seen in Table 3.1. All these platforms represent some tradeoff among the triad of goals mentioned above. For an excellent review and comparison of many of these agent-based modeling toolkits, see Ref [48].

Agent-Based Modeling of Inflammation

The difficulty in engineering safe and effective therapeutic agents directed at inflammation is a primary example of the Translational Dilemma in biomedical research. Because of these characteristics, inflammation represents perhaps the ideal target for systems biology and computational modeling with agent-based modeling. The use of agent-based modeling has dramatically increased since 2000 and is now a generally accepted means of performing computational biology. As is the case when discussing any specific modeling method, it should be re-emphasized that agent-based modeling is only one of an array of methods that can be used to represent and investigate biological systems (such as those covered in other chapters in this book). Each of these modeling techniques has its strengths and weaknesses, and potential modelers need to recognize that the modeling method chosen should be tailored to the question(s) being asked of the model [49]. One of the most effective ways of communicating the capabilities (and limitations) of a particular modeling method is through the use of examples. Since the rest of this book includes detailed descriptions of several ABMs involved in Translational Systems Biology, this chapter will present a few examples of types of ABMs not explicitly covered elsewhere in this book.

Table 3.1 Freeware agent-based modeling toolkits

Toolkit name	Language/Platform	Degree of programming expertise?	Degree of flexibility?	Website
Swarm	Objective C, Java	High	High	http://www.swarm.org
Netlogo	Windows, Macintosh, Linux	Low	Low	http://ccl.northwestern.edu/netlogo/
Starlogo	Windows, Macintosh, Linux	Low	Low	http://education.mit.edu/starlogo/
Repast	Java	Moderate/High	Moderate	http://repast.sourceforge.net/
MASON	Java	Moderate/High	High	http://cs.gmu.edu/~eclab/projects/mason/
SPARK	Java	Moderate/High	Moderate/High	http://www.pitt.edu/~cirm/spark/

ABMs of Inflammation-Related Intracellular Processes

The characterization of intracellular pathways is the traditional focus of systems biology, with a long history of work and achievement in the development of mathematical models of cellular signaling and metabolic control. These models are generally biochemical kinetic models, utilizing deterministic and stochastic differential equations. However, the use of discrete event, particle-based modeling, exemplified by agent-based modeling, has certain applications in this arena. With increasing awareness of the influence of the complex, compartmentalized environment of the intracellular milieu on intracellular dynamics, there is a need to account for issues of molecular crowding and spatial heterogeneity of the reaction milieu and how they affect enzymatic reactions within the intracellular environment. Additionally, the presence of sub-cellular structures, cytoskeletal elements, organelles, and compartments calls for the increasing incorporation of spatial properties and detail. Ridgway et al. [42] used an ABM of intracellular signaling to demonstrate that the reaction dimension determining biochemical kinetics within a prokaryotic cytoplasm was reduced from the expected three dimensions to nearly two, with significant consequences for the dynamic modeling of control loops in which subtle changes in feedback determine the direction of a molecular switch. Pogson et al. [41] developed an ABM of control pathways affecting the transcription factor Nuclear Factor kappa B (NF-kB). These studies demonstrating the importance of the spatial distribution in terms of nuclear translocation of the constitutive inhibitor of NF-kB, I-kappa-B (IkB), and the binding of IkB to actin, a cytoskeletal protein, a mechanism subsequently identified in their laboratory [40]. We developed an agent-based architecture called Spatially Configured Stochastic Reaction Chambers to demonstrate that even an abstract representation of enzyme kinetics could, if sufficient pathway component detail was included, reproduce canonical behavior at the cellular level, as in the effect of preconditioning on the behavior of the Toll-like Receptor 4 (TLR-4) signaling pathway [25]. A screenshot of the SCSRC for TLR-4 can be seen in Fig. 3.2. Similarly, an ABM of NF-kB response to endotoxin utilized molecular-level agents nested within "mega-agents" representing different inflammatory cell types to reproduce recognizable dynamics of endotoxin response, including priming and tolerance at both the transcription factor and cellular activation level [44].

Cell-Level ABMs of Systemic Inflammation and Simulated Trials for Sepsis

The cell-as-agent level of component representation provides perhaps the most intuitive link between the laboratory-derived basic mechanistic knowledge and the structure of an ABM. Some of the earliest examples of biomedical ABMs were focused at this level leading to the realization that even abstract agent rules could produce very recognizable dynamics that could provide deep insights into the essential characterization of a disease process [27, 50]. For example, an early ABM of systemic inflammation and sepsis, the Innate Immune Response Agent-based Model (IIRABM) viewed the inflammatory process as being governed by interactions at

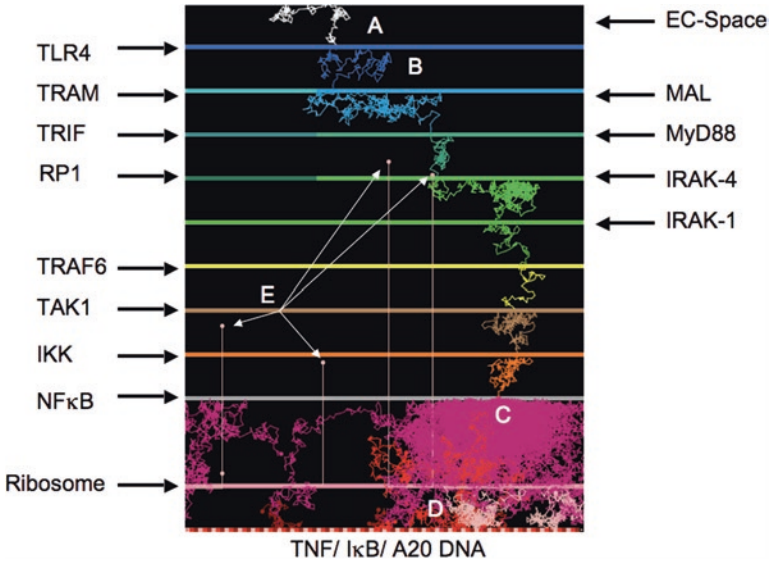

Fig. 3.2 Screenshot of spatially configured stochastic reaction chamber (SCSRC) model of TLR-4 signaling. This figure demonstrates the underlying architecture of the SCSRC as well as the signal trajectory of a single LPS signal agent. Reaction chambers are oriented vertically, and TLR-4 signaling propagates from the "top" of the model (representing extracellular space) toward the "bottom" (DNAs). The various signal transduction proteins are represented as horizontal bars across the model. The trajectory of a single LPS signaling agent as it passed through the various layers of signaling. Note the irregular path of the agent, reflecting the random movement rules that reflect the stochasticity in molecular dynamics. Letter "A" denotes the initial extracellular space where the LPS agent is introduced. Letter "B" denotes the first intracellular reaction space immediately under the TLR-4 border. Letter "C" demonstrates the signal amplification at the NF-κB activation site, as the single signal agent results in multiple NF-κB agents. "Letter D" denotes the DNA reaction space, as additional amplification can be seen in simulated transcription. Letter "E" labels synthesized TNF molecules in the process of transport to the extracellular space, seen as the straight trajectories. (This figure is reprinted with permission from Ref. [25])

the endothelial blood interface [27]. This ABM generated four clusters of distinct trajectories of model-system behavior purely by altering the degree of initial perturbation, trajectories that matched the four primary clinical scenarios associated with systemic inflammatory response. This ABM also demonstrated that the mechanistic basis of inflammation was the same whether the initiating insult was infectious, as in classical sepsis, or tissue damage, as in severe trauma.

The IIRABM was further extended to perform in silico clinical trials based on published and hypothetical inflammatory-mediator-based interventions [28]. Published pharmacologic properties of a series of mediator-targeting compounds were inputted into the ABM simulating a sepsis population. The efficacies of the interventions were then evaluated against a simulated control population. None of the mediator-directed interventions led to a statistically significant improvement in simulated patient outcome, including a set of immune augmenting interventions

(e.g., addition of Granulocyte Colony Stimulating Factor) and combination anticytokine therapy (intended to overcome possible pathway redundancy). While these results were not totally unexpected, the exercise demonstrated that the ABM could be used as a means of assessing the veracity of the proposed intervention: that is, what are the global consequences of intervening in a particular pathway, and is it actually a good idea to intervene at this point? The confirmation that what appeared to be intuitively plausible points of mechanistic intervention did *not* in fact behave as expected when placed in a systemic context demonstrated the potential usefulness of agent-based modeling and dynamic knowledge representation for hypothesis verification. We suggest that one of the primary roles of dynamic knowledge representation is exactly this type of hypothesis evaluation and verification, intended to reduce the set of plausible hypotheses and thereby help direct future investigation by eliminating therapeutic dead-ends.

Having been able to demonstrate what would not likely work, it took several years and significant advances in both computing power and analytical methods to be able to answer what *might work*. During that time ABMs were proposed as having utility as proxy models in order to solve control problems for complex dynamical systems [51]. This concept utilizes the ability of ABMs to map to the architecture of a particular system and takes advantage of the fact the since the ABM is a computational object it can be interrogated at much greater depth and at scale than the real-world system. This led to the use of evolutionary computing/genetic algorithms [30] and model-based deep reinforcement learning [52], operating on the IIRABM, to define the scope of the control problem regarding the treatment of sepsis. This work will be addressed in more depth in later chapters of this book.

ABMs of Multiorgan Inflammation and Failure

The structural/anatomic approach to multiscale modeling can be taken one step further by using the modular property of agent-based modeling to link individual organ ABMs in a multiscale architecture. The approach was introduced in an ABM of the gut-lung axis of systemic acute inflammation and multiple organ failure [12]. This ABM incorporates multiple structural and anatomic spaces, for example, endothelial and epithelial surfaces as aggregated by cell-type into organ-specific tissues and finally to organ-to-organ interconnections and cross-talk (see Fig. 3.3). This architecture also *translates* knowledge across domain specialties (molecular biology to clinical critical care), representing molecular and cellular mechanisms and behaviors derived from in vitro studies, extrapolated to ex vivo tissue experiments and observations, leading to patterns of organ-specific physiology, and finally simulating clinically relevant, interconnected, multiorgan physiology including the response to ventilator support of acute respiratory failure. This ABM also posited certain characteristics of the gut-derived pro-inflammatory compound that is circulated in the mesenteric lymph and induces pulmonary inflammation. Examining the time course of pulmonary inflammation and comparing that to generated factors following intestinal ischemia suggested that the mesenteric lymph inflammatory

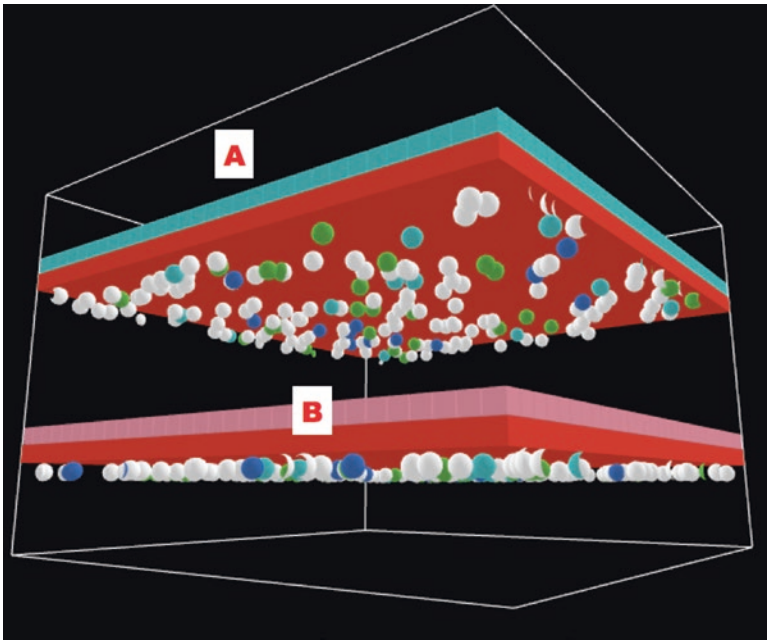

Fig. 3.3 Screenshot of multi-bilayer gut-lung ABM of systemic inflammation. The multiple bilayer topology of the gut-lung ABM is seen with the upper bilayer (Letter A) representing the pulmonary bilayer, with aqua cubes representing pulmonary epithelial cell agents, red cubes representing pulmonary endothelial cell agents, and below are spherical inflammatory cell agents. The lower bilayer (Letter B) represents the gut bilayer, with a similar configuration, the only difference being that gut epithelial cell agents are pink. Circulating inflammatory cell agents move between these two bilayers on a time schedule calibrated to the rate of systemic circulation and gut lymph flow. This ABM represents an aggregate of several submodels including endothelial-based inflammation and epithelial tight-junction protein metabolism. This ABM was able to reproduce the effects of gut ischemia in propagating the development of acute respiratory failure, the salvaging effects of mechanical ventilation, and posited the nature of the gut ischemic product driving respiratory failure as being tied to endothelial cell/tissue damage. (Figure reproduced with permission from Ref. [12] under the Creative Commons License)

compound was not an initial inflammatory cytokine, nor a translocating luminal compound manifesting decreased intestinal permeability, but rather a substance reflecting cellular damage of gut tissue with properties consistent with damage-associated molecular patterns (DAMPs). This last hypotheses remains to be completely confirmed by the sepsis research community, but at this time appears to be consistent with ongoing research in this area [53].

Moving Forward: Scaling Dynamic Knowledge Representation, the Agent-Based Modeling Format (ABMF)

As noted in the Introduction, the Translational Dilemma arises not only difficulties in multiscale representation and instantiation but is also a throughput problem. While computational modeling (including agent-based modeling) can potentially address the former, generating these models, even with a relatively intuitive method as agent-based modeling, is currently a highly specialized, laborious, and time-consuming task. Therefore, developing a scalable global strategy to overcome the Translational Dilemma will require substantially lowering the threshold for the general researcher to engage in computational modeling. We suggest that the process of constructing dynamic computational models can be augmented by leveraging ongoing work in bioinformatics and knowledge representation, primarily related to ontologies [10, 47, 54].

Ontologies are knowledge classification systems that provide a structured vocabulary and taxonomy for a particular scientific domain [55]. Ontologies utilize taxonomic class structures, their properties and the relationships between the constitutive concepts to organize information within the domain. The use of ontologies is well established in bioinformatics, and many bio-ontologies are currently found in an online repository called BioPortal [56], which is managed by the National Center for Biomedical Ontologies (NCBO) [57].

However, despite their usefulness, ontologies/bio-ontologies remain primarily classification systems that define identity relationships between concepts, but have difficulty expressing dynamic and functional relationships that can be used to represent mechanistic rules; this gap is the transition from a descriptive model to a simulation. There has been work, converting ontology-based knowledge representations into dynamic mathematical models of molecular signaling pathways [36, 58–62]. However, useful for representing the behavior of specific pathways, these approaches focus on working within a single ontology (namely the Gene Ontology) and do not deal with the multiscale aspects of biology: that is, the transition of molecules to cells to tissues to the whole organism. Alternatively, ABMs are well suited to be an integrating modeling paradigm since they capture the multiscale organization of biological systems (see Fig. 3.1). We suggest that an ABM-based framework can be used to integrate the knowledge from multiple ontologies describing different aspects of a biological system (components, functions, space, etc.) in order to construct a dynamic multiscale, translational model.

We propose the agent-based modeling format (ABMF) as a framework that leverages and integrates ontological descriptions of biology to facilitate the construction of dynamic, executable knowledge representations with multiscale representational capacity [47]. The ABMF integrates terms and meta-data from BioPortal ontologies into three-level modules formatted around the information and data needed to construct an ABM. These levels are centered on the level of the simulation agent in a “middle-out” configuration [6]. A schematic of an ABMF module can be seen in Fig. 3.4 and is organized in a series of orthogonal descriptive class

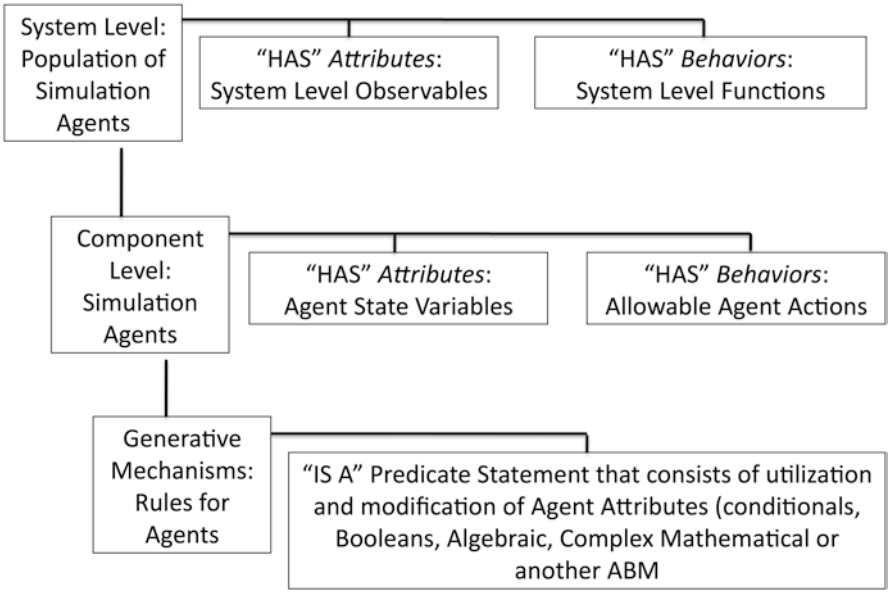

Fig. 3.4 A schematic description of an agent-based modeling format (ABMF) module. The ABMF module incorporates three levels of system representation centered on the simulation agent level, which corresponds to the classical agent-level in an ABM. The system-level corresponds to agent population behavior (including the so-called emergent phenomenon), and the lowest level of organization, Generative Mechanisms, corresponds to agent rules. Inputted Generative Mechanisms can be in the form of any formal model system, including another ABM. This gives the ABMF a recursive structure that allows nesting of ABMF modules and makes it a potential pathway to hybrid computational models that concurrently employ multiple modeling and simulation methods. (Reprinted with permission from Ref. [47])

structures that can be populated with terms extracted from BioPortal ontologies. The modular structure of the ABMF allows for nesting of modules and a recursive description of biological systems; this multiscale organizational recursion has been noted as a property of biological systems [63].

We emphasize that the ABMF is **not** "the" format for an executable ontology layer; we hope that there will be development of similar types of tools, using different modeling paradigms. However, we believe that an agent-based modeling paradigm demonstrates a robust, evolvable approach that can spur future development.

We further note that the ABMF is not a modeling platform, but rather a metastructure that helps collect and organize the components needed to construct an ABM from a biological hypothesis. There is still a significant gulf between the formatting of a biological hypothesis and the ability to construct a computer simulation of that hypothesis. The ABMF provides a pathway toward automation by leveraging the structured vocabulary and inference capabilities of ontologies. Additional text analysis and information extraction technologies can be integrated with an

ABMF constructor and provide a semi-automated way to collect potential parameter values with which to populate a simulation program.

The expression of a conceptual biological model in the ABMF places that biological model into computable form, perhaps facilitating conversion into an executable simulation through the use of a semi-intelligent computational agent. There has been recognition of the importance of ontologies in the development of intelligent system-aided model and simulation generation, with several proposed schema for the development and use of ontology-driven processes [64–66]. The repetitive nature of certain steps of model construction suggests that these steps in the creation and programming of a simulation model can be expressed as task-based algorithms embedded into an intelligent computational agent, which then treats simulation construction as a planning task using formal logical inference. Computational agents have been used in this fashion in bioinformatics for data integration and information flow management [67–71]. We have proposed that the task of converting biological conceptual models into executable simulations, including those associated with the population of the ABMF and subsequent conversion into ABMs, could be carried out by an intelligent computational agent, which we term a "computational modeling assistant (CMA)" [54]. We envision that this type of agent-directed process can semi-automate the specification-mapping work of model construction through the use of ontology-based/traditional predicate logic inference structures to generate simulation code. This will move toward achieving the translational research goal of high-throughput instantiation of conceptual models. Treating the steps of the composition process as a planning task can improve the modularity, robustness, and scaleability of knowledge integration by creating a "middle-ware" discipline, that is, *modeling*, and thereby focusing future development on the algorithmic expression of the mapping rules used in model development that form the CMA's inference instruction set. This allows expansion of the CMA's capabilities and expressiveness while maintaining interoperability with established but ongoing development in the areas of formal semantics/knowledge representation and modeling and simulation methods. We believe that this type of automation advancement offered by the CMA will lead toward the development of cyberenvironments providing scaleable high-throughput hypothesis instantiation and evaluation.

Challenges to the Use of Agent-Based Modeling

As with all modeling methods, agent-based modeling is not without its limitations. One common issue shared with all computational and mathematical modeling methods is that the quality and reliability of the models are directly related to the reliability of the underlying assumptions of the model and the quality of their implementation during construction of the model. This issue can be addressed by emphasizing transparency of both underlying assumptions and implementation details with respect to the construction of an ABM. The ODD protocol, while not developed specifically with biomedical ABMs in mind, provides a useful reference point

with respect to documenting the structure and goals associated with an agent-based modeling project [72].

One shortcoming of agent-based modeling is the difficulty in applying formal analysis to the relationship between the agent rules and the behavior of the system. Due to the combined stochastic behavior of agents and the difficulty in assigning scalar metrics to account for the spatial aspects of an ABM's output, it can be very challenging to evaluate the effect of parameter values and model structure on an ABM's behavior. Alternatively, equation-based models have well-established procedures for analytical tasks such as parameter sensitivity analysis, bifurcation analysis, and behavior-state-space determination. Work on developing mathematical descriptions of ABMs offers the prospect that formal analysis may be available in the future [73]. In the meantime, ABM researchers use a variety of strategies, such as heuristics [5, 28], literature-based constraints [31, 34], and Latin Hypercubes [9, 74] for parameter estimation and sensitivity analysis.

Some of the apprehension associated with the analysis of ABMs can be addressed by viewing ABMs as objects more akin to wet lab experimental platforms rather than more traditional, equation-based mathematical models. Pattern-oriented analysis, in which corresponding patterns of dynamic behavior are used to relate the computational ABM to its real-world referent, allows ABMs to be evaluated much in the same way as wet lab systems or model organisms [24]. From this regard, the stochastic and emergent properties of ABMs reinforce their ability to capture the robustness of dynamic behavior seen in complex systems, thereby allowing more insight into their core organizational structure. However, the fact that they are computational objects provides the opportunity to much more comprehensively characterize their behavior space [75], which can provide a means of gaining fundamental insight into the system being modeled, as has been in the case of sepsis [29]. The ability to run ABMs at scale also provides an opportunity to leverage modern advances in machine learning, artificial intelligence, and evolutionary computing as applied to ABMs as a means of trying to achieve true personalized precision medicine [76].

Conclusion

The Translational Dilemma is the greatest challenge facing the biomedical research community today. Future operational procedures for biomedical science should involve technological augmentation of all the steps of the scientific cycle and allow the knowledge generated from such research to manifest in multiple areas. These include the development of highly predictive, personalized simulations to streamline the development and design of therapies, simulating the clinical application of these therapies in population studies (in silico clinical trials), and predicting the effects of drugs on individuals. We suggest that the agent-based paradigm, incorporating knowledge encapsulation, modularity, and parallelism, can play an important role in the development of this meta-engineering process. Agent-based modeling

can provide an integrative architecture for the computational representation of biological systems. Expanding the tools for AI-augmentation of computational dynamic knowledge representation (such as the ABMF and the CMA) can significantly reduce the threshold for the general researcher to utilize computational modeling and allow investigators to "see" the consequences of a particular hypothesis-structure/conceptual model, such that the mechanistic consequences of each component of the hypothesis can be probed and evaluated. Dynamic knowledge representation enables the instantiation of "thought experiments": the exploration of possible alternative solutions and identifying those that are plausible, that is, consistent with the observed data. These models can aid in the scientific process by providing a transparent framework for this type of speculation, which can then be used as jumping off points for the planning and design of further laboratory experiments and measurements. It is hoped that the increasing use of this type of knowledge representation and communication will foster the further development of "virtual laboratories" and in silico investigations.

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Chapter 4
Integrating Data-Driven and Mechanistic Models of the Inflammatory Response in Sepsis and Trauma

Yoram Vodovotz

Abbreviations

DAMP	Damage-associated molecular pattern molecule
DBN	Dynamic Bayesian network
GM-CSF	Granulocyte-macrophage colony stimulating factor
IFN	Interferon
IL	Interleukin
IL-1ra	Interleukin-1 receptor antagonist
IP-10	IFN-γ-inducible protein of 10 kDa/CXCL10
MCP-1	Monocyte chemotactic protein-1/CCL2
MIG	Monokine induced by γ-interferon/CXCL9
MODS	Multiple organ dysfunction syndrome
ODE	Ordinary differential equation
PCA	Principal component analysis
sIL-2rα	Soluble IL-2 receptor α chain
SIRS	Systemic inflammatory response syndrome
TBI	Traumatic brain injury
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-α

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Introduction

Inflammation is an essential process in maintaining health and responding to disease. Acute inflammation is driven largely by the innate immune system, which not only serves as the first line of defense against invading pathogens but also functions to resolve tissue damage and restore homeostasis upon a variety of inflammatory conditions including sepsis, trauma, wound healing, and many more. However, when inflammation is either insufficient to address the original disruption of homeostasis, or becomes dysregulated and systemic, it can contribute substantially to the morbidity and mortality in these conditions. Dysregulated systemic inflammation also plays a significant role in the pathophysiology of diseases that are not primarily attributed to innate immunity, such as cancer and diabetes. Although the list of diseases is broad and the processes important to each setting may differ in certain respects, the core architecture of the inflammatory response to biological stress is highly conserved [1, 2].

The systemic inflammatory response syndrome (SIRS) is a major driver of morbidity and mortality in the settings of sepsis and trauma/hemorrhagic shock. Sepsis alone is responsible for more than over a million annual hospital admissions, more than 215,000 deaths in the USA per year, and an annual health care cost of over $20 billion [3–5]. Trauma remains the leading cause of mortality and morbidity for individuals under 55 years and accounts for 30% of all life-years lost, with over 190,000 lives lost annually in the USA [6, 7]. In both sepsis and trauma, the acute inflammatory response is concomitant with physiologic manifestations including changes in heart rate and body temperature, responses that act in a concerted fashion in order to help optimize host defense while minimizing tissue damage [8]. Indeed, although a well-regulated inflammatory response is crucial for effective healing and host defense, an excessively vigorous response can become self-perpetuating and lead to organ dysfunction and death [9, 10]. Both sepsis and trauma patients are particularly susceptible to multiple organ dysfunction syndrome (MODS), a poorly understood syndrome that may be partly attributed to excessive and dysregulated inflammation [4, 10]. These vastly different outcomes can be explained by the overall framework of the immune response, which includes a positive feedback loop from inflammation → damage/dysfunction → inflammation that can drive pathophysiology in inflammatory diseases [11–14].

The adverse effects of self-sustaining inflammation are likely responsible for the general perception of inflammation as an intrinsically harmful process [2, 15, 16]. However, in addition to the aforementioned beneficial roles of inflammation in the resolution of tissue injury, recent studies suggest that morbidity and mortality are worse in both experimental animals and trauma patients with low levels of early pro-inflammatory signals [17]. The emerging view of inflammation is indeed more nuanced, casting inflammation as a highly coordinated communication network that allows the body to sense and respond to challenges and subsequently to restore homeostasis [2, 11, 18]. One may consider the complexity resulting from this coordination to be an indicator of a well-regulated and properly orchestrated response,

and consequently a less complex response would be indicative of a pathological dis- or mis-connectivity of the network [14]. Guided by insights from studies on the dysregulated physiology characteristic of sepsis and trauma/hemorrhage, which have reported that a decrease in variability/complexity of heart rate can presage increased morbidity and mortality, we have suggested that well-organized dynamic networks of mediators is crucial to an appropriate inflammatory response [3, 14, 19]. Indeed, such networks are induced early in the response to experimental surgical trauma in mice, and these networks become disorganized and less complex with the addition of hemorrhagic shock to this minor trauma [19]. At the opposite end of the spectrum, trauma patients who survive their initial injuries but then succumb to MODS and die after a prolonged stay in the intensive care unit (ICU) exhibit dynamic networks of inflammation whose complexity rises rapidly as compared to propensity-matched control patients whose networks remain at a much lower degree of complexity [20]. Similarly, elevated inflammatory complexity is observed in complex polytrauma patients with orthopedic injuries [21]. Thus, data-driven computational analyses have supported the intuitive paradigm of adaptive, overexuberant, and insufficient systemic inflammatory responses [14].

The current paradigm for acute inflammation, based in large part on studies of trauma, hemorrhage, or infection, involves a dynamic cascade of cellular and molecular events. Innate immune cells such as mast cells, neutrophils, and macrophages are activated directly by bacterial endotoxin or indirectly by various stimuli elicited systemically upon trauma and hemorrhage [22–25], including the release of damage-associated molecular pattern molecules (DAMPs) [12, 26, 27]. Both DAMPs and pro-inflammatory chemokines and cytokines—primary among them tumor necrosis factor-α (TNF-α) [28–34]—further activate both parenchymal and immune/inflammatory cells, and can affect tissue/organ physiology adversely. These stressed tissues/organs feedback positively to promote further production of inflammatory mediators [14]. We have hypothesized that this behavior could lead to multi-compartment and multi-scale inflammatory "tipping points" [8, 35, 36], and have utilized mechanistic modeling approaches to abstract these complex interactions into models written as systems of equations or rules [22, 24, 25, 37–60]. Thus, we have leveraged both data-driven and mechanistic modeling approaches to gain novel insights into the inflammatory response. Both we [14, 61, 62] and others [63, 64] have called for integrating these two modeling approaches to gain insights into complex biological processes. Below, we detail this overarching vision and its application to complex inflammatory diseases.

A Systems Approach to Inflammation

The complexity and nonlinearity of the acute inflammatory response as described above have largely stymied the development of novel therapies for trauma/hemorrhage and sepsis [61, 62]. Systems biology is a paradigm for tackling complex biological systems in a holistic fashion [65]. Approaches in systems biology span a

broad range of techniques, and can be categorized roughly into correlative or causal methods, with focus on either learning basic principles of system organization and function [66–68] or building predictive computational models [66, 69]. Although there is overlap between these areas, most efforts at elucidating biological mechanisms from high-dimensional data have traditionally focused on particular points along this spectrum of computational approaches. We suggest that gleaning translationally relevant insights into the inflammatory response and its interconnected (patho)physiology will require integration of methods from across this spectrum [19, 22–25, 39, 40, 42, 70], in order to progress from data to models to actionable knowledge and prediction (ideally in an in vivo or clinical context) [26, 35, 62].

Data-Driven (Correlative) Approaches to Dynamic Inflammation Data

Statistically based approaches, with which most biologists and clinicians are generally familiar, include regression techniques that build models predictive within the conditions of the data on which the models were trained [71]. Although these methods cannot provide detailed mechanistic insights, they can be used to understand abstract features of the response, such as the presence of nonlinearities or the identification of factor interactions that affect the response. The main drawback of this class of models is the fact that they often are devoid of mechanistic insight, and their linearity in the parameters can over-fit to the data on which they were trained. Associative methods such as hierarchical clustering may be used to highlight the natural variability, as well as any overlap, across experimental or clinical conditions. Hierarchical clustering is a simple and unbiased clustering method that aims to build a hierarchy of clusters. The limitation is the cluster must be built pairwise; since it is purely based on the similarity between the data, the cluster may lack biological relevance [67]. Hierarchical clustering is used extensively in the genomics field, and was used to discern patterns and co-regulated clusters of gene expression associated with sepsis and trauma/hemorrhage in both animals [72–75] and humans [76, 77]. More recent studies have utilized other metrics to cluster critically ill patients, for example, metrics related to multiple organ dysfunction or other clinical variables [78–80].

Another important data-driven method is principal component analysis (PCA), which reduces a high-dimensional dataset into a few principal components that account for much of the observed variance in the data. When applied to time-series data, PCA may identify the subsets of the variables under study (genes/proteins/etc.) that are most strongly representative of the response. Thus, these principal components may be interpreted as the principal drivers of the observed response and can give some mechanistic insights into the underlying process [19, 67]. In the setting of inflammation, correlative approaches such as PCA could facilitate the development of therapeutics by yielding insights into the mechanisms by which

these therapeutic modalities may function [81]. Similarly, PCA may aid the development of diagnostics by analyzing the inflammatory milieu in the blood resulting from inflammatory spillover, in order to identify the health state of individuals and possibly inform patient-specific interventions [13]. In this regard, a recent study provided proof of concept for the derivation of trauma patient endotypes in the form of "inflammation barcodes," by segregating the clinical outcomes of two small cohorts of trauma survivors based on their dynamic core inflammatory responses within the first 24 h post-injury, using patient-specific PCA (i.e., PCA on the time-series circulating inflammatory mediator data of each individual patient) combined with unsupervised hierarchical clustering [82]. Later studies (Schimunek et al., unpublished) applied this method to a cohort of over 230 trauma patients and involved the analysis of a larger number of inflammatory mediators. This study suggested that three core inflammatory endotypes exist after trauma, which generally match the qualitative phenotypes of adaptive, overly exuberant, or overly damped post-injury systemic inflammation [14].

Gram-negative bacterial lipopolysaccharide (LPS) is both a central mediator of sepsis and a canonical inducer of acute inflammation via toll-like receptor 4 (TLR4). Sepsis involves the systemic spillover of inflammation that normally remains localized in individual organs. We sought to gain insights into (1) early vs. later drivers of LPS-induced inflammation in various compartments, and (2) the systemic spillover from affected organs vs. local production of inflammatory mediators in the blood [83]. Having obtained extensive data on the dynamics of inflammatory mediators at the protein level, data-driven computational modeling of principal characteristics and cross-correlations were performed. A major tool developed for this study was a time-interval variant of PCA (TI-PCA). In addition to verifying known mechanisms in LPS/TLR4-driven acute inflammation, this approach yielded key insights into the progression of inflammation across tissues, and also suggested the presence of TLR4-independent pathways (especially in the gut) [83].

Despite these quasi-mechanistic insights and potential utility for stratifying clinical outcomes, principal components, being linear combinations of the original mediator variables, often do not lend themselves to clear biological interpretations [67]. Principal components do, however, greatly ease dimensionality issues and provide a compact and efficient explanation of the data in terms of meaningful groups of mediator variables. Successful implementation of PCA within this context requires some adjustments. Mediators are measured on widely different scales that need to be appropriately adjusted for meaningful comparisons. This may be done in several ways, taking into account known biological effects. Two mediators may show significant variation within their possibly very different ranges, in which case one can rescale them appropriately. However, this should not be done, for example, if one of the two hypothetical mediators has small variation simply because it is an inert factor. Rescaling inert factors would simply amplify the error in the data. Once this rescaling issue is settled, a PCA can be carried out. In our own studies, we augmented such analysis in two additional ways. We reevaluated the importance of a specific mediator as follows: deem k principal components as being significant (by explaining, as usual, a certain fraction of the total variance). Next, assess the

importance of each mediator in view of these k principal components, by adding the absolute values of the weights associated to that mediator within the k principal components. The higher the sum, the more relevant the mediator. This allows us to rank the relative importance of the mediators. A word of caution: a mediator that is naturally very noisy may be ranked as important by the PCA method, but it need not necessarily be highly relevant to the phenomenon under study. The last point we make is that it is often more convenient to work with only biologically intuitive linear combinations of mediators, rather than principal components. Such intuitive linear combinations are usually suggested by the principal components themselves, from which we may delete certain mediators that appear nonintuitive. This still reduces the dimension, offers good biological interpretation, but the analysis that results is more complicated, since these linear combinations become correlated [26, 67].

In addition to blunt trauma, another clinically important area in which data-driven modeling has yielded novel insights is traumatic brain injury (TBI) [60, 84]. Inflammation induced by TBI can lead to both morbidity and mortality [85, 86]. We obtained both clinical data and data on the dynamic changes in multiple inflammatory mediators in the cerebrospinal fluid of TBI patients. The clinical data on each patient consisted of one-dimensional variables such as age, gender, presence of infection, bleeding, decompression, presence of subarachnoid hemorrhage, and Glasgow Coma Scale (GCS), which quantifies the nature of the initial brain injury on a numerical scale. The Glasgow Outcome Score (GOS) is the outcome variable; we view it as the response variable to study and predict, as a function of the other input variables. The GOS quantifies the state of health of the subject when hospital treatment ceases. While the clinical progression and biological underpinnings of TBI have been studied extensively, accurately predicting a patient's prognosis following a TBI remains a difficult challenge for clinicians [87]. Accordingly, we hypothesized that early TBI-induced inflammation can foreordain similar patients for survival vs. mortality and carried out a study in which we measured inflammatory mediators in the cerebrospinal fluid of TBI patients with subsequent and in silico modeling to gain insights into this process [60, 84].

We next hypothesized that changes in the probability of survival vs. non-survival are related to the dynamics of the inflammatory response, the factors intrinsic to the patient (i.e., key demographic indicators), as well as to metrics related to the injury itself. To test this hypothesis, we developed a method, which we call "dynamic profiling," as a means of assessing the dynamic course of a TBI patient within the hospital environment [84]. In the TBI application of dynamic profiling, a cluster is a subset of TBI patients who share similar characteristics. The set of clusters, recalculated after each set of cytokine readings, forms a partition of the TBI patients. These clusters were determined using the spectral Laplacian and Hartigan's k-means method, resulting in disjoint groups at different stages. Initial clustering was based on GCS score; subsequent clustering was performed based on clinical and demographic information and then further, sequential clustering based on the levels of individual inflammatory mediators over time. These clusters assessed the risk of mortality of a new patient after each inflammatory mediator reading, based on the

existing information in the previous data in the cluster to which the new patient belongs at the time, in essence acting as a "virtual clinician" [84].

Like most biological processes, inflammation proceeds as a series of interacting cascades of signaling events that are often reflected in the production and secretion of inflammatory mediators that, in the healthy state, likely form well-coordinated networks [19, 20, 88–97]. In order to better discern organizational aspects of interacting networks of inflammatory mediators, such as co-regulation or auto-induction, a variety of methods have been developed. Hierarchical clustering and Bayesian methods use high-throughput genomic or proteomic data of several time-points and/or conditions to correlate gene expression patterns with function and infer regulatory networks of correlated genes [98, 99]. Several developments in these methods over the last 20 years have yielded more informative networks that can be more easily translated into mechanistic models [100, 101]. A key point is that any network analysis method must reflect, and yield insights into, the dynamics of a given inflammatory response. For example, we have utilized a relatively simple network analysis method employed over discrete intervals of data to analyze the commonality and differences between experimental surgical cannulation trauma + hemorrhage in mice and the sham procedure (surgical cannulation only). We refer to this method as dynamic network analysis (DyNA) [19]). This method allows for a highly granular dissection of networks over discrete time intervals, similar to the TI-PCA described above. This analysis suggested that the circulating mediators produced in response to the sham procedure were characterized by a high degree of interconnection/complexity at all time-points, while the response to trauma/hemorrhage consisted of different central nodes, and exhibited zero network density over the first 2 h with lesser connectivity vs. sham at all time-points [19]. This same method was used to gain insights into the human blunt trauma, in the context of survival vs. death [20]; nosocomial infection [102]; and the impact of metabolic dysregulation [103], diurnal variation [104], and orthopedic injuries [21] on trauma outcomes.

Among network methods, dynamic Bayesian networks (DBNs) are particularly suited for inferring directed (causative) networks of interactions based on the probabilistic measure of how well the network can explain observed data. Dynamic Bayesian networks provide a good platform for defining potential feedback structures in data in order to increase our knowledge of connectivity in biological processes and may be supplemented by additional experimental evidence and expert knowledge to hypothesize mechanistic models [98, 105]. In the context of trauma/hemorrhage and infection/sepsis, we have applied this methodology to gain insights into how systemic inflammation involves in survivors vs. non-survivors of blunt trauma [20] and TBI [60]; the role of innervation in the inflammatory response to blunt trauma [106]; the impact of injury severity [107] and hypotension [108] on dynamic networks of inflammation; and, in experimental trauma/hemorrhage, into the differential role of TLR4 on myeloid vs. dendritic cells [109].

The translation of in vitro findings to clinical outcomes is often elusive, and this is another arena in which we employed a suite of data-driven modeling approaches [110]. We hypothesized that these methods would help bridge in vitro hepatocyte data and clinical trauma/hemorrhage, in which the liver is a primary site of

inflammation [16]. Primary mouse hepatocytes were cultured under hypoxic or normoxic conditions, and both the cell supernatants and protein lysates were assayed for a variety of inflammatory mediators. Statistical analyses, hierarchical and *k*-means clustering, PCA, and dynamic network analysis suggested that the chemokine monocyte chemotactic protein-1/CCL2 (MCP-1/CCL2) is a central coordinator of hepatocyte-mediated inflammation in mouse hepatocytes. Hepatocytes from MCP-1-null mice had altered dynamic inflammatory networks. Circulating MCP-1 levels segregated human blunt trauma survivors from non-survivors. Furthermore, trauma survivors with elevated early levels of plasma MCP-1 post-injury had worse clinical outcomes as compared to patients with low plasma MCP-1. This study revealed a novel biomarker for trauma outcomes based on an experimental and computational framework for discovery [110].

Dynamic, Mechanistic Modeling of Inflammation

Mechanistic computational models are derived from more detailed biological and physical descriptions of a system and have a rich set of tools for both analysis and simulation. These models, based on causative interactions, can be constructed as ordinary differential equations (ODEs, see Chaps. 2, 7, and 11), rules-based models (RBMs) [111], and agent-based models (ABMs, see Chaps. 3 and 12) among other methods (including hybrid methods), and have the advantage of potentially being predictive outside the range of conditions/time-points on which they were calibrated. Although it is often difficult to parameterize such models, they can unveil emergent phenomena not immediately obvious from the interactions that are encoded in the model. There are several analytic tools, for ODE models especially, that have been developed and used to decipher the organizational principles of networks (or subnetworks), the properties that explain the dynamics and robustness/sensitivity of a given complex system, and, perhaps most importantly, the critical points of control in the system [68]. These tools are particularly important in order to help define the complex interplay between the inflammatory mediators in the blood and other compartments [57, 83]. Tools from dynamical systems theory allow identification of the possible steady state(s) of a system as well as the dynamics of the system's time evolution. These tools have been used extensively to explain (or predict, depending on the context) diverse behaviors such as biostability, hysteresis, and oscillations in a variety of biological systems [112]. Bifurcation diagrams, in particular, can be used to map out the effects of a particular parameter on the possible steady state behaviors of a system, and to indicate the transition from a healthy steady state to a pathological one [24, 39, 113, 114]. The relative importance of parameters can also be quantified by calculating the change in the model output in response to changes in the parameter values using sensitivity analysis [68, 115]. These methods work in a complementary fashion to identify the key points that can be modulated to change the behavior of a system.

The analysis of ODE models of biological systems can be approached from a control theory perspective as well. Achieving robustness and efficiency is the core principle of both evolution and engineering. Indeed feedback, a pervasive biological phenomenon, is also a fundamental component of control strategies [116]. An ODE model is the equivalent of a state space representation of a control system. Thus, it is possible to decompose the biological system into a control structure and analyze the role of each component using control theoretic tools that characterize their robustness and identify the key mediators that modulate the performance of such a control system [117]. These analyses are especially relevant given that the "tipping point" phenomenon in the inflammatory response is likely the result of a failure of the body's control structure to handle stress.

While we wish to navigate through the process of data → data-driven model → mechanistic model → prediction and understanding of the innate immune response, we seek to put it in the perspective of translational applications with a focus on clinical and preclinical settings [61, 62]. Much of the work in systems biology has understandably been in simpler, well-studied model organisms, but even among studies focused on preclinical science, there has been an overall lack of translation to the clinical arena. *Translational systems biology* is a framework with a focus on translational insights for novel diagnostic or therapeutic purposes and predictive mathematical models that inform in silico clinical trials [11, 61, 62, 118, 119]. Initially formulated to deal with the clinical challenge of integrating acute inflammation and organ dysfunction in critical illness, this work expanded to include healing of acute and chronic wounds and infections in various diseases, and rational dynamic modulation of inflammation.

We and others have created mechanistic computational models of acute inflammation in sepsis [24, 37, 38, 42, 120], endotoxemia [22, 39, 40, 44, 45, 50, 121–129], and trauma/hemorrhage [22, 23, 25, 40, 57]. In large part, these models (both ODE and ABM) are based on the typical progression of the inflammatory pathway described in the preceding section. Some of these models are purely theoretical (e.g., [24, 37–39, 42, 122]), while others are based on data either at the protein [22, 23, 25, 40, 57] or mRNA [121, 122, 125–127] level. Similar mechanistic models have focused on related diseases such as necrotizing enterocolitis [46, 130].

Inflammation is an inherently multi-scale process that manifests at the molecular, cellular, tissue/organ, whole-organism, and population levels [36]. Early models of acute inflammation at the cellular level highlighted the nonlinear responses to multiple exposures to the same stimulus (Gram-negative bacterial lipopolysaccharide) [45, 121, 123–125, 129]. Some of these computational studies based on in vitro data suggested molecular control mechanisms that lead to the phenomena of nonlinear responses to repeated inflammatory stimulation at the cellular level [45, 123–125, 129]. One such in vitro study involved mouse macrophages treated with extracellular β-nicotinamide adenine dinucleotide (NAD^+), a ubiquitous intracellular molecule that is anti-inflammatory when given extracellularly [131]. In that study, we hypothesized that extracellular NAD^+ would modulate the anti-inflammatory cytokine transforming growth factor-β1 (TGF-β1). Indeed, NAD^+ led to increases in both active and latent cell-associated TGF-β1 in mouse macrophages.

The time- and dose-effects of NAD^+ on TGF-β1 were complex and biphasic. A statistical model suggested that the effects of NAD^+ on TGF-β1 were nonlinear and this model was capable of predicting not only the levels of active and latent TGF-β1 but also the biphasic dose-effect of NAD^+. Based on these data-driven modeling studies, we inferred that the effects of NAD^+ on TGF-β1 are nonlinear. Accordingly, we created a nonlinear ODE model of interactions we considered the most parsimonious and yet still capable of recapitulating the complex biological phenomena observed experimentally. Model-predicted levels of TGF-β1 protein and mRNA were largely confirmed experimentally, but also suggested the presence of other mechanisms of regulation of TGF-β1 by NAD^+ [51]. These studies highlight the utility of traditional biochemical/pharmacological studies coupled with computational modeling in defining novel biological mechanisms.

Combining Data-Driven and Mechanistic Modeling of Inflammation

We have utilized dynamic data, data-driven modeling, and dynamic mechanistic modeling in diverse contexts. We also utilized both correlative (transcriptomic analysis, PCA, regression) and causative (ODE) models in our in vivo studies on the role of trauma in the murine response trauma/hemorrhagic shock. Initial studies using a literature-based, in vivo-calibrated mechanistic ODE model suggested that the underlying trauma is central in driving the inflammatory response to combined trauma/hemorrhage, both systemically and in the liver [23]. Transcriptomic data supported these model predictions, as indicated by a large overlap between the genes and pathways induced in trauma alone vs. those induced in the setting of experimental trauma/hemorrhage [23]. This ODE model was extended to include details of experimental trauma/hemorrhage in mice (e.g., bleeding rate and target blood pressure), and further validated using a unique, computerized platform for automated hemorrhage that was constructed specifically to test the behavior of this mathematical model [25]. Later, multivariate regression, hierarchical clustering analysis, PCA, and dynamic network analysis all suggested that despite a large overlap at the level of unprocessed inflammatory mediator data (as shown by inconclusive hierarchical clustering of these data), there were major mechanistic differences between surgical trauma alone and trauma/hemorrhage [19].

We also employed data-driven modeling to develop mechanistic models of TBI in order to better understand the inflammatory characteristics of TBI survivors and non-survivors [60]. Similar to our findings based on data-driven modeling in the context of human blunt trauma [20], we found that both survivors and non-survivors had distinct clinical response trajectories to injury. Using PCA and dynamic network modeling, we inferred similar overall characteristics of inflammation in TBI survivors and non-survivors. This led us to hypothesize that clinical outcomes following TBI might be associated not with different inflammatory wiring but rather

with different initial conditions and parameters of inflammation in survivors vs. non-survivors. Accordingly, an ordinary differential equation model of TBI was generated. This model was calibrated separately to the time-course data of TBI survivors vs. non-survivors as a function of initial GCS. Analysis of parameter values in ensembles of simulations from these models suggested differences in microglial and damage responses in TBI survivors vs. non-survivors [60].

In a similar fashion, we created a two-compartment mathematical model of porcine endotoxemia [50], based on an existing mathematical model of mouse endotoxemia [22, 23, 25, 40], in order to further test the hypothesis that a conserved inflammation framework could have radically individual manifestations. Principal component analysis of circulating inflammatory mediators suggested a central role for the cytokine interleukin-1β (IL-1β) in this inflammatory response. Based on this analysis, we constructed a two-compartment ODE mathematical model that encompasses inflammation, lung (patho)physiology, and a damage variable that recapitulates the health of the animal [50]. This mathematical model could be fit to both inflammatory and physiologic data in the individual swine, whose outcomes ranged from a self-resolving inflammatory response with fairly normal lung histopathology and function, through various degrees of dysregulated inflammation and lung damage, to death accompanied by severe lung injury [50]. More recently, we augmented this pig-specific, two-compartment ODE model to include a third "tissue" compartment [57]. This three-compartment mechanistic model was initially calibrated with data from individual surviving trauma patients, data that were used to produce 10,000 in silico patients subjected to virtual trauma/hemorrhage. This study raises the possibility of individualized outcome prediction for trauma patients as well as showing the potential for in silico clinical trials based on a small, but representative, cohort of actual patients [57].

Most recently, we integrated data-driven and mechanistic modeling to define a novel inflammation control architecture (Azhar et al., unpublished observations). We obtained time-course data on multiple inflammatory mediators in the blood of blunt trauma patients. We then used DBN inference to suggest a hypothetical, novel control architecture for systemic inflammation: a three-way switch comprising the chemokines MCP-1, monokine induced by γ-interferon/CXCL9 (MIG), and interferon-γ-inducible protein of 10 kDa/CXCL10 (IP-10). To test this hypothesis, we created a logical (mechanistic) model comprising this putative architecture. This model predicted key qualitative features of systemic inflammation in patient subgroups, as well as the different patterns of hospital discharge of moderately vs. severely injured patients. Thus, a rational transition from data to data-driven models to mechanistic models suggests a workflow for discovery in the context of inflammation.

Conclusions and Future Prospects

We have increased our understanding of the inflammatory response beyond description of its symptoms, and unveiled an ever-increasing complexity underlying this evolutionarily conserved internal communication mechanism [3, 12, 132] that manifests at multiple biological scales [35, 36]. Clinically translational systems approaches to inflammation have the potential for the identification of novel, rationally designed therapies and diagnostics—as well as for gaining new basic mechanistic insights—via combined data-driven and mechanistic modeling.

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Chapter 5
Therapeutics as Control: Model-Based Control Discovery for Sepsis

Gary An, Chase Cockrell, and Judy Day

Introduction

The greatest challenge in biomedicine, termed the "translational dilemma," is the ability to effectively and efficiently translate the output of basic research into the mechanisms of disease into effective clinical therapeutics [1]. The translational dilemma is most notable in complex diseases that involve multi-scale disorders of homeostatic and response pathways such as cancer and sepsis. Yet there remains a tendency to believe that a single agent (or at most a limited combination) given in a regimented fashion is the means of treating such diseases. We believe that this mindset is driven by a traditional, reductionist, and static perspective of disease: that is, a "disease" is a particular entity through its course, and can be treated as that entity across individuals and time. Recognition of the limitations of this approach has led to the concept of "personalized" and "precision" medicine, but we believe that current approaches that use these labels remain insufficient to the goal of tailoring therapies to specific patients. As such we have developed a series of axioms for "real" precision medicine that actually address what one might believe the combination of the words "precision" and "medicine" might mean.

Axioms of Precision Medicine [2]

Axiom 1:
Patient A is not the same as patient B (Personalization)

Axiom 2:
Patient A at time *x* is not the same as patient A at time *y* (Precision)

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Axiom 3:
The goal of medicine is to treat; prognosis is not enough (Treatment)

Axiom 4:
Precision medicine should find effective therapies for every patient and not just identify groups of patients that respond to a particular regimen (Inclusiveness)

These axioms are constructed to directly address the intent of developing a robust methodology to be able to develop therapies/strategies to specifically treat a particular patient at a particular point in their disease process. Axiom 1 addresses the importance of being able to individualize therapy, distinct from the current approach of "precision medicine" that focuses on identifying subgroups of populations' responses to specific therapies but not down to the level of the individual. Axiom 2 recognizes that different interventions may be necessary as a specific patient progresses throughout the course of a disease; intrinsic to this axiom is that it casts "disease" as a trajectory that inherently incorporates dynamics. Axiom 3 emphasizes the ultimate interventional nature of medicine: diagnosis and prognosis are not enough. The goal of medicine is to take a bad prognosis and turn it into a good outcome (i.e., altering a disease trajectory to one of health and recovery). Axiom 4 is to correct the current tendency of "precision medicine" to focus on identifying groups of responders but with no clear path or plan for patients for whom no effective therapy can be identified. Therefore, true precision medicine needs to have a strategy to identify effective controls for those individuals for whom no existing therapy works.

Given these axioms, the goal of precision medicine is developing methods that can, with greater specificity than currently exists, identify an individual's position in disease trajectory space, make some forecast of what that trajectory is, and pose putative controls that can direct that trajectory to a desired end state (i.e. health). Thus, the challenge of precision medicine should be considered a control discovery problem. The discovery aspect of this approach (Axiom 4) distinguishes it from existing "control" applications in biomedicine, which attempt to optimize existing interventions either using classical control theory (i.e., artificial pancreas [3], burn-fluid resuscitation [4], and control of depth of general anesthesia [5]) or via data-driven machine learning (ML) and artificial intelligence (AI), for example, fluid and vasopressor management in sepsis [6]. The primary challenge in modern biomedicine is the development of new therapeutics, not just merely reordering existing ones, and therefore a more comprehensive and responsive strategy calls for a control discovery approach that includes: (1) increased granularity of mathematical descriptions of the control task scaled to the level of pharmacologic intervention; (2) the capability to identify new or novel therapies; (3) addressing the persistent problem of data sparsity; and (4) the ability to assess counterfactuals in evaluating effective control strategies. We propose that model-based control discovery addresses these limitations, specifically that mechanism-based dynamic models can be used as proxy/surrogate systems for a series of control discovery methods that: (1) identify the scope of the control problem for a particular disease; (2) potentially direct concurrent basic science and clinical investigations; and (3) provide insight into

iterative refinement of the models themselves. We recognize that this approach is in its infancy, highly subject to the quality of the mechanistic models used and therefore is currently quite distant from any direct clinical translation; however, we assert that this approach represents the only tractable path forward to achieving the axioms of precision medicine above.

Model-Based Control Discovery: Overcoming Limitations of Current Biomedical Research

Existing approaches to discovering and developing new therapeutic modalities operate by making a series of inferences of potential efficacy based on preclinical experiments, and then attempting to determine clinical efficacy through clinical trials that operate on population-level statistics. As evidenced by the translational dilemma, this approach has significant limitations, not in the least that it insufficiently captures the intrinsic dynamics associated with disease. Appreciation of the dynamic aspects of biological systems is important because these systems evolve over time as their trajectories are influenced by positive and negative feedback structures [7]. These internal, homeostatic control processes are often overlooked during the course of traditional therapeutic design where the reductionist mindset often leads to a naively simplified cause–effect perspective that results in both therapeutic inefficacy and/or paradoxical effects. The only means of anticipating the effects of these intrinsic control properties is through their representation by mechanistic/quasi-mechanistic models that can explicitly represent the dynamics of these systems, and thus characterize the responses to a potential control. Furthermore, the nonlinear nature of biological response pathways and the inherent stochasticity of these processes mean that a single individual can have potentially vastly different states during the course of a disease. As such the ranges of the trajectory and state spaces of biological processes cannot be effectively characterized through either clinical data collection or preclinical experimental investigation; such characterization is only possible with sufficiently granular mechanistic models that can "fill out" the possibility space of the target system [8]. This comprehensive representation capacity is critical for assessing the efficacy of potential control strategies. While the application of an intervention or control may change the direction of a particular trajectory, the efficacy of that change can only be evaluated against the counterfactual "road not taken," for example, the trajectory without the intervention. Assessing counterfactuals for potential interventions/controls not yet implemented can only be accomplished if there is a mechanistic means of generating the dynamic trajectories to be compared. The models used for this type of control discovery should have mechanistic and dynamic representation of the biology at the same scale at which potential interventions would be designed in order to minimize assumptions that could hide the nonlinear and paradoxical effects noted above. Each mechanistically represented component represents a potential target for control, for example, a

mediator with a known mechanistic role, which however is not yet a target for drug development, could be identified as a potential target for additional development. It is in this fashion that these mechanism-based models can serve as proxy/surrogate systems for control discovery [2, 9]. The following sections will demonstrate how this would work using the example of multimodal control of sepsis.

Sepsis as a Control Problem

Sepsis is a disease process that involves the pathophysiological consequences of acute inflammation, often subsequent to severe infection, and is responsible for more than 215,000 deaths in the United States per year with an annual health care cost of over $16 billion [10]. There is currently not a single drug approved by the U.S. Food and Drug Administration that specifically targets the pathophysiology of sepsis [11, 12]. We suggest that this failure represents a fundamental misunderstanding about the nature and role of the inflammatory response in sepsis, namely that the acute inflammatory response is a necessary mechanism for an effective response to injury and infection. We assert that the "pathological" manifestations of inflammation in sepsis can only be appreciated as arising from a context where there is no preexisting disorder of inflammatory homeostasis. This is in distinct contrast to the use (and efficacy) of anti-cytokine therapies in rheumatological disorders, such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease, which arise from an underlying disorder of inflammatory homeostasis [13].

The concept of control is intrinsic to this appreciation of the homeostatic nature of inflammation: a pro-inflammatory response that is augmented by forward-feedback control (to increase the efficacy of the response to the initiating insult) [14, 15] and attenuated by anti-inflammatory mediators [15, 16]. Sepsis manifests when these positive and negative feedback processes are disordered to a point where their ostensibly beneficial function leads to unintended collateral damage to otherwise normal tissue (e.g., remote organ failure). The misunderstanding about the contextual role of pro- and anti-inflammatory mediators manifests in how therapeutics for sepsis were developed and evaluated. Focusing on the clinical disease phenotype in a reductionist manner led to a series of manipulations of various cytokines/mediators, damage-associated molecular pattern molecules (DAMPs), oxygen and nitrogen free radicals, coagulation pathway intermediates, and vasoactive peptides and lipids, none of which resulted in successful Phase III clinical trials. While these failures have led some to question whether anti-inflammatory biologics were properly applied to sepsis [17, 18], these critiques lacked the same understanding of how to approach the challenge of controlling a highly dynamic and heterogeneous process, namely the use of computational models to provide insight into control modalities. Drawing upon lessons from other domains that deal with complex multimodal control, the following sections describe the application of well-established process control engineering to sepsis using model predictive control (MPC) on an ordinary differential equation (ODE) model of sepsis, and then describe the application of

model-based machine learning and artificial intelligence in a manner similar to the game-playing artificial intelligence systems to further define the scale and scope of the control modalities that might be necessary and as a path for control discovery.

Insights from Model Predictive Control of Sepsis

For decades, model predictive control (MPC) has been a widely used methodology for successful control of industrial "plant" processes, with various stages of development in its history (see, e.g., Ref. [19]). The decision to utilize MPC as a tool for control discovery in sepsis stems from the viewpoint of sepsis as a dynamic process not able to be manipulated easily to prevent an unfavorable outcome. In a prior work, [20] an ODE model, termed here the ODE sepsis model, was developed to explore the dynamics of the pathogenesis of Gram-negative bacterial infection. The ODE sepsis model consists of four variables, some of which combine multiple mediators with similar function: a pathogen population P, pro-inflammatory mediators N^*, an integrative variable representing tissue damage/dysfunction and related debris in the form of damage-associated molecular pattern molecules (DAMPs)D, and, finally, anti-inflammatory mediators C_A.

The modeling study of Ref. [20] highlighted not only the nonlinear dynamics of a simplified representation of the acute inflammatory response as a complex system, but also demonstrated through a simple illustration the highly nonlinear response to attempted intervention. Modulation of a particular variable's state at a certain time (i.e., the intervention) during the course of a simulated infection gave rise to a wide variety of *mostly unfavorable* state behaviors that were unpredictable due to the nonlinear response (see Fig. 5.9 in Ref. [20]). If a simplified representation of this complex response was not intuitively obvious to control even though a comprehensive understanding of the dynamics of the system were known, what would it truly take to favorably control the dynamics of clinical sepsis? This question is the translational dilemma for sepsis.

Even in this reduced but still complex system, the predicted outcome of some intervention was impossible to intuit much less to identify a priori *what* intervention(s) could accomplish a particular objective or *when* to implement such intervention(s). Thus, MPC was suggested as being a well-suited approach to investigate such questions and its application to the ODE sepsis model was utilized for selecting appropriate therapeutic targets and developing administration strategies in the correct amounts and at the right times [22, 23].

In MPC, a *predictive model* plays the role of a computational surrogate for the target system and is used to determine effective control measures for a given time step by predicting how prescribed actions will affect the system it represents. The method optimizes the desired objective over a finite time horizon, implementing the subsequent control input(s) in the target system for the current time after which the optimization is repeated for the next time step. A key component of the methodology is that the state of the target system is reevaluated after a given implemented

action via measurements, for example, and these data are used to update the predictive model prior to the next time step (control interval). Thus, a more comprehensive reason to use MPC is that, as a control methodology, it allows for the predictive model to be updated prior to each optimization. The updating occurs before another control intervention is initiated and updating could be with respect to the predictive model's states, parameter values, or even structure. Thus, measurements or observations can be incorporated along the way so that the predictive model can adapt to the target system. For equations-based models, the mathematical theory underlying MPC regarding constrained optimization for a control objective is well established as are the methods for numerically solving nonlinear optimization problems subject to various constraints [24].

The ODE sepsis model, with its nonlinearities ensuring sufficient representational complexity, provides an ideal test system for a proof-of-concept investigation of control discovery for sepsis with MPC, despite the lack of a one-to-one correspondence of its variables to specific biological mediators, especially with respect to the variables N^* and C_A. While direct clinical relevance of the model is limited, this is counterbalanced with the in-depth knowledge of the model dynamics through the ability to perform rigorous analysis. The results of Ref. [20] showed that the model exhibits tri-stability (in the positive octant), for a set of biologically admissible parameter values. These three stable equilibria are translated into clinically relevant outcomes/states: a healthy equilibrium where $P = N^* = D = 0$ and C_A is at a background level, a septic death equilibrium where all mediators, N^*, C_A, and D, as well as pathogen, P, are significantly elevated, and an aseptic death equilibrium where $P = 0$ but N^*, C_A, and D are elevated far above background.

To evaluate the potential of MPC for sepsis using the ODE sepsis model, the following aspects for the framework need to be established: identify the target system(s), define the predictive model, mathematically formulate the desired objective of any intervention, define and incorporate intervention inputs into the model, and define constraints on the control problem. This is precisely what the studies in Refs. [22, 23] set out to do and improve upon, respectively. First, the ODE sepsis model (with a particular parameterization and initialization) will serve as the predictive model for a target system. However, at this stage in the proof-of-concept arena, the target system(s) need to be defined virtually. We therefore define multiple parameterizations and initializations of the model, thereby forming a heterogeneous virtual patient cohort, each representing a target system of the MPC. In other words, each target system represents a virtual patient with a distinct and unique parameterization and initialization of the ODE sepsis model, different from one another and from that of the predictive mode.

When solved/simulated, each parameterization of the system evolves through time and is categorized at the end as one of the three distinct outcomes described above. Thus, the outcome of each virtual patient's disease trajectory is classified accordingly: simulations ending with negligible P are healthy or aseptic, respectively given the ending values of N^*, C_A, and D; and those ending with elevated pathogen are septic. Figure 5.1 illustrates these three possible outcomes determined by the collective inflammatory response of N^*, C_A, and D along with the pathogen

trajectory over time. Note that even though a healthy outcome may resolve on its own after a while (see blue dashed curves in Fig. 5.1), the level of inflammation during the course of disease exceeds a predefined level indicating hyperinflammation by the standards of the model. Thus, from the various unique parameterizations and initializations created, 620 exceeded that threshold thereby emulating the clinical sepsis population known for the presence of hyperinflammation. In order to compare the results of implementing any type of intervention to none at all (i.e., placebo), we calculate the baseline recovery rate for the placebo scenario, which was 40% or a 60% mortality (i.e., aseptic or septic death outcome).

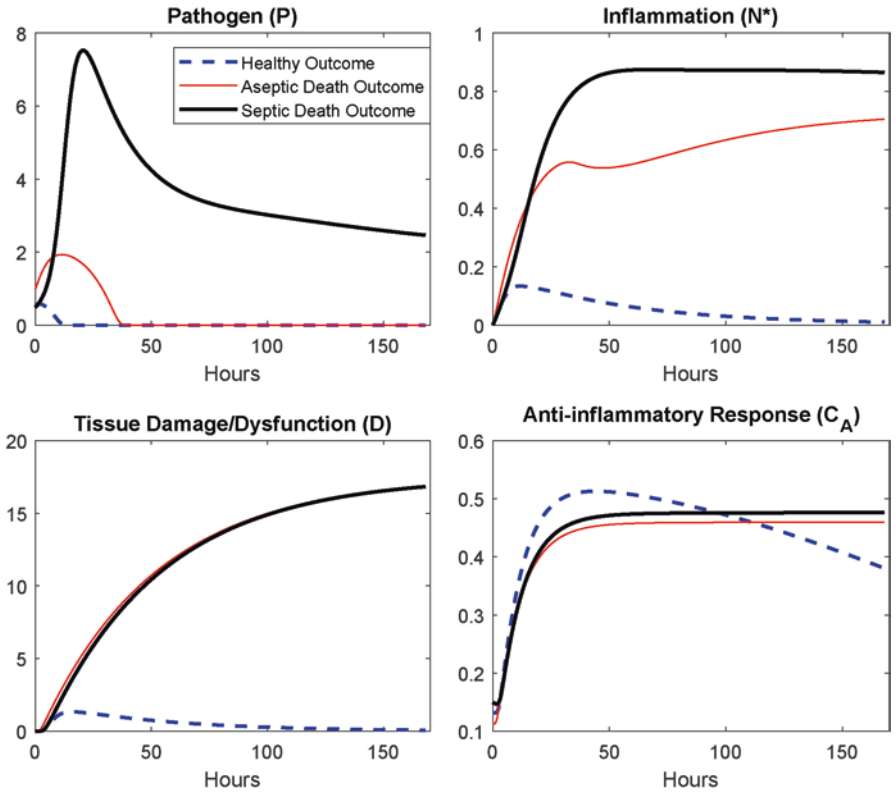

Fig. 5.1 Illustration of the trajectories of Pathogen level (P), Inflammation (N*), Tissue Damage/Dysfunction (D) and Anti-Inflammatory Response (C_A) in three possible patient outcomes: healthy outcome (blue dashed) in which pathogen is eliminated and the collective inflammatory response (N^*, C_A, and D) resolves even after displaying a notable measure of elevation (note for C_A the resolution takes longer than what could reasonably be shown); aseptic death outcome (red thin curves) in which pathogen is eliminated after a period of time but the collective inflammatory response remains elevated; septic death outcome (black thick curve) in which neither the pathogen is eliminated nor is the collective inflammatory response resolved. In any of the possible outcome scenarios here, the goal would be to intervene to help eliminate pathogen and guide the collective inflammatory response to resolution while also not inflicting any harm with the intervention used

Having defined a predictive model and created target systems, we turn to the task of defining a desired objective for any intervention. The objective can be colloquially described as redirecting the virtual cohorts' state trajectories shown in Fig. 5.1 so that they evolve collectively away from any unhealthy outcome and instead evolve toward a healthy outcome. To embody this idea in the constrained optimization problem of MPC, an objective function is mathematically defined to minimize the level of the pathogen (which initiates and drives the immune response, N^*, until eliminated) as well as that of the damage/dysfunction variable (which is involved in a positive feedback look with the inflammatory variable, N^*). Additionally, we incorporate other "costs of control" associated with dosing into the objective function.

The next part of the control discovery framework for this establishes the possible intervention inputs to consider. Given that sepsis had been nearly uniformly viewed as a disease of hyperinflammation, the focus has traditionally been on anti-inflammatory agents in developing therapeutic targets [11], which turned out to be ineffective. The innovation of the control discovery perspective, however, is that it does not limit exploration of only current or past ideas regarding potential therapeutic interventions! Assuming that every aspect of a model is open for modulation in the MPC framework is possible but considering the reduced construct of the ODE sepsis model, two primary therapeutic inputs were considered: pro- and anti-inflammatory. This manifests as two distinct time-varying input functions, one appended onto the N^* equation and another one onto the C_A equation. Even though there are two inputs, there is flexibility in the possible therapeutic modalities in terms of the use of as well as the manner of use for each input in the control explorations. For instance, one or the other input could be exclusively available for use or both therapies available to be used simultaneously. In addition, inputs could either be modulated negatively and/or positively or restricted to positive inputs only. Thus, this framework not only allows for evaluation of a predefined therapy protocol but also will be able to determine the necessity of multimodal therapeutic interventions to achieve the desired result.

Lastly, various constraints were imposed on the control problem, some of which were implemented in stages so as to begin with a broader scope for the control discovery process and then narrow the scope by imposing more realistic considerations. The answer to the question, "Which drug(s), in what amount(s), and at what time(s) will be successful for Patient X," therefore changes depending on the definition/constraints of the problem, giving a spectrum of possible answers that could range from no combination being ultimately successful to an unrealistic titration of multiple modalities being necessary. However, in order to evaluate if this methodology and framework have value for solving the translational dilemma, a progression such as this helps clarify the boundaries and challenges involved.

For example, prior model analysis identified the bioplausible regime of the model with respect to N^* and C_A and these were used to define constraints for the control problem. Clinical scenarios involved measurements of simulated patient trajectories extracted on an hourly basis, and this information was used to update the predictive model. Initial, proof-of-concept studies assumed all system outputs

where measurable and without noise error [22]. Subsequent studies attempted to evaluate potential real-world constraints on measurement technologies by adding measurement noise and restricting the measurable states to a subset, namely N^* and C_A, [23]. In addition, initial results that constrained measurement data to only N^* and C_A clearly showed that knowledge about the pathogen (P) state was critical to the algorithm's success in terms of effectively redirecting patient outcomes. Thus, in Ref. [22], in order to gain that information, the algorithm was provided, at four-hour intervals, low-resolution, binary information indicating that the level of pathogen (P) was high/low or present/absent. This indirect measurement mechanism was improved upon in Ref. [23] by instead using "particle filtering" to perform on-the-fly state estimation to approximate the pathogen state (as well as the damage/tissue dysfunction variable, D) from the measurable states N^* and C_A. Further exploration was done by running several sets of simulations to investigate the effects on outcomes of the virtual cohort of modifying the value of a single parameter in the predictive model, namely the pathogen growth rate (k_{pg}). Thus, some results discussed refer to three different sets of simulation output for the cohort based on the use of one of three k_{pg} values in the predictive model's parameterization. Other parameters of the predictive model could have also been explored in this way, but it was known that the model was particularly sensitive to the pathogen growth rate parameter. Moreover, any parameters of the predictive model could be modified at any/each time step but changing them effectively is a challenging inverse problem that would require on-the-fly parameter estimation.

Following the path taken by the initial work described above, the examination of MPC followed the sequence of scenarios below:

1. Static anti-inflammatory input held at a constant positive input over a certain time period (analogous to attempted clinical interventions [11]).
2. Optimal control-derived treatment regimen of both anti- and pro-inflammatory therapies computed for the predictive model and uniformly applied to the virtual cohorts (no personalization of applied regimen); these simulations were used to assess the robustness of efficacy for each parameterization of the predictive model.
3. Model predictive control (MPC)-derived treatment regimen using anti-inflammatory therapy only and applied to a perfectly matched virtual patient and respective predictive model; these experiments evaluated the efficacy of MPC with the existing paradigm focused on anti-inflammatory interventions.
4. MPC-derived treatment regimen using both anti- and pro-inflammatory therapies and applied to a perfectly matched virtual patient and respective predictive model; these experiments evaluated potentially paradoxical control strategies.
5. MPC-derived treatment regimen using both anti- and pro-inflammatory therapies but only a single predictive model applied across a range of imperfectly matched virtual patients; this set of experiments examined the robustness of the MPC methodology and investigated for three different values of an influential model parameter (pathogen growth rate, k_{pg}) in the predictive model to assess the sensitivity of the approach to the predictive model's parameterization.

Additionally, for this scenario, two implementations (5a and 5b) were investigated with respect to how the states of predictive model were updated.

These implementations can be viewed as in silico clinical trials on the virtual patient cohort, the results of which are summarized in Fig. 5.2, wherein each scenario, 1–5b, described above is given in color-coded rectangular boxes. Under each scenario a circle provides the mortality percentage followed by the percentage harmed, referring to the percentage of patients that would have had a healthy outcome if intervention had not been attempted. Obviously, we wish to do no harm, so this provides an indication of the safety of the intervention relative to the efficacy. Note that scenario 0, that is, "placebo," on the far left of the figure refers to the outcomes of the cohort in the absence of intervention, providing the baseline on which to compare any intervention applied to this cohort. That is, without intervention the patient cohort exhibits 60% mortality. Thus, each intervention is evaluated according to its ability to improve on this baseline while also minimizing the number of patients harmed. Additionally, as will be discussed, the realistic nature of the implementation is of course also a part of that evaluation.

When laying out the progression of these in silico trials as in Fig. 5.2, two initial groups emerge that delineate between the strategies that are the least personalized (scenarios 0–2) and those that are the most personalized (scenarios 3–5b). In short, those with the least personalization apply the same therapy protocol to every patient, even if the therapy protocol was derived from utilizing the predictive model and/or aspects of MPC; and those with the most personalization fully utilize the MPC methodology to apply a unique therapy protocol specified to each patient.

In scenarios 2, 5a, and 5b, three outcomes are reported for each in Fig. 5.2 based on the pathogen growth rate parameter, k_{pg}, used in the predictive model when the scenario simulation is run. The impact of this modification within a scenario is noticeable, where an increase in this parameter essentially has the effect of eliciting a more aggressive pro-inflammatory strategy since the predictive model is assuming there is a more virulent pathogen to deal with. In scenario 2, a nonlinear effect with respect to the induced mortality is present, but the percentage harmed clearly increases with increasing k_{pg}. The lower pathogen growth rate produces the best outcomes under this scenario by harming none and reducing baseline mortality by 20%. However, overall, this result implies that the non-personalized nature of scenario 2, even though a model is being utilized to protocol creation, leaves many patients unreached or worse harmed. This scenario is not utilizing MPC fully and thus serves as a motivation for the methodology applied as it is in scenarios 5a and 5b.

Three separate outcomes based on the k_{pg} value are also given for scenarios 5a and 5b. Furthermore, these scenarios represent the most evolved simulations to date under the MPC framework for the ODE sepsis model and lean toward the more realistic implementations displayed in Fig. 5.2. Scenario 5a is less realistic than 5b due to the indirect measurement mechanism used to help provide information to the algorithm about the pathogen (P) state. Compared to scenario 5a, scenario 5b incorporates additional "realism" by first addressing the indirect measurement issue by instead using particle filtering for state estimation and secondly by including noise

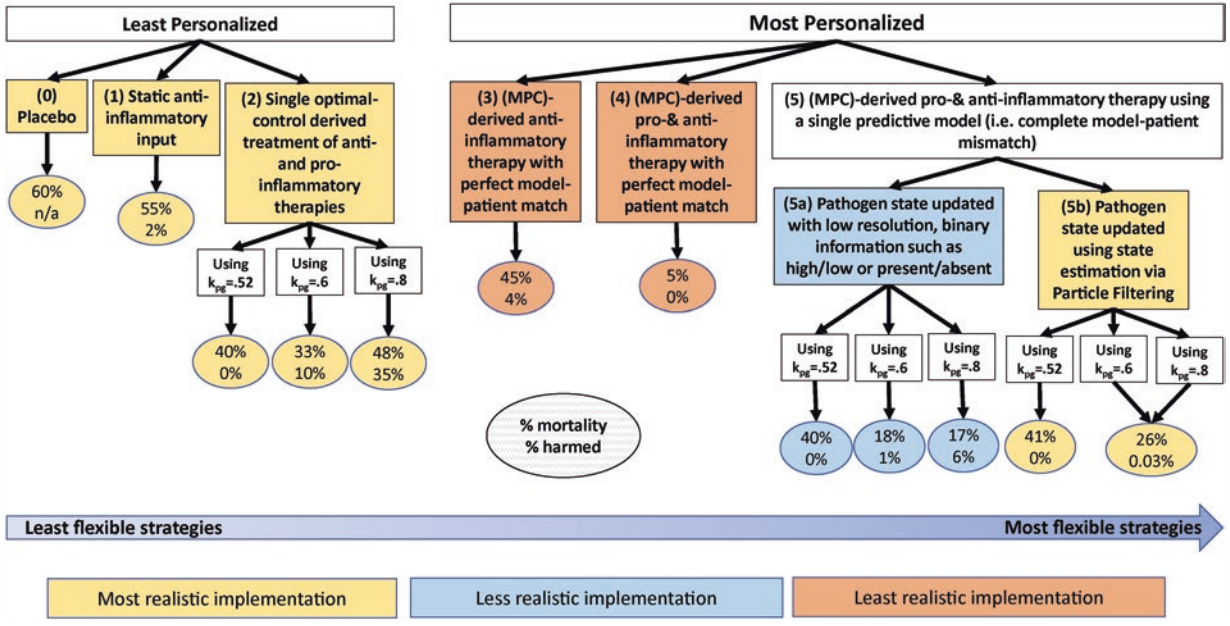

Fig. 5.2 Summary diagram of control discovery progression—scenarios and results from studies [22, 23]. Scenarios 0 through 5b described in the text are shown here in color-coded rectangular boxes. The results of the simulation outcomes for each scenario are given in circles underneath a scenario, showing the percentage mortality followed by percentage harmed. Some scenarios explored three different values of the pathogen growth rate parameter, k_{pg}, in the underlying predictive model. Scenario 1 demonstrated very little improvement over scenario 0 placebo. Scenario 2 demonstrated some efficacy over placebo but when the parameterization of the virtual patients differed greatly from that of the predictive model used to derive the therapy, efficacy was limited. Scenario 3 demonstrated efficacy over that of placebo and, in particular, scenario 1, showing not only the utility of the model predictive control (MPC) approach but also the insufficiency of limiting the controller to an anti-inflammatory treatment alone. With perfect knowledge allowing for an exact matching between the predictive model and the virtual patient, scenario 4 produced very good results with only 5% mortality and no patients harmed, demonstrating the capability of the approach within the speculative arena. Scenario 5 is constructed to provide more realism than that of scenario 4 (e.g., an imperfectly matched predictive model). Not surprisingly, the imposition of additional constraints resulted in higher mortality than scenario 4 but did appear to mitigate harm compared to the other scenarios. A similar trend is seen between scenarios 5a and 5b where additional realism is incorporated in 5b's implementation. Scenarios 5a and 5b, which fully utilize the MPC methodology, represent the most evolved implementation in terms of being the most personalized and most realistic implementations that have the most flexible/customizable strategies among those in this control discovery process on the ordinary differential equation (ODE) sepsis model

in the measurements of the virtual patient's measurable states (N^* and C_A). These modifications in 5b manifested in increases in the mortality rates compared to 5a over the three k_{pg} values from 40%, 18%, 17%, respectively, in 5a to 41%, 26%, and 26%, respectively, in 5b; but the harming rates were maintained at or reduced to 0%, 0.03%, and 0.03%, respectively, in 5b. Scenario 5b demonstrates the potential to infer important, inaccessible information from other measurement data, as the particle filter uses data from measurable states to estimate the nonmeasurable states. Figure 5.3 illustrates the results of the MPC approach using scenario 5b for a single virtual patient whose septic death outcome was averted with treatment. The first column of Fig. 5.3 displays each of the patient's variables plotted without intervention (red dashed) against the resulting time courses for each variable under the MPC-derived therapy from scenario 5b (black solid). The second column of Fig. 5.3 plots the time courses of the patient variable under treatment (black solid, as in the first column) but now against the predictive model's estimate of the patient state along the way as determined via noisy measurements (for N^* and C_A) or via the particle filter estimates (for P and D).

Scenarios 3 and 4 assume a perfectly matched predictive model for each patient, meaning the predictive model's parameterization and initialization are set to be the same as the patient for which it generates the MPC-derived therapy protocol. Hence, there is no need to explore varying k_{pg} values as in scenarios 2, 5a, and 5b. While such assumptions in scenarios 3 and 4 obviously fall into the category of *least realistic implementations* as denoted by the peach shading, they nevertheless demonstrate the capability of the approach within the speculative arena. In other words, if perfect predictive knowledge was known of this complex dynamic process, could interventions as prescribed by the MPC algorithm be found (under reasonable dosing constraints) to reduce the mortality and harmed percentages to 0%? The answer, as shown in the middle of Fig. 5.2, is a definite "no" when only utilizing a single (anti-inflammatory) intervention (scenario 3: 45% mortality; 4% harmed); and the answer is "almost" for scenario 4 (5% mortality; 9% harmed) when given the flexibility to apply two strategically different therapies (pro- and anti-inflammatory). Note that if dosing constraints were relaxed (e.g., no limits to the amount or duration of application and dosing not restricted to positive inputs only) in these two scenarios, the mortality in scenario 3 would be reduced but not eliminated but in scenario 4 it could be reduced to 0% (results not shown).

These results, and in particular those in scenario 5, suggest that our application of the MPC methodology on the ODE sepsis model of Ref. [20] fulfills our criteria for precision medicine. As scenarios 3–5 all demonstrated, dosing regimens could be tailored to a specific virtual patient, fulfilling Axiom 1: Personalization (dealing with different patients/trajectories). The real-time adaptive updating capability of MPC as utilized in scenario 5 fulfils Axiom 2: Precision (different interventions at different time points). Scenarios 3–5, and to some degree even scenario 2, showed the efficacy of the application of MPC fulfils Axiom 3: Treatment (suggesting how potential targets could be used). Finally, scenario 5 showcased that the generalizability of MPC to virtual patients that differed from the predictive model achieved some aspect of Axiom 4: Inclusiveness. To more fully realize Axiom 4, it is likely

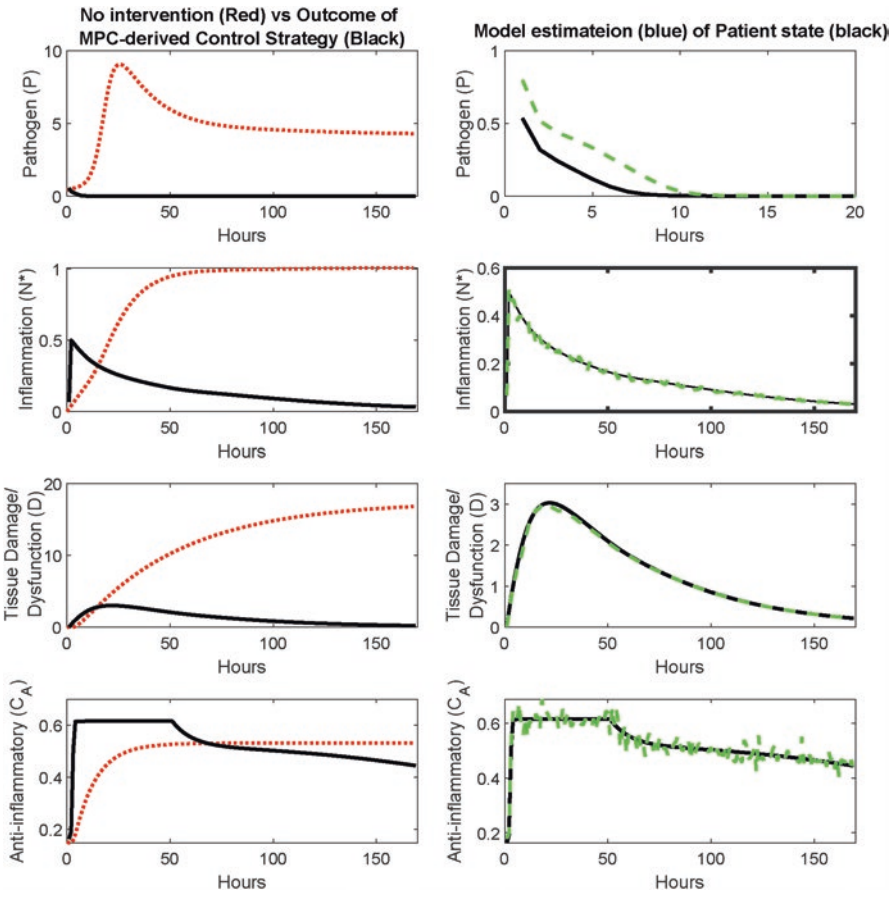

Fig. 5.3 Outcome of a patient-specific, model predictive control (MPC)-derived control strategy applied to a single patient whose outcome without intervention (red dashed curves in first column) would be septic death. The black curves shown in both columns give the time courses of the patient variables under treatment and show successful redirection of the response away from a septic death outcome toward healthy resolution. The second column demonstrates the predictive model's estimate (green dashed curve) of the patient's states along the way as it is updated with direct but noisy measurements of $N^*$and C_A and particle filter estimates of the P and D states

that either (or both) the measurement or control space would need to be expanded in order to eliminate the non-responder group. That expansion process, however, does not yet have a clear path and the challenges of on-the-fly parameter estimation, for instance, to improve the adaptability of the predictive model in scenario 5 present a difficulty of expansion. Additionally, as mentioned for scenario 4 in which complete system information was known, if less restrictions on the control space had been implemented, it would provide evidence that it is theoretically possible with this approach to eliminate the non-responder group. However, we believe from a real world, practical standpoint that it is not possible to achieve this level of

comprehensive knowledge and capability of manipulating the system. Further work is required to maximally extend these methods under realistic conditions/constraints to achieve a minimal level of efficacy.

The control discovery process for the ODE sepsis model clearly demonstrates that multimodal therapeutic strategies are essential for effective modulation of a complex inflammatory response. Overall, MPC showed theoretical efficacy under rather realistic constraints. Given that this work was intended as proof-of-concept application of MPC to sepsis, the translational road to clinical application of this method has a number of barriers. The largest barrier for this method and other model-based control investigations is that implementing any identified control strategy in a real-world setting is limited by the quality of representation of the computational model used to derive the strategy. To translate control insights into actionable steps in control discovery a high-fidelity model would be required, in stark contrast to the current ODE sepsis model that is admittedly a high-level, reduced, abstract representation of the acute inflammatory response with lumped variable/lumped parameters. The granularity of this model used for the proof-of-concept studies in Refs. [22, 23] did produce insight into the necessity of adequately updating the predictive model to account for changes in the system's dynamics after a control interval; but this model cannot effectively direct investigation into the sensing capabilities needed for an effective real-world controller. Therefore, to utilize the MPC framework as a means to solve the translational dilemma in sepsis, it would foremost need to be applied to more complex and detailed computational models of acute inflammation.

While a more detailed equations-based model can be formulated, it still may not capture certain essential features of the real-world system. However, other modeling alternatives such as agent-based models (ABMs) can be more readily constructed to represent a biological system with very high fidelity. Unfortunately, the control optimization methods underlying the MPC methodology require an equations-based representation of real-world systems. This makes it infeasible to simply replace the ODE sepsis model of Ref. [20], used in Refs. [22, 23], with a higher fidelity agent-based model such as the innate immune response agent-based model (IIRABM) [25, 26]. Nevertheless, recent advances in methods for control discovery using agent-based models, in particular the IIRABM, provide promising insight along the road to solving the translational dilemma in sepsis.

Model-Based Control Discovery Using Agent-Based Models

Agent-based models (ABMs) have been used to study and model a wide variety of biological systems [27], as well as serving as platforms for drug development [28]. One of the earliest applications of agent-based modeling to biomedical systems involved modeling the innate immune response agent-based model (IIRABM) [25, 26]. The IIRABM has been used to demonstrate the efficacy of in silico clinical trials as a means of invalidating traditionally designed anti-cytokine/mediator-directed

strategies [26], as well as providing novel insights into the dynamic structure of sepsis that accounted for the heterogeneity seen in its clinical manifestations [29]. The utility of the IIRABM was further extended as a surrogate/proxy system for the investigation of potential control strategies for sepsis [9].

The detailed description of the IIRABM can be found in Refs. [26, 29], but here follows a brief description. The IIRABM is a two-dimensional abstract representation of the human endothelial-blood interface, and models this interface as the site for the initiation and propagation of acute inflammation seen in the systemic response to injury and infection. The IIRABM incorporates the human inflammatory signaling network response (Fig. 5.4) and has been calibrated such that it reproduces the general clinical trajectories of sepsis (see [26, 29] for details). The IIRABM simulates multiple cell types and their interactions, including endothelial

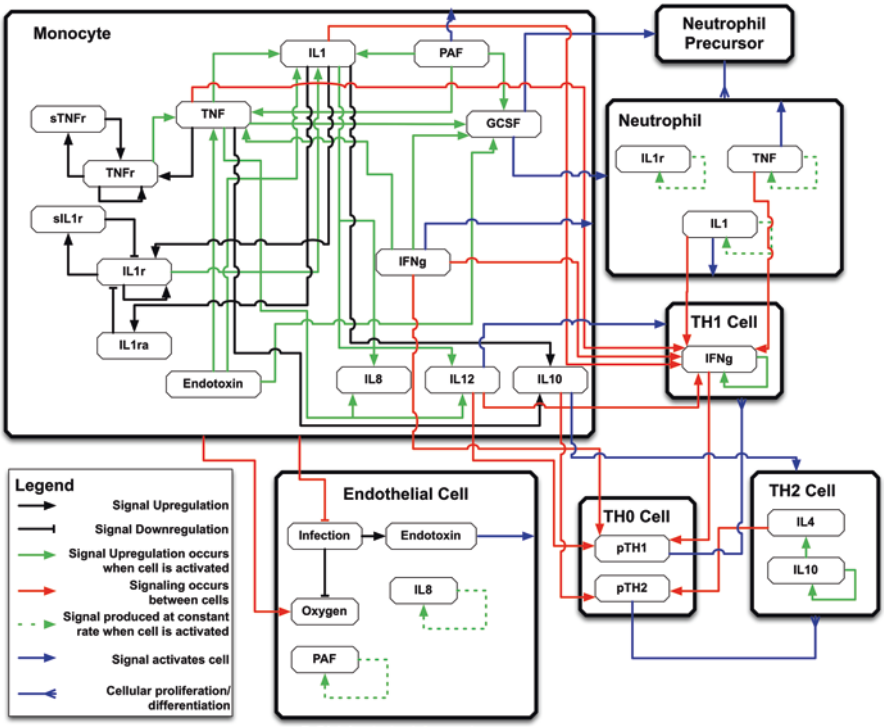

Fig. 5.4 Wireframe diagram of cellular types and mediators present in the IIRABM. Thick black framed boxes represent cellular species, which encapsulate mediator species. *IIRABM* innate immune response agent-based model, *TNF* tumor necrosis factor, *TNFr* tumor necrosis factor receptor, *sTNFr* soluble tumor necrosis factor receptor, *IL-1* interleukin-1, *IL-1r* interleukin-1 receptor, *IL-1ra* interleukin-1 receptor antagonist, *sIL-1r* soluble interleukin-1 receptor, *IL-8* interleukin-8, *IL-10* interleukin-10, *IL-12* interleukin-12, *IL-4* interleukin-4, *IFNg* interferon gamma, *GCSF* granulocyte colony stimulating factor, *PAF* platelet-activating factor, *pTH1* TH1 precursor pathway, *pTH2* TH2 precursor pathway. (Reproduced under the Creative Commons License from Ref. [30])

cells, macrophages, neutrophils, TH0, TH1, and TH2 cells as well as their associated precursor cells. The simulated system dies when total damage (defined as aggregate endothelial cell damage) exceeds 80%; this threshold represents the ability of current medical technologies to keep patients alive (i.e., through organ support machines) in conditions that previously would have been lethal. Infectious insults to the IIRABM are initiated using five parameters representing the size and nature of the injury/infection as well as a metric of the host's resilience—initial injury size, microbial invasiveness, microbial toxigenesis, environmental toxicity, and host resilience. In previous work [29], a sweep over these parameters was used to determine the plausible boundaries for parameter combinations that could be considered clinically relevant; that is, parameter sets that lead to all possible final outcomes when stochastically replicated—complete healing, death by infection, or death from immune dysregulation/sepsis. While the model does not contain a comprehensive list of all signaling mediators present in the human body, all cellular behaviors relevant to inflammation are represented. The named cytokines in this model are those that are most often associated with the available behavior rules in the current literature, even though it does not represent every single cytokine or chemokine known to be involved in inflammation. Because of the breadth of its representational capacity, the IIRABM's core component structure remains valid, despite being nearly 20 years old, and its behavioral validity can even be considered greater than at the time of its initial development as it has predicting aspects of sepsis that have since been recognized in the subsequent years—specifically the temporal concurrence of pro- and anti-inflammatory cytokine responses (as opposed to sequential pro- and compensatory responses) [31, 32] and the clinical importance of the immunoparalyzed recovery phase of sepsis, particularly with respect to its prolonged duration [33–36].

Despite their representational capability to map readily to biological systems, there remain significant limitations to how ABMs are used in biomedical research, particularly in terms of applying formal mathematical methods of analysis, due in larger part to the fact that they cannot be readily converted into analytically tractable sets of equations [9]. Since classical control approaches require such equation-based representation, alternative approaches are needed if ABMs are to be used as proxy systems for control discovery. Fortunately, advances in computational methods and resources (in terms of availability of high-performance computing, or HPC) provide a path forward in terms of using ABMs as proxy systems for control discovery.

The first of these simulation-based methods involves the use of genetic algorithms (GAs), a form of evolutionary computing, to evaluate whether the IIRABM, as a simplified proxy system for sepsis, could be controlled effectively [30]. This approach views the search for effective control as a nonlinear optimization problem [37–39], for example, can a series of system modifications be optimized to achieve a particular goal/system outcome? Genetic algorithms (GA) [40] have been used to optimize a wide variety [41] of nonlinear systems. GAs utilize the evolutionary principles of fitness, selection, and genetic variability to optimize tasks of high combinatorial complexity. A GA utilizes a "gene" composed of various values for a

defined set of parameters within a model and then executes that model to generate an outcome that is compared to a fitness function, which represents the goal of the optimization problem. As in evolution, the "gene" is then shuffled, creating a new set of combinations of parameter values (e.g., the generation of diversity and variation). The model is re-run and compared to the prior version; this is the iterative process of fitness-based selection. The better performing versions are then reshuffled, and the process repeated until a predefined degree of variation between runs is determined to be stable (this is called convergence of the GA). Medical applications of GA include vaccine dosing strategies and protein binding site prediction [42, 43]; the application of GA to the IIRABM as a proxy system for sepsis represents a novel application for model-based control discovery [30]. This work applied clinically realistic constraints in terms of sensor and dosing schedule (spaced at 12-hour intervals) with an exploratory aspect allowing each inflammatory cytokine in the IIRABM to be manipulated up or down a series of specified amounts; each combination of therapies and their sequence of applications thus formed the "gene" of the GA. The rationale for this approach was direct research to potentially fruitful targets for future drug development giving some clinically realistic limits on how those drugs could be delivered. The GA was trained on a single set of parameter values and initial conditions with a mortality of ~80%; the stochasticity of the IIRABM allows the generation of multiple "patient" trajectories from a single set of parameters and initial conditions; the fitness function was to minimize mortality at the end of 90 days. These simulation experiments did demonstrate that sepsis (as represented by the IIRABM) could be controlled with a defined pattern of interventions in a specified sequence that reduced mortality from 68 to 12% in the training set (identical parameter set, initial injury level and random number seed), and then from 82 to 16% for identical parameter set, initial condition but stochastic replicates $N = 1000$. The arrived-at solution required multiple targets of interventions, the combinations of which varied at nearly every dosing interval (Fig. 5.5). The generalizability of the GA-derived regimen was evaluated by applying the GA-derived strategy to other virtual cohorts with different parameter sets/initial conditions but the same baseline mortality (~80%). The arrived-upon strategy did generalize to some degree; however, there was a virtual cohort for which the strategy was ineffective. Analysis of this subgroup showed a separation in cytokine dynamics distinct from the responder group occurring at a consistent time point: Day 4 of treatment (Fig. 5.6). A new series of GA experiments was then applied to this non-responder subgroup to determine if an alternative treatment regimen could be discovered, and it was (Fig. 5.7). Thus, the iterated application of GA fulfills our criteria for precision medicine by addressing Axiom 1: Personalization (dealing with different patients/trajectories); Axiom 2: Precision (different interventions at different time points); Axiom 3: Treatment (an effective regimen); and, via iteration, Axiom 4: Inclusiveness (by identifying an alternative regimen for initial non-responders).

However, while being able to demonstrate utility in identifying potential multimodal control strategies for sepsis, the workflow of utilizing the GA in order to achieve the inclusiveness of Axiom 4 did not appear to be readily scalable, as it required re-examining the non-responder group, identifying specific properties of

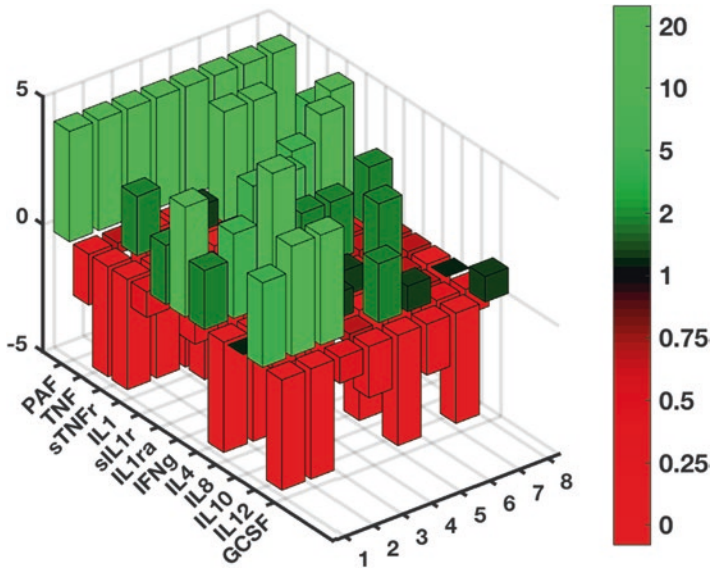

Fig. 5.5 Representation of genetic algorithm (GA)-computed intervention. This figure contains eight bar graphs, each of which represents a single stage of the eight-stage intervention. The cytokines operated on are shown on the *x*-axes and the base-2 logarithm of the augmentation or inhibition strength is shown on the *y*-axes. This sequence of interventions lowered the probability of death from 68 to 12% for the patient upon which the GA was trained; the probability of death was lowered from 82 to 16% for the general population using an identical parameter set. (Figure reproduced from Ref. [30])

that group, and re-optimizing across that population. While it is potentially feasible that some combination of active learning (a machine learning method that is able to refine the search of very large, multidimensional parameter spaces) and GA might be able to semi-automate this process, a truly adaptive robust control strategy would call for a different approach. This led to the investigation of a method that provided a means of adapting to the response of the system as it was being controlled and the application of deep reinforcement learning (DRL) to the IIRABM [21].

Deep reinforcement learning is a type of machine learning that is used to train artificial intelligence systems [44]. While a technical review of DRL is beyond the scope of this chapter, here we provide some basic background information. Reinforcement learning (RL) is a class of machine learning methods for finding near-optimal solutions to high-dimensional control problems not tractable using classical optimal control theory. RL involves training an artificial neural network (ANN) that sits outside a target system and interacts with the target system by changing the values of a prespecified set of components (actuators). The ANN (which is the artificial intelligence agent) obtains information about the state of the target system and is able to provide a series of inputs through a Markov decision process that affects the future states of the system. An RL task becomes a deep RL (DRL) task with greater complexity of the set of possible interactions and

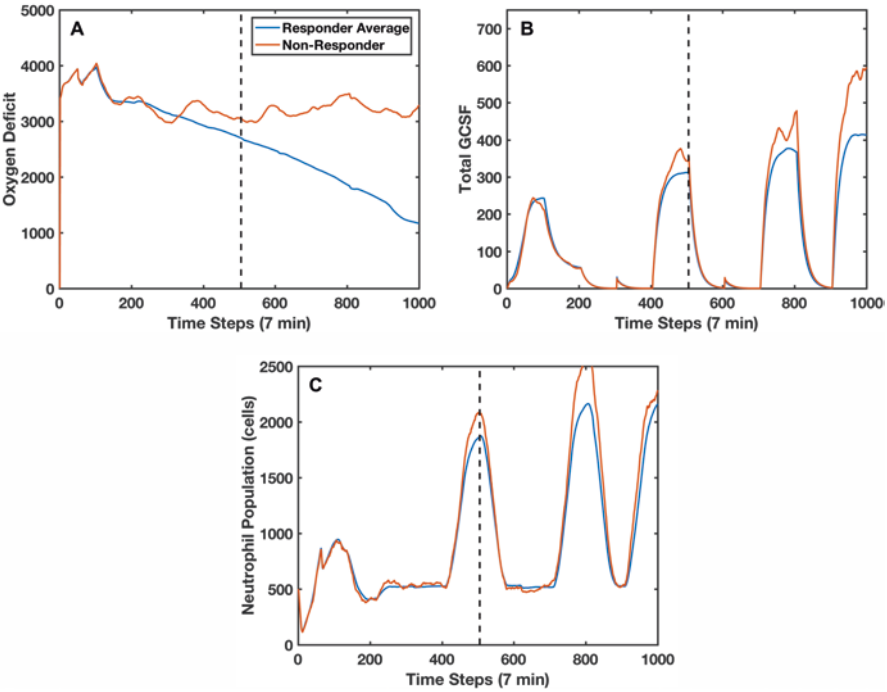

Fig. 5.6 Panel **a** displays the oxygen deficit (an inverse measure of the system's health) for an intervention non-responder (red) compared to the average oxygen deficit for intervention responders (blue) over time. Panel **b** displays the total granulocyte colony stimulating factor (GCSF) for the non-responder and the responder average; panel C displays the total neutrophil population for an individual non-responder and the responder average. In this case, the non-responder does not end up healing due to a hyper-productive response to GCSF pathway stimulation, which leads to a surplus neutrophil population; this patient ultimately dies due to inflammation, which is exacerbated by the applied intervention. (Reproduced under the Creative Commons License from Ref. [30])

trajectories; this is the case of sepsis and the IIRABM as a proxy system. A DRL-ABM system involves the integration of the DRL ANN (controlling agent) and the IIRABM (target system). Under normal circumstances when an ABM is executed, it is initialized and then runs forward without additional external input; with a DRL-ABM system, the DRL interacts with the ABM and adjusts the variables in the ABM as a means of "steering" into a desired trajectory and outcome. The goal of DRL is to train the DRL ANN to identify the best action to execute for each possible state during the simulation; it learns an adaptive *policy* for maximizing a *reward* function (e.g., a patient's health). The integration of DRL with an ABM is of a specific type of DRL termed "model-based DRL." This is the approach that has led to the recent advances in game-playing AIs from Google's Deep Mind project, AlphaGo and AlphaZero, which function by learning on simulated games [45, 46]. In this approach to DRL the controlling AI agent "plays" up to billions of simulated

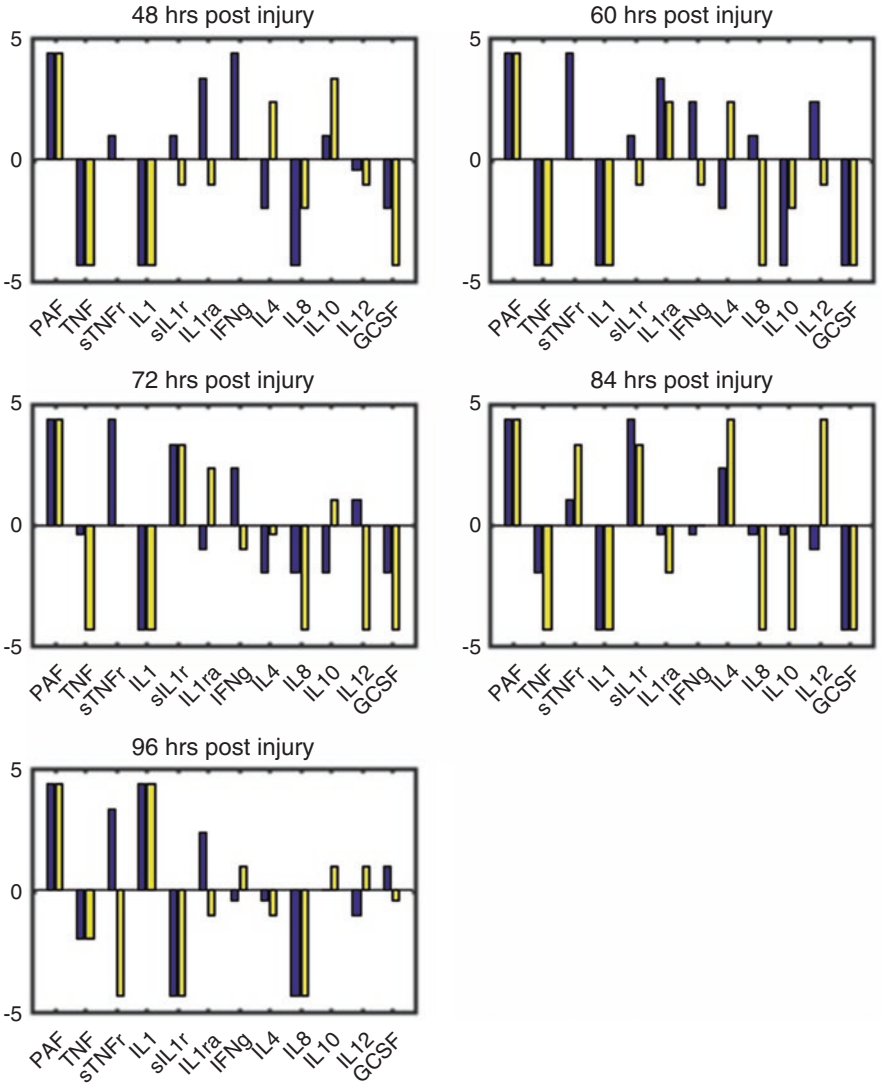

Fig. 5.7 Intervention comparison for responders and non-responder. The set of bar graphs on the left shows the final five interventions in a sequence of eight that showed the greatest success in healing the most in silico patients. The set of bar graphs on the right shows an alternate intervention sequence that was generated by training the genetic algorithm on a patient who was nonresponsive to the original intervention. The cytokines operated on are shown on the *x*-axes and the base-2 logarithm of the augmentation or inhibition strength is shown on the *y*-axes. The in silico patients received identical interventions for the first three time points. At time point 3, a significant deviation from expected behavior is noted in the non-responder. At this time point, the simulation is halted and used to populate the sample set for a new run of the genetic algorithm. When given the original intervention, this patient has a 75% chance of death at 60 h post-injury; the alternate intervention lowers this chance to 8%. (Reproduced under the Creative Commons License from Ref. [30])

games, and notes the outcome of each particular move for each particular board configuration in each particular game, and through the experience of playing all these games is able to derive a probability-based decision tree on what the optimal move would be given a specific configuration of the board. The resulting decision tree is the *policy* the controller agent then uses to interact with any subsequent games it plays. This approach was applied to the IIRABM, treating it as a "game" to be won; the controlling agent observes the state of the IIRABM at each step, and can interact/apply control by manipulating any of 12 cytokines either up or down given a specified range [21].

To our knowledge there is no prior application of simulation model-based DRL in the biomedical arena. Notably, the approach and expected role in biomedical research is a distinct contrast to a publication on the use of reinforcement learning to train an artificial intelligence agent to optimize the current set of interventions for sepsis [6]. That paper is limited by virtue of its reliance on existing data sets of sepsis patients (and, as such, existing therapeutic modalities) and is therefore unable to guide the development of future interventions, that is, new drugs and/or drug combinations. Conversely, the DRL work with the IIRABM mimics the Deep Mind projects insomuch it is simulation based and the trained AI allows for the discovery of novel control strategies (i.e., new drugs, drug combinations, and the timing thereof) based on the much broader control/action space that is grounded on pathophysiological cellular and molecular mechanisms. For the initial proof-of-concept study, the boundaries and action space of the training were set into the speculative region in order to determine if a robust control policy was even possible: the DRL AI had complete knowledge of the state of the IIRABM at every time step and had the ability to manipulate any of the included mediators within a range of up or down.

The DRL control AI was trained on a single parameter set and initial condition combination with 46% baseline mortality rate. The performance of the derived policy plateaued after 2500 training runs with many subsequent runs resulting in successful recovery. Generalizability of the learned policy was tested over a set of 500 different parameter set/initial condition combinations with baseline mortality rates in the range 1–99% for each set calculated over 50 episodes with different random seeds. The patient parameterizations included in this study span the entire space of clinically plausible parameterizations for the IIRABM as previously determined in Ref. [29]. The overall mortality rate (across all 500 patient parameterizations) improved from 46.0 to 0.8% under the policy (see Fig. 5.8). Moreover, no parameter set/initial condition combination exhibited an increase in mortality rate compared to baseline; that is, no cohort was detrimentally affected by the learned policy. These results suggest that despite being trained on a single patient parameterization, the policy generalizes well, as it is robust to variations in parameter set/initial condition combinations.

The nature of the learned control policy is that it is dependent on the particular state of the system, regardless of when during a particular trajectory that state is reached. This means that a consistent policy does not translate into a consistent therapeutic regimen (as is the result of each training session from the GA). Instead, the sequence of optimal actions varies based on the specific dynamics of a particular

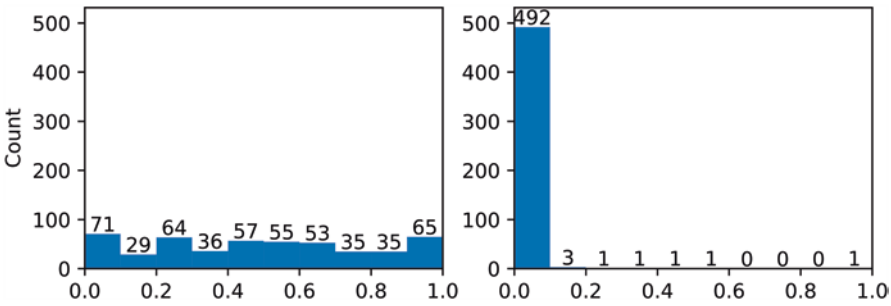

Fig. 5.8 Generalizability of deep reinforcement learning (DRL) discovery sepsis treatment policy. Left panel: Histogram of baseline (standalone antibiotic therapy) mortality rates for 500 test patient parameterizations (N = 50 instances per test patient parameterization). Right panel: Histogram of mortality rates using the learned policy (combination antibiotic cytokine mediation therapy) for 500 test patient parameterizations. (Reproduced from Ref. [21])

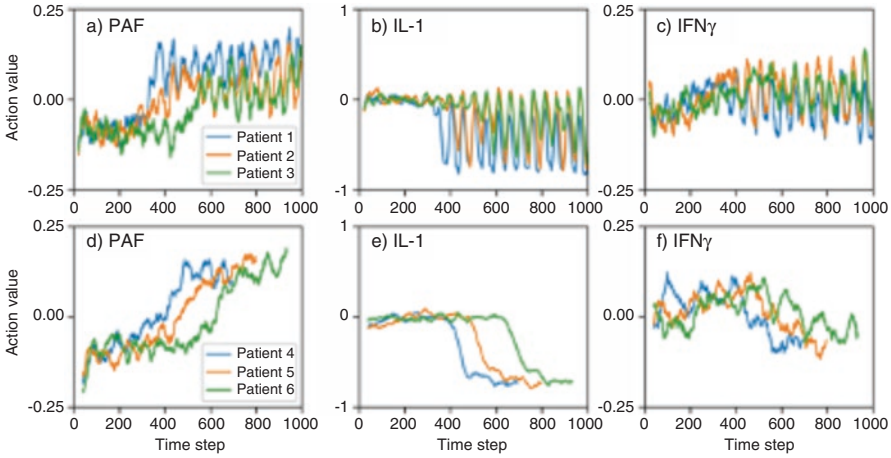

Fig. 5.9 Moving average of action values (window length 20) for PAF, IL-1, and IFN-gamma, selected by the learned policy during test treatment of three patient parameterizations. Top row (**a**–**c**): varying recurrent injury parameter, all other parameters held constant. Bottom row (**d**–**f**): varying initial injury size, all other parameters held constant, with recurrent injury set to zero. All patients healed from the policy's intervention. *IL* interleukin, *IFN* interferon, *PAF* platelet-activating factor. (Figure reproduced from Ref. [21])

run. This adaptive nature of the control was assessed by evaluating the dynamic patterns of the applied control between different simulation runs, which demonstrated a range of different patterns that all resulted in successful outcomes (see Fig. 5.9).

This proof-of-concept work demonstrated that DRL could be used to train an AI to implement a robust policy for sepsis that could heal the vast number of initial parameter conditions corresponding to a clinical population. This approach meets all the criteria established for precision medicine—Axiom 1: Personalization, by generating trajectory/patient-specific actions; Axiom 2: Precision, by providing an

adaptive series of controls that vary over the course of a trajectory; Axiom 3: Treatment, by controlling the system toward a desired goal; and Axiom 4: Inclusiveness, by have a robust policy that is effective across a wide range of parameter sets/initial conditions (patients). Though promising, as noted above, this is very preliminary work; future developments involve applying more clinically relevant constraints on the action space for the DRLAI, as well as developing an iterative workflow that would use the results of the DRL to enhance, improve, and refine the IIRABM.

Despite the promise of this method, it cannot be overemphasized that the strategy computed by either the GA or the DRL is only as valid as the model upon which it was trained. While the IIRABM has been validated [12, 13], it is acknowledged to have an incomplete representation of the acute inflammatory response, and as such is considerably less complex than the real work system: at the current level of refinement of the IIRABM, there is no way that such a trained AI could have clinical utility. Rather, at this point in development, the benefit of these approaches is mainly to aid in defining the scope of the requirements for control of the real-world system, that is, suggestions for intervals of intervention and the number and variance of required targets. In this fashion, model-based control discovery can guide the development of appropriate sensing technologies and classes of specified pharmacological agents.

Discussion

Fulfilling the above-defined axioms of precision medicine can be achieved by framing precision medicine as a control problem solved with the aid of mechanistic dynamic model support. We assert that the effective adaptive control of complex nonlinear dynamical systems requires the controller to have a proxy model of that system in order to predict and implement its control function [24], and that this approach is the only tractable path forward in terms of achieving "true" precision medicine for sepsis. However, we foresee significant cognitive barriers in the biomedical and specifically sepsis communities to the application of model-based control to biomedical therapeutic discovery, not least of which is related to the challenge of developing engineering-grade, mechanism-based biological models. Other domains that have employed engineering-grade proxy simulations are those that are governed by natural laws and first principle model construction; this leads to more readily establishment of the level of trust needed to use these models. Alternatively, the translational dilemma places biological model development in a discovery mode task, not in an engineering mode. Therefore, identifying a sufficiently powerful and representative level of abstraction and detail is a key challenge to the application of model-based control methods in biomedicine; for instance, agent-based models can serve as intermediate-level objects that can bridge between a higher-fidelity mapping, biological organization and function (via the ABMs), and more tractable equation-based system representations suitable for formal control methods [29]. We

assert that the methods presented in this chapter represent a pragmatic and tractable path for the iterative refinement of dynamic computational models that are "good enough" to forecast system behavior and allow for predictive control. An example of this type of iterative model refinement is seen in the evolution of weather models where the integration of model development and model-directed data collection has led to significant improvements in the accuracy and predictive horizon of these computational models. Similarly, as these approaches are applied to biomedical models, the predictive control horizon will increase; data collected from the application and implementation of early versions of these systems will hopefully direct basic science and technological investigations toward improved sensing and actuation modalities that will further improve the capabilities of the simulations that drive these control systems. Therefore, the adoption of this paradigm toward the investigation of treatment for human disease can lead to a shift in the execution of biomedical research such that it comes in line with scientific discovery in the physical sciences that rely upon model- and theory-driven science.

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Part III
Translational Modeling of Sepsis and Trauma

Chapter 6
Disorder of Systemic Inflammation in Sepsis and Trauma: A Systems Perspective

Jillian W. Bonaroti, Kent R. Zettel, Timothy R. Billiar, and Matthew D. Neal

Abbreviations

APC	Antigen-presenting cell
AR	Adrenergic receptors
AT	Antithrombin
BLyS	B-lymphocyte stimulator
Breg	Regulatory B cell
CLP	Cecal ligation and puncture
CLR	C-type lectin receptor
COX2	Cyclooxygenase-2
CRH	Corticotropin-releasing hormone
CTL	Cytotoxic T cells
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DNA	Deoxyribonucleic acid
GM-CSF	Granulocyte-macrophage colony stimulating factor
GPCR	G-protein-coupled receptors
HIF-1	Hypoxia inducible factor-1
HMGB1	High-mobility group box 1
ICAM1	Intercellular adhesion molecule-1
ICU	Intensive care unit
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
MAC	Membrane attack complex

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MAPK	Mitogen-activated protein kinase
MCP-1	Macrophage chemoattractant protein-1
MDSC	Myeloid-derived suppressor cell
MHC I	Major histocompatibility class I
MHC II	Major histocompatibility class II
MIG	Monokine induced by gamma interferon
MIP-1α	Macrophage inflammatory protein-1 alpha
MODS	Multiple organ dysfunction syndrome
MyD88	Myeloid differentiation primary response gene 88
NADPH	Nicotinic adenine dinucleotide phosphate
NET	Neutrophil extracellular trap
NF-κB	Nuclear factor kappa B
NK	Natural killer
NKT	Natural killer T
NLR	NOD-like receptors
NO	Nitric oxide
PAMP	Pathogen-associated molecular pattern
PGI_2	Prostaglandin I_2
PGN	Peptidoglycan
PHD-2	Proline-hydroxylase-2
PMN	Polymorphonuclear neutrophils
PRR	Pattern-recognition receptor
RAGE	Receptor for advanced glycation end products
RANTES	Regulated on activation normal T cell expressed and secreted
RCT	Randomized, placebo-controlled clinical trial
RLR	RIG-I like receptors
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
TF	Inflammation is tissue factor
TFPI	Tissue factor pathway inhibitor
TGF-β1	Transforming growth factor-β1
Th	T helper cell
Th1	T helper type 1
Th2	T helper type 2
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-α
Treg	Regulatory T cell
TRIF	TIR-domain containing adaptor-inducing interferon-β
VACM1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VHL	von-Hippel-Lindau
VWF	Von Willebrand factor

Introduction

The inflammatory response is the host's response to a perceived threat in the form of an invading pathogen or tissue damage and is defined as upregulation of inflammatory cytokines and chemokines, leukocyte adhesion molecules, and infiltration of neutrophils and other immune cells into tissues [1, 2]. This response involves multiple factors that detect the stress response, and many cell types that are responsible for the physiologic reaction to stress (Table 6.1). Homeostasis of the inflammatory response must be closely regulated, as without the inflammatory response, the host would succumb to the threat, while excessive responses will cause cell and tissue damage and death. Trauma and sepsis are distinct entities with some overlapping features. In trauma, the inciting event is tissue damage often associated with local or systemic hypoperfusion. An infectious agent initiates sepsis, which often complicates trauma. Both trauma and sepsis induce systemic changes most often manifested by interrelated immune and physiologic changes. Many of the overlapping features are likely due to the involvement of immune sensing mechanisms both trauma and sepsis have in common.

When the inflammatory response becomes severe and generalized, the pathologic sequelae are manifested in a clinical condition called the systemic inflammatory response syndrome (SIRS). SIRS is diagnosed when the patient presents with two or more of the following criteria: temperature >38 °C or <36 °C, heart rate >90 beats per minute (BPM), respiratory rate >20 BPM or partial pressure of carbon dioxide ($PaCO_2$) <32 mm Hg, or white blood count (WBC) >12,000 or <4000 mm^{-3} or >10% bandemia. Sepsis is defined as the SIRS response to a septic focus [3].

Trauma and severe sepsis are associated with a high rate of immune irregularities. Patients can succumb early after the onset of trauma or sepsis due to a cytokine storm characterized by a hyperinflammatory state, which can lead to early multiple organ dysfunction syndrome (MODS). The appearance of early MODS is correlated

Table 6.1 Host injury inflammatory response

Sensors	Cells	Effectors	Consequences
PRR–DAMP, PAMP Heat shock proteins Hypoxia sensors Mitochondria ROS/RNS targets	Neutrophils Macrophages Lymphocytes Dendritic cells NK/NKT Mast cells Epithelial cells Platelets	Neuroendocrine mediators Cytokines/chemokines Complement Coagulation cascade ROS/RNS	Beneficial 1. Adaptive cell stress responses 2. Initiation of tissue repair 3. Immune defenses Detrimental 1. Immune dysfunction 2. End-organ damage 3. Impaired repair processes

Participants in the systemic inflammatory response divided into sensors of the noxious stimuli, the cells involved, the systemic mediators of the inflammatory response and the consequences of such response

PRR pattern recognition receptor, *DAMP* danger associated molecular pattern, *PAMP* pathogen associated molecular pattern, *ROS* reactive oxygen species, *RNS* reactive nitrogen species

with the development of nosocomial infection and prolonged intensive care unit (ICU) stays. Human data examining the phenotype of circulating leukocytes in severely injured blunt trauma patients indicates that both the upregulation of innate immune pathways and suppression of adaptive immune pathways occur simultaneously, with changes most prominent within the first 12 h after insult. These responses are exaggerated and sustained in patients that go on to develop infectious complications [4]. Changes that separate patients into outcome-based groups, at the transcriptomic level in circulating leukocytes, can be seen within the first 2 h of injury. In a cohort of critically injured patients, those that went on to develop MODS, demonstrated maximal differential gene expression at 2 h compared to the cohort that did not develop MODS [5]. In recent work, four distinct organ dysfunction patterns were identified in post-trauma ICU patients based on MOD scores, with significant differences in clinical outcomes as well as inflammatory patterns between the four groups [6]. A similar study of trauma patients identified three subtypes of MODS based on cluster analysis of Sequential Organ Failure Assessment (SOFA) scores, with each subtype having a different pattern of recovery. Each subtype was also found to be associated with a clinical variable. These included 24-h crystalloid administration, traumatic brain injury, and admission shock severity [7]. Research in sepsis has also identified outcome-based cohorts distinguished by either clinical data or transcriptomic patterns of unseparated leukocytes. In a retrospective review of a large cohort of patients who met Sepsis-3 criteria, four clinical sepsis phenotypes were identified using clustering methods to analyze 29 clinical variables gathered on initial presentation. These phenotypes demonstrated differences not only in clinical outcomes such as in-hospital mortality but also in important biomarkers such as interleukin-6 (IL-6) and IL-10. When these phenotypes were applied to the three prior randomized, placebo-controlled clinical trial (RCTs) of sepsis interventions, varying the frequency of the phenotypes within the patient cohort via simulation-induced changes in the proportion of harm versus benefit for a particular intervention [8]. A study of a smaller cohort of patients admitted to the ICU for all-cause sepsis identified four molecular endotypes of sepsis on the basis of whole blood genome expression profiles. The identified endotypes demonstrated differences in clinical outcomes as well as in the course of host response, with some endotypes predisposed toward immunosuppression, hyperinflammation, or adaptive immunity [9]. The ability to stratify critically ill patients into subgroups may be useful for more tailored research and clinical interventions. Furthermore, incorporation of these phenotypes and endotypes in future trials may prove beneficial in elucidating the nuances of treatment effects in sepsis.

Billions of dollars have been invested to create therapies for severe sepsis and injury. Despite some early promise in animal trials, there has been minimal success in human trials. This is most likely due to the failure to completely understand the complex mechanisms involved in the host response to injury and severe infection. Systems biology holds great promise as a tactic to develop an integrated and predictive model of the human immunoinflammatory response, which may allow a sophisticated, targeted approach to treating injured and infected patients.

Sensing Mechanisms

Evidence continues to mount that the host is programed to initiate inflammatory and cell stress-signaling pathways in response to threats to tissue homeostasis through highly specific sensing mechanisms. These threats are often in the form of invading microorganisms, tissue damage, or reduced oxygen and nutrient delivery. Many of the sensing mechanism have been identified and some are listed in Table 6.1.

Infection

In order for the human immune system to respond to infectious agents rapidly, it recognizes a subset of molecular motifs that are uniquely expressed by microorganisms but are not expressed by host cells. These molecules are known as pathogen-associated molecular patterns or PAMPs. PAMPs are recognized by pattern recognition receptors (PRRs), which include several families of receptors that are expressed on both immune and nonimmune cell types. The toll-like receptors (TLRs) are prototypic PAMP sensors and the microbial molecular motifs recognized by many of the 13 TLRs have been identified. Examples of PAMPs that initiate signaling through TLRs are listed in Table 6.2. TLR signaling involves a number of adapter molecules. Notable among these are the downstream adapters TIR-domain containing adaptor-inducing interferon-β (TRIF) and myeloid differentiation primary response gene 88 (MyD88). These adapters link TLRs to downstream signaling pathways including nuclear factor kappa B (NF-κB), mitogen-activated protein (MAP) kinases, and IFN regulatory fac-

Table 6.2 Toll-like receptors and their respective stimulants

Toll-like receptor	Exogenous molecule (PAMPs)	Endogenous molecule (DAMPs)
TLR-1	Triacyl lipoproteins	
TLR-2	Lipoproteins Peptidoglycan Lipoteichoic acid	HSP-60, 70 HMGB-1
TLR-3	Double-stranded RNA	mRNA
TLR-4	Lipopolysaccharide Fusin protein Envelope protein	HSP-22, 60, 70 HMGB1 Surfactant protein A
TLR-5	Flagellin	
TLR-6	Lipoteichoic acid DNA zymosan	
TLR-7	Single-stranded RNA	
TLR-8	Single-stranded RNA	
TLR-9	Unmethylated CpG-containing DNA	DNA-HMGB1 complexes

Examples of microbial factors and endogenous moieties that are both recognized by pattern recognition receptors
HSP heat shock protein, *HMGB1* high-mobility group box 1, *DNA* deoxyribonucleic acid

tor 3 (IRF3). The lipopolysaccharide (LPS) receptor complex comprised of TLR4 and MD2 is unique among TLRs in that it can signal through both TRIF and MyD88. TLR signaling regulates a range of cellular responses including cytokine and chemokine production to cell stress responses such as autophagy [10]. Other examples of PRR include cytosolic receptors such as RIG-I like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs) [11].

Tissue Damage

Not long after the identification of the role of PRR in the recognition in PAMPs it became apparent that many of the PRRs were involved not only in the detection of microbes but also in the detection of tissue damage [12, 13]. In the setting of tissue damage or even cell stress, PRRs recognize molecules of host origin referred to as damage-associated molecular pattern molecules, or DAMPs. These are molecules within cells or tissue matrix that are normally not available to PRR. However, in the setting of tissue damage or stress, DAMPs are released in quantities adequate to trigger signaling through PRR. Examples of DAMPs that trigger signaling through TLRs are shown in Table 6.2. One of the most well-studied DAMPs in the setting of inflammation and injury is high-mobility group box 1 (HMGB1). HMGB1 is passively released from necrotic cells but can be actively released from several cell types when activated during stress [14]. HMGB1 has been shown to activate immune cells and promote the ongoing inflammatory response by stimulating cytokine, chemokine, and endothelial adhesion molecule production, as well as recruit neutrophils to the site of tissue injury [15]. HMGB1 has been implicated in multiple additional aspects of the inflammatory response, in both pro- and anti-inflammatory capacities, and it's function is highly dependent on location: nuclear, cytosolic, and extracellular [16].

It is notable that the concentrations of DAMPs required to trigger signaling through TLRs are often at least an order of magnitude higher than that seen for PAMPs. This suggests that the threshold for the detection of microbes by PRR is much lower than the detection of tissue damage and perhaps correlates with the magnitude of the threat to the host. However, it seems reasonable to conclude that a common set of receptors is used to detect both infection and tissue damage and that this feature could account for the similarities in the inflammatory response induced by these two very different threats to the host.

Hypoxia/Ischemia

Hypoxic and ischemic conditions also represent a major threat to the host by leading to cellular dysfunction and injury. Whether local or systemic, sustained reductions in perfusion or oxygen delivery lead to the activation of inflammatory and cell stress

signaling. Therefore, it is not surprising that several oxygen-sensing mechanisms are linked to inflammation.

Hypoxia inducible factor-1 (HIF-1) is one of the main mediators of oxygen homeostasis [17, 18]. During periods of normoxia, HIF-1α is degraded by proteasomes. The α subunit is marked for degradation by proline-hydroxylase-2 (PHD-2) and von-Hippel-Lindau (VHL) ligase complexes. In periods of hypoxia, PHD-2 and VHL are inactivated, resulting in a stable HIF-1 cable of binding to coactivators and inducing gene targets. The HIF-1 signaling pathway plays a key role in aspects of the inflammatory response including metabolism, migration, inducible nitric oxide synthase (iNOS) expression, and the antimicrobial activity of polymorphonuclear neutrophils (PMNs) and macrophages [17–19]. One of the key signaling pathways for HIF-1 is through the nuclear factor kappa B (NF-κB) family of transcription factors that regulate inflammation. The expression and release of inflammatory protein-1B by macrophages, which protects PMNs from apoptosis and thereby extending their lifetime, are through HIF-dependent NF-κB activation [17–19]. HIF-1 also plays a role in adaptive immunity. Increased HIF-1α production in T cells induces a shift from a T helper type 1 (Th1) to a T helper type 2 (Th2) phenotype. Furthermore, HIF-1 signaling stimulates the differentiation of regulatory T cells [17].

The mitochondrion acts as a sensor during episodes of ischemia and reperfusion with resultant production of reactive oxygen species (ROS). Under normal circumstances of respiration, mitochondria leak a small quantity of reactive oxygen species (ROS) along the electron transport chain in the form of superoxide radicals ($O_2^{\bullet -}$). Most of these ROS are reduced by superoxide dismutase to hydrogen peroxide and further reduced by peroxidase and glutathione. Ischemia leads to alterations in the mitochondrial electron transport chain and electron leak, causing increased superoxide radical formation [20]. Under prolonged ischemic episodes, the capacity of the cell to reduce these superoxide radicals becomes overwhelmed, resulting in oxidative stress. Under these states, ROS activate NF-κB and activator protein 1. NF-κB regulates inflammatory factors such as iNOS and cyclooxygenase II [21]. Activator protein 1 and NF-κB are essential for induction of many inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-2, IL-6, IL-8, macrophage chemoattractant protein-1 (MCP-1), interferon-β (IFN-β), granulocyte-macrophage colony stimulating factor (GM-CSF), regulated on activation normal T cell expressed and secreted (RANTES), and E-selectin [1, 21].

Cellular Factors of the Inflammatory Response

The inflammatory response to an acute threat involves a response coordinated by cells. The roles of immune cells in driving the initial inflammatory response are well characterized. Less appreciated are the roles of nonimmune cells, which can participate by responding to signals from the immune cells, but also by responding through the same sensing mechanism utilized by immune cells. Here, we will briefly consider the dominant known roles of specific cell populations in the early inflammatory response.

Tissue macrophages are probably among the first cells to send signals in response to infection or injury. These signals include chemokines and cytokines that begin to integrate an influx of cells into the threatened tissue. Monocytes enter the tissues and differentiate into macrophages under influence of the cytokine milieu. These cells play a dual role in the immune response by phagocytizing pathogens and destroying them through oxygen-dependent and oxygen-independent mechanisms, as well as by presenting antigens to T cells, promoting cell-mediated immunity [22]. These cells secrete proinflammatory cytokines to delay neutrophil apoptosis and further enhance the inflammatory response.

After stimulation, a massive PMN infiltration occurs within 4–6 h, followed by monocytes in 24 h and lymphocytes in 48 h [23]. Neutrophils clear the infection by phagocytosis and intracellular killing mechanisms involving reactive oxygen species and the release of proteases, elastase, cathepsins, and matrix metallopeptidase-9 [22]. Neutrophils also secrete cytokines to further attract and activate the immune response, including TNF-α, IL-10, macrophage inflammatory peptide-1α (MIP-1α), IL-12, IL-8, B-lymphocyte stimulator (BLyS), and vascular endothelial growth factor (VEGF) [24]. Neutrophils release extracellular traps (NETs) composed of DNA to snare bacteria in response to TLR4 activation [25, 26]. These cells have a short half-life of 8–12 h unless extended by IL-8 and TNF-α, bacterial components, and complement [22].

In part of the inflammatory response to trauma, the spleen becomes infiltrated with $CD11c^+$/$Gr\text{-}1^+$ myeloid cells that are known to suppress T cell function, termed myeloid-derived suppressor cells (MDSCs). These cells express the enzyme arginase, which convert arginine to ornithine and urea. In trauma, these cells localize with T cells in the germinal centers of the white pulp in the spleen where they suppress T cell function. The expressed arginase activity depletes the local environment of available arginine, which is required for the T cell receptor ζ-chain [27]. T cell proliferative dysfunction and decreased IL-2 production ensues, which can be reversed by arginine supplementation or inhibition of arginase activity.

Lymphocytes can be divided into T cells and B cells. T cells can be further divided into $CD4^+$ helper T cells (Th) and $CD8^+$ cytotoxic T cells (CTLs). In response to injury and inflammation, Th cells are activated by antigen-presenting cells (APC) such as macrophages and secrete cytokines that increase neutrophil infiltration (GM-CSF, IL-3), promote the macrophage and neutrophil response to pathogens (TNF-α, GM-CSF), and delay neutrophil apoptosis (GM-CSF) [28]. Th cells can be further classified into Th1, Th2, and Th17 phenotypes based on the profile of cytokines they secrete. An increase in the ratio of Th2/Th1 and Th17/Treg phenotypes in injury and sepsis has been demonstrated and is associated with diminished resistance to infection [29]. B cells were once thought to have the sole function of producing antibody, but more recent evidence demonstrates that B cells play an active role in modulating the immune response. Regulatory B cells (Breg) are a subset of B cells that produce IL-10, and induce an anti-inflammatory immune response [30].

Severe injury and sepsis lead to a suppressed T cell response. Multiple mechanisms regulate this immunosuppression, including certain cytokines and regulatory T cells. Regulatory T cells (Tregs) are a subset of T cells that have been implicated

in the suppression of T cell response to antigen. The topic of regulatory T cells is too extensive to be completely covered in this chapter. Tregs suppress $CD4^+$ Th cell proliferation and activation after trauma and sepsis and mitigate lipopolysaccharide (LPS)- and peptidoglycan (PGN)-induced TNF-α, IL-1β, and IL-6 production [31, 32]. Tregs inhibit T cell proliferation by cell-contact mediated pathways and produce high levels of the immunosuppressive cytokine IL-10 after injury [33]. While Tregs are upregulated in sepsis and suppress $CD4^+$ Th cell proliferation, Treg depletion did not lead to a survival advantage in cecal ligation and puncture (CLP)-induced sepsis [32].

Natural killer (NK) cells are bone marrow-derived lymphocytes that attack cells through apoptosis-inducing mechanisms. Unlike T and B cells, they do not express clonally specific antigen receptors, but rather detect self-antigens on major histocompatibility class I (MHC I) molecules [34]. They are producers of chemokines and proinflammatory cytokines TNF-α, TNF-β, and IFN [35]. They are activated by the proinflammatory cytokines IL-12, IFN-α, and IFN-β through TLRs, and through direct contact with dendritic cells (DCs) [35]. NK cells, through IFN-γ production, promote Th1 polarization of $CD4^+$ T cells. They activate dendritic cells through IFN-γ, TNF-α, and cell–cell contact [36]. In addition to intensifying the inflammatory immune response, NK cells can also dampen the response through depletion of immature myeloid dendritic cells and activated T cells [36].

Natural killer T (NKT) cells are thymus-derived T cells that contain the T cell receptor, but are dissimilar from $CD4^+$ and $CD8^+$ T cells because they detect antigens expressed on CD1d and contain markers for NK cells [37, 38]. NKT cells can be divided into subsets by the expression of IL-18R or ST2L, which direct the immune response toward a Th1 or Th2 type response by secretion of the cytokines IFN-γ or IL-4 and IL-5, respectively [39]. These cells have been implicated in the T cell-mediated immune suppression after burn injury by high production of IL-4 in addition to lower MHC II and co-stimulatory CD40 expression [40, 41]. Reversing the inhibitory role of NKT cells has been demonstrated by blocking the CD1d signaling [40–42].

Dendritic cells (DC) play an important role in bridging the innate and cell-mediated immune response. These cells phagocytize antigen, migrate to secondary lymphoid organs, differentiate into a mature phenotype, and activate naïve T cells [22]. DCs have much higher capability to present antigen to T cells than macrophages. These cells can be divided into plasmacytoid DCs, and myeloid DCs. Plasmacytoid DCs resemble plasma B cells and are involved in autoimmunity; they are the major subset of DCs responsible for IFN-α response to infection. Myeloid DCs play a major role in IL-12 production to stimulate macrophages and NK cells as well as induce the Th1 response [22]. Sepsis induces an acute expansion of splenic follicular DCs, with an interval decrease through caspase 3 mediated apoptosis [43, 44]. Sepsis also induces an acute decrease in interdigitating DCs similarly through caspase 3 mediated apoptosis [43]. The immature rather than the mature DC population are the targets for apoptotic death [45]. The depletion of DC populations seen in sepsis is also witnessed in trauma and hemorrhage [46]. This apoptotic depletion of splenic and peripheral lymphoid cells has been implicated in part in postinjury

immunosuppression. Several stressors are likely at play including cytokines, ROS, heat shock proteins, and glucocorticoids, and it appears that many of these are dependent on TLR4 signaling [47]. Dendritic cell dysfunction, as indicated by suppressed antigen presentation, is also seen in both sepsis and trauma [43, 46]. Trauma and hemorrhagic shock suppress DC response to LPS with down-regulated mitogen-activated protein kinase (MAPK) activation and suppressed LPS-induced pro-inflammatory TNF-α and IL-6 cytokine production, which is likely due to diminished TLR4 expression [46, 48]. However, there was no change in DC anti-inflammatory IL-10 production in this setting [48].

Often underappreciated is the role of nonimmune cells in the host response to acute infection or injury. Take, for example, the fact that most PRR are widely expressed in nonimmune cell types [49–52]. The role of PRR on parenchymal cells is an underexplored area of inflammation biology. Evidence that PRR on nonimmune cells is important in the setting of acute injury and infection comes from studies using chimeric mice. In fact, the majority of the studies using chimeric mice deficient in TLR4 signaling in either the bone marrow or nonbone marrow-derived cells show that TLR4 on nonbone marrow-derived cells plays an important role in the acute pathobiology of infection [53, 54], ischemic injury [55–57], tissue trauma [58], and hemorrhagic shock [58]. This has been studied in the setting of hepatic ischemic/reperfusion (I/R) injury, where it has been demonstrated that PRRs are essential to initiate a sterile inflammatory response. It has been shown that redox stress and hypoxia can lead to a TLR4-dependent release of HMGB1 from hepatocytes raising the possibility that TLRs may serve as a sensor of redox stress [59]. Work in cell-specific TLR4 knock out mice characterized the role of TLR4 in the sterile inflammatory response during I/R injury and in sepsis models. While TLR4 signaling from both hepatocytes and myeloid cells is required for maximal I/R associated injury, dendritic cell TLR4 signaling serves a protective role [60]. In sepsis, TLR4 on myeloid cells is needed to clear bacteria, while TLR4 on hepatocytes promotes LPS uptake and HMGB1 release [61].

Further supporting the critical role of hepatocytes in the inflammatory response is the fact that hepatocytes are the major source of circulating HMGB1 in sepsis. The hepatocyte-released HMGB1 binds and delivers LPS to caspase-11 in the cytosol of myeloid and endothelial cells through a receptor for advanced glycation end products (RAGE)-dependent mechanism. Caspase-11 activation then results in macrophage and endothelial cell pyroptosis, a form of cell death. This in turn leads to propagation of the inflammatory response [61–63]. Released HMGB1 also plays a critical role in sepsis-induced coagulation by increasing procoagulant phosphatidylserine externalization and tissue factor activity, again through a caspase-11 mediated mechanism. In mice, loss of HMGB1 expression in hepatocytes inhibited intravascular thrombin generation, and in WT mice administration of anti-HMGB1 antibodies attenuated sepsis-induced coagulopathy [64, 65].

Of the nonimmune cells that participate directly in the inflammatory response, endothelial cells are probably the best characterized. At rest, endothelial cells regulate blood flow, inhibit coagulation, control vessel-wall permeability, and inhibit activation of leukocytes. The endothelial cell dysfunction seen in acute inflammation is manifested as derangements in each of these functions. These account for the

cardinal signs of inflammation; increased blood flow causing erythema (*rubor*) and warmth (*calor*), leakage of plasma proteins causing swelling (*tumor*), and leukocyte activation mediating pain through sensory nerve fibers (*dolor*) [66]. Early activation of endothelial cells occurs within minutes and is mediated by G-protein-coupled receptors (GPCRs) and intracellular calcium. Increased intracellular calcium results in production of prostaglandin I_2 (PGI_2) and nitric oxide (NO), both potent vasodilators, allowing increased blood flow and leukocyte delivery. By a similarly GPCR and calcium mediated mechanism, myosin light chain is phosphorylated allowing contraction of actin filaments, relaxation of tight junctions, and exudation of plasma proteins. Intracellular calcium signaling also allows exposure of leukocyte adhesion molecules, such as P-selectin, on the luminal cell surface, leading to leukocyte recruitment. This phase of endothelial cell activation lasts 10–20 min, after which GPCRs become desensitized [66].

A subsequent, more sustained activation is mediated by TNF-α and IL-1 derived from activated leukocytes. Signaling through NF-κB and activator protein-1 transcription factors results in increased expression of leukocyte adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM1), and vascular cell adhesion molecule-1 (VACM1) [52, 67, 68]. Expression of cyclooxygenase-2 (COX2) is also increased, leading to increased production of vasodilatory PGI_2 [66]. Inflammation leads to eventual endothelial cell injury that is the consequence of both the influx of leukocytes and the toxic effects of their secretory granules, as well as the cytokine production from these cells [66, 69]. IFN-γ in combination with TNF-α and IL-1 activates endothelial cell death via caspase-8 and mitochondrial-dependent cell-death pathways [66].

Endothelial dysfunction also contributes to activation of the coagulation cascade during acute inflammation. An intact glycocalyx, composed of proteoglycans, glycoproteins, and plasma proteins, covers the luminal surface of the endothelium and regulates thrombus formation. This structure is disrupted during injury and inflammation, allowing cell–cell interaction and adherence, a key component of the coagulation cascade [70]. Von Willebrand factor (VWF) is produced by the endothelium, and functions to recruit cells and coagulation factors to the endothelium. In normal conditions, large VWF multimers are cleaved by ADAMTS-13. In sepsis, however, ADAMTS-13 is inactivated by mediators such as IL-6, leading to unusually large VWF multimers that are implicated in microthrombi genesis [71]. Additionally, during inflammation the endothelium releases plasminogen activator inhibitor 1 (PAI-1) that acts to suppress the fibrinolytic pathway [71]. In total these processes can lead to widespread microthrombosis and a clinical coagulopathy. The endothelium therefore is an important link between inflammation and the coagulopathy observed during injury and infection.

Platelets play a role in the innate immune response as they influence hemostasis as well as recognize tissue damage, making them a key player in the process known as immunothrombosis. Trauma and hemorrhage induce alterations in platelet behavior that contribute to trauma-induced coagulopathy and secondary organ dysfunction. Platelets express cell surface receptors capable of recognizing DAMPs released after injury, and due to their small size allowing close endothelial contact, are felt to be uniquely capable as sentinel innate immune cells [72]. Platelets express all of the

ten known TLRs, with TLR2 and TLR4 being the best characterized. TLR2 signaling in mouse and human platelets induces aggregation, adhesion, and release of proinflammatory molecules. TLR2 also regulates interactions between platelets and neutrophils [73, 74]. TLR4 signaling similarly induces platelet aggregation and also causes release of immunomodulatory factors including IL-8, RANTES, sCD62p, epidermal growth factor (EGF), and transforming growth factor–β (TGFβ) [75]. Platelets release soluble CD40L from stored α-granules upon activation. CD40L induces adhesion molecule expression and chemokine release from endothelial cells, promoting leukocyte migration [72, 76]. Activated platelets interact in a bidirectional manor with leukocytes, with platelet binding inducing integrin activation, release of granular contents, production of DNA extracellular traps, and induction of gene expression via NF-κB [72]. The overall effect promotes leukocyte migration into injured tissues. Further demonstrating the interface between inflammation and thrombosis is the release of HMGB1 from activated platelets. HMGB1, a prototypical DAMP, is upregulated and released from activated platelets and mediates platelet aggregation, thrombus formation, and innate immune system activation through TLR4 and MyD88-dependent pathways [77]. Platelet-specific HMGB1 knock out mice had increased bleeding times compared to control, and whole blood from these animals demonstrated decreased platelet aggregation. In a trauma/hemorrhagic shock model, the same animals had decreased expression of TNF-α, IL-6, MCP-1, and resistin as well as reduced hepatocellular injury as demonstrated by serum transaminase levels [77]. Platelet-derived HMGB1 is just one of several potential links between thrombosis and inflammation, but may be a promising therapeutic target.

Effectors of the Inflammatory Response

Effectors of the inflammatory response can be broadly classified as pathways activated and mediators produced in response to the sensing mechanisms. The effectors carry out the steps intended to remove the threat and re-establish tissue homeostasis. These induce capillary permeability, fever, tissue injury, and immune cellular responses that are responsible for the phenotype of the inflammatory response. The coagulation and complement cascades, cytokines and chemokines, and neuroendocrine responses are all effectors of the inflammatory response. While these effector systems are presented as distinct entities, in reality there is likely significant overlap and coordination in their downstream effects.

ROS & RNS

Reactive oxygen species can be rapidly produced as part of the sensing mechanisms that initiate the inflammatory response but can also be viewed as effectors when produced in a sustained manor. As previously mentioned, ROS formed from mito-

chondrial stress activate the inflammatory response through NF-κB and activator protein 1. Reactive oxygen species are also actively produced and released by immune cells during respiratory burst. In this process, from nicotinic adenine dinucleotide phosphate (NADPH), oxidase produces superoxide [2]. This can be further reduced by superoxide dismutase to hydrogen peroxide, which myeloperoxidase converts to hypochlorous acid. The major source of these reactive species by this mechanism is phagocytic cells, such as macrophages and PMNs, with the purpose to eradicate infectious agents, but the nonspecific release of reactive oxygen species also results in tissue injury.

Reactive nitrogen species (RNS) become an effector when nitric oxide (NO) is produced by the high-output inducible NO synthase (iNOS). iNOS is not expressed in resting cells but can be upregulated by cytokines and hypoxia in many cell types [2, 78]. Once expressed, iNOS produces NO in a sustained manor. The NO produced can have cell signaling functions to protect cells through promoting perfusion or inhibiting apoptosis or can induce cell toxicity through nitrosative or oxidative stress. When produced in proximity to superoxide, NO can lead to the formation of peroxynitrite, a potent oxidant [79]. We have shown that iNOS contributes to inflammation, organ injury, and immune dysfunction following hemorrhagic shock and trauma [80, 81].

Coagulation Cascade

The coagulation cascade is intimately involved in the inflammatory response and abnormalities in coagulation are a hallmark of sepsis and trauma. Furthermore, there is evidence of cross talk between inflammation and coagulation, where they both have influence on the other [82, 83]. Coagulation as an effector system in inflammation is felt to work to contain the threat. Over activation of the system in extremes can lead to disseminated microvascular thrombosis and clinical coagulopathy [83].

One of the most important initiators of thrombin formation in response to inflammation is tissue factor (TF). During inflammation, cytokines such as TNF-α and IL-1 upregulate TF expression in endothelial cells. In addition, TNF-α, IL-6, LPS, and CD40L induce TF expression in circulating monocytes, which leads to systemic activation of coagulation [84, 85]. Studies have shown that inhibiting TF activity in models of endotoxemia inhibits inflammation-induced thrombin generation [86]. Under normal circumstances, coagulation is controlled by antithrombin (AT), the protein C system, and tissue factor pathway inhibitor (TFPI). During the inflammatory response, these are all impaired. AT levels are decreased as a result of degradation by activated neutrophils and impaired synthesis. Importantly, AT is also depleted by the ongoing thrombin generation. Normally, protein C is activated by endothelial cell-bound thrombomodulin. Activated protein C then works with protein S to degrade coagulation factors Va and VIIIA. During the inflammatory response, there is down regulation of thrombomodulin expression on endothelial

cells by TNF-α and IL-1, causing diminished protein C activation [85, 87]. While these are the major mechanisms involved, there are several more nuanced effects of inflammation on the clotting cascade.

Coagulation modulates the inflammatory response in several ways. Thrombin, Factor Xa, and fibrin activate endothelial cells and stimulate monocytes, causing increased production of IL-6 and IL-8 in both [88]. Thrombin also increases MCP-1 and E-selectin expression in endothelial cells, promoting leukocyte migration. Fibrin and fibrin degradation products activate immune cells through the CD11b/CD18 integrin receptor. This receptor is expressed on circulating monocytes and tissue macrophages. Signaling through the CD11b/CD18 receptor induces production of inflammatory cytokines TNF-α and IL-1β through the NF-κB pathway [89].

There are complex interactions between the coagulation cascade, endothelial cells, platelets, and the immune system during an inflammatory response that go beyond the scope of this section, but further understanding of these mechanisms can shed light on the pathogenesis of organ dysfunction in trauma and sepsis.

Neuroendocrine

The major components of the neuroendocrine stress response to injury include the corticotropin-releasing hormone (CRH) and locus coeruleus–norepinephrine systems. In response to injury, CRH is released through the hippocampus and norepinephrine is released through the hypothalamic–pituitary–adrenal axis. In conjunction, these result in increased cardiac output, heart rate, blood pressure, respiratory rate, blood glucose, and leukocytosis. The catecholamine surge during hemorrhagic shock and resuscitation stimulates the release of the inflammatory modulator, HMGB1, which induces bone marrow neutrophil mobilization through IL-17 and IL-23 induction [90]. The addition of beta-blockade can alleviate the bone marrow mobilization and HMGB1 release, as well as suppress hematopoietic cell growth after hemorrhagic shock [90, 91].

Adrenergic receptors (ARs), including alpha and beta-receptors, are expressed on T cells. In regard to α-ARs, studies have yielded conflicting data, but there is evidence of in vitro T cell activation with increased cytokine production and intracellular redox through α-AR agonism [92]. α-AR stimulation via the sympathetic response leads to NF-κB pathway activation and subsequent production of IL-6, TNF-α, IL-1β, and transforming growth factor-β1 (TGF-β1) production [93]. IL-1β enhances hypothalamic activity and CRH release, which increases sympathetic activity and splenic norepinephrine release in a positive feedback fashion [94]. β-ARs are expressed on T cells, with β_2-ARs being most relevant. When CD4+ T cells are polarized to Th1 and Th2 phenotypes, the expression of β_2-ARs is increased and decreased respectively. β_2-AR agonism in Th1 polarized cells results in increased IFN-γ, whereas in naïve CD4+ T cells, β_2-AR agonism after activation results in reduced IL-2 and IFN-γ production. This suggests both a suppressive and activating role of β-ARs in the T cell inflammatory response [92].

There is antagonism between catecholamines and inflammatory modulators. For example, nitric oxide, the product of iNOS, which is upregulated in sepsis and

trauma, causes vasodilation, while catecholamines cause vasoconstriction. Using hepatocyte culture, catecholamines have demonstrated an antagonistic effect on the production of nitric oxide by hepatocytes by both α_2 and β_1-mediated pathways [95]. These inhibitory effects are greater with epinephrine than with norepinephrine and through β_1 more than α α_2-mediated mechanisms [95]. The mechanism of this inhibitory role is uncertain, though thought to be post-translational.

The cholinergic neuroendocrine response is a vagal mediated anti-inflammatory pathway of the immune response that suppresses the release of TNF-α, IL-1β, IL-6, and IL-18 [96, 97]. This anti-inflammatory response is initiated through central muscarinic acetylcholinergic receptors, transmitted through the vagus nerve to the periphery, where it promotes its anti-inflammatory effects through nicotinic acetylcholinergic receptors on immune cells [96, 98]. This peripheral nicotinic-cholinergic response been used for the treatment of chronic inflammatory conditions such as ulcerative colitis [99, 100]. In vivo models have further analyzed the cholinergic anti-inflammatory pathway in sepsis. Anticholinesterase administration once at the time of CLP reduced the serum IL-6, IL-10, and TNF-α levels, though did not influence survival [101]. Survival benefit was demonstrated in other studies when either anticholinesterase or choline itself was administered two to three times daily after CLP [97, 102]. This indicates that the initial anticholinergic effects are beneficial at reducing the cytokine storm, though prolonged anticholinergic activity is necessary for survival benefit.

Cytokines and Chemokines

Cytokines are hormones that mediate the inflammatory response through cell–cell communication and often with overlapping functions. Following injury, cytokines are produced in response to PRR activation by DAMPs or PAMPs leading to a vigorous inflammatory cytokine response with major contributions from IL-1, IL-2, IL-4, IL-6, IL-8, IL-18, and TNF-α (Table 6.3) [103]. Chemokines are a subclass of cytokines that induce chemotaxis in nearby responsive cells. Macrophage inflammatory protein-1 alpha (MIP-1α) mediates both the acute and chronic inflammatory response by recruiting inflammatory cells and stimulating the production of TNF-α, IL-1, and IL-6 by peritoneal macrophages [103]. Other chemokines involved in the inflammatory response include MCP-1, MIP-1β, RANTES, monokine-induced by gamma interferon (MIG), and IL-8.

Complement

The complement cascade is a defense mechanism activated by antigen–antibody complexes (classical pathway) or microbial surfaces (alternative pathway) that has primary antimicrobial properties in creating the membrane attack complex (MAC) to lyse invading pathogens and assist in phagocytosis by opsonizing bacteria. The complement system also augments the inflammatory response. Cleavage products

Table 6.3 Inflammatory cytokine and chemokine functions

Cytokine	Function
TNF-α	Induces fever. Stimulates NK cells and macrophages/monocytes. Induces synthesis of NO, production of selectins, promotes cell survival, apoptosis, cytokine secretion, PAI, ICAM, thromboxane A2, prostaglandin E2. Delays neutrophil apoptosis
IL-1	Induces fever. Stimulates T cells and macrophages. Induces PMN release from bone marrow. Increases adhesion molecules. Stimulates MCP-1, MIP-1α, and IL-6 production
IL-2	Promotes proliferation and differentiation of T cells into effector T cells, and survival of antigen-specific CD4+ and CD8+ T cells promoting memory T cells
IL-4	Stimulates B cell and T cell proliferation. Promotes B cell differentiation into plasma cells and class switching to IgE. Decreases Th1 cells and the production of IFN-γ and IL-12
IL-6	Induces fever. Regulates growth and differentiation of T and B cells. Increases antibody production by B cells. Inhibits apoptosis of PMNs and mediates hepatic acute phase response
IL-12	Induces T cell differentiation to Th1. Activates NK cells
IL-18	Promotes natural killer (NK) cells and T cells to release IFN-γ. Promotes cell-mediated immunity and inhibits IL-4-dependent IgE production
Chemokine	**Function**
IL-8	Induces neutrophil and granulocyte chemotaxis and phagocytosis. Delays neutrophil apoptosis
MIP-1α	Activate PMNs. Stimulates IL-1, IL-6, and TNF-α production
MCP-1	Attracts monocytes, T cells, and dendritic cells to the site of inflammation

Examples of cytokines of the inflammatory response to trauma and sepsis and their functions
PAI platelet activator inhibitor, *PMN* polymorphonuclear neutrophils, *MIP-1α* macrophage inflammatory protein, *MCP* Monocyte chemotactic protein

of the enzymes in this cascade, C3a and C5a, increase capillary permeability and are powerful neutrophil chemoattractants. The classical and alternative complement pathways are activated after trauma and are implicated in the inflammatory pathway [104–106]. Macrophages are a major source of complement activation through factor B synthesis in response to LPS and DNA [107, 108]. After activation through injury or infection, there is a nonspecific amplification of the cascade through a positive feedback loop, thus mounting a rapid inflammatory and immune response. When complement activation becomes excessive, it can lead to organ injury [3, 104–106]. We have shown that compliment activation is a driver of the early inflammatory response and organ injury in a mouse model of hemorrhagic shock + trauma [109]. C3a levels have shown prognostic value and can serve as an indicator for MODS. The mechanisms by which C3a and C5a contribute to MODS are not entirely clear, but likely involve both the amplification of the immune response as a whole and a direct pathophysiologic effect [110].

Consequences of the Inflammatory Response

In the setting of acute infection or tissue injury, the adaptive roles of the early inflammatory response are to remove the infectious threat, initiate repair processes, and re-establish tissue homeostasis. In trauma, the activation of innate immune pathways probably also occurs to remove microorganisms that invade when barriers are disrupted. There is clearly a threshold at which these processes designed to be adaptive and improve survival become maladaptive and can contribute to adverse outcomes. These adverse outcomes are seen as excessive and sustained inflammation leading to end organ dysfunction and immune dysfunction rendering the patient more susceptible to nosocomial infections. The end effect is a prolonged ICU stay, increased duration of mechanical ventilation, and increased likelihood of death.

Derangements of Systemic Inflammation

Pioneers in the field of immune dysfunction after sepsis and trauma initially proposed a biphasic immune response [111]. This work was largely based on animal studies, which revealed an early proinflammatory response or SIRS followed by a delayed and sustained immune dysfunction. Results from a large multi-institutional observational study in trauma patients has refined the paradigm for the immune response in patients suffering from severe blunt trauma [4] (Fig. 6.1).

In gene array analysis of peripheral blood leukocytes of 167 severe blunt trauma patients, severe injury is shown to induce a "genomic storm" involving 80% of the leukocyte transcriptome within the first 4–12 h, which remains altered for days to

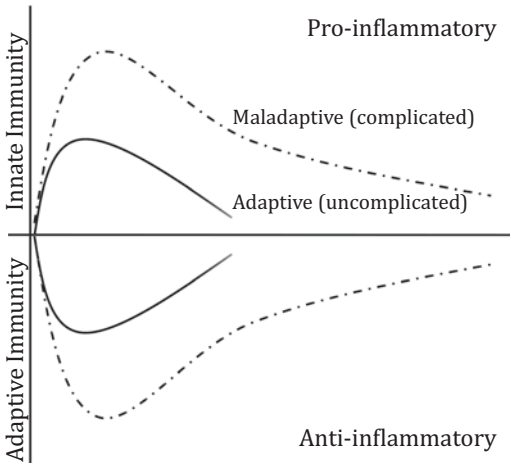

Fig. 6.1 Inflammatory response after trauma. Coexisting proinflammatory and anti-inflammatory responses are rapidly upregulated in circulating leukocytes after injury. Major injury induces a more pronounced response. (Adapted from Xiao W. et al. A genetic storm in critically injured humans, J Exp Med 2011;208:2581–90)

weeks [4]. The genes that had the greatest increase in expression after trauma involved the innate immunity and the inflammatory response, including integrin signaling, leukocyte extravasation, Fcγ receptor mediated phagocytosis, IL-10 signaling, TLR signaling, Ephrin signaling, IL-6 signaling, TREM1 signaling, actin cytoskeleton signaling, and B cell receptor signaling [4]. Those with the greatest decrease in expression involved T cell activation and antigen presentation [4]. On gene analysis comparing uncomplicated to complicated course (recovery in >14 days, no recovery, or death) after trauma, upregulated pathways associated with complicated recovery included IL-10, IL-6, p38 and MAPK signaling [4]. Antigen presentation, T cell proliferation and apoptosis, T cell receptor signaling, and NK cell function were the most downregulated pathways associated with complicated recovery [4]. The difference in gene expression between uncomplicated and complicated recovery was the magnitude of the early response and the time required for gene expression to return to baseline levels [4]. There were no genes that were exclusively expressed or suppressed in complicated vs. uncomplicated recovery [4]. The adaptive immune response alterations occur simultaneous with the acute proinflammatory response to injury.

Excessive Inflammation from Severe Injury

Factors that drive the excessive and sustained immune responses after injury are only partially understood. These include magnitude of insult, gender, age, and some gene polymorphisms [4, 112–115].

Immunosuppression

The delayed response to severe trauma is characterized by suppression of both the cell-mediated and adaptive immune responses. This suppression of the adaptive immune response has been associated with regulator T cells, myeloid-derived suppressor cells, and apoptosis. Suppression of both the cell-mediated and adaptive immune responses have been associated with the conversion of the cytokine milieu from a Th1 to a Th2 response. The conversion of a proinflammatory to an anti-inflammatory response is thought to be beneficial to limit the injury induced by the proinflammatory response, though a prolonged course seen with severe injury leads to increased susceptibility to infection.

Apoptosis

Apoptosis has been implicated as a cause for a depleted lymphocyte response after trauma and sepsis. The degree of T cell apoptosis directly correlates with the degree of sepsis and occurs by both mitochondrial-mediated and receptor-mediated mecha-

nisms in human populations [116]. The T cells most susceptible to apoptosis include the effector memory helper T cells, while the central memory helper T cells remain relatively spared [117]. Blocking mitochondrial-mediated T cell apoptosis by over-expressing Bcl-2 in transgenic mice improved survival in sepsis [118].

Th1 to Th2 Conversion

Helper T cells activate and direct the immune response through cell-mediated and cytokine-directed mechanisms. The two major pathways have been described as Th1 and Th2 responses. Th1 responses are proinflammatory, and promote the cellular immune response, including macrophages, neutrophils, and CD8+ T-cells. IL-2, IL-12, and IFN-γ mediate this response. Th2 responses promote antibody-mediated immunity and are mediated by IL-4 and IL-10. Th2 responses are generally considered anti-inflammatory due to the inhibitory effect of IL-10 on proinflammatory cytokines, such as IL-2 and IFN-γ. Injury and sepsis causes an alteration in T cell function from a proinflammatory Th1 to an anti-inflammatory Th2 response [119]. It has been speculated that the function of the Th2 response is to compensate for and neutralize the proinflammatory-induced tissue injury. When produced in excess, as seen in severe injury, the Th2 response can counteract the Th1 response, making the host susceptible to infection.

Immune Response with Age

Advanced age is well known to be an increased risk factor for mortality and MODS after trauma and sepsis [120–125]. The exact etiology for this has yet to be elucidated, but is thought to be due to altered or excessive immune and inflammatory response to these stresses in the aged. A complex cascade of derangements in cytokine production and response, cellular number and activity, and tissue response characterizes the effect of age on inflammation.

Elderly individuals have a baseline hyperinflammatory state with increased C-reactive protein and cytokine levels of TNF-α, IL-6, and soluble TNF receptor. Elderly also have elevated baseline neutrophil counts [126]. Monocytes in the elderly are in a preactivated state, which release a larger initial amount of cytokines, though there is no difference in the peak cytokine production compared to younger individuals [126]. When healthy elderly and young human subjects were given IV endotoxin, the elderly subjects demonstrated a larger initial TNF-α, soluble TNF receptor, and C-reactive protein response than the younger subjects [127]. Elderly subjects also had a more rapid decrease in monocyte populations and slower resolution of monocyte number and fever after endotoxin exposure [127]. The delayed recovery of leukocytes after infection may make elderly more prone to leukopenia during severe protracted infections. Despite the elevated cytokine level and response to injury, neutrophils appear to be less responsive in the aged. The proliferative

response to GM-CSF and the ability of GM-CSF to delay apoptosis in neutrophils are blunted in the elderly [128, 129]. The etiology for this diminished response is unknown, but could be related to the immune system's tolerance to inflammatory signals from continual immersion in the inflammatory environment of ageing. Our recent study of mediator patterns in young versus elderly trauma patients indicates that the age-dependent differences in response are not just related to the magnitude of change in inflammatory mediators, but also due to major shifts in the actual response pathways involved [130]. Using matched cohorts we showed that older patients had suppressed IL-6 levels early after multiple trauma, but higher and sustained CXC chemokine levels, including MIG and IP-10, after injury [130]. Similarly, a recent study demonstrated elevated levels of matrix metalloproteinase-9 (MMP-9) and its specific tissue inhibitor-1 (TIMP-1) in the serum of geriatric trauma patients in comparison to nongeriatric patients. MMP-9 is stored in granules within PMNs and is a collagenase, acting to mediate vascular permeability and initiate leukocyte migration. TIMP-1 binds MMP-9 in normal conditions and inhibits it. Loss of the balance between these two may play a role in the immunologic derangements seen in the elderly [131].

The combination of atrophy of the thymus with age, which limits the production of new T cells, in addition to replicative senescence due to telomere shortening in the memory T cells, causes a decreased pool of T cells that are less capable to respond to newly and previously encountered pathogens [28, 132]. Elderly have decreased proinflammatory IFN-γ and increased anti-inflammatory IL-4 and IL-10 production by T cells suggesting a natural tendency to an immunosuppressive Th2 response compared to young counterparts [133, 134].

More recently some authors postulate that while "inflammaging," or the established age-related increase in systemic chronic inflammation, does contribute to many disease processes, it is not the mechanism by which the elderly experience increased mortality after injury and infection. Instead, it is suggested that *impairment* in inflammation and protective immunity, particularly in myeloid cell function and myelopoiesis, is implicated [135]. Further investigation into the mechanisms at play is required to better understand outcomes in the elderly.

Treatment Considerations

The clinical course of sepsis and trauma differs between individuals. This suggests that variable activation of inflammatory mediators or differential expression in protective mechanisms leads to poorer outcomes. As both excessive proinflammatory and anti-inflammatory processes are maladaptive, modulating either or both processes are viable options for treating the derangements in the inflammatory response. Various interventions have been investigated including hydrocortisone, immunoglobulins, probiotics, and prostaglandins; however, none have demonstrated a mortality benefit [136]. An integrated and predictive model of the immune response using systems biology should be the focus of therapeutic approaches, but at this

time, such a comprehensive model has yet to be developed. This could be the underlying reason why attempts to address the derangements in the immunoinflammatory response have been unsuccessful. There are two straightforward strategies to attempt to "normalize" the magnitude of the host immune response. These include suppression of the initial hyperinflammatory response or the reversal of the delayed immunosuppressive state.

Mitigating the Hyperinflammatory Response

It is reasonable to hypothesize that suppressing certain key inflammatory pathways early in the host response to injury could have a beneficial effect on both the proinflammatory and counter-inflammatory responses. Complete inhibition, however, may not be desirable as this could result in a loss of the adaptive aspects of a specific mediator or pathway. It is hoped that systems biology analysis will lead to the identification of high value targets for selective therapies. Below are examples of potential targets.

As detailed previously, nitric oxide (NO) is produced in the setting of inflammation by the high-output iNOS in numerous cell types [137]. Nonselective inhibition of iNOS has caused increased injury to liver, while selective inhibition of iNOS has been shown to reduce injury to liver and lung following trauma/hemorrhage and ischemic injuries [2, 137, 138]. The damage associated with nonselective inhibition is thought to be due to inhibition of eNOS, which is organ protective. By selectively inhibiting iNOS and preserving eNOS, the deleterious inflammatory effects can be suppressed, while maintaining the cytoprotective effects of eNOS. N-[3-(aminomethyl) benzyl] acetamidine (1400 W) is a selective iNOS inhibitor that when administered prior to trauma/hemorrhage, attenuates the hepatic damage, reduces inflammatory markers of liver tissue myeloperoxidase activity, and normalizes the levels of TNF-α and IL-6 after trauma and hemorrhagic shock in Sprague-Dewey rats [138]. iNOS inhibition also decreases the HIF-1α expression, which plays an important role in the inflammatory response to hypoxic stressors [138].

Blocking the actions of proinflammatory cytokines or chemokines in the early inflammatory state after trauma and sepsis is another therapeutic approach. One such cytokine studied in animal models is IL-6. Anti-IL-6 administration prior to burn injury improved survival and reduced translocation of gut bacteria acutely after injury [139]. Anti-IL-6 has also been shown to improve delayed type hypersensitivity and splenocyte proliferation after burn injury [140].

DAMPs are a potential target for immunomodulation and studies in mice have explored anti-HMGB1 antibody. Inhibition of HMGB1 in polytrauma mice leads to reduction in levels of IL-6 and IL-10, decreased mucosal permeability, amelioration of splenic MDSCs, reduction in Th1 cytokine expression, and improved 24 h survival [141–143]. The complement cascade is yet another potential target for intervention. C1 esterase inhibition in traumatic hemorrhagic shock in swine led to less circulating TNF, less C3/C5 deposition in tissues, improvement in metabolic acido-

sis, and less tissue damage in kidney, intestine, and lung. Similar studies were proposed in humans but terminated due to lack of feasibility [136, 144]. C3 inhibition in hemorrhagic shock in nonhuman primates via a compstatin compound CP40 led to improved renal function and intestinal edema, and reduced markers of systemic inflammation and coagulopathy [145].

Reversing Immunosuppression

Sepsis and severe trauma are associated with a reduction of the T cell Th1 phenotype. One method to reverse this immunosuppression would be to replace Th1 cytokines. IL-12 is a cytokine produced by macrophages and dendritic cells that induces a Th1 phenotype. Administration of IL-12 in a murine model of CLP after burn injury resulted in improved survival [146]. IL-12 supplementation enhanced IFN-γ production and decreased IL-4 production. IFN-γ supplementation after burn injury also improved survival after subsequent peritoneal sepsis by CLP, though less effectively than IL-12 supplementation [146]. The excessive toxicity of IL-12 therapy in clinical oncologic trials has limited its clinical usefulness [147].

Another method to reverse the immunosuppression would be to inhibit the Th2 response after trauma or infection. IL-4 and IL-10 are known Th2 cytokines, promoting anergy and inhibiting the cell-mediated response. Treatment with anti-IL-10 antibody restores the Th1 cytokine response by T cells to antigen stimulation after burn injury [119]. Though this may have an important role in immunomodulation after trauma and sepsis, IL-10 has been shown to have a dichotomous role in survival after infection. IL-10 is critical to survival of mice in models of endotoxemia and peritonitis while it impairs bacterial clearance and survival in murine *Klebsiella* pneumonia and chronic *Klebsiella* peritonitis models [148–151]. Thus, the dual activities of IL-10 may limit its usefulness as a therapeutic target. Whereas it has a proinflammatory role early, its prolonged expression after the initial injury renders patients more susceptible to infection. For example, delayed inhibition of IL-10 in a two-hit model of CLP and pseudomonas pneumonia has shown to benefit survival and bacterial clearance [152].

One theory to explain immunosuppression following sepsis is the apoptotic loss of immune mediators. IL-7 is an antiapoptotic cytokine that has been studied in multinational clinical trials for HIV, cancer, and hepatitis C, and has demonstrated to induce a greater than twofold increase in CD4 and CD8 T lymphocytes in human subjects. In a murine model of peritoneal sepsis, recombinant human IL-7 improved survival, blocked apoptosis of CD4+ and CD8+ T cells, restored IFN-γ production, and improved immune cell recruitment to the site of infection [153]. IL-7 also improved the innate cellular response by increasing the expression of leukocyte adhesion molecules LFA-1 and VLA-4, improving leukocyte infiltration to the infection site [153]. In vivo, IL-7 is able to restore the loss of delayed type hypersensitivity to recall antigen during sepsis [153]. In a randomized controlled trial, patients with septic shock and lymphopenia were administered recombinant human

IL-7 (CYT107) or placebo for 4 weeks. The treatment was well tolerated and led to a three- to fourfold increase in absolute lymphocyte count as well as CD4+ and CD8+ T cells. T cells persisted for weeks after treatment and there was increased T cell activation [154].

Other mechanisms to prevent the apoptotic loss of helper T cells include inhibiting proapoptotic factors, such as Bid and Bim, and increasing antiapoptotic factor expression such as Bcl-2 and caspase inhibitors. The use of caspase inhibition by N-benzyloxycarbonyl-Val-Ala-Asp(O-methyl) fluoromethyl ketone (z-VAD) has been shown to prevent T-cell apoptosis and improve survival in septic mice [155].

Additional novel treatments, for sepsis in particular, that target immunosuppression include GM-CSF, IFN-γ, and immune checkpoint inhibitors. GM-CSF is a hematopoietic growth factor that stimulates production of monocytes and neutrophils. In four small, randomized controlled trials (RCTs) including 158 patients, GM-CSF administration was associated with improved clinical outcomes and upregulation of functional monocyte markers [156, 157]. IFN-γ is a Th1 cytokine that activates monocytes and increases their antigen presentation capability. A small study in trauma patients demonstrated that inhaled IFN-γ led to increased HLA-DR expression in macrophages and improved clinical outcomes [158]. Larger RCTs have not been pursued to further investigate IFN-γ. Finally, immune checkpoint inhibitors such as nivolumab have been heavily studied in cancer treatment but have potential in infection and injury. Programmed cell death protein 1 (PD1) is a cell surface receptor that acts as an inhibitory check on the immune system, with PD-L1 ligand binding causing reduction of T cell proliferation and activation and IL-2 production. Ex vivo studies in cells from septic patients demonstrated that blockade of the PD1/PD-L1 pathway led to decreased sepsis-induced immune dysfunction [159]. Nivolumab is a monoclonal antibody to PD-1 that inhibits PD-L1 binding. There are limited case reports of nivolumab administration in combination with IFN-γ in sepsis. A recently completed phase 1b randomized study demonstrated safety of nivolumab in sepsis, and further study is warranted [160].

Conclusion

The inflammatory response to sepsis and trauma is a highly integrated and multifaceted interaction of sensors, cells, and effector responses. As an adaptive response, it is designed to promote tissue repair and immune defenses to impending pathogens during times of stress. The inflammatory response is also self-limiting due to the anti-inflammatory component of the response. Individuals who succumb to proinflammatory events such as major trauma and sepsis have a prolonged anti-inflammatory response that leaves them susceptible to nosocomial infections. It was once thought that the anti-inflammatory response follows the initial proinflammatory response to infection or trauma, but recent evidence points out that the anti-inflammatory response is initiated at the time of the proinflammatory response in injured humans. Under circumstances of severe sepsis and trauma, either the proin-

flammatory or the anti-inflammatory response may become excessive and prolonged leading to multiorgan failure, immune dysfunction, and further infectious complications. Decades of clinical and experimental research have enhanced our understanding of the host response. However, the overwhelming complexity of the immune response is the most important barrier to progress toward effective therapies. An important adjunct to a systems-based, computational approach may be the evaluation of the single cell genomics and proteomics of specific cell populations. This approach allows better investigation of differing mechanisms within genotypically identical cells and can even identify new cell types and states. By utilizing these approaches, it is hoped that future targets can be identified in order to modify the immunoinflammatory response to suppress the deleterious effects while maintaining its benefits.

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Chapter 7
Multiscale Equation-Based Models: Insights for Inflammation and Physiological Variability

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Abbreviations

BP	Blood pressure
HF	High frequency
HPA	Hypothalamic-pituitary-adrenal
LF	Low frequency
LPS	Lipopolysaccharide
MSNA	Muscle sympathetic nerve activity
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PAMP	Pathogen-associated molecular patterns
SA	Sinoatrial
TLR4	Toll-like receptor 4

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Introduction

Inflammation is a critical component of the stress response. In response to stressors such as injury and infection, inflammation activates the initial physiological responses aimed at returning to homeostasis. The failure to restore homeostasis subsequent to an inflammatory response can be caused by either an insufficient response that is not strong enough to address the root cause of stress, or an overwhelming inflammatory response that leads to further damage in addition to the original stressor. Dysregulation of the inflammatory response is a component of many pathological conditions, such as sepsis. Although the incidence of sepsis is increasing, with over 2.5 million cases a year, an overall mortality of 12.5% [1] and estimated annual and healthcare expenditures in excess of $20 billion in the United States alone [2, 3], the only drug approved specifically to treat severe sepsis (activated protein C) was recently withdrawn from the market after failing to show improved outcome in a clinical trial [4]. There is clearly a need for more effective clinical tools for the management of inflammatory dysregulation, and novel approaches may be required to achieve this goal [5].

The pervasiveness of nonlinearity, redundancy, and pleiotropy in components of the inflammatory response leads to challenges in reductionist approaches and motivates systems-level approaches toward understanding inflammation [6]. Mathematical modeling is a promising technique because it allows for studying the dynamics of multiple interacting components of a complex system while integrating research from disparate disciplines with the ultimate goal of gaining insight into disease progression and therapeutic interventions [7, 8]. Thus, the potential exists for significant translational innovations based on models of inflammation in optimizing patient care, designing clinical trials, and rationalizing drug development [9, 10]. In all of these areas, issues related to physiological variability are important to consider.

Many components involved in the inflammatory response, such as cytokines, hormones, and autonomic signaling, contain homeostatic rhythmic variability at a wide range of time scales. The disruption of physiological rhythms is often associated with disease, such as changes in patterns of heart beats preceding the onset of sepsis [11, 12] and alterations in circadian variations in plasma cortisol, which are associated with depression [13], obesity [14], psychological stress [15], and cancer [16, 17]. Through studying the origins of these rhythmic signals in homeostasis and their disruption in inflammation, we can work toward understanding their underlying mechanisms and the potential diagnostic utility embedded in physiologic variability [18]. Therefore, when investigating translational applications of systems biology of inflammation, physiological variability represents an important factor influencing the state of the host. For instance, given the circadian time structure underlying many of the physiological responses dysregulated in sepsis, novel therapies should be tested with circadian rhythms in mind because the same treatment given at different times of day could have very different results [19, 20].

In the following sections, we discuss mechanisms through which biological rhythms can exert physiological regulatory effects; relationships between physiological variability and inflammation; and our work on systems-level mathematical

modeling of human endotoxemia, as a surrogate model for systemic inflammation, with a particular focus on accounting for the effects of physiological variability in endotoxemia.

Multiscale Modeling of Human Endotoxemia

The human endotoxemia model is an experimental model that can be applied to evaluate issues related to physiological variability in inflammation. Human endotoxemia consists of injection of endotoxin (lipopolysaccharide, LPS) to healthy human volunteers, allowing for the study of systemic inflammation in vivo in humans [21]. LPS is a component of the outer membrane of Gram-negative bacteria that is recognized by the innate immune system and instigates an inflammatory response. The response to LPS is steps from LPS binding to Toll-like receptor 4 (TLR4), leading to the activation of the innate immune system through the transcription of inflammatory mediators, eventually propagating to the systemic level to induce a wide range of physiological changes characteristic of systemic inflammation such as the release of immunomodulatory hormones, the activation of the autonomic nervous system, and increased body temperature [22]. Human endotoxemia reproduces many of the inflammation-linked physiological changes that occur in critical illness, such as sepsis [21, 23], acute respiratory distress syndrome (ARDS) [24], and trauma [25]. Additionally, endotoxemia alters biological rhythms at multiple time scales, ranging from circadian rhythms [26] and short-term HRV [27–35], allowing for investigation into the relationship between systemic inflammation and biological rhythms. HRV is driven largely by rhythmic patterns in the variability of heart beats [36], and diminished HRV is correlated with disease severity in sepsis [37–42]. In addition to serving as a useful biomarker, the loss of HRV may give insight into disease mechanisms. This reduction in HRV may be driven by a loss of interorgan coupling and communication [22, 43–45].

Here, we describe the iterative development of multiscale models of human endotoxemia, starting with the binding of LPS to its receptor on immune cells and growing to encompass hormonal responses and changes in heartbeat patterns. These three compartments are depicted in the network diagram in Fig. 7.1. Additionally, we identify and discuss areas where physiological variability may play an important role in either governing the response to endotoxemia or giving insight into the state of the system.

Immune Cells

While there are many levels to consider in a model of human endotoxemia, a critical aspect is modeling the initial recognition of LPS and the production of signals that lead to a systemic response. In general, pathogen-associated molecular patterns

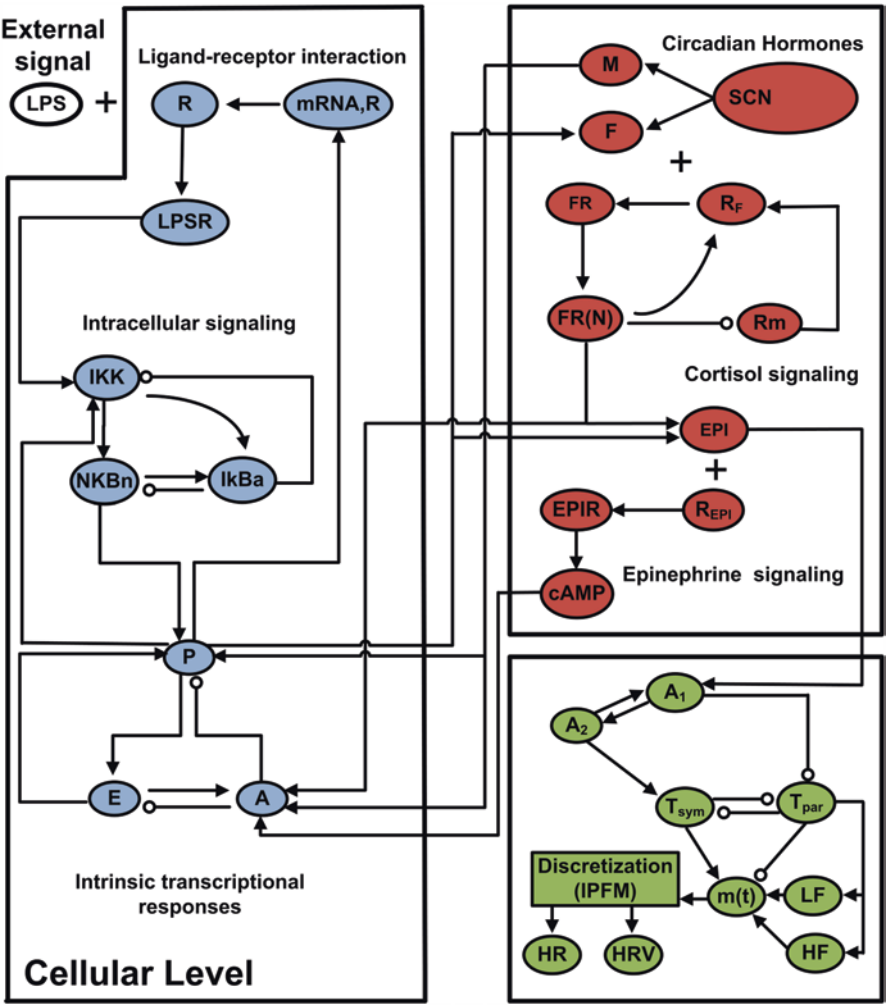

Fig. 7.1 Network diagram of the components of a multiscale model of human endotoxemia, at three levels. At the cellular level (blue), LPS binds to its receptor TLR4 (R) forming the activated complex LPSR. LPSR stimulates NF-κB activity (IKK, NfKBn, and IkBa), which modulates the transcriptional response to inflammation, consisting of proinflammatory (P), anti-inflammatory, and energetic (E) components. At the central level (red), hormonal output both responds to and modulates the progression of the inflammatory response to LPS through both cortisol (F) and epinephrine (EPI) signaling. Furthermore, circadian rhythms in the immunomodulatory hormones cortisol and melatonin (M) impose circadian patterns on many components of the inflammatory response. Finally, at the autonomic level (green), changes in sympathetic (T_{sym}) and parasympathetic (T_{par}) signaling are reflected in modulated patterns of heart rate (HR) and heart rate variability (HRV)

(PAMPs) bind to Toll-like receptors (TLRs) expressed by cells of the innate immune system, leading to transcriptional responses including the production of proinflammatory cytokines; LPS, in particular, activates the TLR4 signaling pathway leading to broad transcriptional changes driven by inflammation-related transcription factors such as NF-κB [46]. The initial transcriptional response to human endotoxemia has been studied experimentally through high-throughput DNA microarrays which simultaneously quantify the state of thousands of gene transcripts in blood leukocytes [47]. This wealth of experimental data allowed for data-driven modeling of the transcriptional responses to LPS without the a priori postulation of which genes are most important in endotoxemia. From this, a dynamical model was constructed to represent transcriptional changes in leukocytes during human endotoxemia [48].

Identification of Key Transcriptional Responses

In order to discover the critical transcriptional motifs in high-dimensional time course microarray data, a systematic computational framework was recently proposed that decomposes the data into elementary set of clusters representing key temporal responses [49]. Given the availability of such high-dimensional data in human endotoxemia experiments [47], we applied this computational approach based on the hypothesis that a specific underlying network structure gives rise to the dynamics of the inflammatory response. Therefore, we sought to identify a set of core transcriptional responses to endotoxemia representative of the dynamic evolution of the host response to LPS under the assumption that related genes responsive to endotoxin undergo concerted changes in their expression profiles.

The clustering algorithm is based on a symbolic discretization of time series data which labels similar temporal expression profiles with the same symbolic motif [50]. Having assigned each gene to a motif, the next task is to select motifs that are so highly populated that the temporal patterns of the genes in that motif are very unlikely to arise by chance. In other words, the goal is to identify highly nonrandom patterns in gene expression profiles putatively caused by a coherent transcriptional regulatory mechanism, thus generating a subset of transcriptional motifs which characterize the host response to LPS. The next step is to reduce this relatively large subset of transcriptional motifs into a smaller elementary set that best characterizes the deviation from homeostasis in human endotoxemia. The global nature of gene microarray data generally results in a roughly log normal distribution of gene expression values [51]. Consistent deviations from this distribution generally result in a motif that is highly enriched relative to baseline. Based on this concept, the transcriptional state of the system is defined as the distribution of expression values at a time point; by comparing the transcriptional state of the system as a whole with the distribution of expression values in a subset of motifs, the motifs that lead to a maximal deviation can be identified. This was done by applying the Kolmogorov–Smirnov test at each time point for subsets of highly populated (as defined above) motifs, and searching through the potential combinations of motifs to identify the

minimum number of motifs that maximally deviate from the overall transcriptional state distribution. This defines a combinatorial optimization problem that was solved through a stochastic simulated annealing optimization algorithm.

Applying the algorithm described above to human endotoxemia data revealed three critical expression motifs, all enriched in genes participating in physiologically relevant pathways: (1) an early upregulated proinflammatory response containing genes related to TLR signaling and members of the NF-κB/RelA family; (2) a late upregulated anti-inflammatory response including components of the JAK-STAT and IL-10 signaling cascades; and (3) a downregulated energetic response comprised largely of genes involved in cellular bioenergetic processes. All of these expression patterns return to baseline within 24 h.

This approach for identifying the key transcriptional signals in human endotoxemia through high-dimensional data analysis is appealing for several reasons. First, it provides in vivo data from a relatively accessible source (blood samples). Thus, the gene expression data reflect complex regulatory properties of the human inflammatory response, such as hormonal and autonomic responses, which cannot be recapitulated through analysis of human cell lines; some of these interactions will be described in later sections of this chapter. Additionally, the ability to gather this type of experimental data in humans rather than in animals means that the results are likely closer to human clinical data, although of course animal studies would allow for a wider range of experimental perturbations and analysis techniques.

Indirect Response Modeling

Having identified the critical components of the transcriptional response to endotoxemia, the next step was to account for these transcriptional patterns in a dynamical model. This is a challenging problem because the precise signal transduction steps leading to the activation of specific genes are not always known, and even if they were, the parameters governing that signal transduction pathway is generally not known. In pharmacokinetic/pharmacodynamic modeling, this problem is often approached through indirect response modeling to quantify indirect relationships between model components [52]. In general, if a compound is modeled by a zeroth order production term and a first order degradation term, such as x in Eq. 7.1a, then indirect effects on these productions and degradation terms can be modeled as in Eq. 7.1b, where $f(y)$ is function (typically sigmoidal) of y representing y's indirect stimulation on the production rate of x.

$$\frac{dx}{dt} = k_1 - k_2 \cdot x \tag{7.1a}$$

$$\frac{dx}{dt} = k_1 \cdot \left(1 + f\left(y\right)\right) - k_2 \cdot x \tag{7.1b}$$

Depending on the sign and placement of this indirect modulation term, it can represent either stimulation or inhibition of either the production or degradation rate of x. Furthermore, it allows for several factors on production or degradation to be combined multiplicatively. Through indirect response modeling combined with a simple model of LPS recognition, we developed an 8-equation model of the transcriptional responses to endotoxemia. Equation 7.2a–7.2d shows the three core transcriptional responses described above, the proinflammatory, anti-inflammatory, and energetic responses, respectively [48].

$$\frac{dP}{dt} = k_{\text{in},P} \cdot \left(1 + H_{P,\text{DR}*}\right) \cdot \left(1 + H_{P,E}\right) / A - k_{\text{out},P} \cdot P \tag{7.2a}$$

$$\frac{dA}{dt} = k_{\text{in},A} \cdot \left(1 + H_{A,P}\right) \cdot \left(1 + H_{A,E}\right) - k_{\text{out},A} \cdot A \tag{7.2b}$$

$$\frac{dE}{dt} = k_{\text{in},E} \cdot \left(1 + H_{E,P}\right) / A - k_{\text{out},E} \cdot E \tag{7.2c}$$

$$H_{X,Y} = k_{X,Y} \cdot Y \tag{7.2d}$$

This results in a model which has the ability to produce dose-dependent responses to LPS, as shown in Fig. 7.2. In response to a low dose of LPS, as is given in human endotoxemia experiments, the transcriptional responses are activated acutely and return to baseline within 24 h. Yet in response to a higher dose of LPS,

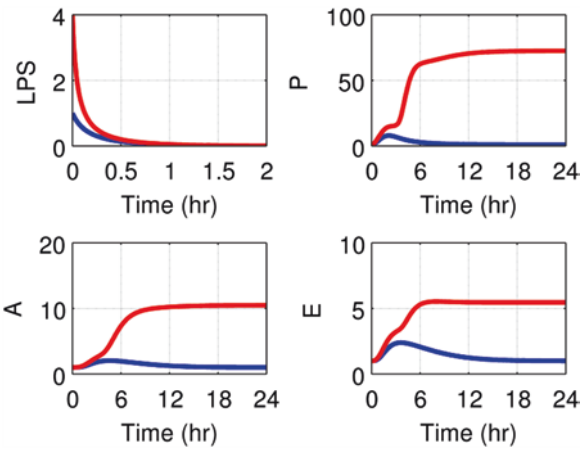

Fig. 7.2 The cellular-level model described more completely in ref. (48) predicts two classes of responses to acute endotoxemia. In response to relatively low doses (blue lines), a resolving response is generated where all components return to baseline within 24 h. Yet in response to larger doses of LPS (red lines), the self-stimulatory nature of the proinflammatory response dominates and leads to a persistent inflammatory state. *P* proinflammatory transcriptional response, *A* anti-inflammatory transcriptional response, *E* energetic transcriptional response

the anti-inflammatory controls are overwhelmed by self-stimulatory proinflammatory signaling, leading to a persistent inflammatory state.

Equation 7.2a–7.2d represents the core transcriptional responses of the innate immune system to human endotoxemia, and indirect response modeling allows for the extension of this core to interact with other systems as will be described in subsequent sections.

Central Control of Immunomodulatory Hormones

In systemic inflammation and in endotoxemia, inflammatory mediators produced by immune cells in response to LPS recognition are secreted into systemic circulation and recognized by the central nervous system, which responds with the release of immunomodulatory hormones such as cortisol (an endogenous glucocorticoid in humans) and epinephrine. Both of these hormones normally undergo circadian rhythms. Glucocorticoids are also commonly used anti-inflammatory drugs. Therefore, there is value in an integrated model relating the pharmacodynamics of glucocorticoids and other hormones with the progression of the human endotoxemia response [53, 54]. Glucocorticoids exert their immunomodulatory effects through binding to the glucocorticoid receptor in the cytosol, translocating to the nucleus, and then acting as a transcription factor for a wide range of glucocorticoid-responsive genes. This glucocorticoid signal transduction pathway has been studied from the perspective of pharmacodynamics, resulting in well-established mathematical models such as the model by Ramakrishnan et al. shown in Eq. 7.3a–7.3d [55].

$$\frac{dR_m}{dt} = k_{\text{syn\_Rm}} \cdot \left(1 - \frac{\text{FR}(N)}{\text{IC}_{50\_\text{Rm}} + \text{FR}(N)}\right) - k_{\text{deg\_Rm}} \cdot R_m \tag{7.3a}$$

$$\frac{dR}{dt} = k_{\text{syn\_}R} \cdot R_m + R_f \cdot k_{\text{re}} \cdot \text{FR}(N) - k_{\text{on}} \cdot F \cdot R - k_{\text{dgr\_}R} \cdot R \tag{7.3b}$$

$$\frac{d\text{FR}}{dt} k_{\text{on}} \cdot F \cdot R - k_T \cdot \text{FR} \tag{7.3c}$$

$$\frac{d\text{FR}(N)}{dt} = k_T \cdot \text{FR} - k_{\text{re}} \cdot \text{FR}(N) \tag{7.3d}$$

The variables in Eq. 7.3a–7.3d represent glucocorticoid receptor mRNA (*Rm*), free cytosolic receptor (*R*), cytosolic glucocorticoid-receptor bound complex (FR), and nuclear glucocorticoid-receptor complex (FR(*N*)), driven by a glucocorticoid concentration *F*. FR(*N*) is then the component that acts as a transcription factor, which we can account for in our core transcriptional response equations by altering Eq. 7.2b to account for the effect of glucocorticoids on anti-inflammatory gene transcription, shown in Eq. 7.4.

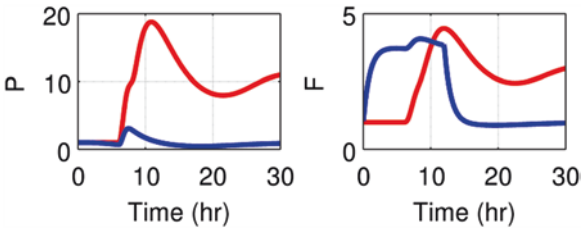

Fig. 7.3 After incorporating the effects of immunomodulatory hormones on the inflammatory response (53), the response to cortisol treatment was evaluated. The two lines show responses to identical doses of LPS at 6 h, but the blue lines represent a system that has been infused with cortisol for 6 h prior to LPS while the red lines represent a simulation of the response to only LPS. This produces divergent outcomes to the same dose of LPS, with cortisol pretreatment exerting a protective effect leading to a self-limited response rather than a persistent inflammatory state

$$\frac{dA}{dt} = k_{\text{in},A} \cdot \left(1 + H_{A,P}\right) \cdot \left(1 + H_{A,E}\right) \cdot \left(1 + H_{A,\text{FR}(N)}\right) - k_{\text{out},A} \cdot A \tag{7.4}$$

Through this integrated model, upstream changes in glucocorticoid levels (either endogenously produced in response to inflammation or exogenously given) propagate through the model and modulate transcriptional processes in immune cells. This allowed us to evaluate the relationship between endotoxemia and cortisol treatment, given either before or after LPS [53, 54]. For example, Fig. 7.3 shows how cortisol infusion prior to LPS can have a protective effect, allowing for a resolving response to a large dose of endotoxemia that otherwise would perturb the system to the persistent inflammatory steady state. It also allowed for the evaluation of the effects of circadian rhythms on human endotoxemia [56].

Circadian Rhythms

Circadian rhythms are present in many components of the inflammatory response [57], including plasma cytokine [58–62] and cortisol concentrations. Melatonin, another hormone with both circadian and immunomodulatory properties, may play a key role in mediating communication between peripheral components of the immune system and the central circadian clock in the suprachiasmatic nucleus [57]. Furthermore, given that melatonin levels peak in the night, roughly at the same time as cytokines, and melatonin stimulates the production of cytokines [63–65], melatonin signaling serves as a plausible mechanism for synchronization between central and peripheral circadian clocks in the immune system, and indirect response modeling can be applied to represent this relationship, as described above. Circadian hormone production is then imposed by varying the production rates of cortisol and melatonin throughout the day [66]. In total, this produces circadian rhythms that

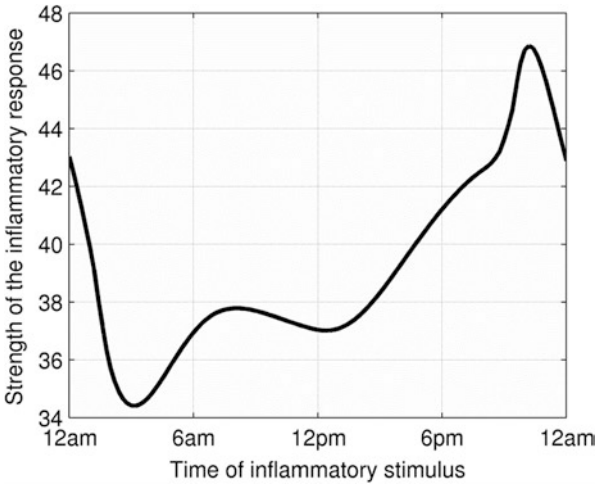

Fig. 7.4 The magnitude of the inflammatory response to LPS has a circadian dependence, as illustrated here by the maximal response of proinflammatory signaling to identical LPS doses given at different times throughout the day (56)

propagate throughout the model, in line with homeostatic experimental data [56]. In response to endotoxemia initiated at different times throughout the circadian cycle, this model predicts that responsiveness of the innate immune system to LPS has a circadian dependence as shown in Fig. 7.4, for instance, decreasing when the anti-inflammatory hormone cortisol is at high levels in the morning.

Ultradian Rhythms

The circadian pattern in cortisol secretion is driven by patterns in rhythmic cortisol secretion at a faster time scale, known as ultradian rhythms. This refers to the pulses of cortisol released roughly hourly, whose magnitude imposes a circadian rhythm. We recently studied a combined model of the hypothalamic-pituitary-adrenal (HPA) axis and glucocorticoid pharmacodynamics [67]. This work revealed differences in mean levels of homeostatic gene expression in response to constant or oscillatory hormone levels, as well as a correlation between homeostatic ultradian rhythm amplitude and peak responsiveness to stress. Similar computational results were identified by Rankin et al. [68]. The importance of glucocorticoid ultradian rhythms is further supported by experimental studies showing differences in expression of glucocorticoid responsive genes when exposed to oscillatory or constant patterns of glucocorticoid concentration [69, 70] as well as more general relationships linking effective physiological function with rhythmic variability in HPA axis output [71].

Heart Rate and Heart Rate Variability

It has long been recognized that critically ill patients, such as those with sepsis, tend to exhibit diminished physiological variability as quantified by HRV [37–42]. Despite this, there is a lack of mechanistic understanding as to why the phenomenon of decrease HRV in disease exists, which limits the translational applications of HRV metrics to observation-based prognostic and diagnostic analysis rather than the development of novel therapies. Given the similarities in physiological changes occurring in endotoxemia and sepsis [21, 23], including the loss of HRV in endotoxemia [27–35], human endotoxemia represents an excellent platform for studying the mechanistic origins and implications of inflammation-driven diminished HRV by applying both experimental and computational techniques.

Modeling changes in HRV driven by inflammation necessitates a multiscale approach. HRV can be quantified by a diverse array of metrics operating on discrete data, a series of heart beat intervals. However, this discrete signal is modulated by continuous variables such as concentrations of inflammatory mediators. Thus, a significant challenge in mechanistic modeling of HRV in endotoxemia is reconciling continuous inputs (e.g., hormone and cytokine concentrations) with discrete, noisy output (the beating of the heart). We approached this problem through a continuous model of autonomic influences on the heart combined with a discrete model to output a series of heart beats, which were then postprocessed to assess HR and HRV [72].

Autonomic Origins of Heart Rate Variability

Cyclic contractions of the heart initiate at the sinoatrial (SA) node, also known as the pacemaker of the heart. The sympathetic and parasympathetic branches of the autonomic nervous system converge at the SA node, allowing for appropriate regulation of heart rate. The SA node is also exposed to oscillations in the output of the autonomic nervous system, which typically occur in characteristic frequency ranges. **High frequency (HF) rhythms** in the frequency range of 0.15–0.4 Hz [73] are largely the manifestation of the breathing pattern and are transduced to the heart by the vagus nerve [74]. **Low frequency (LF) rhythms** in the frequency range of 0.04–0.15 Hz [73]. LF oscillations are generally interpreted as reflecting fluctuations in both sympathetic and parasympathetic activities [74]. At a much longer time scale, **circadian rhythms** in autonomic activity are also apparent in HRV [75].

These periodic signals modulate the firing pattern of the SA node, leading to rhythmic components in HRV. Thus, to mechanistically link inflammation with changes in heart beat patterns, a model of the autonomic modulation of the heart is required. Based on prior work considering the effects of endotoxemia on the autonomic nervous system [76], we constructed a continuous algebraic equation representing effective autonomic modulation of the SA node in homeostasis and in endotoxemia [72], as shown in Eq. 7.5.

$$m(t) = \underbrace{HR}_{\substack{\text{constant}\\ \text{activity}\\ \text{level}}} + \underbrace{k_{\text{circ}}\left(T_{\text{sym}} + \frac{1}{T_{\text{par}}}\right)}_{\substack{\text{circadian}\\ \text{variability}}} + \underbrace{k_{\text{osc}}\left(1 + k_{\text{par,LF}}T_{\text{par}}\right)\sin\left(f_{\text{LF}}t\right)}_{\text{LF oscillations}} + \underbrace{k_{\text{osc}}\left(1 + k_{\text{par,HF}}T_{\text{par}}\right)\sin\left(f_{\text{HF}}t\right)}_{\text{HF oscillations}} \tag{7.5}$$

Equation 7.5 accounts for a baseline activity level modulated by the three rhythms discussed above: HF, LF, and circadian rhythms. The effective modulations imposed by each of these components are driven by levels of sympathetic (T_{sym}) and parasympathetic (T_{par}) activity, which respond to the levels of inflammatory mediators [76].

However, to assess HRV, a series of discrete heart beats is required. Therefore, the continuous Eq. 7.5 must be discretized to output distinct heart beats whose period is modulated by this effective autonomic activity.

Discrete-Continuous Modeling

Cells at the SA node respond to the autonomic nervous system by recognizing the concentrations of autonomic neurotransmitters, leading to altered firing rates. In the absence of autonomic modulation, the heart still beats, just with a regular pattern unperturbed by autonomic rhythms. This type of system can be represented with an integrate-and-fire model, where fluctuations in the propensity for the SA node initiating a contraction depend on the effective autonomic modulation [72, 77, 78]. This is done by repeatedly integrating under the curve defined in Eq. 7.5 and recording the time of a heartbeat occurring whenever a constant threshold is reached. The translation from continuous input signals to a discrete output system is a fundamental aspect of mechanistic modeling of HRV, as physiologically a similar discretization process is occurring. Homeostatic circadian output of this model is shown in Fig. 7.5, illustrating how oscillatory autonomic input produces patterns in both HR and HRV. Based on mechanistic modeling, the resulting discrete list of heartbeats can then be analyzed with the same algorithms that are used for real data. HRV is quantified by time domain, frequency domain, and nonlinear metrics, all aimed at either gaining some specific physiological information from the heartbeat data or optimizing the correlation of the HRV metric with some relevant clinical outcome. Different HRV metrics thus have different information content and have different practical applications. Based on the discrete output of our model, we were similarly able to apply diverse HRV metrics, revealing discrepancies in responsiveness of different HRV metrics to endotoxemia.

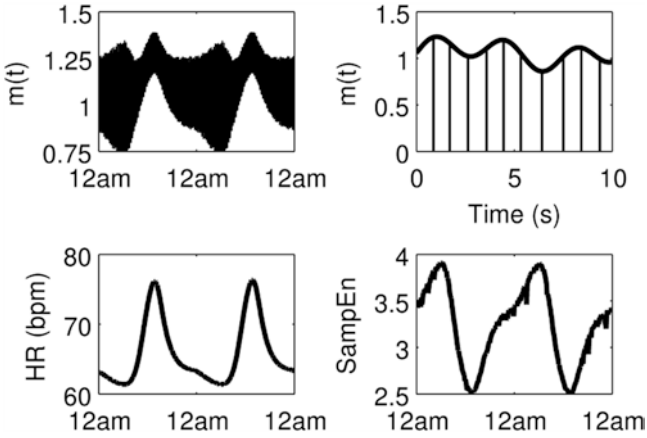

Fig. 7.5 Multiscale rhythmic effects on the heart are depicted here. At the top, $m(t)$ represents the effective autonomic modulation of the heart, which contains circadian (24 h), HF (0.15–0.4 Hz), and LF (0.04–0.15 Hz) components. These combined rhythmic effects produce variability in the discretized beating of the heart, as illustrated by circadian patterns in both heart rate (HR) and heart rate variability (HRV) as quantified by sample entropy (SampEn). These rhythmic components of the output of the heart are altered in response to endotoxemia (72)

Challenges in Translational Modeling of Heart Rate Variability in Endotoxemia

Dysregulation of the inflammatory response is a critical component of many clinically challenging disorders such as sepsis [79]. Through modeling, with a focus ultimately on translational applications, we obtain more fundamental understanding of relevant physiological processes. And by taking advantage of the information embedded in biological rhythms, ranging in time scale from high-frequency autonomic oscillations reflected in heart rate variability to circadian rhythms in inflammatory mediators, we gain insight into the underlying physiology [80]. Although it is difficult to precisely identify how changes in HRV relate to changes in underlying physiology [81], there are already practical clinical applications of HRV analysis. Even going beyond purely phenomenological approaches, our growing mechanistic understanding of the origins of HRV and the dysregulation of physiological rhythms is leading to further insights. For instance, the baroreflex negative feedback loop maintains homeostatic blood pressure while also producing oscillations in blood pressure that are reflected in HRV. In sepsis, the baroreceptor function is altered, producing changes in autonomic output that can be assessed through HRV [37]. Thus, some of the decrease in HRV in sepsis may result from dysregulated baroreflex activity. Studying the mechanisms driving rhythmic patterns in HRV will eventually allow for the maximization of information retrieval from the heartbeat signal, which is particularly of interest when considering changes occurring in the stress response. However, several challenges remain in more broadly developing and

leveraging mechanistic model-based approaches in the context of inflammation-linked diseases.

While endotoxemia experiments have explored changes in HRV in response to LPS [27–35], even in this controlled environment a broad understanding of the mechanisms is lacking. Given the importance of sympathetic and parasympathetic oscillations in driving HRV, changes in autonomic activity in endotoxemia likely play a role in the altered HRV patterns that have been seen experimentally. It is often assumed that sympathetic activity increases in endotoxemia, based on the characteristic physiological changes observed such as increased HR. It is also known that the parasympathetic nervous system plays a role in endotoxemia via the cholinergic anti-inflammatory pathway [82]. However, experimental data on HRV changes in endotoxemia show decreases in both HF and LF rhythms, which is indicative of a decrease in autonomic modulation of the heart [83]. Further investigation into this apparent paradox sheds light on our lack of understanding of autonomic function in endotoxemia and thus also in systemic inflammation in general. Mounting experimental evidence suggests that an answer to this paradox may lie in the sensitivity of the heart to autonomic activity during inflammation because, if the responsiveness of the heart to autonomic signaling is diminished, then HRV will generally decrease. Fairchild et al. showed that pathogen-mediated effects on cardiac function desensitize the heart's response to vagal signaling in mice [83]. In human endotoxemia, Sayk et al. found decreased sympathetic activity measured through microneurography at the peroneal nerve [31]. While peroneal nerve activity may not precisely reflect sympathetic activity at the heart [35], Sayk et al. also found that sensitivity of the heart to drug-induced sympathetic modulation is diminished in endotoxemia [31]. In vitro experiments show that, independent of the autonomic nervous system, interactions between inflammation and cardiac tissue can produce altered beating patterns and also play a role in altering the sensitivity of the heart to autonomic activity [27, 84, 85]. These results suggest that the changes in HR and HRV in endotoxemia may be driven not by changes in autonomic output, but by nonautonomic interactions between inflammation and the heart and decoupling between the heart and the autonomic nervous system.

Godin and Buchman hypothesized reduced physiological variability, such as reduced HRV, is representative of diminished interorgan communication [43]. Given the experimental evidence discussed above, this hypothesis is as relevant as ever. Even in a well-studied and controlled environment like human endotoxemia, the existence of biological rhythms with multiple sources and regulators makes it challenging to determine what precisely a change in HRV means. However, this complexity also means that there is potentially a wealth of information from a wide range of sources embedded in the HRV signal, representing an opportunity to apply computational techniques to reveal as much as possible of the physiologically importance of HRV. Through representing the mechanistic background of HRV in a mathematical model, we can evaluate hypotheses concerning the origins of HRV in homeostasis and the loss of HRV in the stress response by evaluating the relationship between endotoxemia and HRV.

Mathematical modeling enabled us to delineate the complexities underlying the interpretation of HRV complicate understanding the mechanisms that cause vari-

ability [86]. HRV arises largely due to oscillations in autonomic activity which are apparent in the power spectrum of RR intervals primarily in two frequency bands termed low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) [73]. A model to evaluate the relationship between the autonomic nervous system and the beating of the heart requires, at a minimum, four components, as shown in Fig. 7.6: (1) a representation of sympathetic activity; (2) a representation of parasympathetic activity; (3) a combination of sympathetic and parasympathetic activities, representing autonomic modulation of the SA node; (7.4) a method to convert this autonomic modulation into heart beats, which can then be analyzed through the application of HRV metrics, as we have previously demonstrated [72]. Each of these four components is comprised of a multitude of complex interactions and feedback loops, such as autonomic oscillations which arise due to the baroreflex and the respiratory sinus arrhythmia. However, high-level properties of the system can be studied without exhaustively detailing these components. A simple model including these four components was earlier investigated by Brennan et al. in an attempt to gain insight into the relationship between autonomic signaling and Poincaré plots of RR intervals [77]. Chiu et al. analyzed a slightly more complex model that accounts for some of the signal transduction steps between the release of autonomic neurotransmitters and the regulation of SA node activity [78, 87]. The goal was to investigate the relationship between autonomic inputs, such as oscillating frequency and mean levels of autonomic outputs, and the beating of the heart.

The goal of the model presented by Scheff et al. [86] was to investigate the relationship between autonomic inputs, such as oscillating frequency and mean levels of autonomic outputs, and the beating of the heart. It can be succinctly presented as:

$$\begin{aligned}
\mathrm{nor} &= m_{\mathrm{nor}} + \alpha_{\mathrm{nor}} \sin\left(\omega_{\mathrm{nor}} t\right) \\
\mathrm{ach} &= m_{\mathrm{ach}} + \alpha_{\mathrm{ach}} \sin\left(\omega_{ach} t\right) \\
\mathrm{adr} &= \frac{\mathrm{nor}}{k_{\mathrm{nor},1} + k_{\mathrm{nor},2}\mathrm{nor}} \\
\mathrm{cho} &= \frac{\mathrm{ach}}{k_{\mathrm{ach},1} + k_{\mathrm{ach},2}\mathrm{ach}} \\
m(t) &= k_{\mathrm{icpm}} + k_{\mathrm{adr}}\mathrm{adr} - k_{\mathrm{cho}}\mathrm{cho} \\
I &= \int_{t_k}^{t_k+1} m(t)\, dt
\end{aligned} \tag{7.6}$$

The variables *nor* and *ach* represent norepinephrine and acetylcholine, neurotransmitters released by the sympathetic and parasympathetic nerves respectively which modulate the beating of the heart. Each of these variables has a mean level m_k as well as an oscillatory component with amplitude a_k and frequency ω_k. These sinusoids are the source of variability in the model and represent the underlying LF and HF signals apparent in HRV data.

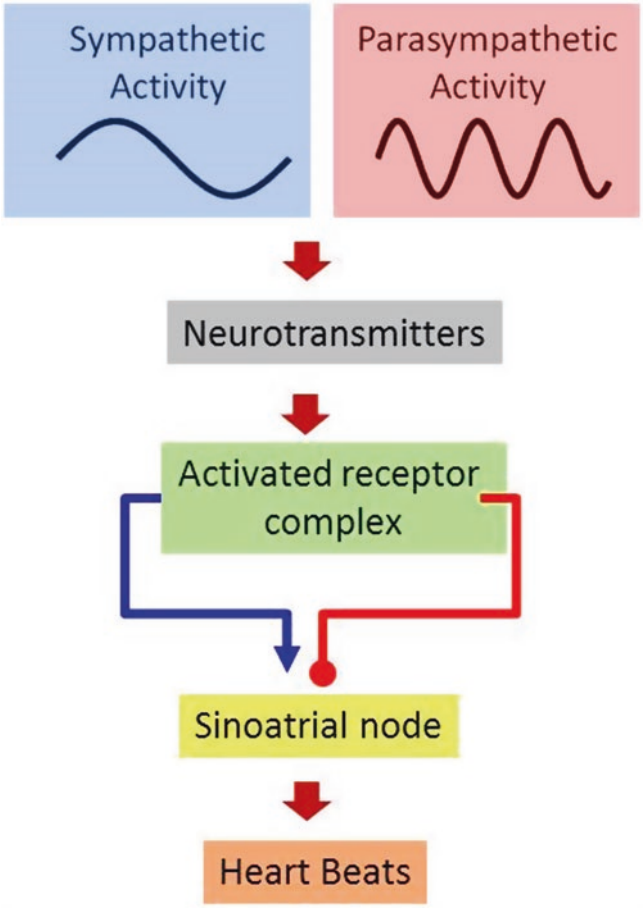

Fig. 7.6 Components of the models linking autonomic activity with heart beats. Sympathetic and parasympathetic nerves impose oscillatory activation of the sinoatrial (SA) node, leading to variability in discrete heart beats. The frequencies of oscillations in sympathetic and parasympathetic activities are derived from the observed frequencies present in the HR power spectrum, the LF and HF bands, 0.095 Hz and 0.275 Hz, respectively

Although more complex models, such as that of Ursino and Magosso, confirmed some aspects of the traditional relationships between LF power, HF power, sympathetic activity, and parasympathetic activity, they also found several other parameters that could confound direct interpretations of these frequency domain HRV metrics [88]. Additionally, they noted how saturation of signal transduction due to sigmoidal functions could also significantly interfere with interpretations of HRV. Zenker et al. also identified this type of saturation as a potential physiologically relevant mechanism that can drive changes in HRV [89], such as the apparent loss of "sympathetic activity" in HRV caused by a real underlying increase in sympathetic activity.

The concept of uncoupling, the loss of interorgan communication, is also important in the context of systemic inflammation, as it has long been hypothesized to play a critical role in disease progression [43] and changes in variability metrics such as HRV have been proposed as metrics for uncoupling [28, 44]. Clinically, similar quantification of uncoupling has been shown to be a marker of disease severity in patients with multiple organ dysfunction syndrome [90]. The most thorough investigation of the autonomic nervous system in human endotoxemia was performed by Sayk et al. through two novel experimental techniques: measuring muscle sympathetic nerve activity (MSNA) to directly quantify sympathetic activity in endotoxemia; and modulating blood pressure (BP) to quantify how BP-induced autonomic signaling is coupled to the heart in endotoxemia [31]. This produced two novel insights. They found that MSNA in the peroneal nerve is suppressed in endotoxemia, which is the opposite of what would have been expected based on changes in HR, and that the autonomic nervous system and the heart were effectively uncoupled in endotoxemia, as autonomic perturbations induced by changes in BP produced no significant effect on HR or HRV even as they significantly altered MSNA.

The fact that sympathetic output of the peroneal nerve is diminished in endotoxemia does not necessarily mean that sympathetic activity at the heart behaves similarly, as different parts of the sympathetic nervous system may respond differently. Thus, it could very well be that sympathetic activity increases [35]. However, the striking uncoupling found between changes in BP and HR requires closer examination. One can imagine that saturation at some point in autonomic signaling pathways could explain a kind of uncoupling in that if the system was responding to very high levels of autonomic activity, such that further changes in autonomic activity were be blunted by the time they reached the SA node and thus were not reflected in the output of the heart. This would only be possible to rationalize with observations of increased HR and decreased HRV in endotoxemia if mean sympathetic and parasympathetic activities were either significantly elevated in endotoxemia (e.g., by increasing m_{nor} and m_{ach}) or if sensitivity to autonomic signaling was significantly decreased. The former is possible, particularly in light of the cholinergic anti-inflammatory response as discussed above. The latter is possible as well, as nonautonomic inflammatory mediators exert regulatory effects on the beating of the heart [27, 85, 91] and crosstalk between the sympathetic and parasympathetic activities can result in altered sensitivities to autonomic neurotransmitters [92].

Additionally, it is possible that the decrease in peroneal MSNA [31] is matched by a decrease in sympathetic activity at the heart. Experiments in rats found that endotoxemia led to increased acetylcholine concentration and decreased norepinephrine concentration in the liver, lending further support to the hypothesis that sympathetic activity is diminished in endotoxemia [93]. In this hypothetical regime, uncoupling between the autonomic nervous system and the heart [31] would mask the effects of diminished sympathetic activity on HR, and the endotoxemia-induced increase in HR could be due to nonautonomic pathways [27, 85, 91]. In terms of Eq. 7.2a–7.2d, this scenario would be equivalent to decreasing m_{nor} to simulate decreased sympathetic activity, decreasing k_{icpm} to simulate a nonautonomic increase in HR, and decreasing both k_{adr} and k_{cho} to represent uncoupling.

Distinguishing between these three mechanisms requires novel experimental work to look more closely at the autonomic nervous system in human endotoxemia [31]. It is important that these issues are approached with an accurate mindset of what the analysis of heart beats can and cannot provide. Signal transduction from the autonomic nervous system to the heart is complex, nonlinear, unintuitive, and often misinterpreted. Simple mathematical models, as discussed here and elsewhere [77, 78, 92, 94], can elucidate issues related to the interpretation of HR and HRV data. Specifically, in the literature related to human endotoxemia, overzealous interpretation of HR and HRV signals is common and may be impeding more fundamental understanding of autonomic function in systemic inflammation. All three hypothetical mechanisms discussed above also allow for the possibility that autonomic modulation of the heart may be substantially different than autonomic activity elsewhere in the body, and the additional complexity in real physiological systems presents even more opportunities for other factors to influence HR and HRV. While quantification of HR and HRV can provide valuable insight into a system, the extent of this insight depends on how well the specific underlying mechanisms in a specific scenario are known, so that physiologically important signals can be accurately identified and isolated.

Conclusions

While the lack of mechanistic understanding of the relationship between HRV and endotoxemia has not fully impeded the clinical application of HRV analysis in inflammation-linked disorders such as sepsis [12], increased mechanistic knowledge, backed both by systems-level experimentation and mechanistic multiscale models, will likely lead to improvements in diagnostic and prognostic applications of HRV as well as potentially novel therapeutic strategies. Just as the realization that the vagus nerve modulates inflammation [95] resulted in the conception of novel therapies based on vagus nerve stimulation [96], greater understanding as to why HRV correlates with disease state may reveal other pathways for therapeutic intervention.

HRV is a particularly appealing metric of physiologic variability due to the ease of noninvasive measurement and well-established correlations with disease state. However, biological rhythms at other time scales are also of importance. For example, LPS given at different times of day both suppresses and synchronizes circadian clock gene expression in peripheral blood leukocytes [26], representing another scale at which the decoupling between oscillators may play a role in inflammation, given the observed relationship between disease and circadian rhythms [97]. Rhythmic oscillations in NF-κB activation [98] and ultradian rhythms in regulate the inflammatory response.

The relationships between physiological variability, interorgan communication, and disease suggest that monitoring variability may reveal disease state; thus, decreases in variability would correspond with disease progression and increases in

variability (toward homeostasis) would correspond with recovery. However, the specific molecular mechanisms driving the loss of variability may be disease specific, paralleling how the presence of variability itself may exert physiological effects through several different mechanisms. Increasingly detailed mechanistic modeling will be required to understand the underlying molecular processes driving the loss of variability, and studying these processes in endotoxemia represents a good first step toward this goal. If these lower-level processes can be linked to readily available observables such as HRV, then this will lead to advances in translational medicine.

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Chapter 8
In Silico Trials and Personalized Therapy for Sepsis and Trauma

Yoram Vodovotz, John Bartels, and Gary An

Abbreviations

ABM	Agent-based model
ICU	Intensive care unit
ISS	Injury Severity Score

Inflammatory Diseases: A Pox on All Our Houses

We are currently faced with a barrage of complex diseases that often coexist in the same patient [1]. In the developing world, the modern disease landscape is a constellation of acute and chronic infections, traumatic injuries, and nonhealing wounds; diseases that are made even more complex due to the impact of malnutrition, war, and displacement [2, 3]. In the industrialized world, we face some of the same challenges with regard to infections, trauma, and wounds, but these diseases are complicated by lifestyles of excess and the attendant metabolic irregularities (diabetes and obesity) [4]. In addition, the generally longer lifespans now being experienced around the world have paradoxically resulted in the rise of aging-related diseases, such as cancer and various neurodegenerative diseases [5]. Given the degree and extent of medical care in the first world, it is virtually guaranteed that a common pathway for patients with this range of diseases is to spend at least some

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time in an intensive care unit with critical illness manifesting with multi-compartment pathophysiological derangements and organ failure [6]. Critical illness can result directly from trauma, hemorrhagic shock, and bacterial infection (sepsis). On its own, trauma/hemorrhage is a leading cause of death worldwide, often leading to inflammation-related late complications that include sepsis and multiple organ dysfunction syndrome (MODS) [7–9]. Sepsis alone is responsible for more than over a million annual hospital admissions, more than 215,000 deaths in the United States per year, and an annual healthcare cost of over $20 billion [6, 10, 11], while traumatic injury remains the leading cause of mortality and morbidity for individuals under 55 years and accounts for 30% of all life-years lost, with over 190,000 lives lost annually in the United States [12, 13]. There is currently not a single approved pharmacological therapy, other than antibiotics, targeting the pathophysiology of critical illness [14].

It is now clear that the acute inflammatory response, with its manifold manifestations at the molecular, cellular, tissue, organ, and whole-organism levels, drives outcomes in all the aforementioned diseases and is central to the pathophysiology of critical illness. Properly regulated inflammation allows for timely recognition and effective reaction to threats to an individual, be it tissue damage resulting from injury or infection from pathogenic microbes. However, when the insult is too great, or repetitive in nature (as seen in chronic inflammatory and autoimmune diseases), inflammation can become disordered and result in ongoing tissue damage and organ dysfunction [15]. We assert that critical illness is the most dramatic manifestation of disordered, dysregulated, and mis-compartmentalized inflammation [6, 16–19]. Thus, the presence of a robust, evolutionarily conserved network of inflammation [20–22], able to respond to heterogeneous insults and tuned for effective containment, yet paradoxically capable of driving and propagating host tissue damage, results in disease states that are fundamentally resistant to reductionist characterization. This property of critical illness is the basis for the lack of effective mechanism-based pharmacologic therapies, and accounts for the fact that even life-saving/perpetuating measures, such as mechanical ventilation or hemodialysis, may have detrimental effects through the induction of additional inflammation [23–25].

Insufficiencies in the Current Process of Drug/Device Design and Executing Clinical Trials

For a therapeutic drug or device to reach its ultimate end user—the patient—a multistep process must be carried out, culminating in approval by regulatory agencies. This process generally consists of years/decades of basic research to identify candidate therapeutic targets, followed by sequential studies to demonstrate safety and some acceptable degree of efficacy (e.g., dosage or timing that results in greatest therapeutic benefit with least harm) in both experimental animals and humans. This process typically concludes with a pivotal (Phase III) clinical trial, which is

randomized (i.e., subjects that meet predecided inclusion and exclusion criteria are recruited into either a placebo or treatment arm in a random fashion) and double-blinded (i.e., neither the clinician nor the patient knows a priori the study arm in which the patient is enrolled) [21, 26–29]. The enrollment into this Phase III trial is usually not individualized in any fashion beyond the set inclusion and exclusion criteria (and, of course, the withdrawal of a patient from the study if certain predecided adverse events occur). This process is considered the *sine qua non* of the scientific method, and it has indeed resulted in numerous drugs and devices available to physicians to treat diseases, though there has been a recent focus on novel, "adaptive" clinical trial designs [30]. However, it is important to recognize the difference between other domains where adaptive trials have been used and that of critical illness: namely that other disease processes, such as cancer and cardiovascular disease, have *known and proven therapies* for which the adaptive trials serve to aid in subgroup selection, dose optimization, and multimodal treatment efficacy. Therefore, while adaptive trial design can be of potential use in critical illness, the seeming goal of finding some subgroup in which an already failed compound can "possibly" be effective is asking the method to answer a question it was not designed to do [31].

There is a fundamental gap between preclinical studies and clinical trials. To begin with, the disease being targeted is usually thought of in a reductionist, static way as a series of discrete "stages" or "syndromes" rather than as a dynamic, stochastic progression of biological events driven by initial conditions and genetically determined parameters that, upon reaching certain multidimensional thresholds, leads to multiple outcomes. This discrepancy leads to the design of drugs that are targeted to ostensibly diagnostic symptoms rather than to underlying causes of the disease as a whole. Next, a highly linear (cause–effect) view of the biological pathways is presumed to underlie the various discrete symptoms, leading to the generation of drugs absent any consideration (at this initial stage of drug development) of impact on other pathways, cells, tissues, and organs. Finally, the statistical approaches commonly used to structure and analyze clinical trials typically make a number of questionable assumptions; for example, that variables are normally distributed, that a marker of patient state is equivalent to a mechanistic driver of that state, and that such a marker of patient state will be altered in a statistically significant fashion as a function of therapeutic efficacy [19, 32]. Below, we discuss how these general features of the healthcare delivery process manifest in therapies for acute inflammatory diseases, with a focus on critical illness.

Inflammation in Critical Illness: Rational Systems Approaches for a Complex Therapeutic Target

The flaws in—and the fragmented nature—of the current healthcare delivery paradigm have led to the recognition of the need to address complex interplay between inflammation and physiology in critical illness, manifesting in divergent group outcomes and heterogeneous individual trajectories [6, 19, 33, 34]. Initially, there was hope for some improvement in this situation through the adoption of "omics" methodologies, with their theoretical capability of interrogating the complete responses of cells and tissues in individuals (and thereby both improving the mechanistic understanding of critical illness in general and enhancing diagnostic and treatment capacities in individuals) [35–42]. While this approach has resulted in key contributions to the understanding of molecular pathways induced by injury and infection in humans [43–47], as these techniques have become more commonplace there has been a growing recognition that more data do not necessarily lead to better—or any—explanations for the phenomena from which those data are derived. Thus, these "omics" methods have not proven to be the panacea for the design of drugs, clinical trials, and diagnostics that they were projected to become. Thus, despite extensive interest in the use of data-driven modeling (colloquially referred to as "artificial intelligence") in clinical trial design [48], from a practical standpoint, there are multiple challenges to implementation of these purely data-driven, descriptive approaches in the healthcare delivery chain [9, 16, 17].

In contrast to data-driven, descriptive modeling, mechanistic computational simulations depict the behavior of biological interactions (e.g., among cells, their products, and the outcomes that result under a given set of conditions) dynamically. Such dynamic computational models and simulations may be used as "knowledge stores" that may be queried as to the emergent behavior of the sum total of known or hypothesized reductionist biological interactions [49–53]; to suggest novel interactions not yet described by experimental data [54]; and to address controversies based on diverse experimental/clinical conditions or other experimental differences among groups studying any given complex biological system [55]. Unlike data-oriented, descriptive models, dynamic mechanistic models offer the possibility of prediction outside of and beyond the data on which they were developed [9, 16, 17, 56, 57]. We have extended the classical systems biology approach to that of Translational (i.e. clinically applied) Systems Biology as systems and computational biology methods have matured and begun to take on characteristics, features, and operating principles of engineering [18, 19, 29, 56, 58, 59].

Indeed, the computational modeling toolset now available for integration into the healthcare delivery pipeline is rich and suited to diverse tasks. Translational dynamic mechanistic modeling used to date in acute inflammation and other phenomena related to critical illness can be divided into two general types: continuous methods, generally employing differential equations (either ordinary or partial) and particularly useful in settings involving data that reflect the mean field approximations of behavior of a biological system, for example, the concentrations of molecules in a

biofluid [57, 60–68]; and discrete methods, most notably agent-based modeling for settings in which spatial pattern/image data are involved, or for prototyping initial computational models of a complex system [54, 69–73]. These various method have their respective strengths and weaknesses [29, 58, 74–76], and have all been used in the setting of critical illness [20, 21, 29, 53, 56, 58, 74].

Dynamic computational modeling has improved our knowledge of the basic biology of inflammation, and, directly or indirectly, led to translational applications in critical illness [6, 9, 16–21, 29, 56, 76, 77]. One key translational application, namely the in silico clinical trial, was pioneered in the arena of critical illness [57, 61, 71, 78]. The potential use of mechanistic computational modeling in the diagnostic arena is evidenced by studies showing the potential to predict the individual inflammatory and pathophysiologic outcomes of individual human subjects [57, 79] and large, outbred animals [80]. Thus, it is now theoretically possible to predict and impact the outcomes of individual critically ill patients using patient-specific computational simulations, likely informed by genetic data and assessment of circulating inflammation biomarkers [53, 56, 57].

Given the multiscale complexity of the disease processes, we suggest that it is imperative to not merely identify candidate molecules, but also to determine if the higher-order, system-level consequences of attempting to intervene in a particular pathway will lead to an ultimately beneficial or detrimental outcome [19, 81, 82]. We have pointed out the need for a computational approach to *dynamic knowledge representation* as a means of hypothesis instantiation and testing [19, 53, 83]. In the context of translating molecular-level mechanistic hypotheses up through the various steps of the healthcare delivery continuum, this process is envisioned as allowing one to determine if the assumptions regarding manipulating a given biological interaction at a given scale of organization (typically the molecular/cellular scale) is likely to behave as expected at another, typically higher scale (e.g., tissue, organ, or the entire organism) [19]. In this way, one may identify effects that would otherwise be considered "unanticipated." Dynamic knowledge representation may be augmented with insights derived from high-throughput/high-content data [53], along with appropriate data analysis and data-driven modeling [22, 56, 59], in order to generate and parameterize mechanistic computational models of disease, patient [56, 57], or population [21, 29, 57, 73].

Dynamic Knowledge Representation in the Context of In Silico Clinical Trials

A key example of the in silico clinical trial as a form of dynamic knowledge representation can be seen in the simulated clinical trials of existing and hypothetical antimediator interventions for sepsis [61, 71, 78], trauma [57], and wound healing [73, 84]. Importantly, these simulated trials were based on the knowledge available at the time the actual clinical trials were performed. Highlighting the power of

computational modeling as a high-throughput test bed for novel therapies, the early in silico clinical trials simulated a series of existing [61, 71] and hypothetical [71] therapies targeting inflammatory mediators-based therapies. In one case, a simulation of neutralizing antibodies to proinflammatory cytokines was implemented in an agent-based model (ABM) [70, 71]. This dynamic computational model reproduced the general disease dynamics of sepsis and multiple organ failure and was used to generate a simulated population corresponding to the control group in a sepsis clinical trial. A similar approach was used in a contemporaneous study focusing on replicating the failed antitumor necrosis factor-α (TNF-α) clinical trials in sepsis, demonstrating that the presence of patient subgroups that were harmed by this drug as well as others that were helped (culminating in no net benefit); this study also suggested means by which biomarkers could identify these subgroups [61].

Importantly, these clinical trials were simulated in such a way that assumed that the proposed interventions behaved mechanistically exactly as had been hypothesized. Therefore, these in silico trials are a form of verification of the underlying hypotheses—either explicit or implicit—that formed the basis for such trials. The way in which these computational simulations were structured avoided the need to invoke factors such as heterogeneity of adjunctive therapy, different pharmacodynamics/kinetics, faulty randomization, or other potentially confounding practical issues commonly used to explain negative outcomes of clinical trials. In line with actual outcomes, and not surprisingly for those studies that were purely hypothetical, none of the simulated interventions demonstrated a beneficial effect [61, 71]. The conclusion drawn from these findings is that, most likely, the underlying conceptual models that informed the development of these therapeutic strategies targeted at blocking individual mediators were flawed, precisely because the hypotheses underlying their selection as therapeutic modalities were flawed in assuming a high likelihood of universal success. That is not to say that—despite this flaw of universal therapeutic efficacy—these mediator-directed therapies would fail. As noted above, one of the studies, an in silico trial of anti-TNF-α therapy using an equation-based model of systemic inflammation, suggested that this type of therapy would work on defined subsets of sepsis patients [61]. Thus, we suggest that flaws in the original hypotheses and assumptions underlying these failed clinical trials would have been exposed through the use of computational dynamic knowledge representation been available and used early and throughout the process of drug development.

As touched upon above, in silico clinical trials offer an unprecedented possibility to transcend the long list of practical limitations—including relatively small cohort sizes, limited availability of measurements, finite study durations, and the presence of confounding factors—that affect real-world clinical trials. However, the interdisciplinary team of clinicians, biologists, and computational modelers that carry out these in silico clinical trials must assure that the base models and implementation of simulated populations represent both the biology and clinical setting.

In addition to providing a check of the plausibility of the underlying scientific basis of a proposed intervention, in silico trials can augment the current process of performing clinical trials in three significant ways [85]:

1. **Enhancement of study group substratification:** The study by Clermont et al. [61] demonstrates the use of an in silico trial to enhance subgroup stratification and candidate patient identification. The finer-grained representation of each simulated patient, in terms of cytokine response trajectories, and how they respond to and without a proposed intervention allows the identification of potential biomarker-defined inclusion criteria for a clinical trial. In essence, this allows each simulated patient to act as his own control with respect to the proposed intervention. This type of analysis is functionally impossible to obtain in clinical trial cohorts that reflect the range of response that would arise in the general population. Furthermore, social or ethical factors that may limit the possible representation of specific groups (e.g., African-Americans, known to be generally under-represented in many clinical trials, or women of child-bearing age, excluded for potential teratogenic risk). As a result, trials are very likely to miss important (positive or negative) effects in subgroups that are sampled inadequately. This mis-sampling can lead to later discovery of adverse events following a promising clinical trial, or in the failure of truly useful treatments in clinical trials that were not properly targeted to the patients that would most benefit from them. By simulating massive virtual cohorts sampled from the space of potential patients, in silico clinical trials can achieve much more thorough sampling of possible patients. The acquisition and analysis of this simulation-generated data can in turn reveal clinical patient subgroups that merit particular attention, and lead to better informed patient selection criteria and more effective clinical trials.
2. **Augmentation and optimization of protocol design:** Protocols for modern interventions depend on multiple complex and often interacting parameters (e.g., dosage levels and timing and frequency of administration). Attempting to determine these parameters experimentally over a wide range of individuals is functionally impossible, and therefore the optimal intervention strategy for an individual patient cannot pragmatically be determined. The inability to anticipate and account for this degree of interindividual heterogeneity will doom a clinical trial to failure at the outset. In silico trials allow a more rigorous computational optimization of these parameters, both on massive populations and for individual patients, and will increase the precision with which protocols can be designed, and therapeutic endpoints defined.
3. **Enhanced characterization of the control group:** Clinical trials rely on control groups against which the effect of a proposed intervention is compared. However, given the vagaries of clinical practice, many control groups may actually compare poorly to the intervention group. Interindividual variability in both underlying biology and clinical practice leads to a situation where the definition of "similarity" between control and intervention patients is often quite crude and imprecise. This situation confounds the ability to truly define the effect of the proposed intervention. In silico trials, however, offer the ideal control group: each simulated patient can be simulated with and without the intervention. Comparison of results against these "perfect" controls thus removes a source of uncertainty that is unavoidable in real trials.

An example of the potential insights obtained from carrying out in silico trials can be seen in the aforementioned in silico trial based on an anti-TNF-α therapy [61]. These simulations recapitulated the general lack of efficacy of the intervention; however, the researchers used the power of computational modeling to evaluate what would have happened in the absence of intervention or in the setting of different doses of the drug. In essence, the placebo group was "cloned" into multiple treatment arms or the placebo arm. Consequently, this in silico analysis suggested specific characteristics of the simulated patients who had been helped by the intervention, had been harmed by the intervention, or had not been affected by the drug, thereby suggesting the possibility of using this in silico approach for deciding on inclusion and exclusion criteria for eventual clinical trials. Thus, the key take-home lesson of this study was that a failed randomized, placebo-controlled clinical trial could possibly have been successful through the use of in silico modeling.

Despite the tangible benefits of in silico trials in gaining insight into the potential efficacy of therapeutic interventions and why such proposed interventions might not be effective, it has been only recently that methods have been developed that can help determine what actually might be effective. Investigation into this challenge led to the perspective of addressing the treatment of sepsis as a control problem, where the goal of therapy is to "steer" a patient's disordered inflammatory state back into a state of health. ABMs have been proposed as proxy systems to aid in the development of control strategies [86], and this has led to the use of both genetic algorithms/evolutionary computing to define the scope of the task of controlling sepsis [87], and the application of model-based Deep Reinforcement Learning to train an artificial intelligence (AI) agent to control sepsis [88]. The details of this work are presented in Chap. 5 of this book.

Dynamic Knowledge Representation at the Individual Level: Optimization of Diagnosis and Therapy

It may be argued that the ultimate test of dynamic knowledge representation is that of characterizing the drivers of dynamic patient state to a degree sufficient to identify and treat the individual patient [6, 56, 81, 82]. To do so, a robust, mechanistic computational model (presumably the same one used for in silico clinical trial) must be adapted to reflect the temporal dynamics of inflammation and organ damage/dysfunction in the individual patient [57]. From a practical standpoint, model parameters that alter the patient's dynamics (e.g., comorbidities, prior health history, and relevant genetic traits) are modified over known or presumed ranges in accordance with known biology [56, 57]. The applications of this approach are myriad. Of most direct connection to the in silico clinical trial, individual-specific models could be used to generate much larger cohorts of virtual patients, which in turn could be used to make in silico clinical trials more realistic.

As an example of this approach, we constructed a multicompartment, equation-based model, consisting of the "tissue" (in which physical injury could take place), the "lungs" (which can experience dysfunction), and the "blood" (as a surrogate for the rest of the body) in order to simulate traumatic injury and subsequent inflammation and organ dysfunction [57]. This model was calibrated initially with data on approximately 30 individual trauma patients, all survivors of moderate blunt trauma. Based on these individual trajectories of both inflammatory and physiological variables, normal and uniform distributions were created. These distributions were sampled repeatedly to create a population of 10,000 virtual trauma patients, where each patient is defined by his/her parameter values in the mathematical model. Each patient was then subjected to simulated low, moderate, and severe trauma. These virtual populations of trauma patients exhibited realistic and partially overlapping distributions of "damage" recovery times [which we equated with intensive care unit (ICU) lengths of stay] and total "damage" (which we equate with degree of multiple organ dysfunction). These virtual patients were queried as to the parameters driving the above distributions and found that for patients with a low Injury Severity Score (ISS), parameters related to IL-1β were the predominant drivers, while IL-6 was the main driver of outcome in patients with moderate or severe ISS. However, while real patients could be segregated based on IL-6 single-nucleotide polymorphism into high- versus low-IL-6-producing subcohorts, and while tuning up IL-6 production in silico could turn virtual survivors into virtual nonsurvivors, the net effect in both virtual and real patients was negligible (demonstrating the difficulty in extrapolating linearly in complex diseases such as critical illness). Moreover, while only data from trauma survivors were used to calibrate the in silico trauma model, simulations of virtual populations predicted the appearance of approximately 4% nonsurvivors [57]. These predictions were in line with the actual mortality in this population [57, 89]. These results demonstrate the utility of mechanistic models with regard to predicting emergent phenomena, and suggest the possibility of determining novel basic mechanisms in trauma, of individualized outcome prediction for trauma patients, and of virtual clinical trials based on a small number of actual patients [57].

The aforementioned studies highlight some of the particular advantages that mechanistic models afford: virtual cohorts can be generated of any required size, and each individual patient's disease state can be tracked at an extremely high level of resolution (limited only by the resolution of the model) for as long as required [57, 87, 90]. When information is available about the approximate distribution of these characteristics in real populations, this information can be used in the generation of a virtual patient population to ensure that the composition of simulated cohorts mirrors reality.

Another application of this approach involves in silico "testing" of multiple therapeutic modalities on individuals. As an example of this application of dynamic mechanistic modeling, an ABM of vocal fold inflammation and healing was calibrated to the early levels of inflammatory mediators present in the laryngeal secretions of individual humans subjected to experimental phonotrauma, and could predict the later levels of these mediators in an individual-specific fashion [79].

Importantly, these individualized ABMs were utilized to predict the likely efficacy of a "rehabilitative" treatment, namely resonant voice exercises, both in patients who had in fact received this treatment and in patients who did not [79]. A similar process could be employed to evaluate the specific efficacy of a drug modulating an aspect of inflammation or healing [57, 73], thereby forming the basis of a much more realistic in silico clinical trial.

Conclusions and Perspectives

What is clear now is that the biocomplexity of pathophysiological processes underlying the systems-level diseases that represent the greatest health risk today, such as cancer, diabetes, atherosclerosis, Alzheimer's, sepsis, and wound healing, confounds the use of traditional experimental methods. These reductionist experiments and data-oriented descriptive methods are unable to evaluate and test multiscale causality, an essential and critical step in the design and development of therapeutic interventions for systems-level diseases. The complexity and dimensionality (in terms of multiple factors and variables) of these biomedical issues, particularly in terms of translating mechanisms across scales of organization, essentially precludes this approach. Reliance on only these traditional methods can produce, at best, "one-off" products based on fortuitous discovery, but does not provide a robust and sustainable strategy. The Scientific Method mandates that it is the ability to evaluate mechanisms and causality sufficiently in a multidimensional, high-throughput world—as is potentially possible with dynamic computational modeling and the application of principles from Translational Systems Biology—that forms the crux of the translational dilemma [19]. The use of dynamic computational modeling can provide a framework that allows the introduction of "theories" into biomedicine, in order to facilitate the translation of robust conceptual structures and architectures across experimental platforms as well as into the differences among individual patients [19, 79, 91]. Specifically, we assert that the computational approaches described in this chapter, with an explicit goal of addressing the challenges of implementing the last stage of getting a therapy to the bedside, represents a necessary step in the future of obtaining and implementing effective therapeutics for the complex diseases that challenge us today and in the future.

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Chapter 9
Computational Modeling of the Coagulation Response During Trauma

Evan J. Tsiklidis, Christopher C. Verni, Talid Sinno, and Scott L. Diamond

Abbreviations

ADP	Adenosine diphosphate
DIC	Disseminated intravascular coagulation
FEM	Finite element method
LB	Lattice Boltzmann
LKMC	Lattice kinetic Monte Carlo
NN	Neural network
ODE	Ordinary differential equation
PAS	Pairwise agonist scanning
PAS-FC	Pairwise agonist scanning-flow cytometry
PBRC	Packed red blood cells
PDE	Partial differential equation
PRP	Platelet-rich plasma
TAT	Thrombin-antithrombin
TBI	Traumatic brain injury
TEG	Thromboelastography
TF	Inflammatory tissue factor
TIC	Trauma-induced coagulopathy

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Introduction

Hemostasis is the body's primary response to vessel injury. During trauma, this pathway may be pushed to its biochemical and physical limits to stop blood loss. Additionally, dramatic changes in inflammation, platelet function, and blood biochemistry set the stage for trauma-induced coagulopathy (TIC). Excessive blood loss (hemorrhagic shock) in combination with tissue trauma, NETosis, endothelial dysfunction, and excess fibrinolysis are evolving components that drive a multiscale pathogenesis and high-dimensional risk for these patients. This chapter reviews computational modeling efforts to help quantify the interplay of hemodynamics and bleeding. First, the hemodynamics from the systemic circulation to the damaged vessel scale are presented. Second, simulation tools (bottom-up and top-down) to describe the platelet function and clotting under flow are presented in the context of bleeding.

Multiscale Modeling of Bleeding During Trauma

Although tube flow simulations provide insights into intravascular clotting on a surface, particularly for thrombotic episodes, these simulations do not predict the evolution of the trauma patient as a whole. Tsiklidis et al. recently developed a multiscale model of a trauma patient by coupling a circulatory system model to a branching vascular tissue model of local bleeding [1]. The circulatory model was based on the pioneering work of Ursino et al. [2, 3], where the hemodynamic system is represented by lumped, zero-dimensional (0D) resistances and capacitors with a baroreflex model for blood pressure control. The baroreflex provided a negative feedback loop to respond to the loss of blood pressure by increasing heart rate and peripheral resistance. Increasing heart rate maintains cardiac output and increasing peripheral resistance (via vasoconstriction) drives blood pressure to increase. Transcapillary refill is another process in the hydrated patient that occurs during hemorrhage, where ~0.5–1.0 L of fluid from the interstitial space moves into the capillaries, increasing blood volume and helping to maintain blood pressure [4]. This refill process is driven by the Starling forces, in particular capillary hydrostatic pressure. While beneficial to maintain blood pressure, refill would contribute modestly to dilutional coagulopathy. Changes in hematocrit, either by physiological response or by resuscitation therapy, will affect blood viscosity, hemodynamics, and platelet margination to the wall where they are needed [5].

A visual overview of the model (Fig. 9.1) shows the linkage of the systemic circulation to a site of vascular damage where blood can exit the patient driven by prevailing upstream blood pressure. The exiting blood drives a hemostatic response at the site of the severed vessel(s).

At the tissue scale, a branching vascular network must be constructed to match physiological conditions prior to wounding. This required specification includes:

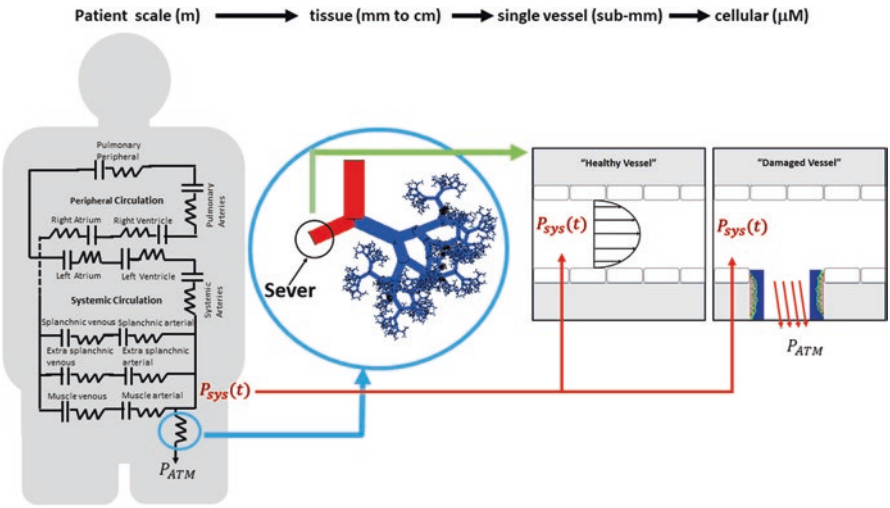

Fig. 9.1 Multiscale model of a trauma patient that spans over six orders of magnitude. Lumped resistance parameters in the global hemodynamic module can be decomposed into tissue-scale vascular networks. An initial wound distribution in the vascular network is specified and then the model can simulate the dynamic patient response of blood volume and blood pressure over the course of a bleeding episode. (Reprinted with permission [6])

(1) a rule governing how the vessels bifurcate into daughter vessels, (2) a rule for the angle between daughter vessels, and (3) a length-to-diameter ratio for a wound distribution upon the blood vessels to simulate damage. The model was used to simulate the complete severing of blood vessels, where the outlet pressure boundary condition is set to atmospheric pressure. Importantly, the local upstream depressurization occurs as a result of the imposed atmospheric boundary condition. Along with vasoconstriction, this depressurization of the wounded vasculature reduces the driving force for blood loss. Declining blood pressure due to hemorrhage also reduces the driving force for blood loss. Distinct from bleeding to the atmosphere, internal bleeding into finite compartments with essentially static whole blood clotting (hematoma) involves complicated boundary conditions that remain poorly studied. A different boundary condition is the partial sever (crushed vessel) condition where blood can exit through the damaged wall or can flow down the vessel. Once the damage is large enough, the majority of blood will exit the vessel since downstream resistance to flow is higher.

At the single vessel scale where vessels are severed upon injury, both vasoconstriction and hemostatic blood clotting must be quantified, either by full simulation or by phenomenological models based on data. Hemostatic clotting is flow dependent, and so by imposing a concentric growth rate along the diameter of the bleeding vessel, the simulation was able to predict the patient evolution under different conditions (wounding patterns, variable hematocrit, with and without vasoconstriction, etc.) as shown in Fig. 9.2. Of special note, the wall shear stress at the exit of a severed vessel can exceed 5000 s^{-1}, a condition where von Willebrand factor

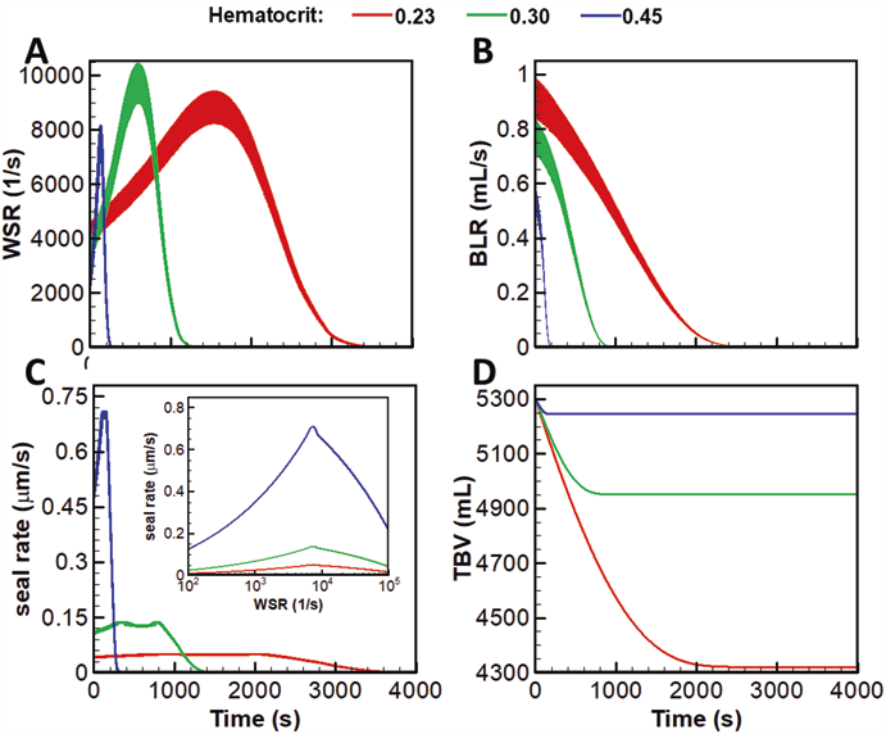

Fig. 9.2 (**a**) Wall shear rate, (**b**) blood loss rate, (**c**) seal rate, and (**d**) the total blood volume evolutions for the same injury pattern as a function of hematocrit. This difference in response suggests that hemodilution could be a strong contributor to TIC. (Reprinted with permission [1])

unfolding may be essential for platelet capture. Importantly, this model does not consider the effects of inflammation or of sepsis but could be extended for this [7, 8].

Canuto et al. [9] developed a multiscale model for the study of trauma and other stressors by coupling a 0D global hemodynamic model with 1D models of the major systemic arteries. They simulated traumatic bleeding by replacing the end of an artery with an atmospheric pressure sink and once 10% of the total blood volume had been lost, they modeled tourniquet application by reducing the blood vessel area to just 1% of the baseline value over a 10 s window. This had a dramatic effect on the patient, allowing the patient to maintain blood volume and blood pressure. The importance of applying a tourniquet to a bleeding trauma patient, when possible, immediately after a penetrating injury was highly correlated with survival probabilities [10].

In other work, Hirshberg et al. developed a model to examine transfusion guidelines and how transfusion may be insufficient and contribute to dilutional coagulopathy in a trauma patient [11]. The washout equation had been used to guide transfusion decisions previously but had not accounted for the, often, long delay between initial trauma and transfusion products, nor the fact that trauma patients

undergo pressure-driven hemorrhage with a variable blood loss rate as opposed to a constant blood loss since blood pressure decreases with the loss of blood volume. To account for these differences, the model was constructed to simulate a clinical scenario where an exsanguinating patient underwent four distinct phases: a no-fluid stage, a prehospital fluid stage, an emergency room stage, and an operative stage where transfusion of blood products begins. By simulating different protocols, the model predicted that the optimal ratio of fresh frozen plasma to packed red blood cells (PBRC) is 2:3 and 4:5 for platelets to PRBC.

Coagulation Modeling

The simulation of blood coagulation on a surface under flow conditions has progressed in stages through the efforts of many laboratories over many years (for review see: [12, 13]). Kinetic models have focused on the extrinsic pathway initiation of plasma clotting (isotropic), the signaling within activating platelets, and the assembly of platelet deposits on a surface with or without coagulation under flow conditions. However, hemostatic clotting is defined by clotting that prevents blood from leaving the vessel, while thrombosis is confined within the vessel (the exception being hemorrhagic stroke). Microfluidic experiments to mimic bleeding [14] and computational models of bleeding represent relatively new advances in the recent years.

The Hockin-Mann model [15] describes the isotropic generation of thrombin following the treatment of fibrinogen-free plasma with exogenously added tissue factor (typically 1–100 pM inflammatory tissue factor [TF] initial condition). Since the model did not include the contact activation pathway, it does not model coagulation in the absence of tissue factor. Chatterjee et al. extended and modified the Hockin-Mann model with a Factor XIIa source term to predict clotting in convulxin-activated human blood (with or without TF) [16]. The "platelet-plasma" model modeled platelet activation based on the measured dose response to evolving thrombin levels. Kuharsky and Fogelson constructed an ordinary differential equation (ODE) model of the extrinsic pathway to account for coagulation and blood flow, which has been shown to be a strong regulator of coagulation [17]. The model simulated blood flow over a 10 μm TF patch with a specified number of binding sites. The transport of proteins into the evolving clot was modeled by imposing flow-dependent mass transfer coefficients. The model showed a very strong dependence on surface TF concentration, a result verified experimentally with whole blood perfusion [18]. Leiderman and Fogelson extended Kuharsky's work to a partial differential equation (PDE) formulation where the concentration gradients in the thrombus were determined [19]. The model continued to demonstrate the importance of flow and physical coverage of the surface TF as important mechanisms for inhibiting coagulation. Models of coagulation (from TF to thrombin to fibrin) with flow typically have grown in complexity, with associated computational cost. A highly reduced ODE model [20] was able to predict thrombin–antithrombin (TAT), F1.2,

and fibrinopeptide generation under flow in the presence or absence of fibrin. The binding of thrombin to fibrin (i.e., antithrombin I activity) through a high-affinity site was an essential feature of the model.

Platelets are essential to both hemostasis and thrombosis with extremely complex metabolism driven by receptor signaling. Bottom-up models of platelet signaling have treated calcium regulation by adenosine diphosphate (ADP) [21, 22] or thrombin [23]. Flamm et al. developed a stochastic, patient-specific model of thrombosis under 2D flow over tissue factor surface conditions [24]. The model used the lattice kinetic Monte Carlo (LKMC) method to solve for the stochastic platelet motion and binding events, the finite element method (FEM) to solve for the concentration profiles of soluble agonists as a function of time and space, the lattice Boltzmann (LB) method to solve for the velocity profile over the growing clot, and a neural network (NN) model to predict platelet activation states. The NN was trained via the pairwise agonist scanning (PAS) method (described in more detail later in this chapter), where individual and pairwise combinations of agonists are used to predict a donor's platelet calcium concentration over time [25, 26]. Interestingly, the model was highly predictive of microfluidic experiments of whole blood flowing over a collagen patch and predicted a ranked order of sensitivity to drugs, such as indomethacin, aspirin, and iloprost. Lu et al. extended the model to include a time-dependent thrombin flux at the TF surface since this is validated by experimental measurements and is more efficient than solving the entire coagulation cascade continuously [27].

Coagulation During Bleeding

While these models of coagulation aid in understanding clot formation on a surface, they primarily relate to thrombosis and are not suitable for simulating bleeding, hemostasis, or TIC. There are several key differences that must be accounted for before they can be used in these cases, however. Blood flowing into a region of vessel damage will exit the vessel as a clot builds up to stop flow. The blood that pools around the wound extravascularly experiences stasis, has prevailing hematocrit, and will undergo contraction. The clot at the wound wall-blood boundary experiences high shear stresses and is platelet rich with fibrin polymerizing on the wound boundary. A core–shell morphology is typical of these wounds as visualized in animal models [28] or in microfluidics with human blood [29]. These laser injury models of clot structure, to date, have typically used healthy animals.

During trauma, there are dramatic changes in coagulation proteins and platelet function. Trauma may present as a consumptive coagulopathy with excessive fibrinolysis, endothelial glycocalyx shedding, excessive inflammation, and neutrophil NETosis. Furthermore, some injuries, typically traumatic brain injury (TBI), can lead to defective platelets, which are believed to be a key contributor to TIC [30]. In moderate to severe injuries (injury severity score > 15), elevated levels of D-dimer and high prothrombin times have been observed [31]. A model of a severe trauma patient experiencing TIC must account for these differences.

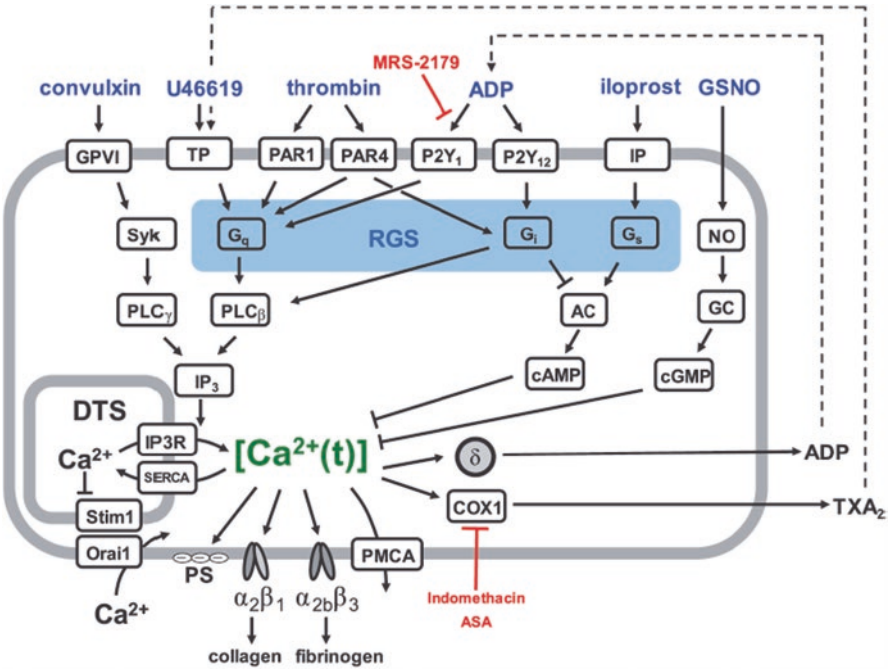

Fig. 9.3 Platelet signaling pathways. Common surface receptors are activated by specific ligands (blue) leading to intracellular signaling cascades, which all converge on increases or decreases in calcium concentrations. Calcium mobilization then facilitates inside-out platelet activation to promote platelet aggregation and other hemostatic phenomena. (Reprinted with permission [26])

Common platelet activators include collagen (lab analog: convulxin), ADP, thromboxane A2 (lab analog: U46619), and thrombin, the latter of which also plays key enzymatic roles in coagulation (e.g., fibrin polymerization and cross-linking). Following these "outside-in" binding events, cytosolic calcium concentrations rise via two different mechanisms [32, 33], which then leads to "inside-out" activation of additional receptors important for platelet aggregation via the plasma protein fibrinogen among other phenomena [32]. A relatively simplified schematic of external and internal platelet activation with common ligand–receptor interactions is shown in Fig. 9.3.

Data-Driven Development of Subject-Specific Platelet Function Profiles

Chatterjee et al. designed the dual experimental–computational technique known as PAS for phenotyping platelets [25]. Essentially, platelet-rich plasma (PRP) was prepared via centrifugation following the collection of whole blood from a consenting

donor or patient and incubated with a fluorescent dye that tracks changes in intracellular calcium concentration upon agonist stimulation. In order to generate sufficiently diverse training data for the machine learning algorithm, single and pairwise combinations of six different platelet agonists (or inhibitors) at low, medium, and high concentrations were prepared with liquid handling machines and then dispensed into the cell suspensions for dynamic data collection. In all, the combinatorial space amounted to 154 unique conditions, and the experiment lasted about 2 h from start to finish. Next, a multilayer supervised NN model could be trained by using the agonist concentrations and corresponding calcium time series as input–output pairs, and then predictions of responses to higher order or more complex stimulation conditions could be generated. Once trained, the NN models serve multiple purposes: (1) phenotypic development of individualized platelet function profiles for each subject included in the study and (2) input into the multiscale models of platelet aggregation discussed previously [24, 27]. The general workflow from initial experiments to NN training to multiscale simulation to validation against microfluidic experiments in shown in Fig. 9.4. There have been other reports of the computational simulation of platelet deposition and activation, such as that developed by Sorensen et al. [34, 35], but few if any feature capability to predict distributions and effects of multiple stimuli on the overall hemostatic state.

Initially, the PAS method was developed using mostly nonphysiological activating agents [25], but then it was expanded to include the effects of thrombin by Lee et al. for more representative simulation of the hemostatic response [26]. This approach reliably interrogates multiple platelet signaling pathways and, with simple experimental implementation, can accurately predict platelet reactivity in the presence of multiple stimuli working in concert. Platelet activation is a highly dynamic process with several contributing factors, which sheds light on the importance of understanding how these factors work synergistically (or antagonistically) during clot development. Until recently, only healthy human subjects (no previous medical conditions or medications) had been studied with the PAS-NN method.

Verni et al. developed a modified version of PAS with fewer (n = 31) agonist conditions, comparing healthy donors with coagulopathic trauma patients [36]. The data indicated a severe level of platelet dysfunction across multiple signaling pathways, as shown in the heatmap form in Fig. 9.5, which was supported by several previous reports [37–39]. These other groups typically characterized only the platelet function on the basis of single-agonist stimulation or with clinical assays, such as thromboelastography (TEG), which provide useful but limited information. Demonstration of the utility of this high-throughput assay technique in a trauma patient population opens the door for studying countless other patient cohorts.

The principles of PAS can be applied in other assays as well. The original design relied on intracellular calcium mobilization as the primary readout, which is universally recognized to be a correlate of platelet function and activation. However, other markers of activation exist downstream of calcium mobilization and are well studied among groups in the platelet biology community. For example, upon initial stimulation, the surface integrin $\alpha_{IIb}\beta_3$ converts to an activated state to enable platelet aggregate formation, α granules fuse to the plasma membrane and release contents

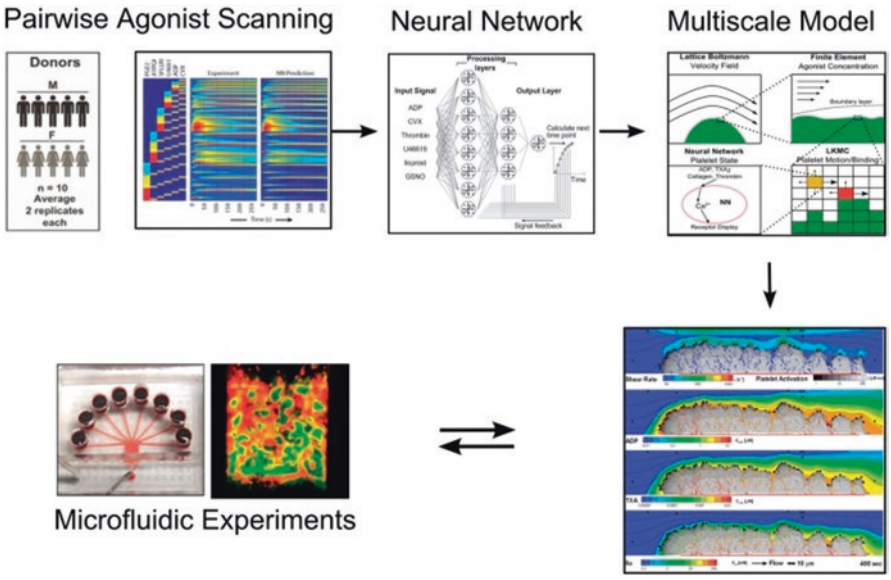

Fig. 9.4 Connection between experimental measurement and numerical prediction. PAS experiments were performed and used to train a NN ensemble to predict calcium response for an average healthy person and was incorporated into the multiscale model to predict platelet activation. An effective boundary flux term was imposed. Thrombus growth dynamics can be predicted by the multiscale model and were compared against microfluidic experiments on healthy human blood conducted under identical conditions. (Reprinted with permission [22])

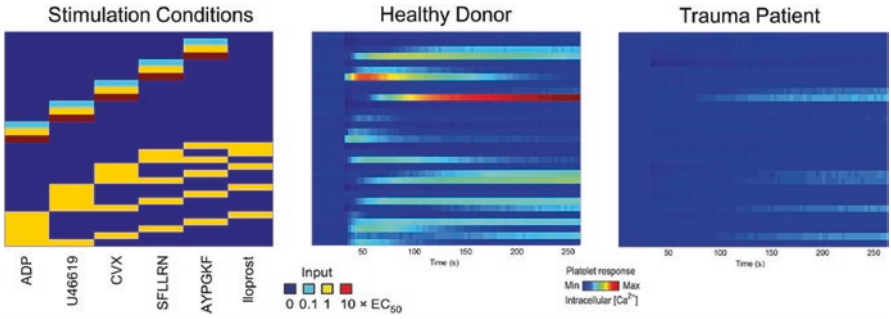

Fig. 9.5 Representative calcium responses to the array of agonists are shown for a healthy donor ("normal" phenotype) and a trauma patient ("dysfunctional" phenotype). Five platelet agonists (ADP, U46619, convulxin, SFLLRN, and AYPGKF) and one platelet antagonist (iloprost) were prepared according to the concentration map shown. Low, medium, and high doses (0.1×, 1×, and 10× EC50) of the five platelet agonists as well as pairwise combinations of the full six compounds at medium doses (1× EC50) resulted in 31 total conditions, including a null buffer control. (Reprinted with permission [36])

such as P-selectin, and phosphatidylserine (PS) is exposed on the membrane to provide a charged surface for facilitation of coagulation [40, 41].

Observation of multiple biomarkers simultaneously is difficult with the traditional well-plate reader technology but can be achieved by using flow cytometry. Jaeger et al. developed a PAS analog known as pairwise agonist scanning-flow cytometry (PAS-FC) in which each of the activation events discussed above was tracked as a function of combinatorial agonist stimulation [42].

Machine learning provides access to understanding basic biological mechanisms and, perhaps more significantly for the progress of biomedicine, developing diagnostic applications as well. The work by Verni et al. with respect to trauma patients presents a framework to understand platelet dysfunction and potentially influence transfusion strategies employed in the emergency room [36]. Currently, work is being performed to fully simulate a trauma patient's hemostatic state, with the influence of patient-specific NN models and extension to 3D geometries.

Additionally, other machine learning algorithms can be developed that consider relative influences of various clinical variables (e.g., vital signs, demographics, previous medical history) to make predictions of patient outcomes and inform appropriate treatment regimens. Yoon et al. have published an approach for diagnosing disseminated intravascular coagulation (DIC). DIC is notorious for its lack of accepted biomarkers, and so using readily available patient data to train machine learning models certainly carries significant weight. For example, the DIC diagnostic NN model rank-ordered importance of 32 variables and identified platelet count, D-dimer content, and clinically assigned scoring systems as parameters with the most direct implication for the development of DIC [43]. Such models, with sufficient learning, have the potential to be used in real time to obtain a rough understanding of patient prognosis based on the previous cases and perhaps provide hints towards the best method of treatment. Ultimately, all of this work lends itself to the long-term goal of personalized medicine for countless disease states.

Conclusion

The multifactorial nature and ever-increasing complexity of physiological responses to blood vessel injury have been the focus of several efforts of computational modeling. Some groups have chosen to focus on the role and interplay of various coagulation proteins, while others have strived to understand the mechanisms of blood cell (specifically platelet) function and activity. The ultimate goal of simulating hemostasis as completely as possible is becoming more achievable with integration and development of models that span multiple orders of magnitude and combine several individual components together. Applying data-driven modeling approaches that are dependent on bench-scale experimentation and postsimulation verification has proven to be an incredibly valuable method that takes advantage of the power of the machine learning technology. Additionally, recent advances have begun to model non-healthy populations (e.g., patient cohorts experiencing coagulopathy

and acute inflammation) in an effort to better understand patient prognoses and potentially guide diagnosis and treatment strategies. Using the examples discussed above as motivation for the future, there should be great optimism for development of similarly structured models for other clinical conditions and diseases.

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Part IV
Translational Modeling of Organ/Tissue Specific Inflammatory Disease Processes

Chapter 10
Disorders of Localized Inflammation in Wound Healing

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Abbreviations

ADP	Adenosine diphosphate
CD	Cluster of differentiation
CDK	Cyclin-dependent kinase
CGD	Chronic granulomatous disease
COX	Cyclooxygenase
CTAP-III	Connective tissue-activating peptide III
CXCL	Chemokine (C-X-C motif) ligand
CXCR	C-X-C chemokine receptor
DAMPs	Damage-associated molecular patterns
ECM	Extracellular matrix
EGF	Endothelial growth factor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
FERMT3	Fermitin family homolog 3
FGF	Fibroblast growth factor
G6PD	Glucose-6-phosphate dehydrogenase

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Gp1b	Glycoprotein 1b
GpIIb-IIIa	Glycoprotein IIb-IIIa
GRO-α	Growth-related oncogene α
HIF	Hypoxia-induced factor
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LAD	Leukocyte adhesion deficiencies
LO	Lipoxygenase
MBV	Matrix-bound nanovesicles
MMPs	Matrix metalloproteinases
NADPH	Nicotinamide adenine dinucleotide phosphate
NAP-2	Neutrophil activating peptide-2
PDGF	Platelet -derived growth factor
ROS	Reactive oxygen species
SLP	Secretory leukocyte protease inhibitor
TF	Tissue factor
TGFα	Transforming growth factor
TIMP	Tissue inhibitor of matrix metalloproteinases
tPA	Tissue plasminogen activator
VEGF	Vascular endothelial growth factor
vWF	von Willebrand factor

Introduction

The objective of this chapter is to provide an overview of the soft tissue wound healing process and disorders that can affect normal wound healing. Other chapters will provide greater detail of individual phases and specific disorders of wound healing.

Wound healing can be classified as a well-delineated six-step process: hemostasis, inflammation, epithelialization, angiogenesis, matrix deposition, and remodeling [1]. Working knowledge of this process is useful for understanding where and how dysfunction can occur. While the wound healing process is a dynamic interaction of multiple factors, each step has a beginning and an end and is characterized by specific mediator molecules and cells. This chapter seeks to outline classic normal epithelial tissue wound healing as well as causes and consequences of abnormal wound healing.

Hemostasis

Hemostasis, the first step in wound healing, is the body's immediate response to prevent life-threatening hemorrhage. This step is composed of both a primary and a secondary phase, each of which is essential for mature clot formation. In the primary hemostasis, two rapid physiological "reflexes" occur: vasoconstriction and formation of the platelet plug. Vasoconstriction mediated by vascular smooth muscle cells narrows the vascular lumen to diminish blood flow [2]. Simultaneously, platelets traveling through the vessel are exposed to subendothelial collagen, become activated, and adhere to the site through platelet-expressed glycoprotein (Gp) 1b and collagen-bound von Willebrand factor (vWF) [3–6]. As additional circulating platelets arrive, they attach to the developing platelet plug in an adenosine diphosphate (ADP) mediated positive feedback loop [7]. This ADP loop induces expression of the glycoprotein (Gp) IIb-IIIa, a glycoprotein complex on the surface of platelets that allows platelets to bind fibrinogen and additional platelets to form a temporary platelet plug [7]. Beyond forming the temporary platelet plug in primary hemostasis, platelets continue to contribute to the wound healing process by secreting endothelial growth factor (EGF) and transforming growth factor-alpha (TGF-α), two important chemotactic and angiogenic peptide growth factors whose presence is critical to the subsequent steps in the wound healing process [8]. Platelets also secrete platelet derived growth factor (PDGF), which is critical in catalyzing the next phase of the wound healing process, that is, inflammation [9].

While the immature platelet plug forms, the enzymatic cascade of secondary hemostasis begins to develop. The rupture of the blood vessel endothelium exposes tissue factor (TF), present in the subendothelial extracellular matrix (ECM), to encounter circulating clotting factors found within the blood plasma, which in turn initiates the extrinsic pathway of the clotting cascade [10]. Similarly, the collagen present in the vessel basement membrane triggers the initiation of the intrinsic pathway [10]. Together, the intrinsic and extrinsic pathways convert clotting factors from inactive to active forms and converge on the cleavage of prothrombin to thrombin (its active form) that in turn converts platelet-bound fibrinogen to fibrin [10]. In this active form, fibrin forms cross-links that assemble the stable and mature blood clot with substantial structural integrity.

Predictably, the sheer volume of necessary components required for hemostasis foreshadows the existence of hemostatic disorders. These disorders follow the same primary/secondary distinction as the native hemostatic process. Disorders of primary hemostasis include, but are not limited to, congenital defects in vascular structure, vWF deficiency, or impaired platelet function. Clinically relevant pathologies, such as Ehlers-Danlos disease (characterized by vascular fragility), von Willebrand disease (varying degrees of dysfunctional or absent vWF), thrombocytopenia (low platelet count), Glanzmann thrombasthenia (deficiency of Gp11b-111a), Bernard-Soulier syndrome (deficiency of Gp1b), and myeloproliferative disorders such as aplastic anemia, all represent primary hemostatic disorders [11–14]. The etiology of secondary hemostatic disorders is

similarly also either congenital or acquired. Clinically relevant congenital disorders of secondary hemostasis include inherited clotting factor deficiencies, such as hemophilia A, B, and C [15]. Acquired deficiencies include nutritional deficiencies, such as vitamin K deficiency (vitamin K is an essential vitamin and cofactor for clotting factor function (including factors II, VII, IX, X), or hepatic and renal insufficiencies, where clotting factors are unable to be produced or lost in the urine) [16]. Both conditions manifest as a disruption in the clotting cascade and the subsequent inability to form a mature and stable blood clot.

Regardless of etiology or pathology, hemostatic failure represents a significant cause of morbidity and mortality as it can lead to infection, electrolytic imbalance due to fluid loss, and hemorrhage [17]. While the blood clot is traditionally thought of as a mechanism for hemostasis, it is also an important contributor to innate immunity by preventing the entrance of pathogenic organisms [18]. When an epithelial wound loses its barrier function, the underlying tissue is at increased risk of invasion by pathogens. Bacterial contamination and infection destabilize the subsequent steps of the healing process [19]. Stated differently, a hemostatic failure is associated with increased morbidity and mortality.

Plasmin is responsible for dissolving the fibrin plug [20]. It attaches to the clot in its zymogen form, plasminogen, and it is activated by circulating proteins, such as tissue plasminogen activator (tPA) and factor VII, part of the extrinsic cascade. Active plasmin then dissociates the fibrin clot with its serine proteolytic activity. This eventual clot dissolution is crucial for resolution of hemostasis. While insufficient hemostasis provides grave short-term risks, an excessive hemostatic response and failure to dissolve the clot can pose equally dangerous long-term risks. Specifically, an excessive platelet response leads to intensified activation and aggregation that can lead to vessel occlusion (thrombus) and ischemic injury to tissues that are supplied by the respective vasculature [21]. Furthermore, high shearing force can cause the thrombus to detach from the vessel wall with the risk of systemic embolization [21]. Such events illustrate the importance for balance of the hemostatic process and its role in local tissue wound healing.

Inflammation

The immune system's first responding cells to a site of injury are circulating white blood cells (leukocytes) called neutrophils. There is a notable baseline presence of neutrophils in circulation and, like platelets, neutrophils near the site at the time of injury immediately emigrate from the vasculature and begin recruiting additional circulating neutrophils through a positive feedback loop. This mechanism is so robust that by 24 h after injury, neutrophils can easily comprise 50% of the cell population at a wound site [22].

Neutrophil extravasation occurs by diapedesis and is mediated by several chemokines, endothelial surface proteins, and neutrophil surface proteins. PDGF released from the platelet plug is one of the first neutrophil chemoattractants produced upon

formation of the clot [23]. Platelets concurrently release chemokine-connective tissue-activating peptide III (CTAP-III), which is converted by neutrophils into neutrophil activating peptide-2 (NAP-2) [24]. Together, PDGF and NAP-2 recruit additional circulating neutrophils to the site of injury [24]. Simultaneously, endothelial cells and pericytes near the wound site begin to produce and secrete growth-related oncogene α (GRO-α, also known as CXCL1), which in turn facilitates neutrophil diapedesis [25]. These three ligands bind through CXCR2, or the interleukin (IL)-8 receptor, which promotes increased recruitment, production, and release of immature neutrophils from the bone marrow [26]. Importantly, as a group, these cytokines mediate the phenomenon of desensitization, in which increasing ligand concentrations desensitize or downregulate the expression of their own receptors. Therefore, the neutrophil inflammatory response is to some degree self-limiting because of this desensitization, which is important in mitigating the risk of chronic inflammation. The transient unresponsiveness to CXCR2 can be reversed by high levels of IL-8, which is a ligand to both CXCR1 and CXCR2 in the tissue [27]. This delicate balance is important because the response can revitalize the inflammatory response if it is prematurely dampened, or it can reanimate the response when there is no longer a need for it.

Neutrophils possess a potent antimicrobial arsenal, but these same molecules in excess can destroy endogenous cells that are critical for wound healing. Neutrophils release reactive oxygen species (ROS) that are unstable oxygen-based free-radicals that can damage cell membranes, enter surrounding cells, and damage essential cell functions. Reactive oxygen species are an extremely efficient mechanism for neutralization of pathogens but can also cause self-damage [28]. Similarly, neutrophils release a variety of proteases into the wound site, such as elastase, cathelicidin G, and various matrix metalloproteinases (MMPs) [29]. These proteases damage microbes, aid in wound debridement (removal of damaged tissue), and begin to degrade the associated extracellular matrix (ECM) that allows for more cell infiltration [28]. However, the same proteases can exacerbate inflammation, delay reepithelialization, impair the provisional matrix formation, and reduce wound strength. In other words, sustained neutrophil activity and tissue residence create an impasse at the inflammation stage of wound healing, preventing progression to the subsequent stages; therefore, there is the need for redundant self-limiting mechanisms [30]. One such mechanism involves the process of apoptosis through a cyclin-dependent kinase (CDK) activated, caspase-mediated pathway [31]. This pathway inhibits the secretion of inflammatory products and allows for the neutrophils to be engulfed and neutralized by tissue resident macrophages and peripherally recruited monocytes/macrophages [32]. Failure to remove neutrophils can play a substantial role in progression to nonhealing, chronic inflammation.

Circulating monocytes arrive at the wound site within the first 24–48 h following the injury, mature into macrophages within the tissue, and have two principal roles: phagocytosis of extracellular debris and secretion of essential factors that shape the subsequent wound healing response. These roles can be further subdivided into four distinct functions—promotion of inflammation, anti-inflammatory immunomodulation, angiogenesis, and matrix synthesis.

Upon recruitment to the wound site, pro-inflammatory cytokines, interferons, bacterial endotoxins, or damage-associated molecular patterns (DAMPs) promote several events, including: (1) polarize naïve macrophages toward a pro-inflammatory phenotype, called M_1-like or classically activated phenotype. M_1-polarized macrophages phagocytose apoptotic neutrophils, (2) secrete a large number of pro-inflammatory cytokines (IL-1, IL-6, IL-12, tumor necrosis factor-alpha (TNF-α)), (3) promote the recruitment of inflammatory T-lymphocytes, and (4) produce nitric oxide via inducible nitric oxide synthase (iNOS) [33]. These cytokines shape and support the inflammatory response that is required to properly clean the wound site. However, if unbalanced, these same cytokines and cellular products can promote unresolved inflammation. One solution to excessive or prolonged pro-inflammatory activity is the transition to the alternate pro-remodeling macrophage phenotype, known as an M_2-like, or alternatively activated phenotype. These M2-like macrophages secrete anti-inflammatory cytokines, such as IL-10, as well as growth factors, such as TGF-β and vascular endothelial growth factor (VEGF) [34]. VEGF is important as it is essential in a future wound healing step, namely, angiogenesis. Thus, this shift in the macrophage activation state is critical in the resolution of wound healing. The phenotype transition is facilitated by particular cytokines, such as IL-4, lipid mediators, and the release of ECM signaling molecules and matrix-bound nanovesicles (MBV) [35–47]. Importantly, the macrophages remain at the site of injury throughout the repair process, acting as a continual production source for critical downstream cytokines.

The lipid mediator profile reflects the immune cell function at the wound site. While each lipid mediator class has its own primary function, they can be categorized as pro-inflammatory or anti-inflammatory. Lipid mediator molecules are derived from three essential fatty acids: arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid [48]. The latter two of these, eicosapoentaenoic acid and docosahexaenoic acid, give rise exclusively to anti-inflammatory lipids-D type resolvins, and E type resolvins and protectins, respectively [48]. On the other hand, arachidonic acid also gives rise to pro-inflammatory eicosanoids (prostaglandins, leukotrienes, and epoxyeicosatetraenoic acid) and lipoxins, which are anti-inflammatory. It follows, then, that the metabolism of arachidonic acid into either eicosanoids (through the cyclooxygenase (COX) pathway) or lipoxins (through the lipoxygenase (LO) pathway) is critical in creating the inflammatory context of a wound [49]. The balance of COX and LO activity is in turn dictated by microenvironmental cues at the wound site.

Logically, any disruption or dysregulation to these immunologic processes can lead to substantial deficiencies in wound healing. Beginning with the initiation of inflammation at the wound site, errors in wound healing can result from a failure of peripheral neutrophils to migrate to the site of injury and respond appropriately. The first site of failure is in the physical attachment of neutrophils to the vessel wall. In conditions known as leukocyte adhesion deficiencies (LAD), there are mutations in the cell–cell adhesion molecules (integrins) that prevent both the attachment of neutrophils to the endothelium and the leukocyte adhesion and extravasation into the tissue. Examples of LAD include LAD-I (deficiency in CD18, the common

chain of the 2 integrin), LAD-II (decreased Sialyl-Lewis-X on neutrophil that binds selectin on endothelial cells), and LAD-III (mutation in fermitin family homolog 3 (FERMT3) gene resulting in impaired activation of integrins) [50, 51]. Once in the tissue, the neutrophil response can be affected by both congenital and acquired etiologies. The neutrophil oxidative burst essential for killing invading pathogens can be altered by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase deficiency (chronic granulomatous disease (CGD)) or glucose-6-phosphate dehydrogenase (G6PD) deficiency [52, 53]. Additionally, a decreased number of neutrophils (neutropenia), as seen in a wide variety of conditions ranging from medication related, hematological malignancies, to idiopathic etiology, can markedly affect wound healing. In all these conditions, neutrophil function and response to tissue injury is significantly impaired, resulting in an increased risk of infection and chronic nonhealing wound formation.

Conditions that compromise the integrity of the immune system, including autoimmune disease and ageing, also negatively affect the host response to tissue injury and repair. For example, patients with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematous, and anti-phospholipid syndrome have a substantial increase in the incidence of chronic nonhealing wounds compared to the general population. These patient populations report the highest incidence of nonhealing ulcers among those with the autoimmune disease [54–58]. Specifically, in these conditions, the excessive vasculature inflammation (vasculitis) leads to dysregulated inflammation at the site of injury [54–58]. Additionally, patients with autoimmune diseases present with a prolonged pro-inflammatory phase of wound healing that can exacerbate tissue damage and lead to inefficient healing and repair. For those not affected by autoimmune diseases, normal ageing as well as conditions of advanced ageing, such as Down syndrome and progeroid syndromes, also lead to impairments in inflammation during wound healing. The cause of compromised wound healing in ageing is believed to be a result of increased IL-6 and TNF-α produced by invading immune cells and decreased levels of pro-healing growth factors such as TGF- β [59–61]. This combination of excess inflammation and decreased anti-inflammatory mediators seen in autoimmune diseases and ageing results in progression to nonhealing wounds and failed tissue remodeling.

Epithelialization

Epithelialization of the wound site begins shortly after the beginning of the immune response. For proper epithelialization, which reestablishes an epithelial-stromal barrier, epithelial cells and their respective progenitor cells must migrate and proliferate. Native epithelium, the outermost layer or luminal component of a tissue, mainly consists of epithelial cells bound to each other through desmosomes and to the ECM through hemidesmosomes. After tissue insult, these cell-to-cell and cell-to-ECM connections disassociate, allowing epithelial cells to move freely into the wound site. This migration and coinciding proliferation are promoted by

transforming growth factor-alpha (TGF-α) and epidermal growth factor (EGF), secreted by local platelets and macrophages of the late inflammatory response [62]. These signals bind to the epithelial surface expressed EGF receptor (EGFR), a tyrosine kinase receptor. An inability to signal through EGFR (e.g., inappropriate localization of the receptor to the membrane or mutations in the cytoplasmic signaling domains) is sufficient to create a chronic wound [63]. Additional factors regulating epithelial cell migration are MMPs and tissue inhibitors of MMPs (TIMP) activity in the microenvironment. Matrix metalloproteinases are a class of extracellular enzymes that facilitate the degradation of the structural proteins of the ECM, a process that is essential in facilitating the movement of cells through the matrix, and thus epithelialization. Furthermore, digestion of the ECM by MMPs releases matrix-bound growth factors and matrix-bound nanovesicles, and this further contributes to this process [35, 36, 47]. Therefore, a well-regulated MMP/TIMP ratio is critical for maintaining appropriate levels of keratinocyte migration and proliferation. Dysregulated MMP/TIMP ratios are indicative of a chronic wound.

Angiogenesis

Another critical aspect of wound healing is the formation of new blood vessels, which are essential for delivering oxygen and nutrients to cells as well as for removing waste and carbon dioxide. Inability to re-vascularize the wound in this manner recapitulates the inflammatory cascade, due to hypoxia, creating a chronic wound. Broadly, angiogenesis consists of the development of capillary sprouts by endothelial cells that permeate through the granulation tissue formed in the wound bed, creating new capillary structures. This temporospatial regulated process is a result of interactions between endothelial cells, angiogenic factors, and the extracellular matrix.

There are several known soluble factors with angiogenic activity. Fibroblast growth factors 1 and 2 (FGF-1, FGF-2) have long been considered primary angiogenic factors in wound healing [64]. While other angiogenic cytokines have since been identified, FGF-1 and FGF-2 occupy an interesting space in angiogenesis due to the fact that, lacking any transmembrane region in their molecular structure, they are not secreted molecules. Rather, these cytokines are released from disrupted fibroblasts at the wound site, thereby acting as an immediate pro-angiogenic response to tissue injury. TGF-β is another potent angiogenic factor. TGF-β is secreted by M_2-polarized macrophages and stored in a latent form in the extracellular matrix; therefore, it plays a role in promoting angiogenesis later in the wound healing process relative to FGF-1/2 [65]. Likewise, vascular endothelial growth factor (VEGF), which is important for both vascularization and vascular permeability, is induced by hypoxia at later stages of the wound healing process [66]. HIF-1α is the oxygen-regulated subunit of the hypoxia-induced factor 1 (HIF-1). In hypoxic conditions, HIF-1α is no longer continuously degraded, and upon binding the

constitutively expressed HIF-1β relocates to the nucleus to activate several pro-angiogenic genes, such as VEGF [67]. In summary, FGF plays an important angiogenic role in the first 3 days of wound healing and shifts to TGF-β and VEGF, via HIF-1α, from day 3 onward.

Integrins are cell surface receptors that mediate interactions between the ECM and a cell and are obligate heterodimers comprised of an α and β subunit. One particular integrin $\alpha_v\beta_3$ is critical for wound repair due to its ability to recognize the three major components of the provisional matrix—fibrin, fibronectin, and vitronectin. It has been demonstrated that on day 3 of wound repair, $\alpha_v\beta_3$ expression is localized to the wound periphery in stromal microvessels [68]. On approximately day 4, capillaries expressing high levels of $\alpha_v\beta_3$ begin to bud off from the microvessels and extend into the provisional matrix and granulation tissue. It has been shown that the provisional matrix lacking fibrin and fibronectin is associated with $\alpha_v\beta_3$-negative endothelium, suggesting that the provisional matrix composition dictates the expression of $\alpha_v\beta_3$ and therefore $\alpha_v\beta_3$-mediated migration of neovasculature [69]. As neovasculature matures into distinct vascular capillary structures, blood flow is restored to the healing wound site.

Failure or impairment of angiogenesis results in severe pathology and associated morbidity for patients. The most common clinical examples of deficient angiogenesis are in chronic nonhealing cutaneous ulcers, severe epithelial trauma, and skin and gastrointestinal infections [70]. Hyperglycemia seen in patients with diabetes mellitus leads to endothelial dysfunction and poor vessel responsiveness to pro-angiogenic factors such as VEGF and the angiopoieten1/angiopoieten2/Tie2 complex [70–72]. In this chronic state of impaired angiogenesis, the development of chronic cutaneous and mucosal ulcers is common and represents significant clinical morbidity and reduced quality of life. There is evidence to suggest that bacterial pathogens can produce inhibitors of angiogenesis, resulting in chronic infections and nonhealing cutaneous and mucosal ulcerations [70, 73, 74]. The ultimate consequence of impaired angiogenesis is a failure to re-vascularize the healing tissue, which in turn exacerbates the local inflammatory environment and promotes chronic wound development.

Provisional Matrix Formation

The deposition and maturation of new ECM is essential for proper healing of tissue [75]. The extracellular matrix represents both a structural scaffolding for cells and an initiator of biochemical signaling that regulates cell behavior. This ongoing, bidirectional cross talk between cells and ECM, known as "dynamic reciprocity," is evident throughout the wound healing process [76]. The wound matrix is characterized as either early or late provisional matrix, depending on its composition and origin. The early provisional matrix is fibrin rich and derived from the structure of the mature platelet plug. In contrast, the late provisional matrix is derived from

resident cells of the wound and matures following the production of the hemostatic plug. During matrix maturation, the fibrin of the early provisional matrix is replaced with fibronectin and proteoglycans. Fibronectin plays critical and varied roles in tissue repair from regulating the dynamics of cell adhesion to signaling the deposition of additional collagen. Fibronectin also has cryptic cytokine binding sites that help modulate the interaction of these cytokines with cells [77].

During inflammation, neutrophils release serine proteases, such as elastase and cathepsin G, MMP, which are responsible for degrading various matrix proteins present in the wound site, including elastin, fibronectin, laminin, vitronectin, and various collagens [78]. The serine proteases are inhibited by α1-antitrypsin and secretory leukocyte protease inhibitor (SLPI). MMPs are inhibited by tissue inhibitors of metalloproteases (TIMP) [79, 80]. Matrix turnover is, in part, regulated by the ratio of matrix proteases and their respective inhibitors. In situations of prolonged inflammation, the balance of proteases and their inhibitors is destabilized, and the rate of matrix degradation can exceed the rate of matrix synthesis; thus, the provisional matrix cannot properly mature, contributing to propagation of the inflammatory phase.

Remodeling

The remodeling phase of the wound healing process begins 2–3 weeks following injury but can persist for as long as a year or more. The goal of effective tissue remodeling is to restore the endogenous tissue structure and function and the tensile strength of the tissue [81]. In other words, remodeling is the process that attempts to restore normal tissue structure–function relationships. Remodeling involves degradation of provisional matrix, rich in collagen III and proteoglycans such as hyaluronic acid and fibronectin, and the deposition of a strong coll1agen I-rich matrix. The newly synthesized collagen I triple-helix fibers are organized in parallel bundles and cross-linked to each other, which contributes to the overall strength of the wound [82].

Conclusion

Wound healing is a complex and tightly regulated physiological process designed to minimize tissue damage and restore functionality. Throughout the six steps of hemostasis inflammation, epithelialization, angiogenesis, matrix deposition, and remodeling, the sheer quantity of cells, cytokines, and other mediating molecules involved provide several opportunities for disordered wound healing. A thorough understanding of the normal physiology and concomitant pathology of wound healing is essential for developing a framework for new therapeutic approaches and model-based precision medicine.

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Chapter 11
Equation-Based Models of Wound Healing and Collective Cell Migration

Julia Arciero and David Swigon

Abbreviations

ABM	Agent-based model
ECM	Extracellular matrix
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
GGH	Glazier–Graner–Hogeweg
IEC-6	Rat small intestine epithelial cell
KGF	Keratinocyte growth factor
MAPK	Mitogen-activated protein kinase
MDCK	Madin–Darby canine kidney
ODE	Ordinary differential equation
PDE	Partial differential equation
ROS	Reactive oxygen species
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

Introduction

Wound healing is a physiological process of repair of a tissue that has been structurally damaged. The most common wounds disrupt one of the epithelial tissues that protect the internal and external surfaces of the body and act as barriers against invasion by microorganisms. Such tissues include skin, corneal tissue, and the

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epithelial lining of the digestive tract (including the mouth and esophagus), respiratory tract (including the alveoli), urinary tract, and reproductive organs. Any disruption of these tissues can lead to serious health conditions or developmental abnormalities; for example, a wound in the gut epithelium can lead to necrotizing enterocolitis, which is the leading cause of death from gastrointestinal disease in preterm infants. Internal wounds may also arise due to physical overexertion or blunt force trauma.

The ability to heal wounds is closely related to the regenerative ability of the organism to restore the function of many organs. Wound healing generally proceeds in four stages, although these stages differ in details depending on the location of the wound [1–4]. The **first stage** is hemostasis, characterized by the leakage of fluids out of broken blood and lymphatic vessels and the delivery of inflammatory cells and platelets to the wound. The platelets trigger vasoconstriction to reduce blood loss and form a blood clot to fill the wound. The clot contains fibrin molecules that provide an extracellular matrix (ECM) for the migration of leukocytes and fibroblasts, which are cells responsible for eliminating pathogens and repairing the tissue, respectively. The **second stage** (2–3 days for skin wounds) is an inflammatory reaction marked by neutrophil recruitment followed by macrophage infiltration. Neutrophils phagocytose necrotic tissues kill bacteria that enter the wound and produce chemoattractants to recruit macrophages. Macrophages secrete pro- and anti-inflammatory cytokines that regulate inflammation and trigger the phagocytosis of pathogens and cell debris. Macrophages also secrete growth factors necessary for the third stage of wound healing. The degree of inflammation that ensues is directly related to scar formation [5]; for instance, the lack of inflammation in embryos leads to scarless wound healing. The **third stage** of the process (3–10 days for skin wounds) is the recovery of the tissue via cell migration and proliferation. The wound is also infiltrated by fibroblasts, which initiate the formation of the collagen matrix to provide mechanical strength for the disrupted tissue and keratinocytes to regulate the re-epithelialization process. In addition, new capillaries are grown by extension and sprouting in a process called angiogenesis, and the development of acute granulation tissue is initiated. This transitional granulation tissue replaces the provisional wound matrix and is characterized by a high density of fibroblasts, granulocytes, and macrophages. The **fourth stage** of the wound healing process is tissue remodeling, which can take anywhere from 21 days to 1 year for skin wounds. During this process, the formation of granulation tissue ceases, and collagen III, which forms a basket weave-like structure in the extracellular matrix, is replaced by collagen I, which is stronger and oriented in parallel bundles. Furthermore, the wound contracts and decreases the surface of the developing scar.

These four stages have been observed, measured, and assessed in a wide range of experimental and clinical scenarios. In some cases, shallow wounds are studied using in vitro experiments known as scratch wound assays (depicted in Fig. 11.1), which are designed to track the migration and proliferation of a monolayer of cells. In a scratch wound assay, cells are cultured (typically on a glass coverslip), grown to confluence, and then scraped with the tip of a pipette to create a gap that represents a wound in the tissue layer. Medium is continuously perfused across the cells,

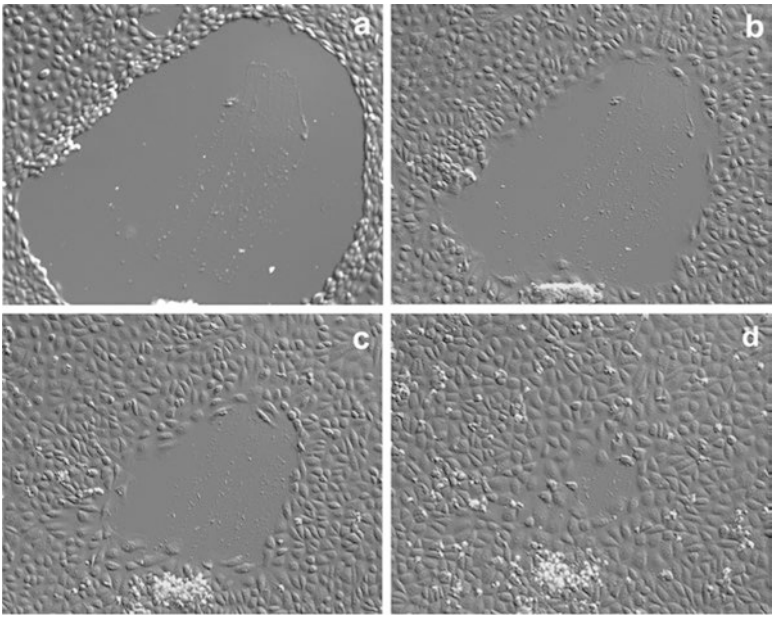

Fig. 11.1 Scratch wound assay of intestinal epithelial cells. A large space void of cells denotes wound; the surrounding region is the epithelial layer, which remains connected during the closure. Panels (**a**–**d**) show the progression of wound closure after 0 min, 125 min, 250 min, and 500 min, respectively

and the motion and deformation of cells in the layer are analyzed. Other in vitro assays include three-dimensional organ explant cultures or three-dimensional sprouting invasion assays from mesenchymal cells overlaid onto a three-dimensional ECM or implanted as a multicellular spheroid [6]. Using such assays, numerous studies have evaluated how extracellular stimuli, geometric anisotropy of substrates, or intracellular processes activate cell migration and trigger cell proliferation [7–11]. Measurements of the physical forces driving cell migration indicate that traction forces arise many cell rows behind the leading wound edge and extend across large distances [9]. Trepat et al. [9] demonstrated that individual cells at both the leading wound edge and inside the sheet engage in a "tug-of-war" that integrates local force generation into a global state of tensile stress. Mechanical forces exerted by epithelial cells were measured by du Roure et al. [11] using a technique that combines microfabrication of flexible substrates and multiple-particle tracking microscopy. Because each micropillar deflection is independent of its neighbors, the measured traction forces under the cells are uniquely determined.

In clinical settings, the progression of wounds, such as skin wounds or diabetic foot ulcers, are quantified according to measures such as the absolute wound area remaining, percentage of initial wound area remaining, wound volume remaining, or wound perimeter remaining. Some of these measurements can be difficult to obtain the given location of the wound or type of data needed, and, as a result,

currently there is no universally accepted measure of wound healing [12]. In addition, although the wound area is an obvious measure of wound closure, predicting healing time based on the percent of wound area healed tends to bias small wounds, and predicting healing time based on the absolute wound area healed tends to bias large wounds [13]. A reliable measure of wound healing time is of particular interest to both physicians and patients in order to determine effective treatment methods for various wounds.

Ultimately, a wound is considered healed once tissue functionality has been fully restored via the migration of cells into the wounded region and the proliferation of new cells to restore the original density of the tissue. Observations from multiple wound scenarios have shown that cell migration and proliferation as well as the overall healing time for a wound are affected by factors such as wound geometry, tissue type, cell–cell interactions, and the stage of the healing process (epithelialization, contraction, or proliferation). Combining these observations with mathematical modeling techniques may help to unravel the key aspects governing wound healing.

Modeling

Mathematical modeling of wound healing is used to aid in the understanding of the complex processes involved in wound healing by providing a platform for testing various hypotheses regarding the interaction of wound healing components. Equation-based models describe biological processes by formulating interactions of individual biological components using a system of differential equations for variables that measure the concentrations of cells and chemicals in time and space. The equations are constructed using knowledge deduced from experiments and are calibrated using experimental data (e.g., data could be used that describes the dependency of cell migration speed on the concentration of a chemoattractant). The equations are solved using a variety of analytical and numerical techniques and are used to predict the dynamics of cell populations within a wound. There are two classes of equation-based models in common practice: models based on ordinary differential equations (ODEs) and models based on partial differential equations (PDEs). The main difference is that ODEs can describe the time-dependence of the wound healing process but not its spatial variability, while PDEs can describe both. In addition, there are also stochastic models that can include fluctuations in chemical concentrations and other random effects [14, 15]. A summary of these different types of models, many of which are described in more detail in this review, is provided in Table 11.1.

Equation-based models are beneficial in situations in which it is reasonable to assume that the components of the system are either of the same kind and respond identically to stimuli or of different types but their response depends only on the number of components at a given spatial location at a given time. For example, when fibroblasts are rebuilding the collagen matrix, the speed of rebuilding depends

Table 11.1 Summary of equation-based wound healing models

Model	Type	Examples	Phenomenon modeled	Tissue type
ODE	Reaction-Diffusion	Cukjati et al. [16]	Wound area healing	Endothelium, etc.
		Johnson [17]	Wound area healing	Arteries, veins
		Bardsley et al. [5]	Wound area healing	General
		Baker et al. [18]	Wound area healing	Diabetic ulcers
		Jercinovic et al. [19]	Wound area healing	Pressure ulcers
		Menke et al. [20]	Fibroblasts; oxygenation	Dermis
		Maini et al. [21, 22]	Migration; proliferation	Peritoneal
		Sherratt et al. [23, 24]	Migration; proliferation	Epidermis
		Sheardown et al. [25]	Migration; proliferation	Cornea
		Dale et al. [26]	Migration; proliferation	Cornea
		Tremel et al. [27]	Migration; proliferation	Fibroblast cells
		Gaffney et al. [28]	Migration; proliferation	Cornea
		Chen et al. [29]	Migration analysis	Tumor
		Dale et al. [30]	Migration analysis	Scar tissue
		Wearing et al. [31]	Migration; proliferation	Dermis
		Javierre et al. [4]	Migration; proliferation	General
	Continuum Mechanical	Vitorino et al. [32]	Migration; proliferation	Endothelium
		Lee et al. [33]	Migration	Kidney cells
		Xue et al. [34]	Migration, oxygenation	Cutaneous
		Qi et al. [35]	Migration	Epithelium
		Arciero et al. [36]	Migration	Epithelium
PDE	Cell Signaling	Posta et al. [37]	MAPK activity	Epithelium
		Dale et al. [38, 39]	Fibroblasts; collagen	Scar tissue
		Murray et al. [40]	Wound contraction	General
		Palecek et al. [41]	Cytoskeleton-integrin	General
		Gaffney et al. [42]	Diffusion	Cornea
		Tranquillo et al. [43]	Migration; proliferation	Fibroblasts, ECM
		Olsen et al. [44]	Fibroblasts; proliferation	Scar tissue
		Sherratt et al. [45]	Wound contraction	Epithelium
		Murray et al. [46]	Morphogenesis	ECM
	Angiogenesis	Chaplain et al. [47]	New capillary formation	Tumor
		Anderson et al. [48]	New capillary formation	Cornea tumor
		Pettet et al. [49]	New capillary formation	Soft tissue
	Chemotaxis	Hillen et al. [50]	Chemical migration	General
		Schugart et al. [51]	Fibroblasts; oxygenation	Cutaneous
		Dallon et al. [52]	Collagen deposition	Dermis
		DiMilla et al. [53]	Receptor-ligand binding	General
ABM		Walker et al. [54, 55]	Migration; proliferation	Epithelium
		Bindschadler et al. [56]	Migration; proliferation	Scratch wound
		Ouaknin et al. [57]	Migration; chemotaxis	General
		Fozard et al. [58]	Collective cell migration	Epithelium
		Byrne et al. [59]	Cell expansion	General

on how many fibroblasts are present, but not on where precisely each fibroblast is and how it moves about. In such a case, one may invoke the *continuum hypothesis* and assume that there is a density function $f(x,t)$ that depends on space and time, which describes the mass (or molar) density of the cellular component (cell or a chemical). The density can be understood in a statistical or probabilistic sense. In the first case, the quantity f is equal to the average number of components per unit volume centered at position x and measured at time t for a series of identical experiments; in the second case, f represents the probability of finding a component in that volume at time t. The use of the continuum hypothesis implies that we can only predict the behavior (motion and state changes) of population-averaged properties, not of individual molecules or cells. In contrast to ODE models, models satisfying the continuum hypothesis preserve the possibility of properties varying in space.

By choosing a continuum formulation, we substantially reduce the amount of information needed to specify the state of the system. Imagine a 1 cm wound with 10^8 fibroblasts. In order to specify the fibroblasts' positions in 3-space, we would need 3×10^8 numbers. However, to define their spatial distribution it may suffice to use a grid of spacing $100 \times 100 \times 100$ nodes (10 nodes per mm) that results in 10^6 values for the density and a 300-fold reduction in the amount of data needed to characterize the system. A coarser grid would result in even higher simplification and speed-up in the computation of the dynamical behavior of the system. Even more important is that we need not be concerned with the details of the motion of individual cells; instead, the motion can be described in one of several standard ways (diffusion, chemotaxis, or convection) that we describe below.

The time–rate of change of the density variable is expressed using an equation that involves partial derivatives of f with respect to the independent variables $\boldsymbol{x}$ and t. The most basic partial differential equation one can construct is the law of conservation of the number of cellular components in a given volume:

$$\frac{\partial f}{\partial t} = -\nabla \cdot \mathbf{J} + \sigma$$

Here, $\mathbf{J} = (J_x, J_y, J_z)$ is a vector-valued variable that represents the flux of the component (migration or transport of the component away from the position $\mathbf{x}$); σ represents the local source or decay of the component; and $\nabla \cdot \mathbf{J} = \partial J_x/\partial x + \partial J_y/\partial y + \partial J_z/\partial z$ is the divergence of $\mathbf{J}$.

By using different relations between $\mathbf{J}$ and f, one can account for different types of transport such as (1) the *convection* of cells or molecules in the direction of velocity $\mathbf{v}$, defined by $\mathbf{J} = f\mathbf{v}$, and (2) the *diffusion* of cells or molecules, resulting from the random motion of cells or molecules in all directions and defined by $\mathbf{J} = \nabla f$. A special case of convection is the *chemotaxis* of cells in which the direction of motion is defined by a gradient of a chemoattractant molecule with concentration c, that is, $\mathbf{J} = f \nabla c$. The source σ is a function of f (expressing self-regulation of the component) and possibly other components (expressing the influence of components on

each other). For example, in models describing the mechanics of a tissue, the variables are the mass density of the tissue, ρ, and the momentum of the tissue, $\rho\mathbf{v}$; the flux of the momentum is the stress tensor, $\mathbf{T}$.

The system of PDEs formulated from known properties and interactions of cellular components forms the core of a PDE model. The remaining parts of the model are the definitions of the *domain* in which the equations are valid, the *initial values* of all variables across the domain, and the *boundary conditions* imposed at the boundary of the domain. The boundary conditions are typically defined as one of two types: (1) Dirichlet conditions, representing a constant source or sink of the quantity and prescribed as a fixed value of the quantity at the boundary and (2) Neumann conditions, representing the flux of a quantity across the boundary and prescribed as the derivative of the quantity along the normal to the boundary.

The analysis of a PDE model starts with an examination of the existence and uniqueness of solutions. Contrary to strong results in the theory of ordinary differential equations, there are no generic results apart from the Cauchy–Kowalevski theorem guaranteeing the local existence of a solution for a Cauchy problem. Global existence can be proved for a diffusion equation and systems of diffusion equations with the same diffusion constant. But in general, every system must be analyzed individually using techniques such as maximum principle, weak solutions, variational formulation (see, e.g., [60]). The next step in the analysis may be to search for special solutions that enable one to reduce the dimension of the equation. Such special solutions can be (1) a *steady-state solution* in which f is independent of t, (2) a *self-similar solution* that is invariant under a rescaling of the spatial and time variables, or (3) a *traveling wave solution* that represents solutions invariant under the transformation $\mathbf{X} \rightarrow (\mathbf{X} - \mathbf{V}t)$. These special solutions are then analyzed for stability, that is, invariance under a small perturbation. Stable solutions are of particular interest to various applications since they describe the observed behavior of the system. If solutions do not converge to steady-state solutions or traveling waves, they may approach singularities or blowups.

Once all information is extracted using analytical tools, numerical solutions of the system can be obtained. Numerical solutions of ODEs can be found using standard integration packages such as CVODE (available in C or FORTRAN) or MATLAB suite of integrators. A convenient free standalone program that allows the user to explore solutions of ODE systems is XPP. Numerical solutions of PDEs are obtained by converting the PDE system into a system of algebraic equations by transforming the derivatives into finite differences (finite difference methods) or by transforming the equations into a variational form formulated as integrals over appropriate test functions with simplicial support (finite element methods). User-friendly software packages have been developed that enable researchers with little knowledge of numerical mathematics to solve various types of PDE problems—see, e.g., the FEMLAB, or Matlab Partial Differential Equation Toolbox.

ODE Models

The simplest ODE models of wound healing are phenomenological: they try to capture the time-dependent closure of the wound by formulating an equation for the wound area or circumference as a function of time and fitting the constants in the equation to observed data [5]. The majority of such models are based on linear or exponential functions that involve two parameters [17–19]. However, these are not sufficient to describe the initial delay of the healing process that is noticed in wound healing experiments. Cukjati et al. [16] formulated several ODE models by considering two, three, and four-parameter functions of chronic wound healing and assessed their goodness of fit to 226 chronic wounds. They used a set of five criteria to qualitatively and quantitatively assess the model (statistical analysis of goodness of fit) and concluded that a delayed exponential model with three parameters is the most adequate for representing the healing process.

Mechanistic ODE models are based on the formulation of equations describing the concentration of various components of the wound healing process. An example is the model of Menke et al. [20] that focuses on the second stage of the process (inflammation) by using an extension of an ODE model of inflammation [61, 62] to include tissue damage, pathogen level, inflammation, fibroblast concentration, and tissue oxygenation. The model is used to simulate impaired wound healing in hypoxic skin wounds with varying levels of contamination. Pathogen growth is assumed to depend on tissue oxygenation levels. The skin is assumed to have a baseline level of circulating fibroblasts that increases in response to tissue damage and inflammation. The authors classify wounds as healed, nonhealing, or chronic and find parameter ranges for each type of outcome. Impaired wound healing is simulated in hypoxic wounds with varying levels of contamination, and the model is used to suggest possible components to target in therapies, such as the fibroblast death rate and the rate of fibroblast recruitment.

PDE Models

PDE models of wound healing describe the spatial dependence of various components involved in the healing process and can predict the shape of the wound. Most existing PDE models focus on the third and fourth stages of wound healing process, that is, on the repair of the epithelial layer and the remodeling of the scar tissue.

Reaction–diffusion models. The repair of the epithelial layer is a combination of two mechanisms: migration of epithelial cells into the wound and cell proliferation. The simplest PDE model of wound closure that can be constructed is one that consists of a single equation for cell concentration with wound closure interpreted as a traveling wave of cell concentration. The equation commonly used in that interpretation is the Fisher–Kolmogorov equation, which is a reaction–diffusion equation with proliferation given by a logistic term. Maini et al. [21, 22] verified the

validity of using the Fisher–Kolmogorov equation in a medical context by using a scratch-wound assay (for an example of a scratch wound assay, see Fig. 11.1) and comparing model predictions with multiple experiments.

Both the migration and the proliferation of epithelial cells are regulated by growth factors. The first model to account for such chemical control was developed by Sherratt and Murray [23]. The model consists of two nonlinear reaction–diffusion equations that track epithelial cell density and a chemical regulating mitosis (the epidermal growth factor, EGF) in the context of epidermal wound healing. The epidermis is assumed sufficiently thin and thus the tissue is modeled as two-dimensional. The mitosis chemical is modeled as both an activator and inhibitor, and the effect of these two chemical behaviors on cell migration is investigated. The model was further analyzed in [24] by providing details for traveling wave solutions for cell density and chemical concentration. The results for the wound radius as a function of time were shown to be consistent with experimental measurements. Clinical implications of the model were studied in [63], in particular the dependence of healing time on wound shape (e.g., cusped, ovate, and rectangular) and the dependence of predicted wound contours on the character of the growth factor.

The Sherratt–Murray model was extended to corneal epithelial wounds by Sheardown and Cheng [25] and by Dale et al. [26] who used the model to study the impact of increased mitotic and migratory activity due to an epidermal growth factor. They also predicted the wound healing rate if the growth factor was applied only topically to the wound and found that the factors affecting migration include cell migration, cell-to-substrate adhesion, and cell mitosis. They noted that the model predicted a lag time immediately after wounding.

Tremel et al. [27] modified the Fisher–Kolmogorov equation to include the effects of cell density-dependent diffusion on wound healing. They assumed that diffusivity decreases with increasing cell density in order to capture contact-inhibited movement between cells so that cells slow, stop, or change direction when they encounter another cell in their path. In their study, cell tracking was performed on groups of cells in a wound healing experiment; in the images, it was observed that the cells initially located close to the wavefront traveled significantly greater distances than cells starting farther behind the wavefront. Also, while the overall cell movement was directed, a significant amount of random motion was observed.

Several studies have modeled the influence of physiological electric fields on wound closure. In those studies, the PDE problem was formulated with a *free boundary*, that is, a boundary whose position is changing in time, and this change is governed by an additional equation. For example, Gaffney et al. [42] described the evolution of the free boundary problem for a system of two reaction–diffusion equations for cell density and chemical stimulus in the context of corneal wound healing. The formulation predicts a linear relation between the wound healing speed and the physiological electric field strengths over a physiologically large range of electric field strength. Spatial and temporal data on mitotic rates measured during corneal epithelial wound healing in a rat were studied by Gaffney et al. [28] who argued that earlier models were not adequate for the study of cell kinetics. Chen and Friedman

[29] analyzed the Gaffney model [42] and applied a similar approach to predicting tumor growth [64].

In a subsequent paper, Dale et al. [30] presented a complex model for scar tissue formation in deep wounds and focused on the role of key chemicals in determining the quality of healing. The authors described wound healing as a traveling wave and investigated the factors controlling the speed of the wave. A more complex model accounting for the effect of the keratinocyte growth factor (KGF) was proposed by Wearing and Sherratt [31] who found that high KGF levels decreased the speed of healing but increased the cell division rate at a greater distance away from the wound edge. A comprehensive review of wound healing models of Sherratt and collaborators is given in [65, 66].

Javierre et al. [4] also modeled the re-epithelialization of the basal membrane of the epidermis by cell mitosis and migration in the presence of a generic epidermal growth factor. The diffusion, depletion, and production of the concentration of the growth factor in the model are determined by a reaction–diffusion equation. The model assumes that cells become motile if the accumulated growth factor concentration exceeds a threshold value. A sigmoid function is used to relate cell mitosis and the growth factor concentration. Since cell migration is interrupted when the growth factor concentration drops below a threshold, cell motility is dose-dependent in this model. Moreover, the wound closure rate is assumed proportional to the local curvature of the wound edge. Javierre et al. [4] analyzed the roles of diffusion, closure rate, and wound geometry on healing kinetics and concluded that healing is always initiated at regions with high curvatures, and that the evolution of the wound is sensitive to multiple physiological model parameters.

Continuum mechanical models. For the success of wound closure during the third stage of wound healing, it is essential that the epithelial cells migrate collectively, in synchrony, so that the coverage of the wound is continuous without the formation of any holes in the remaining sheet. Cell migration at the single-cell level has been studied extensively over many decades [67–71]. In brief, each cell moves by a cyclic mechanism that proceeds through stages involving the formation of a lamellipodium, translocation of the nucleus in the direction of motion, and detachment of the trailing edge [69, 72]. This mechanism is regulated by a complex signaling and regulatory network responsible for the underlying processes of actin polymerization and depolymerization, motor protein activation, and integrin formation and release.

Although the study of individual cell migration has been pursued vigorously, there is less understanding of the interactions that drive and synchronize collective cell migration in wound closure. Several mechanisms of closure have been proposed: (1) a leader cell mechanism, (2) cooperative traction force mechanism, (3) steered migration mechanism, (4) differential adhesion hypothesis, and (5) differential interface tension hypothesis. In the leader cell mechanism [6], the cells at the edge of the wound are believed to change their phenotype and direct the migration of other cells toward the wound. In the cooperative traction force mechanism, cells near the edge of the layer exert coordinated forces that result in a cumulative stress within the layer and motion of cells toward the wound [9]. In the steered migration

mechanism, the direction of autonomously migrating cells is changed in a gradual fashion by forces exerted on them by neighboring cells [73]. The differential adhesion [74] and differential interface tension [75] hypotheses stipulate that the cell layer evolves to minimize either the adhesion energy or surface tension of the constituent cells, which leads to the eventual wound closure.

As described earlier in this chapter, a typical experimental method used to study collective cell migration is the scratch-wound assay (Fig. 11.1). Farooqui and Fenteany [76] studied wound closure in Madin–Darby canine kidney (MDCK) epithelial cell layers and established that submarginal cells exhibit protrusive and migratory behavior similar to that of marginal cells. They found that the general direction of the coordinated cell movement was toward the center of the wound and the cell velocity within a sheet was found to be inversely proportional to the distance from the wound edge. Wound closure was shown to occur even if the motility of edge cells was inhibited, but it occurred at a slower rate [77]. Coordinated cell movement toward the center of a scratch wound assay is depicted in Fig. 11.2. In the left panel, the starting positions of all cells of the scratch assay are denoted by blue dots. In the right panel, colored lines define the trajectories of all the cells, with the blue dots indicating the starting point of the cell, as in the left panel. The trajectories indicate the tendency of the cells to migrate toward the center of the wound.

Vitorino and Meyer [32] studied growth factor-induced migration of endothelial cell monolayers and proposed that the fibroblast growth factor (FGF) led to directed migration of leader cells but did not control cell migration and coordination of the follower cells. Mechanically, the robust and dynamic coupling of cells to one another and to the substrate is accomplished via adherens junction proteins, desmosomal proteins, and integrins [6, 78]. The cells in the interior are connected to the cells at the boundary by tight junctions, which prevent separation of the cells in the layer [79]. The level of adhesion between the cell and the substrate, moderated by integrins, was found to control the speed of wound closure [41]. The effects of substrate stiffness on cell traction forces were quantified for epithelial cells and fibroblasts, and it was shown that cell movement could be modulated by changing the

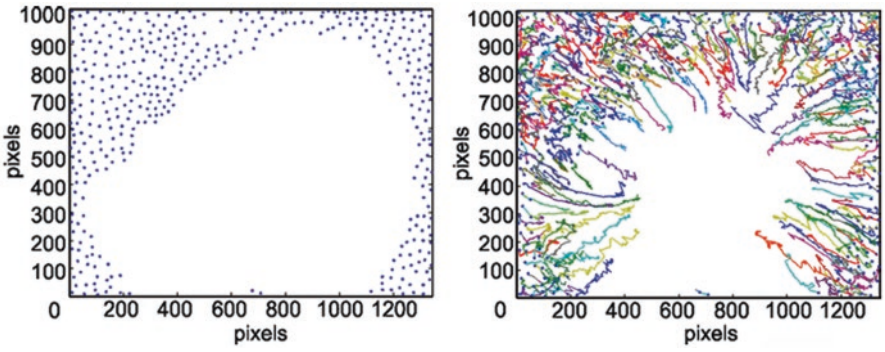

Fig. 11.2 Left panel: the initial position of all cells in scratch wound assay is indicated by blue dots. Right panel: cell trajectories of every initial cell position over the course of several hours

stiffness of the substrate [8]. Trepat et al. [9] found that traction forces, applied by moving MDCK cells on the substrate, were smallest in the center of a cell colony and largest at the edge of the colony of cells moving radially outward. They estimated that tension in the cell layer increased with distance from the edge of the cell colony and argued that accumulated traction stresses were balanced by the forces within the cell sheet; the interplay of these two stresses was described using a tug-of-war model. In several studies, a release of tension was observed within the cell layer once a wound was induced [7, 80]. Block et al. [80] compared cell-sheet migration in wounds induced by different methods and hypothesized that the release of spatial constraints initiates a healing response. However, this hypothesis is difficult to verify experimentally since it is hard to eliminate all possible methods (such as biochemical communication) that may contribute to collective cell migration.

All the models described so far represented migrating cells using reaction–diffusion equations for cell density. Such equations are based on the diffusion mechanism for cell migration that provides no guarantee of continuity of the cell layer. The process of collective cell migration is complex and requires fundamentally different, mechanics-based models. Lee and Wogelmuth [33] developed a model in which an MDCK cell layer was represented as a viscous liquid with orientation, similar to a liquid crystal; the layer orientation was equated with the direction in which the cell exerts a crawling force. They formulated equations of the balance of forces on the cells and, using numerical solutions, were able to reproduce not only wound closure dynamics, but also the irregular, undulating, progression of the edge of the layer typical for scratch-wound assays, without the need to specify leader cells. Xue et al. [34] developed a continuum model of ischemic dermal wounds with the wound boundary represented as a free boundary that moves with the velocity of the extracellular matrix at the wound edge. The model was used to predict how ischemic conditions may impair wound closure.

Mi et al. [35] recently developed a one-dimensional continuum mechanical model of a migrating IEC-6 enterocyte cell sheet to study the influence of lipopolysaccharide (a protein found in the coat of Gram-negative bacteria) and integrin concentration on wound closure during experimental necrotizing enterocolitis. The model predicts low migration speed at high and low integrin concentrations and high velocity at medium concentrations, in agreement with experimental observations [41]. It also predicts that the edge velocity decreases with time, in accord with our experimental observations but contrary to the behavior of reaction–diffusion models. However, the model is only appropriate to situations in which the wound has a simple geometry with two long parallel wound edges. In a follow-up study, Arciero et al. [36] designed a two-dimensional model of cell layer migration that captures the same primary interactions driving the motion of the cell sheet, namely, the elastic coupling between cells in the layer, the adhesion of cells to the substrate, the force generated by lamellipodia both in the interior and at the wound edge, and the proliferation of cells within the layer, but has the additional benefit of being applicable to an arbitrary wound geometry. Figure 11.3 shows a model schematic of a wounded region and the model predicted contours for the closure of an experimental scratch wound at 30-min intervals until the wound is completely closed.

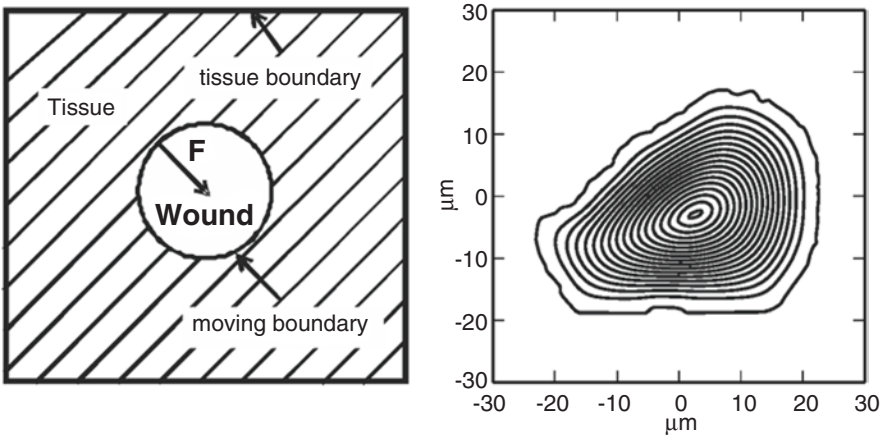

Fig. 11.3 Left: schematic of a circular wound surrounded by tissue. The force of the lamellipodia at the edge of the wound is denoted by F. Right: model-calculated contours of wound edge (initial position is outermost contour) every 30 min until wound closure

In Arciero et al. [36], the cell sheet is represented as a compressible inviscid fluid, and therefore individual cells are not distinguishable. The leader and follower cells are accounted for in an average manner by including a focused traction force applied by the lamellipodia at the edge of the sheet. The two-dimensional character of the problem requires us to use Eulerian independent variables. The physical laws governing the mechanics of the layer then yield a partial differential equation problem with a moving boundary that is known as the Stefan problem in other contexts [81, 82]. The problem is solved numerically using a level set method, and the basic properties of solutions are analyzed. The model is calibrated for two scenarios: the closure of a wound and the expansion of a cell colony. Parameter values in the model are fit to data from a scratch-wound assay as well as to data from a cell colony expanding radially outward [9]. Cell proliferation is neglected in wound closure simulations but is included in colony expansion simulations. The model successfully reproduces cell density and edge migration velocity data from both types of experiments.

Cell signaling models. Models that are developed to understand both the mechanical and biochemical aspects of cell migration can help to determine which phenomena are primarily responsible for initiating cell motility following an injury and what factors regulate the speed and direction of cell migration. In general, the regulation of wound healing by biochemical signals and feedback pathways remains poorly understood. Posta and Chou [37] developed a mathematical representation of ligand-mediated intercellular signaling mechanisms related to the cell migration of epithelial monolayers. Experiments have indicated the need for mitogen-activated protein kinase (MAPK) activation for coordinated cell movement following an injury. The model reproduces two waves of MAPK activity that have been observed experimentally and that may depend on reactive oxygen species (ROS) and

competition between a ligand (such as a growth factor) and ROS for the activation of the epithelial growth factor (EGF) receptor. The resulting traveling wave solutions of the model are consistent with MAPK patterns observed experimentally.

Models of the fourth stage of wound healing, that is, the remodeling of the scar tissue, are primarily concerned with the factors that determine the final size of the scar. Two key features of the scar tissue attract attention: details of collagen composition (relative proportion of type I and type III collagen) and orientation of the fibers. The balance between the two types is regulated by different isoforms of transforming growth factor-beta (TGF-β) protein and was studied by Dale et al. [38] who developed a reaction–diffusion model. The model predicted that different ratios for fetus and adult tissues depend on the secretion of the different isoforms of TGF-β. In a follow-up paper [39], Dale et al. used the model to determine whether fibroblast cells enter the wound area from the surrounding unwounded dermis or from the underlying subcutaneous tissue and gave reasons favoring the latter. The orientation of fibers in the wound tissue was analyzed in a series of papers by Dallon et al. [52] who employ agent-based, as opposed to equation-based, models. In particular, fibroblasts were modeled as discrete entities and the extracellular matrix was assumed to be a continuous entity composed of collagen and a fibrin-based blood clot. The following interactions were captured by the model: fibroblasts orient the collagen matrix, fibroblasts produce and degrade collagen, and fibrin and the matrix direct the fibroblasts and determine the speed of the cells. The model was used to predict how multiple cellular phenomena play a role in collagen alignment during wound repair.

Wound contraction is also an important component of wound closure, especially in animals. Contraction is primarily caused by myofibroblasts that exert traction forces on their environment. Experimentally, this process has been studied on collagen gels. The contraction was first studied mathematically by Murray et al. [40] who adapted a general model of tissue biomechanics to a wound healing situation. Subsequently, Tranquillo and Murray [43] investigated the interplay between cellular, biochemical, and biomechanical phenomena that result in wound contraction. They modeled fibroblast migration and proliferation as well as the deformation of the ECM and formulated an extended model that accounts for the influence of an inflammation-derived mediator on traction, growth, and chemotactic properties of fibroblasts in order to predict the qualitative features of a contracting wound. A similar model was also used by Olsen et al. [44] to study failures in wound closure due to fibro-proliferative disorders, such as keloid and hypertrophic scars. All these models describe tissue as a linear viscoelastic material. For embryonic epidermal wound healing, Sherratt [45] developed a model involving actin filament network formation and wound contraction, based on a mechanochemical model for the deformation of epithelial sheets proposed by Murray and Oster [46].

Angiogenesis models. Angiogenesis in a growing tissue has been studied in the context of wound healing or tumor growth. The process of capillary ingrowth is essential to healing since it helps to maintain high levels of metabolic activity by increasing blood supply. The biology of angiogenesis has been studied mostly in the context of cancer growth, but the biology applies equally well to wound healing.

Tumor angiogenesis has been modeled by Chaplain and Sleeman [47] and continued by Anderson and Chaplain [48]. Chaplain and Byrne [83] reviewed the similarities of wound healing and tumor growth, and Olsen et al. [84] studied the interactions between endothelial cells and soluble regulators (such as growth factors), as well as the insoluble ECM substrate, which consists primarily of collagen. Pettet et al. [49] developed a model of angiogenesis during wound healing that includes contributions of capillary tips, capillary sprouts, fibroblasts, macrophage-derived chemical attractants, oxygen, and ECM. The model reflects the dependence of macrophage activity on local oxygen concentration, which is the major difference between the process in wounds and tumors, and is able to reproduce the failure of wounds to heal when the proliferation rate of endothelial cells is too low. A new version of the model was compared with experimental data by Byrne et al. [85].

Chemotaxis models. The directed movement of cells and organisms in response to chemical gradients, known as chemotaxis, plays an important role in several aspects of physiology, including embryonic development, inflammatory cell migration, wound healing, new vessel formation, and tumor growth. The deterministic Keller–Segel continuum model is a well-established method for representing the chemotactic behavior of cell populations since it is able to capture key phenomena that are often lost on discrete or single-cell level models. Hillen et al. [50] analyze ten models that are variations of the Keller–Segel model in order to determine which model components relate most directly to biological observations of chemotaxis. Their analyses include the determination of the existence of model solutions and the identification of long-time behavior of solutions and the form of steady-state patterns.

As an example of a chemotaxis model in the context of wound healing, Schugart et al. [51] presented a PDE model of wound healing that focuses on the release of angiogenic growth factors (e.g., VEGF) by inflammatory cells. In particular, the growth factors are assumed to interact with fibroblasts to produce collagen and other components of the ECM, which in turn facilitates the migration of cells into the wound. A circular wound is considered in this theoretical study, and thus the model is solved over a radial cross-section of the wound. Model results suggest that a hypoxic wound environment cannot sustain vascular growth, that hyperoxia promotes wound angiogenesis and healing, and that there is an optimal level of hyperoxia beyond which the beneficial effects of oxygen may be reversed.

Agent-Based Models of Cell Migration

Various types of agent-based models (ABM) have been used to test wound healing hypotheses and to isolate factors that may direct cell sheet migration. Since a detailed description of wound healing ABM is presented in a different chapter of this book, we here focus on ABM models of collective cell migration, as such models are often used as a basis for the development of equation-based models of wound healing.

A detailed model of the dependence of cell speed on adhesion-receptor/ligand binding was proposed by DiMilla et al. [53]. Walker et al. [54, 55] used an agent-based model to simulate the wounded epithelial cell monolayers and suggested that simple rules are sufficient to qualitatively predict the calcium-dependent pattern of wound closure observed in vitro. Khain et al. [86] built upon the work in [54, 55] and considered a simple discrete model that focuses on the effects of three key processes, cell–cell adhesion, diffusion, and proliferation, on wound healing in the context of a scratch wound assay. Different cell behavior was predicted by the model depending on the adhesion strength and the proliferation rate. The model is defined by a list of rules that dictate the conditions under which cells can proliferate or migrate, depending on the number of nearest neighbors to the cell.

Bindschadler and McGrath [56] used an ABM to simulate cell migration in which cells responded to crowded conditions by decreasing their cell division rates and moving to less crowded areas. The model predictions were consistent with experimental rates of closure. Ouaknin and Bar-Yoseph [57] used the Glazier–Graner–Hogeweg (GGH) model to simulate the collective movement of cells, taking into account adhesion energy, deformation energy, and stochastic behavior of the system. The model results were similar to experimental behavior obtained by Poujade et al. [87], in which leader cells progressed faster than the rest of the cell layer and a fingering morphology emerged. Fozard et al. [58] developed an ABM for epithelial monolayers and used it to derive an equation-based continuum model in the limit of a large number of cells. Relating agent-based and continuum models may help to estimate model parameters and justify model assumptions. Fozard et al. [58] assumed that the energy dissipation of individual cells was due to the drag between the cell and substrate, as well as due to the internal viscosity of the cells (which was not accounted for in the model presented here). Active cell migration and cell division were not included in their model, and a more complex formulation of cell–cell and cell–substrate adhesion could provide additional mechanical insight. The continuum model yielded results consistent with the ABM for even a moderate number of cells. Byrne and Drasdo [59] also derived a continuum model from their ABM for the growth of cell aggregates on compact monolayers. Growth was assumed to be governed by contact inhibition, and cells were assumed to proliferate. The continuum model agreed with the ABM in the prediction of initial and asymptotic growth regimes for the radius of the colony and the cell population size. A detailed description of agent-based models of wound healing is provided in the next chapter.

Applications of Wound Healing Models

Both equation- and agent-based theoretical models of wound healing have important applications that extend beyond the context of wound healing. The mechanisms and techniques used to describe migration and proliferation of a cell layer can be

used to predict wound closure time as well as to describe the mechanical processes governing morphogenesis, tumor growth, and colony expansion.

Predicting wound healing time. Three commonly used methods for estimating wound closure time in clinical practice are the absolute area reduction method, percent area reduction method, and linear parameter method [13]. The absolute area reduction method estimates the time rate of change in wound area as the ratio of the difference between the current wound area and the original wound area to the total change in time. The percent area reduction method estimates the rate of change in wound area as the difference in wound areas between two consecutive time points. The linear parameter method assumes that the average velocity of the wound edge over the wound contour is constant in time and uses the value of a linear healing parameter, which is defined as the ratio of the difference in wound areas to the average perimeter for two consecutive time points, to predict overall closure time for wounds. Recently, Arciero et al. (accepted) introduced two additional methods for calculating healing time in which the time rate of change of wound area is not constant but is proportional to the square root (square root method) or the first power (proportional area method) of area. These methods were shown to provide better estimates of closure time than the three previously established methods since they both converge to the correct closure time as more data are available and they provide relatively accurate predictions at early stages of the closure process. While these two methods were shown to be useful for predicting a range of wound healing times for superficial epithelial wounds, other clinical aspects may be required to obtain accurate closure time predictions for wounds of various types and sizes. A comparison of the predicted healing times of these five methods is provided in Fig. 11.4.

Morphogenesis. Cell and tissue mechanics are important components dictating embryonic development and organ shape within a body. In particular, at the tissue level, force production and viscoelastic material properties of tissues determine the direction and speed of tissue movements as structures are sculpted. Integrating

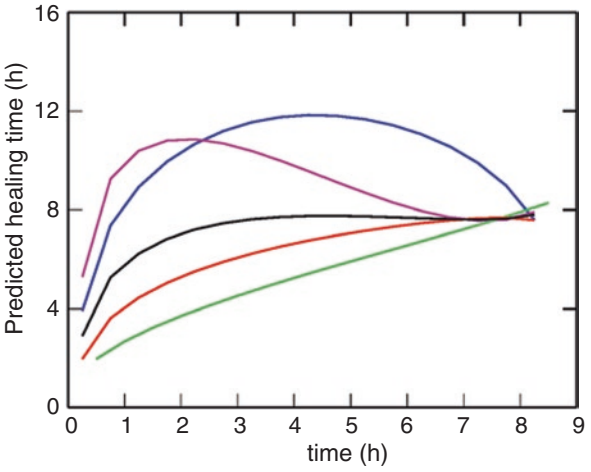

Fig. 11.4 Comparison of predicted healing times for a scratch wound assay using five different methods absolute area reduction method (green), percent area reduction (red), linear parameter method (blue), square root method (black), and proportional area method (magenta). The predicted healing time is shown as a function of time

intracellular force generation with the local micro-mechanical environment directs molecular-mechanical processes and cell differentiation [88]. Significant advances have been made in morphogenesis experiments, and the use of mechanical and theoretical analyses in this field is beginning to gain momentum. The combination of these experimental and theoretical techniques may help to answer three important questions in the field of morphogenesis outlined by Davidson et al. [89]: (1) Are mechanical properties of the embryo important to morphogenesis? (2) At what scale are mechanical properties shaped? (3) Can the processes that generate force be separated from the processes that make tissues stiff?

Tracheal branching morphogenesis and mammary gland development are two examples in which morphogenesis of branched tubular organs or terminal end buds can be studied. Tracheogenesis occurs without mitosis, and thus collective cell migration can be studied in this context without interference from cell proliferation. It has been concluded that the pattern of tracheal branching emerges from the interplay between an extracellular chemoattractant and collective decision making that uses a negative-feedback loop to restrict the number of cells that respond to this chemoattractant [6]. Mammary gland development occurs via the branching morphogenesis of terminal end buds; this branching is unique from most other systems due to the absence of leader cells at the tip of the bud. Instead, the cells at the bud tip for a blunt-shaped multilayered bulb with cells continually exchanging positions [6].

Cancer. Several models originally developed for wound healing has been employed to simulate expansive growth and cell migration of tumors [59, 83, 90]. Both discrete and continuous approaches have been used that consider the effects of mitotic inhibitors, nutrient depletion, cell cycle, and new capillary formation on tumor growth [90]. For example, Tracqui [90] developed models that relate cell motility and traction forces and that are used to simulate the transition from a homogeneous distribution of cells on a tumor surface to a nonhomogeneous density pattern that may correspond to a pre-invasive stage of the tumor.

Colony expansion. Models for wound healing can be also transformed to simulate the process of cell colony expansion [36]. Trepat et al. [9] recorded the cell density of a canine kidney cell population as a function of distance from the leading edge of the cell layer at 24-h time intervals. Growth of the layer plays a prominent role in the context of colony expansion, and Poujade et al. [87] observed that cell proliferation by a colony of cells occurred almost exclusively within the band where cells were originally seeded, potentially due to the longer presence of cells in the originally seeded region or modifications made by cells to the underlying substrate. When applied to a cell-colony scenario, the model in [36] predicts an increase in cell density when approaching the center of the cell colony. The results also suggest that in the experiments of Trepat et al. [9], as in those of Poujade et al. [87], the cells proliferate only in the region originally seeded by the cells.

Conclusion

A multitude of mathematical models of wound healing have been developed in an attempt to understand the qualitative and quantitative aspects of the process. Although many of the models differ substantially in scope, the mechanical and mathematical principles underlying all of the models are related and can be applied to multiple biological systems. The choice of model type depends on the information desired. Certain models are appropriate at a cellular level (e.g., to simulate individual cell motion) while other models are more beneficial on a tissue level (e.g., to represent collective migration).

The study by Stolarska et al. [91] provides a perfect example of differentiating among model types while also highlighting model similarities. In the study, three different cell and tissue mechanics models are presented: a continuous model of an arbitrarily deformable single cell, a discrete model of the onset of tumor growth, and a hybrid continuum-discrete model of later stages of tumor growth. Three essential processes involved with cell migration are captured in the single-cell model: the controlled spatiotemporal remodeling of the actin network, the generation of traction forces to move the cell body, and the construction and destruction of focal complexes or focal adhesions. Cell-level details are incorporated into their tissue-level model, including how an individual cell reacts to forces on it, how cells interact mechanically with their surroundings, and how growth and division are described and how stress affects growth. And thus, predictions obtained across multiple levels of mathematical modeling can be used to gain insight into wound healing processes.

Byrne and Drasdo [59] compare the benefits of using a biophysical agent-based or a continuum mechanical model to track the expansion and migration of cells in a dense monolayer. Single-cell based models permit a higher degree of spatial resolution than models composed of locally averaged quantities; however, large cell population sizes are not amenable to investigation using agent-based models. Ultimately, conditions under which spatiotemporal behavior of the different models agreed were identified in order to determine how to relate the parameters in the different models. The same growth pattern for dense and sparse cell aggregates was obtained using both models.

Khain et al. [86] commented that most theoretical models of wound healing employ reaction–diffusion equations for the cell density and a growth factor. However, in their study they demonstrated that simple discrete models can be applied to wound healing and yield the results obtained from reaction–diffusion equations when proliferation is small. Since biologically reasonable rates of perfusion are small compared to rates of diffusion, both continuum and discrete models provide good predictions of the velocity of a wound edge.

Whether an ODE, PDE, or ABM wound healing model is used to describe the migration of cells in response to an injury, all three model types aim to accomplish three main objectives: to track the cell response and position following the induction of a wound, to understand the role of tissue growth factors in the healing process,

and to predict the time required for a wound to heal. As described in this chapter, the particular choice of theoretical wound healing model dictates the specific phenomena or elements that are most likely to be understood and uncovered.

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Chapter 12
Agent-Based Modeling of Wound Healing: Examples for Basic and Translational Research

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Abbreviations

ABM	Agent-based model
ATP	Adenosine tri-phosphate
DAMP	Damage-associated molecular pattern
DFU	Diabetic foot ulcer
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
GTPase	Guanosine tri-phosphate hydrolase
HMGB1	High-mobility group protein B1
I/R	Ischemia/reperfusion
IEC	Individual epithelial cell
IL	Interleukin
IVSABM	In vitro scratch agent-based model
LM	Laminin
MASON	Multi-agent simulation of networks
mTOR	Mammalian target of rapamycin
Rac1	Ras-related C3 botulinum toxin substrate
ROS	Reactive oxygen species
SCI	Spinal cord injury
SECM	Simulated extracellular matrix

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SPARK	Simple platform for agent-based representation of knowledge
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha

Introduction

Wounds, whether developed in hospital or present on admission, pose a great threat to a patient's health. Wounds provide an opportunity for pathogens to invade, and also divert resources that the body could be using to restore health elsewhere. Furthermore, the inflammatory response incited as a consequence of tissue trauma can lead to extreme complications, especially when this inflammatory response becomes dysregulated.

Breakdown of the wound healing process at any level, leading to both acute and chronic failure of healing, is of interest across medical specialties. In patients with significant co-morbid conditions including diabetes, obesity, or steroid use, these problems are compounded. Not only do wound healing problems cause morbidity to the individual patient, there is also a significant cost to the healthcare system as a whole [1]. Accordingly, there has been significant interest and research effort directed at understanding how wounds heal, with the goal of improved strategies and resources for prevention and treatment of wound-related complications.

However, the overwhelming complexity of the molecular and cellular healing machinery defies study using traditional experimental methods. Much information has been elucidated regarding the roles of various individual components, but these components are generally studied using in vitro systems that are only abstractions of their actual biological reference systems. Adding to the difficulty in obtaining a systems-level view of the healing process is the disparate and often ambiguous information present in the literature. Moreover, while wound healing is well-studied in animal systems [2, 3], only recently have experimental methodologies emerged that may allow for the study of the time courses of wound healing in humans [3]. Even in these settings, it is difficult to collect time courses of primary samples from humans suffering from chronic wound healing diseases without possibly disturbing the very process being measured. Perhaps more importantly from a translational standpoint, it is essentially impossible to modulate all possible mechanisms of inflammation and wound healing in an attempt to find novel therapies.

Wound Healing and Inflammation

Wound healing involves multiple cell types, intertwined signaling pathways, and numerous control and regulatory mechanisms [4, 5]. In general, the process can be divided into three phases: inflammatory, proliferative, and remodeling. The

inflammatory phase of wound healing begins immediately after injury and primarily involves release of mediators to invoke both hemostatic and inflammatory responses [4]. The clotting cascade is initiated first, and the hemostatic plug is assembled on exposed collagen within the wound [4]. In addition to their role in controlling hemorrhage, platelets within the plug release mediators such as platelet-derived growth factor which set the inflammatory cascade in motion. These factors act as chemotactic agents to recruit inflammatory cells, primarily neutrophils, as well as local vasodilators to allow passage of the cells into the damaged tissue [6]. The presence of infectious agents such as bacteria prolongs and exacerbates the inflammatory response, which prevents progression to the proliferative phase and can lead to nonhealing. The inflammatory phase of wound healing will be described in more detail below.

The proliferative phase consists of several key steps to form a temporary yet durable wound closure, which can later be remodeled into the final scar. One of the earliest steps is re-epithelialization, which occurs via several mechanisms. Re-establishment of the epithelium restores its immunologic and barrier function, which is critical to the overall healing process by preventing further infection and propagation of the inflammatory response [7]. Thereafter, angiogenesis occurs and allows for sufficient nutrient delivery to enable deposition of granulation tissue into the wound bed [4].

Remodeling begins once primary wound closure has been achieved, and is a much more prolonged process. Collagen is deposited into organized networks by fibroblasts, and the wound is gradually contracted by the action of myofibroblasts to form the mature wound. Abnormalities of remodeling lead to chronic wound problems such as keloid and hypertrophic scar formation. Although clearly clinically important, this phase of wound healing will be underemphasized in this chapter, as the focus is more on understanding and modulating the acute wound healing environment.

As the initial phase of wound healing, inflammation is critical to successful wound healing. However, inflammation can also cause chronic tissue injury via a positive-feedback loop incited by incidental cell damage [4, 5, 8] and in extreme cases can even lead to distal organ dysfunction and death [9]. As a well-coordinated communication network, inflammation allows organisms to deal with a rapidly changing and often hostile environment. The mechanisms that have evolved to carry out these complex tasks are redundant, robust, and highly context dependent[1] [10]; for example, the same cytokine may have opposite effects depending on other factors present in its local environment. Inflammatory mediators such as interleukin (IL)-6 [11], IL-10 [12], transforming growth factor-β1 (TGF-β1) [13], and nitric oxide [14, 15] all modulate the wound healing response in a highly context-dependent manner. Conversely, while the functions may remain constant, biological redundancy allows for variation in the players that pass individual messages that comprise those macro-functions. For example, not only does the pro-inflammatory response consist of complex signaling by a cascade involving many mediators, but any given mediator participating in each role of that chain may also change from organism to organism or situation to situation.

These intracellular signaling networks and their products, including diffusible molecular mediators, are in essence the carriers of information in the network of inflammatory communication and, therefore, possible targets for intervention. The problem, as mentioned above, is that any given pathway or mediator may exert either beneficial or detrimental effects in a dynamically varying fashion based on the nature of the wound and the particular aspects of the individual patient. Selecting likely therapeutic targets for wound healing—in a rational fashion that takes into consideration this complex system as a whole—is thus a tremendously difficult task.

Agent-Based Modeling

Computational techniques are useful for amalgamating data and generating hypotheses in the study of complex biological phenomena. A mechanistic computational model based on literature knowledge could be validated experimentally or clinically, and in turn may have applications in diagnosing/predicting the wound healing trajectories of individuals or possibly in the design of novel therapeutic modalities for wound healing. Differential equations are the classical method for modeling biological processes and have been used since the late 1980s to explore all phases of wound healing, from inflammation [16, 17], to wound closure [18, 19], to tissue remodeling [20], incorporating terms for mechanical stress, population dynamics, and biochemical signaling [21]. This compendium of equation-based models has resulted in important insights into the wound healing response and has advanced our understanding of wound healing as it is supposed to work, as well as suggesting underlying pathological mechanisms and testing therapies *in silico* [17, 20]. However, while extremely useful, these continuum models cannot be readily used to create tissue-realistic simulations that involve stochastic biological effects, a capability of growing importance given the increased availability of spatial, non-invasive data (such as clinical photographs and real-time dynamic microscopy) along with physico-mechanical information. We suggest that this need can be met, in a complementary fashion, through the use of agent-based modeling.

Agent-based modeling is an object-oriented, rule-based, discrete event computational modeling technique that is well-suited for integrating and synthesizing such data, thereby providing a useful, translational tool for examining the greater wound healing picture. In an agent-based model (ABM), agents representing cellular or molecular components of a system populate a "virtual world," in which their simulated behaviors are governed by rules extrapolated from known knowledge regarding their biological behaviors. This is called dynamic knowledge representation, and can be used for integrating such disparate and scattered information, bridging gaps in the current knowledge base, and generating and instantiating novel hypotheses [22–24]. A significant amount of component-level mechanistic detail can be included in a set of agent rules, and qualitative overall system behaviors examined using a visual interface.

Because the rules in an ABM define local, concurrent interactions and are frequently probabilistic, simulation experiments with an ABM can often lead to non-intuitive and paradoxical behaviors. Such simulated outcomes mirror the translational gulf between basic science and clinical therapeutics [25]. However, as opposed to the seemingly insurmountable translational hurdle facing the traditional biomedical community, the ability of ABMs to cross this translation divide provides an opportunity for researchers to augment their ability to evaluate (and potentially discard) hypotheses that do not pass at least an initial "eye test" in terms of the generated system level output.

ABMs offer another advantage in this role of dynamic knowledge representation intended to increase the efficiency of integrative and translational research. Compared to more traditional mathematical models, ABMs can be more intuitive to non-mathematicians, and therefore be more accessible to the general community of biomedical researchers. This makes agent-based modeling a particularly attractive tool to the biologist or clinician for dynamically representing their hypotheses and allowing them to carry out "thought experiments." [26] This process can lead to the generation of novel, clinically relevant hypotheses that can then be examined in an iterative investigatory loop.

Further reducing the threshold for adoption of agent-based modeling is the fact that the development of a biomedical ABM very often involves the use of an existing software toolkit/development environment. This allows biologists to focus on the implementation of their biological concepts as opposed to the detailed issues related to software design. ABM toolkits that have been used by biomedical researchers include NetLogo [27], Repast [28], and MASON [29]. More recently, the agent-based modeling software Simple Platform for Agent-based Representation of Knowledge (SPARK) [30, 31] was developed to facilitate tissue-realistic modeling in biological contexts. This type of software may be particularly useful as the goal of agent-based modeling expands beyond dynamic knowledge representation to augment basic research to more translational applications.

Agent-Based Modeling of Wound Healing

The ability of ABMs to represent spatial relationships and tissue patterning effects makes this class of models an appealing approach for modeling the biology of wound healing. As with other applications of biomedical agent-based modeling, simulations generated by an ABM concerning wound formation and evolution are marked by global, system-level morphological outputs, that is, spatial patterns with identifiable temporal trajectories. Tracking these morphological features—along with numerical data concerning both the temporal trajectories of individual components (mediators and cell populations) and "experimental/epidemiologic" output derived from performing a series of simulation runs—provides a rich space of output features to which hypotheses can be examined, evaluated, and falsified.

In general, ABMs are developed by following a consistent series of steps. This process typically involves the initial integration of various sources of knowledge, guided by the expertise and intuition of the researcher into a putative hypothesis structure that is then instantiated into the ABM. The ABM must then be calibrated and validated, a process in which the spectrum of behaviors of the ABM is evaluated following iterative manipulation of its parameters (calibration). A separate step (validation) involves determining if the ABM behaves plausibly when compared against data not used in its construction. Finally, simulation experiments must be carried out in order to see if the ABM exhibits properties not previously described in the reference system. This process involves the generating perturbations (the addition of external factors or simulations of knockouts/knock-downs) to the model.

There are several examples of this multi-step process in the context of ABMs of wound healing. In general, these ABMs can be divided into: (1) those intended to provide greater insight into how the system works (as an adjunct to translating and integrating knowledge in a basic science setting), mostly involving the examination of intracellular signaling and gene regulation [32–35], and (2) those focused on characterizing the global system level properties arising from generative mechanisms and what might be done to potentially control them (as an adjunct to translational science and the rational development of clinical therapeutics) [23, 25, 36–41]. In the sections below we will highlight examples of both use cases.

Agent-Based Modeling for Basic Science Knowledge Integration: An ABM of Epithelial Restitution

A wound damages the epithelial tissue layer and exposes underlying tissues to potentially detrimental factors in the external environment [42]. Restitution (reapposition of opposing sides of the damaged epithelium) is the initial phase in the re-establishment of the epithelial barrier and occurs quickly and independently of cell proliferation [7]. Understanding the dynamics of this complex process requires integrating the disparate knowledge present concerning these pathways. Specifically, a substantial amount of study of epithelial restitution and healing has focused on two pathways: transforming growth factor-β1 (TGF-β1) and epidermal growth factor receptor (EGFR). However, despite extensive experimental work on both of these signaling pathways, there is a startling paucity of work concerning the intersection and integration of these two canonical systems. In order to integrate these two areas of study by identifying putative points of crosstalk, as well as investigating the consequent dynamics of epithelial wound healing arising from this synthesis, Stern et al. [33] developed an ABM in NetLogo [27] that is an in silico analog of an in vitro scratch assay. A scratch assay involves scratching a confluent epithelial monolayer with a pipette tip or other instrument to create a reproducible linear defect and evaluating the subsequent healing dynamics time-lapse microscopy with or without fluorescent staining [43]. This ABM was termed the in vitro scratch

agent-based model (IVSABM) and consisted of agents representing individual epithelial cells (IECs) existing within a simulated extracellular matrix (SECM) to generate an in silico analog of an in vitro cell culture. Such in vitro systems are a mainstay of basic science research, but to date most computational/mathematical models developed on the information generated from these cell culture systems are not able to capture the spatial patterning present, thereby losing a rich source of vital information contained in cell culture experiments. Since agent-based modeling is particularly well suited to reproduce exactly this type of output, the visual output of the IVSABM constituted a major advantage in its use as a means of integrating basic science-derived mechanistic knowledge (see screenshots below). Rules governing IEC agent and SECM behaviors were extracted from information present in the literature, and based on this information putative points of crosstalk were hypothesized and instantiated in the IVSABM. This would allow the IVSABM's overall system dynamics to be examined against data from traditional experiments and allow the falsification of non-plausible hypothesis structures.

Model Construction and Overall Architecture

The population of IEC agents represents a single confluent epithelial cell layer, with each agent occupying a distinct space (patch) on the grid. Intracellular proteins and cell surface receptors were assigned as agent state variables, and diffusible factors, such as secreted mediators, were assigned as patch variables. IECs interact with their environment via surface receptors and secrete factors that then become patch

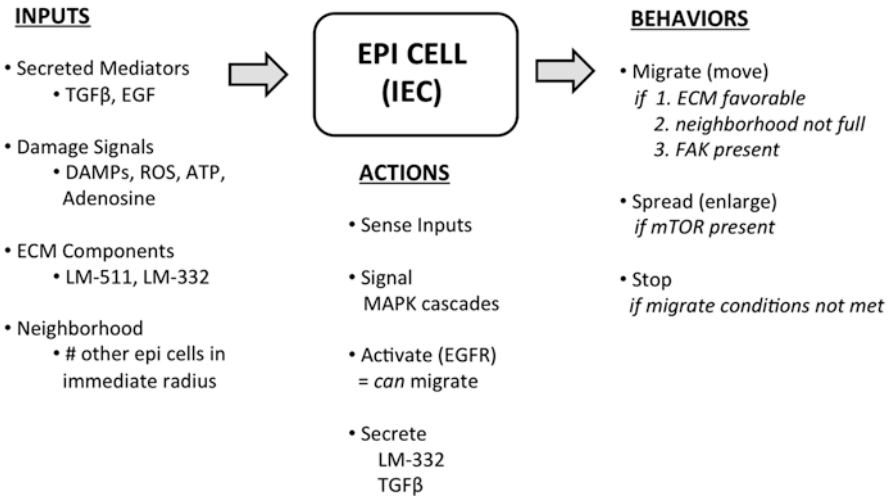

Fig. 12.1 State diagram for IEC agent in the IVSABM. This schematic depicts the inputs, outputs, and actions of an IEC agent. (Reprinted with permission from Ref. [33])

variables in the SECM. For an overview schematic of the components and interactions in the IVSABM, see Fig. 12.1. At baseline all IECs have intact tight junctions with all of their immediate neighboring cells. When a "scratch" is introduced, this results in a linear defect of cells and matrix across the center of the grid. IECs at the wound edge were considered to be "damaged" and able to elaborate damage signals, reactive oxygen species (ROS), damage-associated molecular pattern proteins (DAMPs), and extracellular ATP, into the media as stimulatory variables for IECs. During each simulation run, IECs signal through their surface receptors in response to values of available stimulatory ligand on the patch they occupy. Bound receptors are no longer available to interact with additional ligands, but after signal transduction to intracellular components they are re-constituted to allow re-activation of the pathway if further ligand is available. Binding of a ligand to a receptor also removes the ligand from the patch it is on. Intracellular signaling cascades were modeled without signal amplification as 1:1 interactions, in which downstream molecules are assigned a value equal to their corresponding upstream molecule with each iteration of the simulation. Levels of intracellular molecules decremented at a set rate in order to simulate the actions of phosphatases and other degradative pathways. "Migrating" IECs move onto adjacent open patches in a semi-random manner, which leads to re-population of the damaged area of the grid. As IECs move away from their neighboring IECs, tight junctions are broken; these tight junctions are re-formed when migrating IECs come back in contact with other IECs. Migration pauses when migrating IECs reach a distance of greater than 1 patch from any neighbor and resumes once the trailing cells have closed the gap. This mimics the "sheet-like" movement behavior of cell monolayers undergoing restitution as observed in vitro. The IVSABM is considered to be "healed" when IECs from one side of the scratched monolayer become apposed with cells from the opposite side and the stimuli for signaling mechanisms cease.

As noted above, epithelial re-apposition has largely been studied, as have most biological processes, on an individual, pathway-by-pathway basis, with particular attention paid to signaling from TGF-β and activation of EGFR. These two canonical pathways are each recognized as critical and necessary components of epithelial cell restitution; however, cross-pathway interactions between the two have been poorly characterized. Following a review of the literature, both of these pathways were instantiated in the IVSABM (see Fig. 12.2 for a depiction of the components of the TGF-β1 and EGFR pathways included in the IVSABM). Based on this knowledge, three hypothetical mechanisms for interaction and control between the two pathways were proposed at the level of integrin-EGFR cross-phosphorylation and activation (see Fig. 12.3 for a depiction of these potential mechanisms):

1. *Mechanism 1*: Laminin-332 (LM-332), an extracellular matrix molecule secreted at the wound edge by IECs, binding to integrin α3β1 leading to intracellular signaling through Src.
2. *Mechanism 2*: Direct signaling from LM-332 binding to an as-yet unidentified receptor to Rac1 independent of EGFR activity.

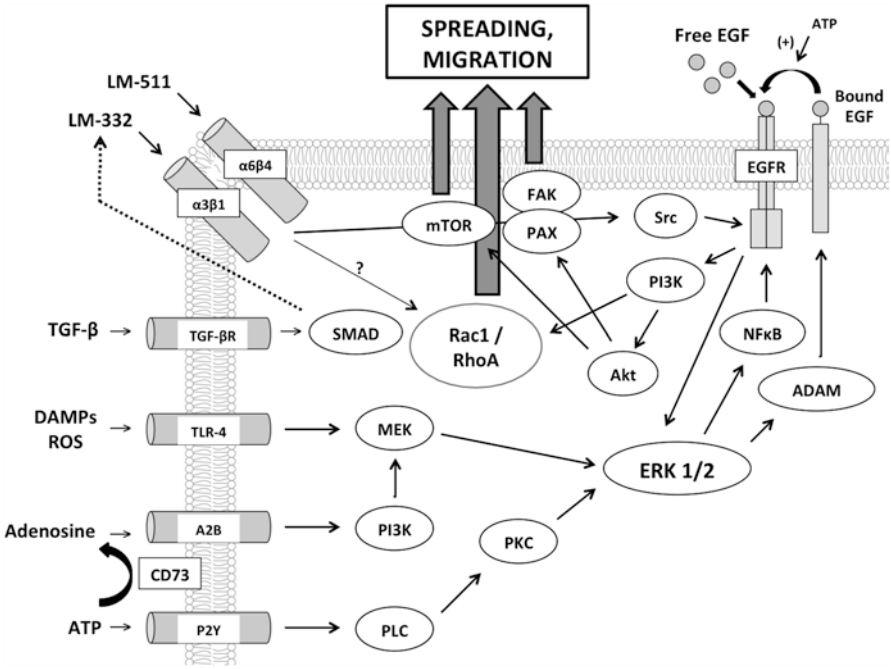

Fig. 12.2 EGFR and TGF-β pathways instantiated in the IVSABM. A depiction of the pathway components and interactions incorporated into the IVSABM. (Reprinted with permission from Ref. [33])

3. *Mechanism 3*: Extracellular binding of an EGF-like domain of LM-332 directly to EGFR.

These mechanisms were instantiated into the IVSABM code to examine their respective plausibility (see *Simulation Experiments* below). Rac1, a small GTPase known to be an end-effector of epithelial migration and restitution [44], was used as a quantitative marker for healing capacity among IEC agents.

Model Calibration: System-Level Dynamics

Baseline wound healing was examined in a series of calibration/validation simulations. IECs interacted with the extracellular environment and produced effector molecules leading to a migratory phenotype. DAMPs, ROS, and ATP produced by the initial scratch injury-initiated stimulation of IECs, leading to an initial surge of EGFR signaling. Subsequently, EGFR activity was maintained at a relatively constant level by a signaling loop from laminin–integrin interactions. IECs from opposing edges of the monolayer migrate in a sheet-like pattern inwards through the described intracellular, cell–cell, and cell–matrix interactions. When the IECs

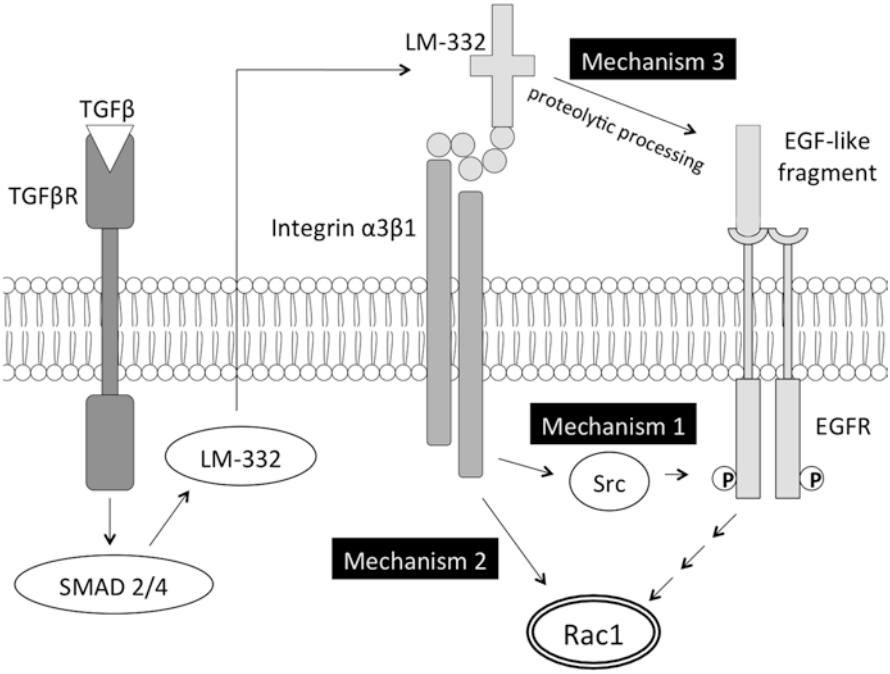

Fig. 12.3 Proposed mechanisms of crosstalk between EGFR and TGF-β pathways. *Mechanism 1*: Laminin-332 (LM-332), an extracellular matrix molecule secreted at the wound edge by IECs, binding to integrin α3β1 leading to intracellular signaling through Src. *Mechanism 2*: direct signaling from LM-332 binding to an as-yet unidentified receptor to Rac1 independent of EGFR activity. *Mechanism 3*: extracellular binding of an EGF-like domain of LM-332 directly to EGFR. (Reprinted with permission from Ref. [33])

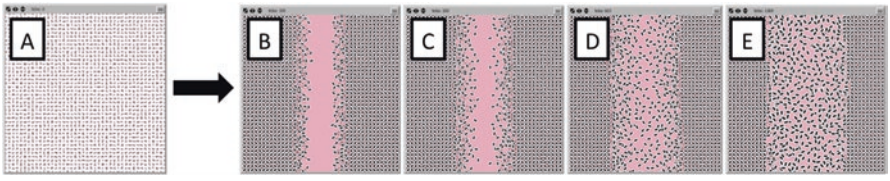

Fig. 12.4 Screenshots of IVSABM demonstrating successful healing of the scratch wound. The intact simulated monolayer prior to induction of the scratch wound is represented in panel **a**. Panel **b** depicts the scratch wound as a linear defect across the center of the IEC monolayer. During the course of the simulation run IECs migrate inwards to close the wound space until the two sides are re-approximated (**c**–**e**), resulting in a healed monolayer. (Reprinted with permission from Ref. [33])

re-attain "confluence," migration ceases and the wound is considered healed. The quantitative timescale of the IVSABM is such that one iteration of the simulation corresponds to approximately 1 min. The IVSABM was successfully calibrated such that the condition using serum-based media, which takes approximately 24 h (1440 min) to heal [45], heals in approximately 1300–1400 ticks. Figure 12.4 demonstrates successive screenshots of successfully healing simulations.

Additionally, since EGFR activation is absolutely required for epithelial healing in vitro [46], a simulated knockout of EGFR in the IVSABM should similarly lead to a lack of restitution. When a functional EGFR knockout IVSABM was studied, the healing capacity was indeed completely abolished. In the computational code, signals must go through EGFR in order to reach the effector molecules for migration (Rac1) and spreading (mTOR). This must in fact be the case *in vitro* as well; otherwise, there would be some minimal level of healing seen through pathways which circumvent EGFR. As such, the ABM represents at least a minimally sufficient overall mechanism for wound healing, and a plausible integrative construction of the major signaling pathways involved.

Simulation Experiments

As noted above, a primary benefit of computational dynamic knowledge representation is the ability to falsify clearly implausible or incorrect hypotheses; doing so automatically increases the efficiency of the experimental workflow by directing resources to more fruitful investigations. Therefore, the simulation experiments with the IVSABM involved introducing perturbations known to generate a particular biological outcome, and then evaluating the model's consequent behavior to see if it matched what is observed in the real world. Toward this end, simulation experiments were designed to determine if simulated knockouts could meet the necessary/sufficient criteria for EGFR and TGF-β1 in terms of overall system behavior and healing for each of the three putative points of crosstalk between TGF-β1 and EGFR—*Mechanism 1*: LM-332 binding to integrin α3β1 leading to intracellular signaling through Src; *Mechanism 2*: direct signaling from LM-332 binding to an as-yet unidentified receptor to Rac1 independent of EGFR activity; *Mechanism 3*: extracellular binding of an EGF-like domain of LM-332 directly to EGFR. As noted above, simulated EGFR knockouts did not heal; therefore, simulation experiments were performed with simulated TGF-β1 knockouts, looking at wound closure rates as well as levels of Rac1. The advantages of an ABM's ability to produce a visual/spatial output are immediately evident upon examining the visual output of the IVSABM at the end of the simulation runs. Figure 12.5 demonstrates IVSABM screenshots of simulations run with Mechanism 1, Mechanism 1 + 2, Mechanism 2, and Mechanism 3. Of these, the failure of Mechanism 3 to heal is readily apparent, and therefore that hypothesis can be discarded. More information about the outcomes seen in these simulations can be obtained by plotting the tabular data generated by the IVSABM, specifically levels of Rac1. In these simulations, Mechanism 1 produces accurate wound healing dynamics, suggesting that produced levels of Rac1 are sufficient for healing. Furthermore, this satisfies the condition that EGFR must be absolutely necessary since there is no way for these cells to signal directly from integrins to Rac1. Mechanism 2, where integrin signals lead directly to Rac1, also produces plausible healing dynamics, but occurring with substantially higher levels of Rac1. However, Mechanism 2 does not satisfy the necessity of EGFR,

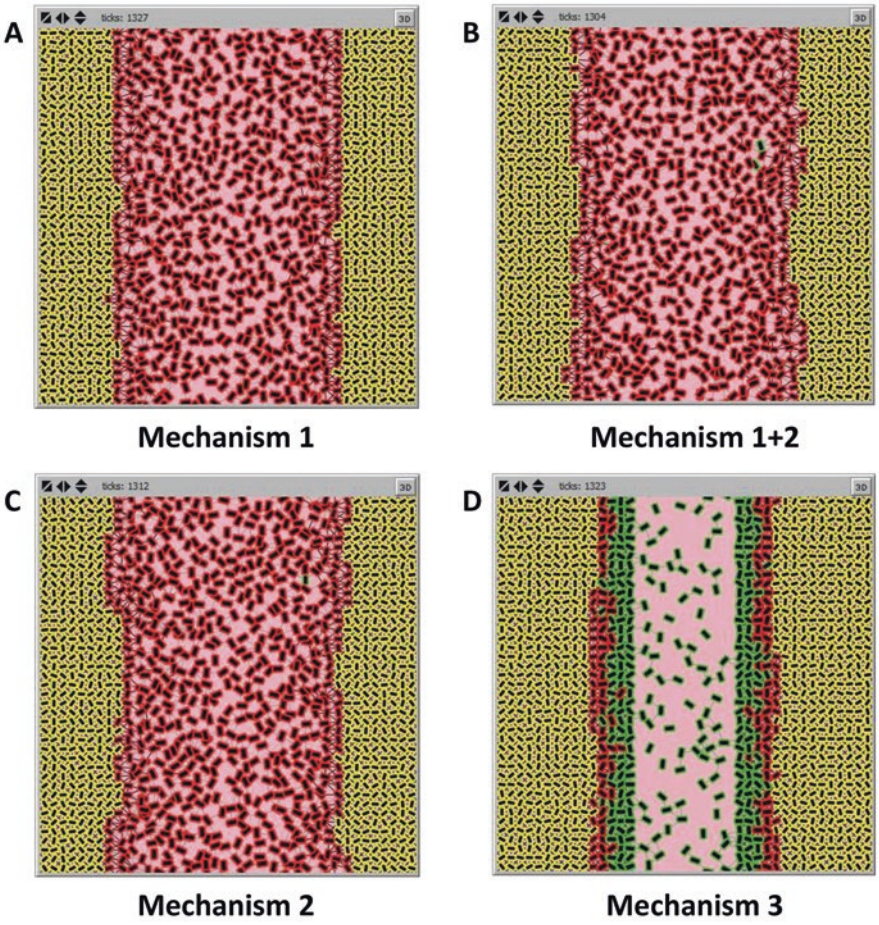

Fig. 12.5 Screenshots of IVSABM simulation experiments evaluating different hypothesized mechanisms for crosstalk between EGFR and TGF-β pathways. Due to insufficient healing of the monolayer Mechanism 3 can be rendered implausible and therefore discarded

which suggests that while integrin signaling leads to pro-motility effectors, there must be an intermediate step involving EGFR. Therefore, Mechanism 2 is deemed implausible by not fitting with known observations and can be discarded. Mechanism 3, where a fragment of LM-332 binds directly to EGFR, does not lead to complete wound healing in the ABM, as is seen in Fig. X5. Analysis of the visual output in conjunction with the generated Rac1 levels provides more information about why Mechanism 3 fails: this mechanism satisfies the EGFR requirement, but at levels of LM-332 that are normally sufficient for healing there is insufficient activation of EGFR downstream pathways. Given these findings, Mechanism 1 seems to be the most consistent with current knowledge and can therefore serve as a guide for

additional experimentation to further refine mechanistic knowledge of restitution and suggest potential failure points associated with disease phenotypes.

Agent-Based Modeling as a Clinical–Translational Aid: An ABM of Pressure Ulcer Formation in Spinal Cord Injury Patients

Pressure ulcers are a common complication of hospitalization and are especially common in spinal cord injury (SCI) patients [47]. In acute care in the United States, pressure ulcers affect 2.5 million patients per year, costing up to $1.5 billion. As many as 60,000 of these patients die each year from complications due to pressure ulcers. There are several accepted theories about how ulcers form, but the current standard of care is labor-intensive and patients still develop ulcers daily. Serial, non-invasive wound imaging combined with ABMs could, in theory, allow for the investigation of both space- and time-dependent dynamics via visually realistic, mechanistic simulations.

Accordingly, an ABM was created in which pressure ulcer formation was simulated as arising from alternating pressure, as a patient might experience when being turned into and out of a position where pressure over a bony prominence reduced local perfusion. In the model, repeated cycles of ischemia followed by reperfusion cause tissue damage, inducing inflammation, which leads to additional damage caused by pro-inflammatory positive-feedback mechanisms in a cyclical fashion (Ziraldo et al, submitted). The ABM was calibrated against serial images of post-SCI pressure ulcers obtained from patients following Institutional Review Board approval and informed consent. Cell-level behaviors encoded in the ABM led to tissue-level phenotypes described in the literature [48, 49]. The model also recapitulated visual patterns of ulcer formation in SCI patients, while it was only calibrated on mechanistic, not visual data.

Model Architecture

This ABM was built using knowledge from the literature, as an extension of an existing ABM of pressure ulcer formation [50]. The agents that comprise this model are cells and tissues (neutrophils, macrophages, epithelial cells, blood vessels), with each cell type represented by its own class of agent. Data layers are employed to simulate diffusible cytokines, free radicals, oxygen, oxidase enzymes, and sometimes drugs. The model architecture consists of a layer of tissue cells, fed by blood vessels throughout the tissue, carrying oxygen and inflammatory cells to the field. The basic model depicts a tissue region composed initially of healthy cells. In the absence of perturbation, the tissue remains intact for a reasonably long time (decays

on a timescale much slower than the processes we are simulating). Pressure is simulated by a constriction of the blood vessels, decreasing the amount of material that can flow through them. Without oxygen, tissue cells are compromised and their health begins to decline. This stress leads them to release diffusible "danger signals" (damage-associated molecular patterns [DAMPs]), mediators that stimulate the inflammatory response [51].

I/R Mechanism: Implementation and Validation

In addition to ischemic injury (lack of oxygen reduces the health of epithelial cells), the model incorporates an additional method of tissue injury on pressure release: reperfusion injury. Ischemic cells build up the capacity to produce damaging free radicals upon reintroduction of oxygen. This is modeled by the accumulation of oxidase enzymes inside ischemic cells. When pressure is released and oxygen again perfuses these cells, oxygen free radicals will be formed in proportion with the concentration of oxidase present in that cell. Free radicals cause damage to the immediate cell and those they encounter via diffusion, and they do so in a stepwise manner: epithelial cells show no sign of damage from radicals until they have accumulated a certain threshold of insults. At that time, their health is drastically reduced. Varying the length of pressure cycles (turning rate) and measuring total tissue damage after a fixed period of ischemia revealed that all else being equal, a period of ischemia will cause less tissue damage than the same period of ischemia followed by a reperfusion event. These results agree with in vivo studies carried out in rats, wherein ischemia reperfusion was simulated using a compression via a magnet and a steel plate surgically implanted under the epidermis [48].

Inflammation Mechanism: Implementation and Validation

Three diffusible mediators represent the canonical early pro-inflammatory response, the canonical slower pro-inflammatory response, and the canonical anti-inflammatory response, each of which is secreted by activated neutrophils or macrophages (type I or II). This version of the model contains both neutrophils and macrophages, which are initially in a resting state. They are activated by local concentrations of mediators in a threshold-dependent manner. DAMPs, another data layer, above a certain local concentration will activate nearby neutrophils to produce early pro-inflammatory mediators (called TNF-α hereafter). At a certain threshold of local TNF-α concentration, resting macrophages will be activated to a type I phenotype and begin secreting longer-acting pro-inflammatory mediators (called IL-1β hereafter). TNF-α also causes damage to nearby epithelial cells, thus re-stimulating the pro-inflammatory response. Local concentration of IL-1β above a threshold activates macrophages to type I (pro-inflammatory) phenotype and above

a higher threshold; IL-1β induces macrophages to adopt a type II (anti-inflammatory) phenotype. Active type II macrophages produce anti-inflammatory mediators (called TGF-β1), which above a threshold cause further activation of type II macrophages.

The rules governing the inflammatory mechanisms in the model are based on dynamics of acute inflammation, so these dynamics were tested in a simulated acute wound, without repeated pressure cycles. In a successful incorporation of these mechanisms, tracking activation of neutrophils and macrophages would reveal cellular dynamics similar to those found in settings of acute inflammation. Since the mediators in the simulation represent amalgams of several mediators, it makes more sense to validate dynamics at the cellular level. An initial injury was created in the center of the tissue, pressure removed, and the dynamics of inflammatory cells were tracked in the field. Because the model is calibrated to real clock time, these results are directly comparable to measurements from published sources, and qualitative agreement was sufficient. The relative timing of peaks of cell populations was as expected. Interestingly, a single set of initial conditions and parameter values was able to give rise to two distinct outcomes. In all simulations, the initial injury was sufficient to incite the inflammatory response. In approximately 70% of cases, this inflammatory response became self-sustaining and led to an ulcer. In the remaining 30% of simulations, however, the inflammatory response resolved early enough that the tissue suffered minimal damage beyond the initial injury. This variation mimics variation in a population of patients who may present with the same intensity of disease, receive the same treatment, but experience very different disease progressions.

Sensitivity Analysis and In Silico Trials

Sensitivity analysis was used to explore the range of behaviors possible from the model. This group of methods gives the modeler a measure of which parameters account for the greatest amount of the variance in the model's output. For this model, parameters were partitioned according to which mechanistic cause of damage they could be attributed (I/R injury or pro-inflammatory ancillary damage). These experiments revealed that either increasing oxygen availability or the rate at which pressure is applied and released (simulating a patient being turned) led to predictions of improved outcomes, but that changing inflammatory parameters only led to modest improvement (Ziraldo et al, submitted).

The ABM was then used as a platform to investigate potential treatments in silico. To examine the effects of suppressing acute inflammation in manner more severe than tweaking parameters, a trial of corticosteroid application was simulated in silico. While this might be a controversial treatment plan in the clinic because corticosteroids are broadly considered to put one at risk for chronic, non-healing wounds [52], simulating this treatment strategy virtually allowed us to assess potential benefits without any negative consequences. In this in silico clinical trial of

steroid treatment, steroids applied at early enough time points and at a high enough dose were effective in stemming the local inflammatory response, leading to predictions of improved outcomes in the early stages of pressure ulcer formation and progression. However, as suggested by the sensitivity analysis mentioned above, suppressing inflammatory damage was not sufficient to prevent an ulcer from forming. The ischemia/reperfusion injury was eventually enough to cause an ulcer in the tissue.

In this ABM, damage-associated molecular pattern (DAMP) molecules were key signals that led to inflammation following tissue injury. The DAMP high-mobility group protein-B1 (HMGB1) has emerged as a therapeutic target for inflammatory diseases [53–55]. Accordingly, an in silico trial of a putative, neutralizing anti-HMGB1 antibody therapy was implemented. In these simulations, this strategy was not successful in stemming the inflammatory response, whether incited by repeated ischemia/reperfusion injury or an initial acute tissue injury. Mechanisms encoded in the ABM allow activation of the inflammatory response via cell damage, without an explicit diffusible signal (Ziraldo et al, submitted), which we hypothesize may account for the apparent non-effect of the anti-HMGB1 antibody treatment. Together, these in silico trials and sensitivity analyses led to the conclusion that while inflammation is definitely an aggravating factor in pressure ulcer formation, ischemia is the most prominent cause of tissue injury.

Discussion and Conclusions

In silico mechanistic models provide unique opportunities to study inflammation and wound healing dynamics, which is of obvious clinical importance. Recent ABMs not only have allowed mechanistic insight, but also provide an inexpensive and accessible platform for hypothesis testing, simulating clinical trials, designing patient-specific therapies, and developing diagnostic tools. By providing a means of synthesizing disparate aspects of biomedical knowledge, mechanistic computational modeling provides a plethora of opportunities to continue exploring, make new insights, and ultimately help patients [5, 23, 33, 41, 56–59].

These systems have been extensively studied and are indeed well-characterized, but in proceeding beyond the understanding of simple mechanistic detail to examination of whole system dynamics, computational models appear to have a useful role. The models presented here represent different levels of resolution of knowledge: molecular detail leading to cellular level dynamics in the epithelial restitution model, and cellular detail leading to tissue level dynamics in the pressure ulcer model. Furthermore, the models represented an even wider range of resolution of outputs. The decision of what resolution to model each system is determined by the available literature and how it relates to the modeling goals of the individual modeler. For example, epithelial restitution has primarily been studied in the context of the relative importance and contributions of various signaling pathways to epithelial behavior. Hence, the goal of this model was to integrate known data at this level of

resolution and provide information regarding amalgamated cellular behavior—exactly what the primary literature has been unable to achieve via traditional studies. Similarly, with regard to the pressure ulcer model, the impact of the early inflammatory response on ulcer progression was the goal of the modeler and this was achieved by integrating cellular and tissue-level detail. Both of these represent significantly important contributions, in that they fit into "knowledge gaps" in their respective knowledge bases.

Incorporating physically and physiologically relevant information into ABMs, such as blood flow in tissues and forces between cells, allowed for new mechanistic predictions. In the case of the former, a simple approximation of tissue blood flow differences between non-injured subjects and SCI patients, when juxtaposed on a stochastic model of inflammation and tissue injury, led to the prediction of higher propensity to ulcerate in SCI patients vs. controls. Even visual information can be deceiving, as illustrated by agent-based simulations of restenosis. Static cross-sections of arterial restenosis suggested that neointima entered through a break in the external elastic lamina of a coronary artery, and therefore the degree of hyperplasia was thought to be proportional to the size of the rupture [60, 61]. However, time courses yielded by dynamic simulations revealed an alternative possibility. Infiltrating cells appeared to be actually pushing on the edges of the ruptured external elastic lamina, forcing it wider. This insight might impact the analysis of histological samples of balloon-injured arteries and may also affect the design of novel therapies aimed at mitigating these physical forces.

Incorporating tissue realism into ABMs of pressure ulcer formation has raised the possibility of non-invasive diagnostics and possibly therapy based on serial macroscopic images of non-healing wounds. Due to the individualized and context-dependent nature of the inflammatory response, a tool that could help clinicians decide the best course of treatment based only on visual information could be revolutionary. Such an in silico diagnostic could improve patient care. This model has also been used to simulate a variety of treatment strategies, paving the way for fast, inexpensive early stage trials of newly designed treatments.

There are, of course, limitations to this approach. Simulations of biological processes are necessarily approximations. In order to balance computational cost with a model's utility, models must be parsimonious, including the smallest number of elements that will still yield a model that is useful for gaining mechanistic insights, clinical applications, or both. Often, modeling choices can be driven by the availability of data rather than which elements might be most critical to or informative of a process. These choices are subjective, and one can always find a justification to include additional elements in a given model, as long as those elements increase the validity or utility of the model. An implication of this simplified version of reality is that a model can only be useful to a certain level of resolution. It is important to keep in mind the scope a model can reach when probing it for insights.

Each of the models here represents a framework upon which further work can be added. The modular nature of ABMs allows more detail to be added as more information is gathered—for example, when a new pathway is elucidated and published in the scientific literature, this can be easily incorporated into an established model.

If the model has been well validated, then changes seen in the model output with new information blocks can be enlightening with regard to both the plausibility of the new discoveries and the accuracy of the model itself. In fact, model outputs that are incongruent with expectations are often the most useful, as they provide the equivalent of scientific falsification and allow more accurate model calibration going forward.

The next frontier for tissue-realistic mechanistic computational modeling is really to form bridges between visual simulation outputs and clinical images. Image analysis methods could allow comparisons of simulations of pressure ulcers to photos of patients' wounds, extracting pertinent details that cause some simulations/subjects to resolve their wounds, while others progress to deranged inflammation and inappropriate healing. At the molecular level, models that can use concentrations of mediators as inputs to or outputs from equation-based models will have an advantage. Eventually, the goal is to link together local dynamics with the larger context of a patient's body, and thus truly allow for rational, patient-specific diagnosis and therapy.

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Chapter 13
Multiscale and Tissue Realistic Translational Modeling of Gut Inflammation

Chase Cockrell and Gary An

Abbreviations

ABM	Agent-based model
Akt	Protein kinase B
BMP	Bone morphogenetic protein
GEC	Gut epithelial cell
Hh	Sonic hedgehog homolog
HPC	High-performance computing
IBD	Inflammatory bowel disease
I-FABP	Intestinal fatty acid binding protein
PTEN/PI3K	Phosphatase and tensin homolog/Phosphoinositide 3-kinase
RIP	Receptor interacting protein kinase
SEGMEnT	Spatially explicit general-purpose model of enteric tissue
TLR	Toll-like receptor
Wnt	Wingless-related integration site

Introduction

Inflammation in the gut is associated with a range of pathophysiological processes, including inflammatory bowel disease [1], gut-derived sepsis [2], environmental enteropathy [3], necrotizing enterocolitis [4], and cancer [5]. The mucosal surface of the gut is also the major interface between an individual and their microbiota. There is an intimate bidirectional relationship between the architecture and function of the gut mucosa, and as such the histological characterization of the mucosal architecture is a central feature in the diagnosis of intestinal disease [6]. This relationship between the control of tissue patterning via morphogenic pathways and the

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threat-response nature of inflammation can become disturbed to produce pathological conditions and associated with characteristic histological changes. Given the multiscale complexity of these cellular and molecular processes, agent-based modeling can serve as a useful means of integrating wide-ranging knowledge concerning mucosal dynamics and gut inflammation while reproducing the spatial relationships noted histologically in various conditions. The ability to generate different patterns (corresponding to different disease processes) from a shared set of generative processes (as is the case in the real world) can serve as a translational bridge between the knowledge acquired through basis biomedical research and clinically relevant phenotypes.

The dynamics of the intestinal mucosa has been the subject of much computational modeling, and how these projects have examined tissue patterning of the mucosa falls into two general categories: (1) those examining de novo morphogenesis of the mucosal architecture via modeling the generative processes governing crypt–villus growth [7–10] and (2) models with a fixed crypt architecture within which there is examination of morphogen spatial distribution [11, 12], cell type distribution [13–15], and homeostasis [14, 15]. What is more unusual, however, are projects that have the capability to reproduce variations in the mucosal architecture that mirror histological changes used to define different disease states clinically; this is an important representational capability if such a model is to have translational utility. One such model is the spatially explicit general-purpose model of enteric tissue (SEGMEnT) [16]. SEGMEnT incorporates the growth and migration dynamics of gut epithelial as well as how those processes interact in the face of inflammation while being able to reproduce variations of morphologic topology via alterations in the crypt–villus configuration and tissue architectural features such as the location of the crypt–villus junction, relative and absolute sizes of the crypt and villus. This capability allows for comparing SEGMEnT's output with various histological phenotypes representing different states of health and disease in the gut.

The Spatially Explicit General-Purpose Model of Enteric Tissue (SEGMEnT)

General Description

SEGMEnT is a cell-level agent-based model (ABM) [16]. ABMs are object-oriented, discrete event, rule-based computational models consisting of populations of computational entities (agents) that follow programmed rules governing their behavior with respect to the environment and interactions with other agents [17–22]. SEGMEnT represents the three-dimensional structure of the enteric mucosa by wrapping a two-dimensional interaction surface representing the epithelial layer of the intestinal crypts and villi over an array of rectangular prisms that reflect the volumes of the crypts and villi (Fig. 13.1). SEGMEnT includes the following cell

types: gut epithelial cells (GECs), including their subtypes of stem cells, differentiating and mature enterocytes, and two main lineages of inflammatory cells, neutrophils and macrophages/monocytes. As with many ABMs, SEGMEnT organizes its cell-agent rule structure into a series of functional modules: (1) intracellular morphogenic signaling pathway, (2) intracellular inflammatory signaling pathway, (3) cell state transitions for proliferation, differentiation, and movement, and (4) spatial diffusion of morphogens to define gut epithelial cell behavior for the homeostatic maintenance of the spatial architecture of the enteric mucosa (Fig. 13.2). Figure 13.2 depicts cellular processes (Fig. 13.2a), processes linked to GEC type (Fig. 13.2b), spatial location in terms of the crypt–villus architecture (Fig. 13.2c), and expected gradients of different morphogens [wingless-related integration site (Wnt), bone morphogenetic protein (BMP), sonic hedgehog homolog (Hh), β-catenin, and protein kinase B (Akt)] are shown in Fig. 13.2d [16]. SEGMEnT employs an expandable architecture that will allow the subsequent addition of functional modules to allow representation of additional enteric features.

SEGMEnT was used to investigate two general types of potentially pathogenic conditions: (1) those resulting from acute insults/perturbations, namely local tissue injury resulting in a wound and the response to enteric ischemia-reperfusion, and (2) chronic conditions that result in a persistent alteration of the homeostatic setpoint of the crypt–villus architecture. For this latter case we use the example of colonic metaplasia of the ileal pouch following restorative surgery for ulcerative colitis, thought to be induced by chronic, low-level inflammation due to fecal stasis [23–25]. In the course of model development, it became apparent that the

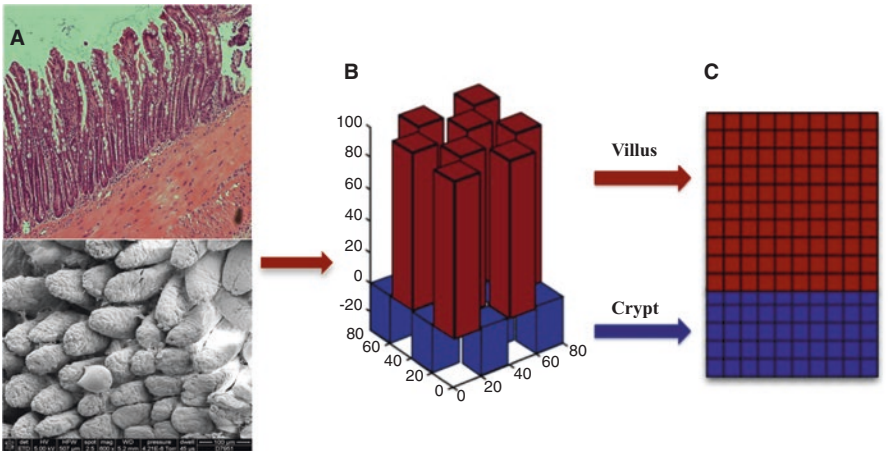

Fig. 13.1 SEGMEnT topology. Panel **a** contains a histology cross section of ileal tissue (top) and scanning electron microscopy of the mucosal surface of ileum (bottom). These images are juxtaposed with Panel **b**, which is the topology used by SEGMEnT where crypts and villi are represented with a matrix of rectangular prisms. Each individual crypt or villus is then "unwrapped" onto a two-dimensional grid (Panel **c**), on which signaling interactions, morphogen diffusion, and physical cellular actions take place. (Reproduced from Ref. [16] under the Creative Commons License)

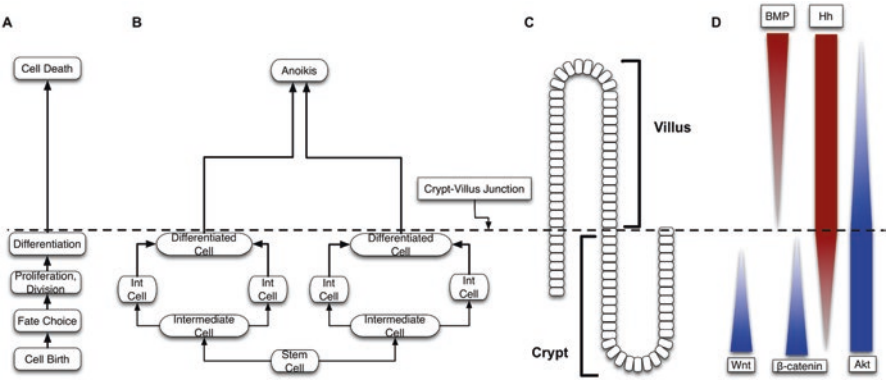

Fig. 13.2 Overall schematic of SEGMEnT, depicting cellular processes (Letter **a**), processes linked to GEC type (Letter **b**), spatial location in terms of the crypt–villus architecture (Letter **c**), and expected gradients of different morphogens (Letter **d**). Biologically realistic gradients for wingless-related integration site (Wnt), bone morphogenetic protein (BMP), sonic hedgehog homolog (Hh), β-catenin, and Akt are shown. (Reproduced from Ref. [16] under the Creative Commons License)

recognized links between inflammation and tissue patterning in the gut were not sufficient to generate the metaplasia phenotype. This insufficiency in the current state of knowledge led us to hypothesize a link between inflammation and the induction of apoptosis through the phosphatase and tensin homolog/phosphoinositide 3-kinase (PTEN/PI3K) pathway, a relationship present in other tissues [26] but not previously suggested in the gut. We demonstrate that SEGMEnT, with this putative mechanism, is then able to generate colonic metaplasia of the ileum as seen after surgery for ulcerative colitis. SEGMEnT is the dynamic knowledge representation of a minimally sufficient set of components, mechanisms, and interactions for the maintenance of enteric mucosal architecture (morphogenesis) and the effect of inflammation.

Behaviors: Calibration and Validation

Multicellular biological systems exist in states of dynamic equilibrium where the ongoing interactions between the different constituent cells lead to a stable overall multicellular pattern [27, 28]. Achieving this baseline stability is therefore the initial level of validity an ABM needs to display. For SEGMEnT this means being able to maintain the dynamically stable configuration of gut epithelial cells as they are generated, migrate and eventually undergo apoptosis, as well as reproducing the appropriate spatial distribution of molecular signaling gradients reported in the literature.

Ileal tissue was targeted for baseline calibration and validation. The baseline homeostatic crypt–villus configuration in the ileum consists of a crypt depth approximately 1/4 the villus height (approximately 1 mm) [29]. Spatial gradients exist for

morphogens such as Wnt, which is highest at the base of the crypt and zero at and above the crypt–villus junction [30–35]. The BMP molecule exists everywhere in an isotropic distribution [36], while the gradient of BMP activity is initiated at the crypt–villus junction progressively maximizes toward the tip of the villus [36–38]. Other morphogens included in SEGMEnT, Ephrin-B ligand/EphB receptor, Hh, and β-catenin, also exhibit spatial gradients [30]. Furthermore, the cellular components of the epithelium undergo a complete renewal every 5 days. SEGMEnT was calibrated to produce stable cell populations over time with a 5-day turnover of enterocytes as they move from the base of the crypt (where they are generated by stem cells) and migrate to the tip of the villus where they eventually undergo apoptosis and slough. The crypt–villus junction is defined by the point at which undifferentiated enterocytes transition to become differentiated. This functional switch is defined by the end of the Wnt morphogen gradient and the degradation of β-catenin after the activation of the β-catenin destruction complex (see Fig. 13.2d). A screenshot of the profile of a SEGMEnT crypt–villus complex at homeostasis is seen in Fig. 13.3 (reproduced from Ref. [16]). Similarly, the distribution of both extracellular and complexed BMP with suppression of BMP complexing in the crypts, and transition to BMP activity at the crypt–villus junction with maximal BMP activity at the villus tip, was able to be reproduced compared to histological data [16].

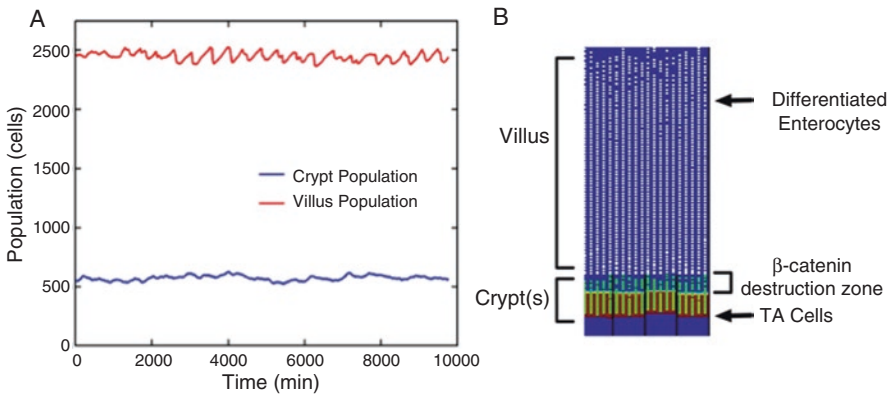

Fig. 13.3 Baseline healthy ileal tissue dynamics. Panel **a** displays murine intestinal tissue stained for β-catenin to be compared with the output from a simulation for healthy baseline ileal tissue at homeostatic equilibrium (Panels **b** and **c**). Panel **b** demonstrates stable cell populations in the crypt and villus over time with complete renewal of the epithelial tissue every 5 days of simulated time. Panel **c** displays a SEGMEnT screenshot at homeostasis. Note that this 2D projection is the unwrapped 3D topology seen in Fig. 13.2. All subsequent SEGMEnT screenshots are presented in this fashion to aid in the interpretation of its behavior, but are simulated in a 3D model. Differentiating GECs in the crypts are seen as green circles; white circles represent differentiated GECs on the villi. The red shading in the background represents areas of wingless-related integration site (Wnt) activity; the absence of Wnt is seen as a blue background. Note that at the crypt–villus junction there is a region of undifferentiated GECs with no Wnt; this is the zone after the activation of β-catenin destruction complex and before all the β catenin has been destroyed, at which point differentiation is triggered and occurs. (Reproduced from Ref. [16] under the Creative Commons License)

Selective pathway component knockout and/or inhibition studies are a mainstay of molecular biology [39]. The results of these types of experiments are often used to infer the mechanistic role of the targeted pathway component. From the standpoint of modeling and simulation, this approach can be viewed as model component sensitivity testing and form a ready set of real-world experimental reference sets against which to evaluate the plausibility of a dynamic computational model. A series of simulation experiments were performed with SEGMEnT replicating three knockout/inhibition conditions: Wnt inhibition, Hh inhibition, and PTEN inhibition. The results of these simulations were then compared to existing corresponding published experimental results, showing respectively: (1) Wnt inhibition resulting in progressive loss of crypt, then villus cells and sloughing of the entire mucosa, and (2) Hh inhibition producing increased Wnt activity and crypt hyperplasia. PTEN was chosen to be included into SEGMEnT to provide a link between the morphogenic pathways and inflammatory signaling via a down regulation of Hh [35, 40]; as such SEGMEnT provides an example of dynamic knowledge representation by instantiating the hypothesis that upregulated PTEN that is responsible for the downregulated Hh, with subsequent effect on GEC apoptosis. Thus, simulation of PTEN inhibition demonstrates a "null" consequence of SEGMEnT's PTEN representation on baseline morphogenesis; we hypothesize that its role would only become significant in the perturbation case involving inflammation. The simulation experiments demonstrate an experimentally demonstrated transient effect on the crypt GEC population and which subsequently recovers with compensation via Hh [40].

Having been able to generate baseline homeostatic behavior and replicated knockout/inhibition experiments, then next set of SEGMEnT simulation experiments focused on reproducing clinically relevant pathophysiological processes known to involve the effect of inflammation on the mucosal histology.

Wound healing is a fundamental and highly conserved function of multicellular organisms, and part of the basic homeostatic capability of the gut is to heal epithelial damage up to a certain point without negative consequence. Figure 13.4 depicts the simulation of localized epithelial injury in SEGMEnT. A tissue wound/injury is induced by having all cells on the villi to die simultaneously by necrosis (Fig. 13.4a Arrow 1). After the injury, the loss of Hh signaling and Wnt inhibition results in rapid growth of undifferentiated GECs in the crypt (Fig. 13.4a, Arrow 2), which migrate back up the crypt to reconstitute the villus (Fig. 13.4a, Arrow 3). Necrotic cells are cleared by inflammatory cells (see Fig. 13.4b–d) and allow restoration of

Fig. 13.4 (continued) Panels **b**–**d** display three screenshots from a simulation of localized epithelial damage/injury. Undifferentiated transit amplifying (TA) cells are shaded in blue. Differentiated enterocytes are shaded in red. Necrotic cells are shaded in black. Panel **b**, captured at $t = 750$ min, demonstrates that the epithelial insult results in necrotic cell death throughout the majority of the villus. Panel **c**, captured at $t = 2250$ min, shows that the necrotic cells continue to damage surrounding tissue until clearance by macrophages and neutrophils (not explicitly represented in these screenshots). Panel **d**, captured at $t = 4500$ min, demonstrates the recovered tissue architecture after regrowth of the epithelial cell populations

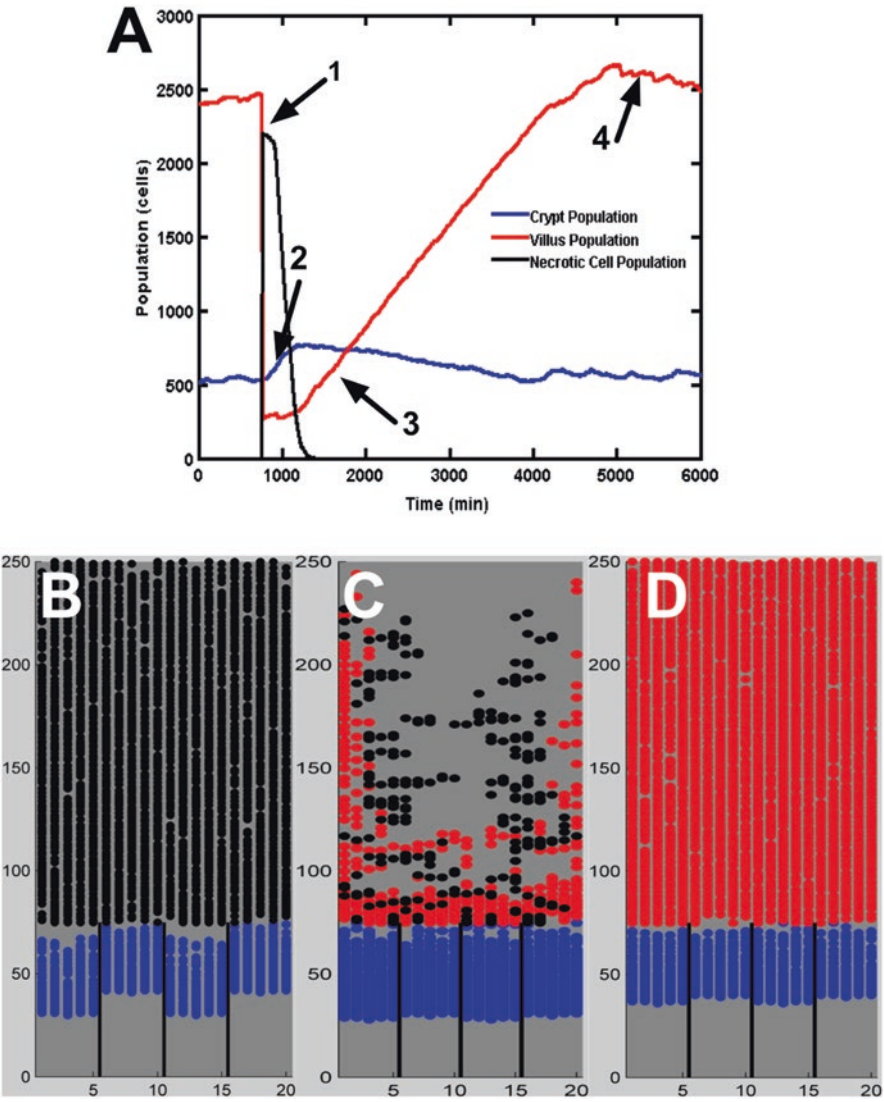

Fig. 13.4 Local injury to the gut mucosa and subsequent reconstitution of crypt-villus architecture. Panel **a** displays populations of living gut epithelial cells (GECs) on the crypt and villus, and necrotic cells upon local tissue injury and subsequent healing. Injury is induced by causing necrosis of an entire villus, resulting in a rapid drop of villus GEC population and spike in necrotic cell population (Arrow 1). In the time period directly subsequent to villus death the crypt grows rapidly; this is due to the sudden loss of Sonic Hedgehog Homolog (Hh) signaling as most of the differentiated cells on the villus have died. The death of the villi cells reduces the Wnt inhibition of the surviving crypt GECs, resulting in a growth spike in the crypt population (Arrow 2) that precedes the reconstitution of the villus population (Arrow 3). All during this process the inflammatory response is clearing the necrotic cells, allowing the regulatory functions of the morphogen pathways to normalize, leading to regrowth of the villus back to the homeostatic state (Arrow 4)

the regulatory functions of the morphogen pathways, with subsequent regrowth of the villus back to the homeostatic state within 24 h (Fig. 13.4a, Arrow 4).

Another clinically relevant means of creating intestinal mucosal injury is via intestinal ischemia/reperfusion (I/R). Intestinal ischemia can occur when the body diverts blood away from the mesentery in shock states, when there is arterial obstruction due to abnormal blood clotting or following certain types of major surgery. Correcting these disturbances leads to a reperfusion phase with a rapid influx of blood and circulating immune/inflammatory cells that encounter an endothelial surface that has been primed by the ischemic period [41]. This leads to a dramatic change in the enteric mucosal tissue, with activation of tissue inflammation, cellular death, and turnover [42]. Reperfusion also leads to sloughing/shedding of ischemic cells into the intestinal lumen. Other researchers have noted that this sloughing of damaged GECs is actually an adaptive protective mechanism: by removing necrotic cells that can stimulate inflammation, their sloughing reduces the forward feedback loop that can propagate inflammation [43, 44]. We implemented a proposed mechanism for GEC sloughing in SEGMEnT, the accumulation of intestinal fatty acid binding protein (I-FABP) in ischemic cells [43], and then tested this hypothesis by simulating the contrafactual condition by artificially reducing the sloughing rate of the GECs and measuring its effect on the degree of crypt/villus injury. We first performed simulations across a range of ischemic periods from 30 min to 6 h, followed by reperfusion; these resulted in tissue disruption and recovery patterns consistent with what has been reported in the literature [43]. Up to 5 h of ischemia the simulated mucosa was able to recover normal crypt/villus architecture; however, by 5 h of ischemia the protective effect of sloughing is insufficient to prevent a persistent alteration in the tissue architecture, with perpetually stunted villi. At 6 h ischemia there is nonrecoverable injury, with necrosis widely present in both villus and crypt and propagating inflammation and leading to viable crypt and villus populations dropping to zero, consistent with the recognized response of the gut to ischemia reperfusion injury [41, 42]. We then tested the hypothesis that GEC sloughing is protective to I/R injury by performing simulations where FABP-mediated sloughing is deactivated. The lack of sloughing increases the number of necrotic cells in place, leading to increased inflammatory stimuli and the propagation of inflammation-mediated damage. This phenomenon is seen in the conversion of readily recoverable ischemia at 30 min and 3 h, to correspondingly persistent effects on the tissue architecture at 30 min ischemia and tissue necrosis with 3 h of ischemia. These results reinforce the plausibility that sloughing has a protective effect [43].

Another known biological process that leads to potentially pathogenic alterations of the intestinal mucosa is metaplasia. Metaplasia is the reversible transformation of one type of tissue architecture into one resembling another type of tissue. It is distinct from neoplasia or dysplasia in that the cells themselves do not exhibit dysfunctional features, but rather alter their differentiation path toward a different terminal cellular phenotype. Metaplasia therefore can be seen as an adaptive response to an abnormal growth environment and is often seen in chronic diseases. One example of intestinal metaplasia is seen in the small intestine following definitive surgery for ulcerative colitis resulting in the creation of an “ileal pouch.” Definitive surgery for

ulcerative colitis involves complete removal of the colon and rectum, and the rectal vault is reconstructed by creating a looped pouch of the terminal ileum. This ileal pouch then serves as a neo-reservoir for the stool in an attempt to more closely approximate normal bowel habits for these patients. However, this altered configuration and function produces changes the tissue environment for the ileal mucosa in the pouch. Even though the composition of the intestinal contents entering the pouch may not be considerably different than before, in the presurgical situation those intestinal contents transit relatively rapidly past the ileal surface while now those contents are subject to stasis, leading to an alteration of the environment for the ileal mucosa and can result in *colonic metaplasia* of the pouch epithelial tissue [23–25]. While not a true conversion to colonic tissue, the metaplastic epithelial architecture exhibits defined changes that more closely resemble colonic tissue: a change in the crypt–villus relationship where the crypts deepen and the villi become shortened and an increase in the relative population of goblet cells (mucous producing cells) [23–25].

Chronic, low-level inflammation has been associated with colonic metaplasia, and has been implicated as a mechanism driving the alterations seen in the mucosal architecture [25]. However, the known connections between inflammatory signaling and the morphogenesis pathway [45–50] did not produce the appropriate tissue architecture alterations, that is, increasing crypt depth and shortened villi, consistent with the metaplasia phenotype. We recognized that the key function that needed to be invoked in order to produce the shift in crypt–villus architecture was apoptosis of GECs, which would affect the height of the villi. However, known effects of inflammation on cell-death pathways are primarily either antiapoptotic (i.e., NFκB) or pronecrotic (i.e., receptor interacting protein kinase, or RIP), neither of which would generate or is associated with a colonic metaplasia phenotype in the ileal pouch. To address this mechanistic gap we identified a potential link between inflammation and the induction of apoptosis is through the PTEN/PI3K pathway [26] and hypothesized that GEC apoptosis could be induced via the PTEN/PI3K pathway. This hypothetical mechanism would potentially generate the appropriate crypt–villus morphology of pouch metaplasia by increasing the rate of GEC apoptosis thereby shortening the villus height, and inhibiting Hh production to reduce inhibition on the Wnt pathway and increase the size of the proliferative compartment in the crypt.

The effect of fecal stasis in the pouch was simulated as prolonged low-level inflammation mimicking increased toll-like receptor (TLR) signaling from overgrown microbiota. The effect of this condition on the crypt–villus architecture was evaluated in terms of alterations of the crypt–villus ratio as well as absolute changes in both crypt and villus dimensions. Figure 13.5a displays crypt and villus GEC populations when the system is exposed to chronic low-level TLR4 signaling (an abstraction of fecal stasis). This upregulation leads to an increased rate of apoptosis, shortening the villus, as well as an inhibition of the Hh pathway, which leads to an increase in the size of the proliferative compartment. Figure 13.5b displays output from SEGMEnT when simulating conditions leading to colonic metaplasia. Crypt hyperplasia and villus atrophy are clearly evident compared to normal ileal

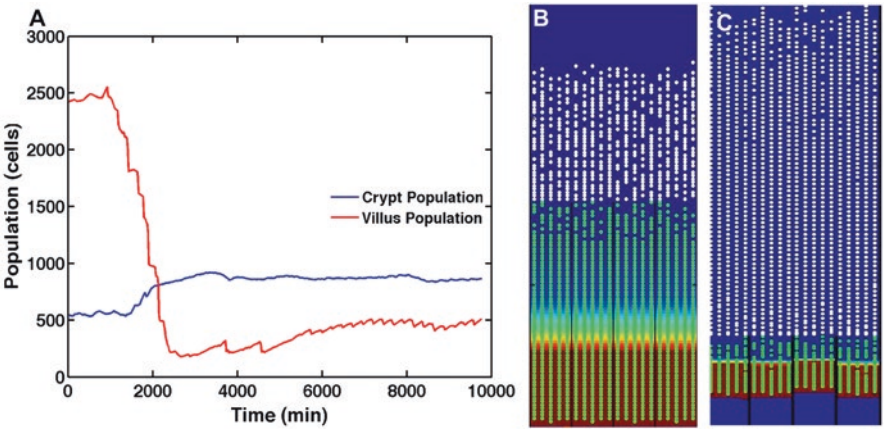

Fig. 13.5 Colonic metaplasia in the ileal pouch. Panel **a** displays average crypt and villus gut epithelial cell (GEC) populations after exposure to sustained low-level toll-like receptor (TLR4) stimulation and signaling (an abstraction of fecal stasis). This low-level upregulation of inflammation communicates via our hypothesized phosphatase and tensin homolog (PTEN) mechanisms, leading to increased apoptosis, shortening the villus, as well as an inhibition of the sonic hedgehog homolog (Hh) pathway, which increases the size of the proliferative compartment (i.e., crypt). Panel **b** displays a screenshot from SEGMEnT when simulating conditions leading to colonic metaplasia. Crypt hyperplasia and villus atrophy are clearly evident (compare with normal homeostatic condition in Panel **c**), along with a shift in the villus to crypt height ratio that matches the alterations reported in colonic metaplasia. (Reproduced from Ref. [16] under the Creative Commons License)

crypt–villus configuration (Fig. 13.5c), with a villus to crypt height ratio that matches the alterations seen in colonic metaplasia [25].

Anatomic Scale: Whole Organ Simulation with SEGMEnT_HPC

A natural evolution of the use of ABMs is being able to create cell-for-cell level representations of entire organs, that is, anatomic scale ABMs. The utility of anatomic-scale is evident in intestinal disease where multiple biological/organizational levels interact to generate clinically relevant system phenotypes: cellular tissue patterning; locoregional phenomena, including tumor growth and surgical wound healing; and organ-level distribution of disease as seen in inflammatory bowel disease. The regional nature of intestinal disease suggests that shifts in the heterogeneous distribution of microenvironments plays an important role in the pathogenesis of disease; in fact these shifting microenvironments are intrinsic to the function of the intestinal tract, which involves the transit of nutrients and waste contents throughout its length. This in turn suggests that effective characterizing clinically relevant disease phenotypes needs to capture these multiscale phase

transitions that occur as microlevel cellular and molecular interactions cascade and manifest at anatomic scale. However, the recognition of the scale of such an endeavor is daunting when one realizes the actual number of cells present: the intestinal tract over 500 billion cells. Fortunately, the capabilities and availability of parallel high-performance computing (HPC) platforms, from cloud computing resources to stand-alone exascale supercomputers, have grown significantly in the last decade. This makes tractable the implementation of ABMs representing millions and billions of cellular agents. As a clinically relevant anatomic-scale example we extended the simulation of intestinal metaplasia performed on the original SEGMEnT to the representation of the clinical entity, ileal pouchitis. Given that the progression of pouchitis and the spatial distribution of the stimuli that drive it (fecal stasis, microbial overgrowth, and chronic inflammation) are not homogenous throughout the entirety of the pouch, anatomic scale simulations are required in order to plausibly simulate the complicated interplay of ileal and rectal (colonic) tissue with a dynamic microbiome. The original version of SEGMEnT could represent up to 1.4 mm^2 area of tissue on a single processor with 2 GB of memory. Therefore, we undertook an HPC implementation of SEGMEnT, called SEGMEnT_HPC, and used it to simulate ileal pouchitis/metaplasia at clinically-relevant anatomic scale [51].

The design of SEGMEnT_HPC would discretize the surface of the pouch into individual patches of mucosa of a size that could be implemented on a single processing core of the HPC machine. This geometric partitioning allowed us to minimize the need to deal with load-balancing and latencies associated with the core-to-core communication. Figure 13.6 depicts the multiscale nature of the SEGMEnT_HPC implementation of an ileal pouch. Figure 13.6a presents an illustration of the ileal "J-pouch." Figure 13.6b illustrates how the j-pouch is modeled as a cylinder and distributed to the processing cores. Figure 13.6c shows how crypt and villus topologies are represented on a single processor. Figure 13.6d shows how these topologies are unwrapped to form a series of two-dimensional grid. Finally, Fig. 13.6e displays the actual biological system that SEGMEnT_HPC represents. Crypts and villi that are located on the spatial processor boundary contain an additional buffer in order to maintain continuity with neighboring processors. The buffer extends all grids by one cell length in order to synchronize cellular motion and chemokine diffusion across processes.

Figure 13.7 displays 16 mm^2 cutouts from a healthy tissue sample (Panel a), tissue with an applied ulcer (Panel b), and tissue recovering from severe inflammation (Panel c). Pouch metaplasia simulations were performed on 12,672 processors. The simulated pouch had a diameter of 3.5 cm and a length of 6 cm, which is approximately ½ of anatomic scale [52]. Inflammatory potential of the stool was calibrated such that it maximized at the value necessary to induce the metaplasia demonstrated in Ref. [16]. Figure 13.7d shows a 16 mm^2 area of epithelial tissue from the above simulation of pouch metaplasia. This tissue section has been unwrapped such that it is presented as a rectangle.

SEGMEnT_HPC demonstrates the clear potential to scale up to anticipated exascale HPC platforms. We estimate that SEGMEnT_HPC would require 450,000

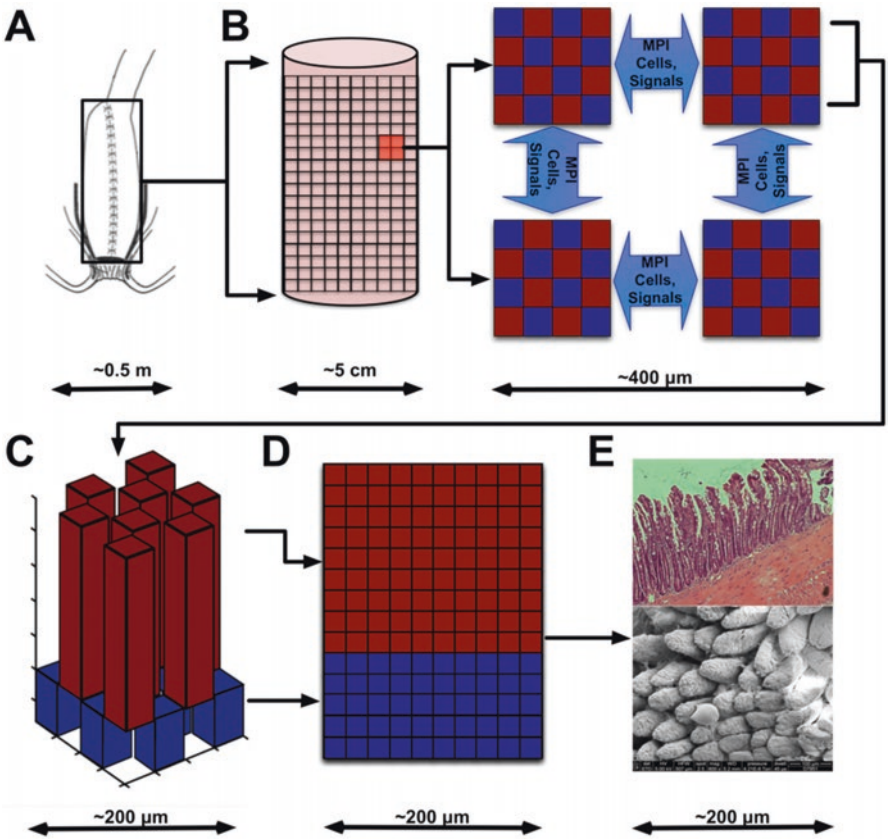

Fig. 13.6 Panel **a** presents an illustration of the ileal "J-pouch." Panel **b** illustrates how the J-pouch is modeled as a cylinder and distributed to the computational processes. Panel **c** shows how crypt and villus topologies are represented on a single processor. Panel **d** shows how these topologies are unwrapped to form a series of two-dimensional grid. Finally, Panel **e** displays the actual biological system being which SEGMEnT_HPC represents. (Reproduced from Ref. [51] under the Creative Commons License)

processing cores for 30 h (at the current temporal resolution) to simulate the healthy colon for 1 year. We estimate that the simulation of the small and large intestines together for 1 year would require approximately 2.4 million processing cores for 40 h. However, in order to plausibly simulate inflammatory bowel disease, additional cell types will have to be added to SEGMEnT. A module representing the adaptive immune system will be necessary to simulate inflammation on a time scale greater than a few days. We anticipate that this will incorporate multiple T-cell lineages as well as further characterization of the functional properties of the microbiome, including microbial population dynamics.

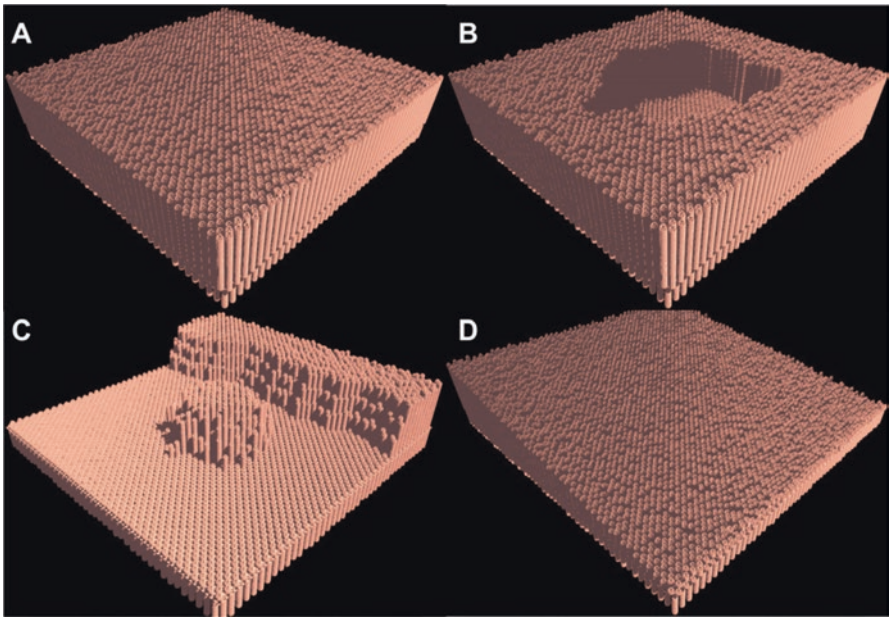

Fig. 13.7 Tissue renderings: These panels display 16 mm^2 tissue sample cutouts (Panels **a**–**c**) and a 20 mm^2 cutout (Panel **d**). These renderings are a postprocessing output of SEGMEnT_HPC. Cellular spatial location information for select processing cores is written to file. These coordinates are then mapped from their two-dimensional grids back onto the topology that they represent. The entire simulation is not rendered due to size. Panel **a** presents healthy tissue in homeostasis. Panel **b** presents tissue with an applied circular ulcer, which spreads slightly due to inflammation. Panel **c** presents tissue recovery after inflammatory stimuli has been removed. Panel **d** presents tissue that has been exposed to an inflammatory gradient as described in the sample output section above. (Reproduced from Ref. [51] under the Creative Commons License)

Challenges in Anatomic Scale Modeling: Buffering

It should be noted that, at present, nearly all HPC systems with >10,000 processing cores are distributed memory systems [53], meaning that each processing core has a unique allocation of memory, only accessible by that core (or potentially other cores on the node). This can introduce complications for cells located at the boundaries of the discretized space. Consider Fig. 13.6, Panel c: one can see how, upon differentiating and exiting a crypt at the boundary of the panel, the cell would then begin to climb the villus simulated by a different processing core. In order to keep a proper accounting of all cellular populations and diffusible cytokines, crypts and villi that are located at the boundary of the discretized space have their grids extended by a single cell length. These buffers are synchronized using Message Passing Interface (MPI) at each time step, and all cellular or molecular information is exchanged between processing cores at that time.

Challenges in Anatomic Scale Modeling: Load Balancing

When simulating systems in which the spatial population distribution is approximately homogenous, load balancing is of minimal concern; however, in systems such as cancer, in which the spatial density of cells can increase significantly (due to both tumor and infiltrating immune cells). This will result in an increased workload for the processors responsible for that discretized spatial region, and allow other processors to idle, which can become a significant waste of both time (the model is not efficiently exploiting parallelism) and resources (the machine is using electricity but producing nothing). This must be balanced against the cost to transfer large data structures over the HPC network—performing a perfect load balance upon each time step of the simulation would likely end up costing more than if one juts accepted and ignored the inefficiencies.

Conclusion

The primary challenge in biomedicine is being able to translate basic science cellular and molecular knowledge into clinically relevant and effective interventions. Dynamic computational modeling in the context of Translational Systems Biology aims at crossing this gulf by using these models as means of dynamic knowledge representation and making explicit the link between putative generative processes and the system level behaviors seen in the clinical setting. A key step in this process is the ability to identify intermediate metrics by which the validity of the computer models can be assessed. Tissue histology is an example of just such a metric, and it plays a particularly significant role in the diagnosis and classification of intestinal disease. Being able to represent the spectrum of histologically identifiable tissue patterning is therefore a key feature of Translational Systems Biology models of intestinal disease, and SEGMEnT fulfills these criteria:

1. SEGMEnT replicates the dynamically stable morphology and cellular populations of the healthy ileum, while qualitatively matching the spatial distribution of molecular signaling gradients consisting with the existing qualitative histological criteria utilized and reported in the literature [30–36, 38].
2. SEGMEnT reproduces the effects of inhibition of various signaling pathways by successfully representing the resulting phenotypes in terms of alterations of the crypt–villus architecture [30–36, 38].
3. SEGMEnT demonstrates the ability to recover from local wound injury as would be expected from real intestinal tissue.
4. SEGMEnT reproduces the mucosal response to ischemia/reperfusion, including the protective role of enterocyte sloughing.
5. SEGMEnT is able to generate the effect of chronic inflammatory simulation from microbial overgrowth seen in ileal pouchitis, resulting in colonic metaplasia, and can do this at clinically relevant anatomic scale. In so doing, SEGMEnT

demonstrates the plausibility of PTEN as a cross-talk nexus between inflammation and epithelial patterning in the continuum effect of inflammation on the genesis of colonic metaplasia.

SEGMEnT remains an ongoing project, with future areas of development related to incorporating our existing ABMs on necrotizing enterocolitis [54], gut derived sepsis [55] and, importantly, intestinal cancer, both in terms of oncogenesis [56] and as a platform for evaluating potential therapies [57]. It is our hope that the ongoing, concurrent development of multiscale dynamic models, such as SEGMEnT, enhanced utilization of high-performance computing and model-directed basic science and clinical studies, will form the fundamental structure for biomedical research on the pathophysiology and treatment of gastrointestinal disease in the future.

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Chapter 14
Data-Driven Modeling of Liver Injury, Inflammation, and Fibrosis

Ruben Zamora and Yoram Vodovotz

Abbreviations

APAP	Acetaminophen
APAPo	APAP overdose
DBN	Dynamic Bayesian Network
DyNA	Dynamic Network Analysis
HMGB1	High Mobility Group Box 1
IL	Interleukin
MCP-1	Monocyte Chemotactic Protein-1/CCL2
NAC	N-acetylcysteine
PALF	Pediatric Acute Liver Failure
PCA	Principal Component Analysis
T/HS	Trauma/Hemorrhagic Shock

Introduction

The effective translation of basic mechanistic knowledge into clinically relevant therapeutics is fraught with difficulties, especially in the context of "systems" processes/disorders that involve inflammation [1, 2]. Over 15 years ago, the United States Food and Drug Administration (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html) outlined the ever-rising expenditure on research and development concurrent concomitant with a steady decrease in therapeutic drugs and devices. We refer to this as the Translational Dilemma [1, 2]. Translational Systems Biology is a potential a framework by which to translate high-throughput data to

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practical clinical outcomes, via the intermediary of intertwined mechanistic and data-driven computational models [1–4] at multiple scales of organization.

Nowhere is this need more acute than in the context of liver failure. Many biochemical processes key to metabolism are located exclusively or primarily in the liver, and more specifically in the hepatocytes that comprise 80% of this organ [5]. Liver failure is a prototype of complex disease that has defied therapeutic modulation [6]. Our work has focused on deciphering key aspects of the multiscale complexity of liver failure through an iterative combination of computational modeling and experimental studies. We suggest that the complexity of this biology, as well as the lack of clear mechanisms that account for how a fibrotic liver relates to a failing liver, requires analysis methods beyond typical statistics. We have described previously our systems biology approach to characterize the inflammatory response to stress in clinical acute liver failure [7–9], liver tissues [6, 10–12], and isolated liver cells [6, 9, 13, 14]. Below, we discuss the lessons gleaned from these studies, as well as our attempts at bridging across preclinical/in vitro studies to clinical insights and biomarkers using data-driven computational modeling. While this chapter is focused on the liver, the methods and approaches are likely to be informative and useful to investigators in other fields as well.

Data-Driven Modeling: Clinical Insights into Pediatric Acute Liver Failure

Our primary application of data-driven modeling in the context of liver disease involves examining the systemic inflammatory responses of pediatric acute liver failure (PALF) patients [7–9]. PALF is a complex, catastrophic, rapidly evolving clinical syndrome. The clinical trajectory of PALF is dynamic and the precise onset of disease rarely identified, with an exception being acute ingestions (e.g., mushrooms, acetaminophen [APAP]). Patient outcome is reflected, in part, by the interaction among etiology, disease severity, supportive management, and treatment. Yet, outcomes vary among children with seemingly similar etiology, disease severity, and treatment. It is therefore likely that a multitude of factors are involved in the pathophysiology of PALF. Such factors likely include a complex interaction among the inflammatory milieu, end-organ damage, immune activation, potential for liver regeneration, and clinical interventions [15, 16]. Perhaps for these reasons, this is a relatively under-studied disease.

We hypothesized that dynamic networks of immune/inflammatory dysregulation drive outcomes in PALF, and that analysis of these dynamic networks and principal inflammatory characteristics would shed insights into the clinical diversity inherent in PALF. In our initial studies [7], we assayed a multitude of inflammatory mediators on stored serum samples obtained from approximately 50 children in the PALF Study Group (PALFSG). Data were subjected to Dynamic Bayesian Network (DBN) inference to suggest connectivity among inflammatory pathways as well as

potential central control nodes, using data obtained serially over 7 days and outcomes defined at 21 days. DBN is a probabilistic network discovery algorithm that shows a single graph structure for a full time course of data, and can indicate potential feedback loops [17, 18]. These studies focused on three core clinical outcome groups: spontaneous survivors, nonsurvivors, and survivors with liver transplantation. Whereas unprocessed inflammatory mediator levels assessed over time did not distinguish among PALF outcomes, DBN analysis revealed distinct chemokine-related networks that segregated spontaneous survivors from nonsurvivors. The DBN pattern identified in patients who underwent liver transplantation was more like that seen in spontaneous survivors than in those who died, suggesting the possibility of a permissive inflammatory milieu (or, intriguingly, that some liver transplant patients may have survived even without a transplant) [7].

This work was extended to include serum samples from over 100 children enrolled in the PALFSG, again collected over the first week following enrollment [8]. Despite heterogeneity in individual inflammatory responses as in our prior study [7], DBN inference largely recapitulated those prior results. However, since additional inflammatory mediators were assayed, DBN inference identified a common network motif with the damage-associated molecular pattern molecule high-mobility group box 1 (HMGB1) as a central/hub node in all groups. This finding suggests a common central inflammatory network response in PALF. The networks in spontaneous survivors and liver transplant recipients were similar, and differed from those of nonsurvivors (as in the initial study [7]). In addition to DBN inference, we also utilized Dynamic Network Analysis (DyNA, an alternative, nonprobabilistic dynamic network inference algorithm) [19]. This analysis suggested similar dynamic connectivity in spontaneous survivors and liver transplant recipients versus a more highly-interconnected network in nonsurvivors that increased in complexity with time [8].

The next PALF study focused on a specific etiology of acute liver failure in children, namely APAP overdose (APAPo) [9]. As mentioned above, we assayed multiple inflammatory mediators in serial serum samples from over 100 study participants, including 13 PALF survivors with APAPo+N-acetylcysteine (NAC, the frontline therapy for APAPo), 8 non-APAPo+NAC, 40 non-APAPo non-NAC, and 12 nonsurvivors. Based on both DBN and DyNA, HMGB1 was a dominant mediator in dynamic inflammation networks in all subgroups, associated with a threshold network complexity event 1–2 days following enrollment that was exceeded in nonsurvivors versus survivors. We thus hypothesized that differential HMGB1 network connectivity after day 2 is related to the putative threshold event in nonsurvivors, and this was borne out in in vitro studies (see below) [9]. Importantly, this study highlights the value of a computational approach to the discovery and contextualization of molecular phenomena, including in the process of "reverse translation" from clinical to experimental data and back.

Most recently, we have focused on delineating the age-related differences in systemic inflammation associated with PALF (Zamora et al, unpublished observations). Age-specific differences in the ontogeny of immune/inflammatory responses to viral infections and vaccines have been characterized. However, there are no data

exploring age-specific immune/inflammatory responses in PALF. Accordingly, we measured relevant inflammatory mediators in daily serum samples collected over the first week following enrollment from five distinct PALF cohorts (all spontaneous survivors without liver transplantation): infants (<1 years old), toddlers (1–2 years old), young children (2–4 years old), older children (4–13 years old), and adolescents (ADO, 13–18 years old). Among those groups, we observed significant ($P < 0.05$) differences in alanine aminotransferase (ALT), creatinine, and 17 circulating inflammatory mediators. Furthermore, we found different dynamic patterns and overall dynamic inflammatory network complexity as follows: older children > infants > toddlers > adolescents > young children. This is an interesting and not necessarily intuitive result since it is not linear with age, and merits further study. Thus, distinct inflammatory trajectories and networks are associated with age-specific clinical markers in PALF, and this is likely an important area for future studies involving data-driven modeling.

Data-Driven Modeling: Tissue-Scale Insights in Liver Tissue Preservation

In PALF as well as multiple other liver diseases, liver transplantation is often the sole viable treatment option. As is the case with other organs, the number of transplants that can be performed is limited not just by the number of acceptable donor grafts, but also by the viability of these grafts at the time of transplantation. Unfortunately, the current standard of cold and hypoxic preservation often leads to the loss of organ viability between procurement and transplant. Extracorporeal organ preservation with machine perfusion providing oxygenation, in which organs are isolated in an ex vivo environment over an extended timespan, is a concept that has led to the development of numerous alternative preservation protocols designed to better maintain viability [6].

We carried outs several studies focused on gaining novel insights into inflammatory and metabolic changes associated with a novel, ex vivo normothermic machine perfusion system in preclinical studies of porcine livers [11, 12]. In initial studies, we utilized principal component analysis (PCA) to help suggest principal differences in the metabolomic responses of porcine livers preserved with standard cold static preservation versus a novel, normothermic machine perfusion solution. Those studies suggested that machine perfusion enhanced antioxidant defenses and improved carbohydrate metabolism as compared to cold static preservation [11].

In subsequent studies, we focused on defining dynamic networks of inflammation in both liver tissue and the perfusate of the machine-perfused versus cold, static preserved porcine livers [12]. We measured 14 inflammatory mediators and carried out DBN and DyNA studies. These studies pointed to an NLRP3 inflammasome-regulated response in both treatment groups, driven by the pro-inflammatory cytokine interleukin (IL)-18 and the anti-inflammatory mediator IL-1 receptor antagonist

(IL-1RA). This finding is especially interesting given the extensive interest in the role of the NLRP3 inflammasome in liver diseases [20]. Both DBN and DyNA suggested a reduced role of IL-18 and increased role of IL-1RA with machine perfusion, along with increased liver damage with cold static preservation. These network modeling studies also suggested that machine perfusion led to a pattern of resolving inflammation, as compared to persistent, IL-18-associated liver damage under cold, static liver preservation [12]. An important aspect of this work was the potential for repeated monitoring of the inflammatory status of livers to be transplanted via analysis of inflammatory mediators in the perfusate in the machine perfusion setting [6].

Data-Driven Modeling: Cellular-Scale Insights into Hypoxia, Ischemia, and Hemorrhagic Shock

Hepatic ischemia and hypoxic stress are major factors affecting liver cells in relevant clinical settings such as trauma/hemorrhagic shock (T/HS) [21], ischemia/reperfusion injury [22], and liver transplantation [6]. The liver plays a significant role in inflammation and innate immunity. In the normal adult murine liver, hepatocytes represent the largest pool of parenchymal cells, comprising approximately 70–85% of liver volume [23]. Recent studies suggest that a large variety of immune/inflammatory cell types also reside in the liver [24]. Numerous studies, mostly using primary murine hepatocytes and mouse cell lines, have been carried out to study the inflammatory and stress response of hepatocytes in vitro [25]. There have also been several important systems biology studies using primary hepatocytes and hepatocyte cell lines that have elucidated novel features of signal transduction [26–29]. We studied mouse hepatocytes cultured under both normoxic and hypoxic conditions and defined core, dynamic networks of inflammatory mediators both intracellularly and in the secretome [30] (see also below). Those studies pointed to a central role for the chemokine MCP-1/CCL2 since it, along with interleukin (IL)-1α, were central/hub nodes in those networks. We extended those studies by demonstrating that both mouse hepatocytes and hepatic stellate cells respond to hypoxic stress by releasing a somewhat similar, but not identical, repertoire of cytokines and chemokines in vitro, suggesting that the secretory machine in hepatocytes might be either more limited at baseline or disrupted following stress [13]. Furthermore, we showed the presence of more complex autocrine inflammatory networks in hepatic stellate cells as compared to hepatocytes, which supports the notion that these two cell types not only coexist but also provide a microenvironment for each other in which hepatic stellate cells play the "messenger" role as opposed more of a "responder" role for hepatocytes [13].

The role of inflammatory networks formed by cytokines and chemokines released by isolated primary human hepatocytes in vitro has not been investigated extensively, however. Recently, we assessed the secreted, protein-level chemokine and cytokine responses of primary human hepatocytes. Our findings suggest that

distinct dynamic response networks are evoked in hepatocytes in response to hypoxia as compared to isolated cells cultured under normoxic conditions [14].

Traversing In Vitro and Clinical Data Using Computational Modeling

An important barrier to clinical translation of promising therapies is the discordance between in vitro and in vivo/clinical phenotypes, and we have proposed that computational modeling may aid in this regard [1, 31]. As an example of the use of data-driven modeling in this context, we carried out studies in isolated mouse hepatocytes cultured under normoxic or hypoxic conditions, to simulate (albeit very imperfectly) the scenario of liver ischemia in the setting of T/HS [30]. These studies involved multiple data-driven modeling tools including dynamic network analyses as well as PCA, along with a novel meta-clustering approach by which to coalesce these various analyses to glean novel insights about the complex spatiotemporal dynamics of this experimental system [30]. As noted above, these studies pointed to the chemokine MCP-1/CCL2 as a central inflammatory regulator in this system. Using hepatocytes derived from MCP-1-null mice, we found that MCP-1 appeared to regulate the central inflammatory cytokine IL-6 [30]. Since IL-6 has been long associated as an outcome biomarker in the setting of T/HS, we assessed the patient-specific circulating MCP-1 versus IL-6 levels in blunt trauma patients and found two main groups based on this analysis. This, in turn, led to defining MCP-1 as a biomarker of adverse clinical outcomes in trauma, independent of the presence of hemorrhagic shock/hypotension [30]. Later studies with larger patient cohorts have reinforced the role of MCP-1 as an outcome biomarker in trauma [32]. Thus, we point to this workflow as a rational means by which to define novel disease biomarkers based on in vitro data.

Acetaminophen (APAP)toxicity is the most common distinguishable cause of Acute Liver Failure in both children [33] and adults [34]). Liver injury, commonly due to APAP overdose (APAPo), occurs when inherent mechanisms to detoxify APAP, including conjugation with antioxidants such as glutathione, are overwhelmed and lead to the formation of reactive oxygen species that form damaging adducts with vital intracellular proteins. N-acetyl cysteine (NAC), the supplement form of the amino acid cysteine, serves to replete intracellular glutathione stores and is the clinically established treatment of acute liver injury due to APAP toxicity. However, a potential mechanism to support the use of NAC in non-APAP ALF has not been established [35].

As mentioned above [9], we recently identified a putative temporal threshold of inflammatory network connectivity in which HMGB1 was implicated as a hub node involved in nucleating systemic inflammatory responses in PALF. Interestingly, HMGB1 is also a potential mediator of APAP-induced hepatotoxicity and anti-HMGB1 antibodies and knocking out HMGB1 in the liver have been shown to

reduce hepatic inflammation and liver injury in mouse models of APAP toxicity [36]. An important aspect of our work involved demonstrating that PALF patients suffering from APAPo and APAP-treated primary mouse hepatocytes shared core dynamic network phenotypes [9]. Our preliminary studies in PALF patients [9] also suggest that the inflammatory dynamic networks, specifically the HMGB1 connectivity in the context of APAP toxicity, are largely dependent on (or reflect) the use of NAC and open the possibility, in a more general context, for the use of analysis of dynamic networks of inflammation to differentiate patient etiologies leading to novel therapeutic opportunities for PALF.

Conclusions and Future Prospects

In this chapter, we have focused on liver failure as a prototypical inflammatory disease that has a multifaceted etiology and, with exceptions such as APAP toxicity, usually has an unclear origin and an indeterminate etiology. This complexity has resulted in a dearth of diagnostic and therapeutic options [37, 38]. The studies described in this chapter, however, have resulted in some novel insights that may ultimately lead to clinical benefit. These insights include the finding that, at the dynamic network level, inflammatory networks in PALF patients and in mouse hepatocytes are strikingly similar. This finding potentially opens the way to inferring the clinical impact of modulating some of those networks based on studies in cells. Another key insight at the network level is the similarity between PALF patients that went on the recover and those patients that received a transplant. While ethically complicated, this finding suggests that it might be possible to forego liver transplantation in some cases, or that patients who have successful responses to their transplant have inflammatory networks similar to those patients who survive spontaneously. Finally, another network-level insight may be of benefit in the context of the perfusion of livers for transplantation, a setting in which monitoring the evolution of dynamic inflammatory networks may give insights into the likelihood of successful transplant for any individual liver. While further work is clearly needed and warranted, these insights suggest that data-driven modeling may have an important place in the toolkit of liver researchers.

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Chapter 15
Temporal and Spatial Analyses of TB Granulomas to Predict Long-Term Outcomes

Louis R. Joslyn, Marissa Renardy, Caleb Weissman, Nicole L. Grant, JoAnne L. Flynn, J. Russ Butler, and Denise E. Kirschner

Abbreviations

ABM	Agent-based model
BCG	Bacillus Calmette–Guérin
CFU	Colony forming units
GIS	Geographic information systems
IFN	Interferon
IHC	Immunohistochemically
IL	Interleukin
kNN	*k*-nearest neighbors
LHS	Latin hypercube sampling
MLR	Multinomial logistic regression
Mtb	Mycobacterium tuberculosis
NHP	Nonhuman primate
PET-CT	Positron emission tomography–computed tomography
SVM	Support vector machines
TB	Tuberculosis
TDA	Topological data analysis

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TGF-β	Transforming growth factor beta
TNF	Tumor necrosis factor
t-SNE	t-Distributed stochastic neighbor embedding

Introduction

Worldwide, tuberculosis (TB) kills over 4000 individuals per day and about one quarter of the world's population is infected with Mycobacterium tuberculosis (Mtb), the causative agent of TB [1]. The most common form of TB is adult pulmonary TB, a chronic lung disease for which drug treatment requires a long course of multiple antibiotics. Although the Bacillus Calmette–Guérin (BCG) vaccine is administered at birth in most countries, it has variable efficacy against pulmonary TB in adults. Infection is established via inhalation of Mtb into the lungs, where immune cells called macrophages take up the bacteria. The subsequent immune response leads to the formation of organized immune structures, called granulomas, that reside within lungs and thoracic lymph nodes and are the pathological hallmark of TB [2, 3]. They serve to immunologically restrain and physically contain bacteria that cannot be easily cleared [4]. However, there are currently no methods to systematically quantify the spatial characteristics of granulomas for comparison across individuals or between species. Understanding the formation, function, and spatial development of granulomas is key to not only preventing infection but also aiding intervention strategies.

Within the granuloma environment, immune cells, bacteria, and necrotic dead cells interact throughout the course of infection. This interaction can lead to different granuloma outcomes, including bacterial growth and escape, bacterial sterilization and control of infection, or a dynamic balance between bacteria and the immune response, wherein a single granuloma may remain for years or decades [5, 6]. Different granuloma outcomes can lead to different clinical outcomes for the host. Furthermore, multiple granulomas develop within humans and nonhuman primates (NHPs) and occur in varying numbers and with different behaviors. During early time points, NHPs with controlled infection had an average of 7 granulomas, while those with an active infection had an average of 12 granulomas [7]. In almost all latent hosts, some granulomas persist providing a niche for bacterial persistence and survival [8]. Importantly, recent evidence suggests that when as few as one granuloma cannot control bacterial growth, that could be sufficient to drive the onset of active disease [7, 9]. Since granulomas arise as inflammatory responses to the invading mycobacteria, it is equally important to control the activities of the host response and to control the bacterial burden. Classifying the temporal and spatial evolution of granulomas could lead to further our understanding of the underlying mechanisms that drive toward uncontrolled bacterial growth and inflammation. Further, knowing the fates of the granulomas, and predicting their individual outcomes early on, could aid in designing precision medicine approaches for TB.

Spatially, most granulomas exhibit the following architecture: a central necrotic core, surrounded by a dense population of macrophages, followed by a ring of T cells, macrophages, and B cells [10, 11]. Granulomas have typically been loosely classified as caseous, non-necrotizing, neutrophilic, mineralized, and fibrotic [12]. However, there are currently no methods to systematically quantify the spatial characteristics of granulomas for comparison across individuals or between species. Such quantification and eventual automation would allow for integrating cross-species data and provide a faster, more reproducible analysis of TB pathology. Further, connections between granuloma structures and granuloma outcomes have yet to be elucidated.

As granulomas are difficult to study in vivo and in vitro [13, 14], systems biology approaches have been vital to understanding the formation and function of granulomas [15–23]. For example, Ray et al. illustrated the role of multiple TNF activities within a granuloma using a stochastic and discrete computational model of granuloma formation [15]. Additionally, Bru et al. showed that the chemokine concentration within the granuloma environment is a crucial component in the ability of a granuloma to attract immune cells and thereby control infection [17]. Mathematical and computational models can integrate diverse datasets from various experimental studies in order to test hypotheses, make predictions, and provide an in silico platform to run virtual experiments to test aspects of a complex biological system that may not be currently accessible in the wet lab or clinic. In particular, computational modeling provides an opportunity for systematic investigation of the temporal dynamics and spatial structures of granulomas.

Here, we seek to identify the spatial and temporal characteristics of both in silico and in vivo granulomas and to connect these features with granuloma outcomes. Our approach couples a collection of systems biology approaches including in silico granuloma modeling, topological data analysis, and machine learning in an effort to understand granuloma dynamics across time, quantify granuloma structure, and predict how granuloma structure affects long-term control of bacterial growth. We pair this approach with immunohistochemistry and geographical information systems to apply the in silico predictions to in vivo derived data as a proof-of-concept to test our approach for future application.

Methods

We use computational modeling of granulomas to create a repository of 1500 simulated granulomas. We then classify the granulomas in our repository based on their temporal behavior from day 100 to day 200 postinfection. In parallel, we use topological data analysis to quantify the spatial structure of the granulomas at day 100 postinfection. We apply our spatial analysis to an immunohistochemically (IHC)-stained image of a granuloma from an Mtb-infected macaque. From this spatial information, we use machine learning to predict the temporal classification of granulomas. This allows us to predict the likely future behavior of an experimental

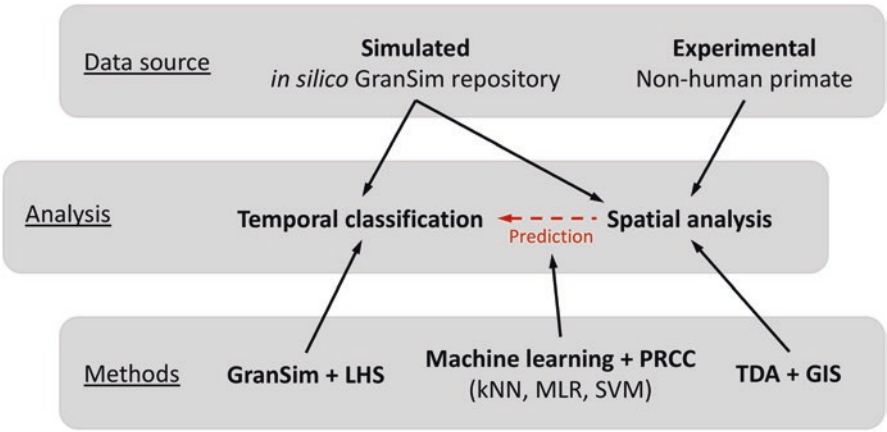

Fig. 15.1 Spatiotemporal framework overview. Flow chart showing the different data sources and methods used for temporal classification, spatial analysis, and prediction of granuloma behavior. *LHS* Latin hypercube sampling, *PRCC* partial rank correlation coefficients, *kNN* *k*-nearest neighbors, *MLR* multinomial logistic regression, *SVM* support vector machines, *TDA* topological data analysis, *GIS* geographic information systems

granuloma had the macaque not been necropsied. The different types of analyses and data sources are summarized in Fig. 15.1.

GranSim: A Hybrid Agent-Based Model

GranSim is a hybrid agent-based model (ABM) for studying the dynamics of individual granulomas in silico, and is used to analyze the complex cellular and molecular dynamics that occur within granulomas [24]. The immunobiology of Mtb infections has been widely documented in previous studies [24–26], and *GranSim* has been developed and continuously curated to recapitulate known immunobiology based on nonhuman primate (NHP) data since 2004 [15, 16, 20, 27–31]. An in-depth description of *GranSim* rules as well as an executable file can be found in previous published work and on our website: http://malthus.micro.med.umich.edu/GranSim/.

Briefly, *GranSim* describes the cellular behavior of macrophages (resting, activated, infected, and chronically infected), T lymphocytes (interferon [IFN]-γ-producing, cytotoxic, and regulatory), and bacteria (intracellular replicating, extracellular replicating, and extracellular nonreplicating) across time as infection progresses. Simulations begin with one infected macrophage and one intracellular Mtb placed in the middle of a 2 mm × 2 mm gridspace that represents a 2D section of lung tissue that is the same size, with resting macrophages distributed across the grid at a specified density. We also have a version of *GranSim* in 3D ([32], http://malthus.micro.med.umich.edu/3D-GranSim/); however, we use the 2D version here

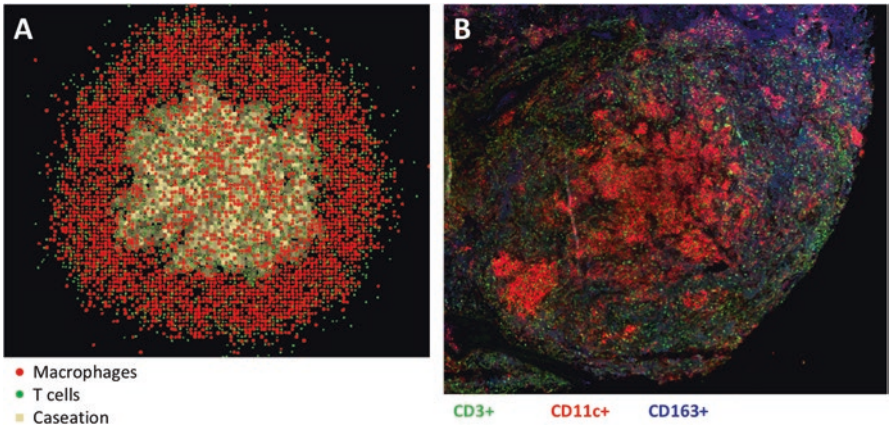

Fig. 15.2 Simulated and experimental tuberculosis granulomas. (**a**) Simulated granuloma generated by *GranSim* at day 100 postinfection. (**b**) Immunohistochemically stained image of an experimental granuloma taken from an NHP necropsied at day 37 postinfection. CD3 is a marker for T cells and CD11c and CD163 are markers for different types of macrophages. This granuloma has 1529 colony forming units (CFU) of M. tuberculosis

due to the 2D nature of experimental granuloma images. Each grid compartment is 20 μm × 20 μm. One grid compartment can hold a single macrophage, two T cells, or a T cell and a macrophage. Bacteria and immune cells are simulated as individual "agents" that interact and move around the lung grid across time, according to a set of rules specific to each agent. In addition to cell dynamics, *GranSim* simulates the molecular dynamics of the degradation and diffusion of the cytokines tumor necrosis factor (TNF), transforming growth factor beta (TGF-β), interleukin 10 (IL-10), and chemokines (CCL2, CCL5, CXCL9/10/11) using partial differential equation representations. An example of a simulated granuloma generated by *GranSim* is shown in Fig. 15.2a, along with an image of an experimental NHP granuloma in Fig. 15.2b.

Generating a Repository of Simulated Granulomas

To provide a breadth of granuloma outcomes and behavior, we created a repository of 1500 simulated granulomas. Many methods can be used to sample parameter space. Here, we utilized Latin hypercube sampling (LHS), as previously described in [33], to generate a repository of simulated granulomas that are biologically reasonable according to bacterial burden [measured as colony forming units (CFU)] and immune cell counts. Using uniformly distributed model parameters within biologically reasonable ranges, we generated 500 distinct parameter sets. For each of the 500 parameter sets, 3 replications were performed to account for stochastic

effects. Thus, in total we simulated *GranSim* 1500 times. Simulations were run for a virtual time of 200 days postinfection.

Immunohistochemical Staining of NHP Tissue Samples

Lung granuloma samples were isolated and excised from the lungs of cynomolgus macaques infected with Mtb during positron emission tomography–computed tomography (PET-CT)-guided necropsy. Following formalin fixation and paraffin embedding, tissue samples were sectioned with a microtome into 5 μm pieces and placed on slides for IHC staining. Slides containing tissue sections were hydrated by submerging in xylene twice for 5 min each, followed by 5 min in 100% and 70% ethanol. Once rehydrated, slides were boiled in 1× Tris/EDTA antigen retrieval buffer for 6 min in a pressure cooker. Slides were cooled to room temperature and then incubated for 30 min in a humidified chamber with 1% BSA in PBS, then with primary antibodies for CD3 (Dako A0452, 1:100 dilution) or CD11c (Leica, clone 5D11, NCL-L-CD11c-563, 1:30 dilution) for 1 h at room temperature or overnight at 4 °C. Following primary incubation, slides were washed three times in 1× PBS and incubated in a humidified chamber with secondary antibodies Cy3 AffiniPure Fab goat antimouse IgG (Jackson Immunolabs, 115-167-003) and AF488 donkey antirabbit IgG (Life Technologies, A21206) for 1 h at room temperature, then washed three times in 1× PBS. CD163 (Lab Vision, clone 10D6, MS1103S1, 1:30 dilution) was stained using a Zenon labeling kit for mouse IgG1 AF647 (Thermo Fisher, Z25008) at a 1:1:1 ratio of components A, B, and diluted antibody and incubated overnight at 4 °C in a humidified chamber. Slides were washed three times in 1× PBS and mounted using DAPI prolong gold antifade reagent (Thermo Fisher, P36931) and imaged using a Nikon epifluorescent microscope.

Using GIS to Identify Locations of T Cells and Macrophages in an IHC Image of a Granuloma

Previously, we used geographical information systems (GIS) technology for image analysis [34]. Herein we use a similar approach, but with the goal of providing a spatial analysis that identifies the locations of T cells and macrophages rather than providing only overall counts of each immune cell type. The microscopy image of the immunohistochemically (IHC) stained granuloma (above) was imported in ArcPro 2.5 (ESRI) as raster (pixel) red, green, and blue (RGB) images. To identify the 2D locations of T cells and macrophages, we used a combination of supervised (Iso-cluster analysis) and unsupervised (maximum likelihood analysis) learning to classify the raw RGB image. We utilized these classification techniques because IHC-stained images of granulomas contain partial expression and mixed expression

of staining markers. Thus, we were able to separate this type of "noise" from true cellular marker expression. This technique produced a large number of classes (over 100). The noncell classes were manually collapsed into "background," thereby isolating the cell classes. In the next step, we converted the raster classified image into a vector image containing polygons that represent T cells and macrophages. Stained granuloma preparations often contain large numbers of dead cells and cellular debris. We removed these artifacts by using queries that excluded dead cells and cellular debris. We then located polygon centroids and identified *xy*-coordinates for each centroid, for use in downstream analysis for each cell type.

Methods for Classification and Analysis of Granulomas

Temporal Classification

The primary goal of our temporal analysis is to determine whether a simulated granuloma becomes *sterilized* (i.e., a granuloma kills bacteria completely), *stable* (i.e., infection is controlled within the granuloma), or *unstable* (i.e., infection is uncontrolled within the granuloma). To bin granulomas within these categories, we first considered the temporal data from our 1500 simulations, including bacterial burden, immune cell counts, chemokines and cytokines, granuloma size, caseation, and a number of other granuloma statistics. We used this data to determine what factors of Mtb infection within a granuloma were most indicative of individual granuloma outcome. From this analysis, we empirically determined that bacterial burden over time is the best indicator of both stability of a granuloma and how well the immune response controls infection. Additionally, we incorporated granuloma size in this analysis as a proxy for overall immune response at the site of infection. Thus, granulomas were binned based on two features: changes in their bacterial burden and their diameter over the last 100 days of infection.

Spatial Analysis

In addition to classifying simulated granulomas based on temporal dynamics, we aim to quantify their spatial structure. We consider the output from *GranSim* in the form of *x*–*y* coordinates of cell positions on a 2D spatial domain. Simulated granuloma cell locations are recorded at 100 days postinfection. The coordinates of an immune cell are given by location of the grid compartment containing a cell. The majority of cells in a granuloma are macrophages and T cells, and thus we focus our analysis on these two cell types, knowing that others could be added into the analysis if desired. Due to random effects in both actual and simulated granulomas, the spatial positions of cells in two distinct simulated granulomas may differ while the

overall structure remains the same. Further, since *GranSim* simulations account for only small pieces of tissue from anywhere inside the lung, spatial coordinates are not comparable across different domains. Similarly, IHC granuloma images are taken from small sections of NHP lung tissue. Thus, we wish to compare the spatial structure of different granulomas while allowing for variation in cell positions. This leads us to consider the topological features of the granulomas, rather than the explicit spatial coordinates of cells.

To quantify granuloma structure, we utilize topological data analysis (TDA). TDA is a set of tools for analyzing the structure of data, where data points are viewed as a noisy sample from a topological space. In recent years, TDA has been used to understand the global behavior of biological agent-based models, particularly relating to aggregation and swarming of organisms [35, 36]. It has also been used to analyze a variety of biomedical phenomena such as liver lesions, brain artery trees, and murine tumors [37–39]. This type of analysis can be similarly used to study agent-based models for aggregation of immune cells during inflammation, such as *GranSim*. One common tool in topological data analysis is *persistent homology*, in which topological features of data are quantified across a range of spatial scales [40, 41]. We follow the approach of Topaz et al. (2015), which we summarize below. A more detailed description of the process for computing persistent homology in the context of agent-based models, as well as a summary of the underlying mathematics, can be found in [35].

First, spatial data are transformed into a simplicial complex, which can be thought of as a generalization of a network. In this work, we use *witness complexes*, which use subsamples of data and are better suited for large datasets [42]. The complex is created by choosing a distance parameter ϵ and considering data points (in this case, cells) as nodes, or 0-simplices, in the complex. If two nodes are within ϵ of each other, they are connected by an edge (1-simplex). If $k+1$ nodes are pairwise connected to each other, they form a k-simplex. The topology of this simplicial complex can then be described by its *Betti numbers*. For each non-negative integer k, the kth Betti number gives the number of k-dimensional "holes" in the complex; for example, the 0th Betti number represents the number of connected components in the granuloma and the first Betti number represents the number of topological circles. The R package *phom* was used to construct the simplicial complexes and compute the 0th and first Betti numbers for macrophages and T cells separately using a variety of spatial scale parameters ϵ, varying from 20 μm (one grid compartment) to 400 μm (20 grid compartments), in increments of 20 μm. The same range for spatial scale parameters was used for both simulated and experimental granulomas. The upper bound of 400 μm was chosen because we empirically observed that distances between groups of cells and radii of empty regions in granulomas are typically much smaller than 400 μm. Thus, each granuloma is represented by a vector of Betti numbers corresponding to macrophages and T cells at a range of spatial scales. This process is summarized in Fig. 15.3.

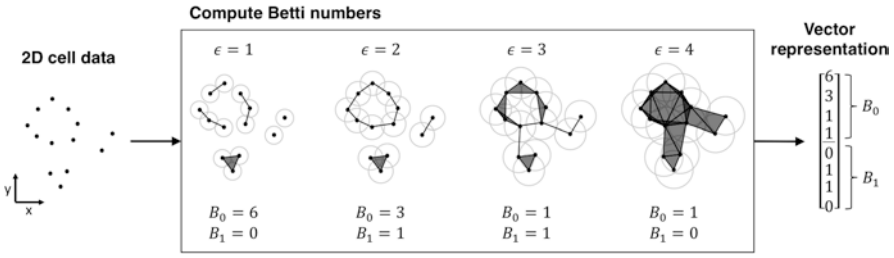

Fig. 15.3 Graphical demonstration of topological data analysis applied to a simple hypothetical "granuloma." The granuloma is represented by 2D spatial coordinates of the involved cells (left). Betti numbers are computed by constructing simplicial complexes from the 2D data for different values of the spatial scale parameter ϵ (1–4 from left to right). Corresponding Betti numbers B_0 (numbers of connected components) and B_1 (numbers of holes created by connected components) are shown for each simplicial complex. A granuloma can then be represented by a vector of Betti number values

Prediction of Future Temporal Behavior from Spatial Structure Analysis

We use the spatial structure of a granuloma at day 100 postinfection to predict its temporal behavior over the next 100 days (until day 200 postinfection). Each granuloma is represented by its vector of Betti numbers at day 100, as discussed above (Fig. 15.3). We applied three different classification algorithms to predict the temporal classification of a granuloma using Betti numbers as input: *k*-nearest neighbors (kNN), multinomial logistic regression, and support vector machines (SVM). For each machine learning algorithm, 70% of the simulated granulomas were randomly selected to be used in the training set, and the remaining 30% of the granulomas were used in the test set.

We calculated an F_1 score to gauge the accuracy of each predictive algorithm with each granuloma class. The F_1 score is defined as $F_1 = 2 * \frac{\text{precision} * \text{recall}}{\text{precision} + \text{recall}}$, and is a measure of a test's accuracy which uses both *precision* and *recall* of the test to assess the test's accuracy [43]. Precision is the number of true positives divided by the number of true and false positives. Recall is the number of true positives divided by all the instances that are within the predicted group. In other words, precision is the number of instances correctly predicted divided by the total number of instances predicted. Recall is the number of instances correctly predicted divided by the total instances.

We perform correlation analysis to determine which spatial characteristics are most related to the temporal classification of a granuloma. Since relationships may be highly nonlinear, we used partial rank correlation coefficients (PRCC) [44]. We first enumerated the temporal classifications as 1 = sterilized, 2 = stable, and 3 = unstable and created a matrix of the 0th and first Betti numbers for macrophages

and T cells at all spatial scales. Using the Betti numbers as inputs and the enumerated temporal classifications as outputs, we utilized the R function epi.prcc to compute the correlation coefficients and corresponding *p*-values. Since Betti numbers for very close spatial scale parameters may be identical, we added a small amount of normally distributed random noise to the Betti numbers to avoid issues of matrix singularity.

Results

Temporal Classification of Simulated Granulomas Based on CFU and Lesion Size

Three distinct groups of granulomas were identified based on bacterial load and granuloma size among 1500 *GranSim* simulations: *unstable*, *stable*, and *sterilized*. *Sterilized* granulomas were defined as granulomas with zero colony forming units (CFU), that is, granulomas in which the bacteria had been completely cleared. We tested a large variety of cutoffs between *stable* and *unstable* granulomas. The final cutoffs were empirically chosen to be conservative in that the *unstable* classification captures granulomas with even moderate growth. We used this conservative classification methodology in an effort to minimize underestimation of the severity of an Mtb infection. CFU doubling over 100 days was used as the cutoff to differentiate granulomas based on their bacterial load. Granuloma size growth (increase in diameter) of 25% was used as the cutoff to differentiate granulomas based on our proxy for overall immune response (inflammation). Thus, *stable* granulomas were defined as nonsterilized granulomas whose CFU less than doubled over the last 100 days of the infection and the diameter grew by 25% or less. *Unstable* granulomas were defined as granulomas whose CFU more than doubled in the last 100 days of infection or whose diameter grew by more than 25% in the last 100 days of infection (simulation was run for 200 days). This temporal classification is summarized in Methods section and in Fig. 15.4a.

We identified 597 *unstable* granulomas, 411 *stable* granulomas, and 492 *sterilized* granulomas in our simulated repository of 1500 granulomas (Table 15.1). CFU and cell counts for these simulated granulomas are shown in Fig. 15.4b alongside experimental data from nonhuman primate granulomas [45]. Cell counts are scaled from the two-dimensional simulation to three dimensions in order to match whole-granuloma flow cytometry data, using the methodology presented in [46]. While macrophage counts are somewhat overestimated in our simulations, CFU and T cell dynamics are well-captured.

After obtaining these three separate granuloma classes, we examined a variety of factors implicated in the immune response to Mtb and granuloma formation that are present in *GranSim*. We considered levels of TNF and IFN-γ, as well as cell counts for T cells and macrophages. All of these measures were different between *unstable*,

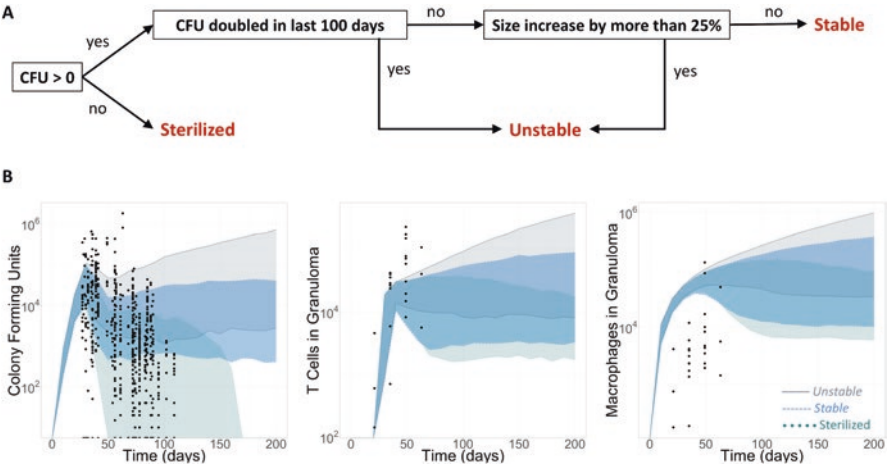

Fig. 15.4 Temporal classification of simulated granulomas. (**a**) The description of our classification of simulated granulomas based on temporal statistics. (**b**) Temporal output of simulated granulomas by category. Bacterial and cell counts for *sterilized* (green), *stable* (blue), and *unstable* (gray) simulated granulomas shown together with an experimental dataset derived from nonhuman primate granulomas (black dots) [45]

Table 15.1 Average levels of key immune factor levels for each granuloma class by temporal analysis of CFU and lesion size. *Unstable* granulomas have higher levels of caseation, lymphocytes in the granuloma, and proinflammatory cytokines than *stable* and *sterilized* granulomas. *Stable* granulomas have much lower levels of caseation, lymphocytes in the granuloma, and proinflammatory cytokines than *unstable* granulomas, but greater levels than *sterilized* granulomas. Cell counts represent the number of cells in a 2D slice through the center of a granuloma. Cytokine values represent the number of molecules on the simulated lung tissue grid

	Unstable	Stable	Sterilized
Number of runs	597	411	492
Caseated compartments	648	86	21
Average TNF concentration	105,720	11,737	1971
Average IFN-γ concentration	156	103	68
Average T cells in gran	1742	439	183
Average macrophages in gran	5950	2246	928

stable, and *sterilized* classes of granulomas, as we determined via our above classification. More severe granulomas tended to have higher levels of TNF and IFN-γ and higher numbers of immune cells (Table 15.1). We also examined the number of caseated compartments in the core of these granulomas. Intuitively, our results suggest that larger and less controlled (*unstable*) granulomas tend to have higher levels of caseation on average.

Spatial Analysis of Simulated and Experimental Granulomas

Using techniques from topological data analysis, we quantified the topological features of each granuloma at a variety of spatial scales in the form of Betti numbers. To visualize the topological data for our set of 1500 simulated granulomas, we utilize *t*-distributed stochastic neighbor embedding (*t*-SNE), a technique for visualizing high-dimensional data by giving each datapoint a location in a two- or three-dimensional map [47]. A two-dimensional *t*-SNE of the topological data is shown in Fig. 15.5a along with examples of the corresponding simulated granulomas. This reveals some distinct clusters of simulated granulomas. Some clusters are visually quite different from others; for example, one cluster consists entirely of simulations that did not result in a granuloma at day 100 (lower left of Fig. 15.5a). On the other hand, some granulomas across different clusters appear similar (e.g., top left and top right of Fig. 15.5a). This may be due to the choice of hyperparameters for the *t*-SNE, or may be due to topological differences at small values of a spatial scale parameter. Since these Betti numbers may indicate differences at the cellular scale, this may not be immediately evident when looking at the tissue scale of entire granulomas.

As a proof of concept, we analyze a single experimental IHC image. A granuloma was obtained from a NHP infected with Mtb and necropsied at 37 days postinfection, and a section of this granuloma was stained for CD3 as a marker for T cells and CD11c and CD163 as markers for macrophages (Fig. 15.1b) [48]. Since this image is used purely for proof of concept, we disregard differences such as timing and focus solely on spatial characteristics. We applied GIS analysis to the IHC-imaged granuloma to identify the cell positions for macrophages and T cells. The IHC granuloma and corresponding plot of cell positions are shown in Fig. 15.5b.

Once we obtained x–y coordinates for macrophages and T cells in the IHC image via GIS, we applied our topological data analysis methodology to represent the IHC granuloma as a vector of Betti numbers. We applied *t*-SNE to the set of 1501 granulomas (1500 simulated + 1 IHC) to visualize where this granuloma fits among our repository of simulated *GranSim* granulomas (Fig. 15.5c). The IHC granuloma has topological features consistent with the range covered by our simulated granuloma repository. Further, the nearest simulated granulomas, shown in Fig. 15.5d, are all classified as *unstable*.

Spatial Structure Predicts Future Temporal Granuloma Stability

We used the Betti number data from the topological data analysis as input to three different machine learning methodologies (kNN, multinomial logistic regression, and SVM) to predict temporal classifications at a later time point for each granuloma in our database. We integrate the spatial structure of a granuloma at day 100 postinfection with machine learning techniques to predict its temporal behavior in

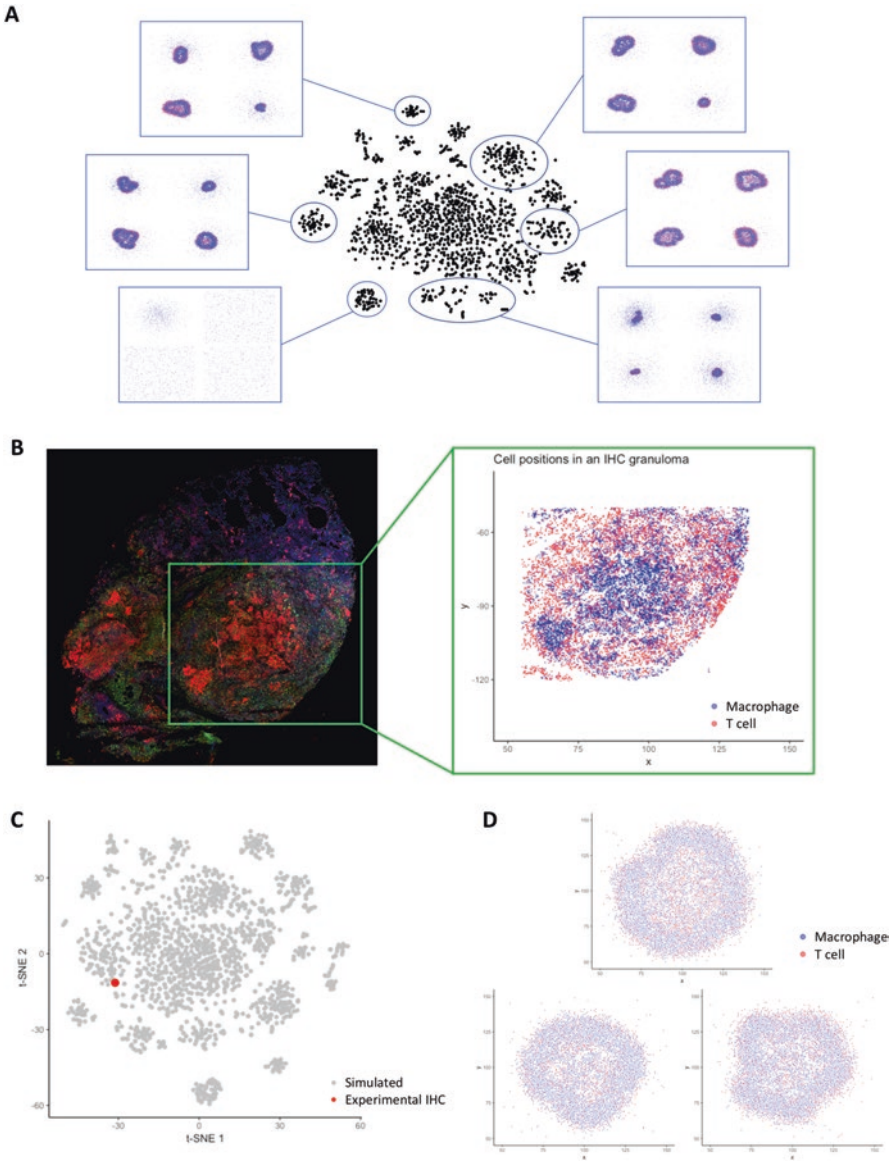

Fig. 15.5 Visualizing spatial clustering of in silico and in vivo granulomas. (**a**) A two-dimensional representation of the Betti number data resulting from TDA applied to the *GranSim* repository. Each black dot corresponds to a single simulated granuloma at day 100 postinfection. Examples of corresponding simulated granulomas are shown for several apparent clusters in the t-SNE. (**b**) Experimental IHC image of an NHP granuloma (green = CD3, red = CD11c, blue = CD163). Cell positions for macrophages and T cells in the granuloma are determined using GIS software (see the Methods section). (**c**) Visualization via *t*-SNE of where the IHC granuloma fits within our simulated granuloma repository in Betti number space. (**d**) Plots of simulated granulomas that are closest to the IHC granuloma in Betti number space. All of these granulomas were *unstable*

Table 15.2 Predicting future granuloma classifications at day 200 by three machine learning techniques. F_1 scores for *k*-nearest neighbors (kNN), multinomial logistic regression (MLR), and support vector machines (SVM) in classifying sterilized, stable, and unstable simulated granulomas based on topological data. Scores are highest for *unstable* granulomas and lowest for *sterilized* granulomas

	kNN (62%)	MLR (62%)	SVM (62%)
Sterilized	0.63	0.67	0.65
Stable	0.28	0.29	0.18
Unstable	0.76	0.74	0.77

terms of CFU and lesion size over the next 100 days (i.e., from day 100 to day 200 postinfection). In particular, we predict whether the granuloma will be classified as *sterilized*, *stable*, or *unstable*. We calculated overall accuracies to be 62% for each of kNN, multinomial logistic regression, and SVM (Table 15.2, first row).

F_1 scores (defined in the Methods section) were computed to determine the accuracy of each test independently for the three different temporal classifications of granulomas. As shown in Table 15.2, the F_1 scores demonstrate that the algorithms perform the worst for *stable* granulomas. This is primarily due to structural similarities between *stable* and *sterilized* granulomas resulting in unreliable predictions. The structural similarity between *stable* and *sterilized* granulomas may be due to a lack of healing mechanisms in *GranSim*, among other factors.

Clinically, it is more important to correctly identify *unstable* granulomas than to discriminate between *stable* and *sterilized* granulomas, since *unstable* granulomas are more likely to lead to active disease [49]. Thus, we group together *stable* and *sterilized* granulomas as *controlled* granulomas. Using this grouping, all three algorithms perform well in discriminating between *unstable* and *controlled* (i.e., *stable* or *sterilized*) granulomas. The accuracy increases to 78%, 77%, and 80% for kNN, multinomial logistic regression, and SVM, respectively. Table 15.3 shows accuracies and F_1 scores for discriminating between controlled and *unstable* granulomas.

Identifying Spatial Characteristics that Correlate with Granuloma Severity

We utilized partial rank correlations to determine which Betti numbers were significantly correlated with temporal classification. We found the first Betti numbers for macrophages and T cells at the smallest spatial scale parameter value ($\epsilon = 20$ μm) at day 100 postinfection are significantly correlated with our temporal classification of long-term granuloma behavior ($p < 0.001$). The first Betti numbers represent the number of holes in the cell distributions for a granuloma when cells are connected using a small radius. The strongest correlation was for the first Betti number for T cells at $\epsilon = 20$ μm. While this analysis does not reveal any mechanistic explanation, we hypothesize that these "holes" may correspond to nonuniform caseous regions

Table 15.3 Predicting future granuloma classifications at day 200 by three machine learning techniques after combining stable and sterilizing granulomas. F_1 scores for k-nearest neighbors (kNN), multinomial logistic regression (MLR), and support vector machines (SVM) in classifying controlled (*sterilized* or *stable*) versus *unstable* simulated granulomas based on topological data

	kNN (78%)	MLR (77%)	SVM (80%)
Controlled	0.81	0.80	0.82
Unstable	0.76	0.74	0.77

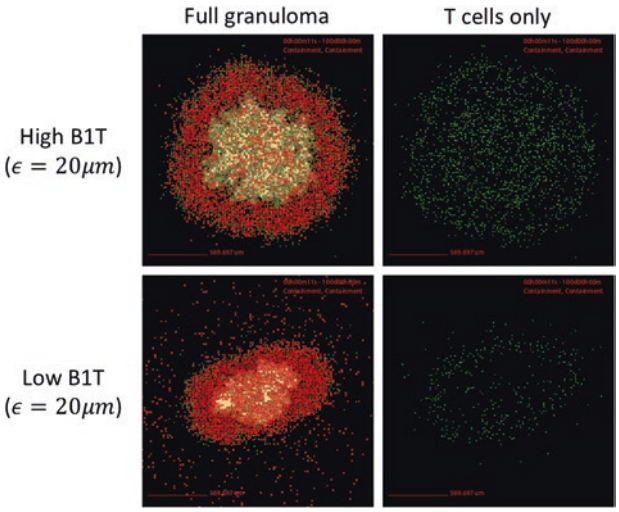

Fig. 15.6 Examples of simulated granulomas with high (top) and low (bottom) first Betti numbers for T cells at a small spatial scale. Full granulomas are shown on the left, where red indicates macrophages, green indicates T cells, and beige indicates caseum. The T cell distribution is shown on the right. High first Betti numbers (B1T) for T cells at this scale correlate with uncontrolled granulomas, while low first Betti numbers correlate with controlled granulomas

in the granuloma, and indicate that there is not a clear lymphocyte cuff in granulomas that do not control infection (Fig. 15.6).

Discussion

In this work, we employed a suite of system biology approaches to classify and predict granuloma outcomes. We performed in silico modeling to understand granuloma dynamics across time and classify granulomas based on their ability to control bacterial growth and inflammation. Further, we used topological data analysis to quantify the spatial structure of granulomas. We then used three different machine learning approaches to predict future granuloma stability from present granuloma structure and obtained 80% accuracy in predicting whether a granuloma would

remain controlled over the next 100 days. Finally, we paired this approach with immunohistochemistry and geographical information systems software to apply the analysis to an in vivo-derived dataset.

While we applied this analysis to TB granulomas, the same type of analysis could be applied to any biological system for which imaging and agent-based modeling can be performed. For example, MRI images of the brain could be coupled with modeling approaches to predict neurological outcomes; in fact, geometric analysis has already classified such data [50]. Additionally, solid tumor modeling and imaging could be paired using a similar framework to predict the impact of potential treatment protocols.

While not explored in this work, model calibration and validation is another potential application of the temporal and spatial analysis of simulations. For example, by classifying different temporal dynamics and quantifying the structure of simulated granulomas, we ensure that parameter ranges are selected that reflect a variety of biological behaviors. Further, these techniques could be used to calibrate model parameters to match a particular type of granuloma instead of representing a broad range of outcomes. Spatial analysis of granulomas can also provide a model validation step by showing, in a quantifiable way, that the spatial structures generated by our model are consistent with imaging performed by ex vivo wet lab studies. One additional gain from the ability to predict granuloma outcomes from spatial structures is potential savings in computational cost by allowing us to simulate short-term simulations in place of long-term ones.

While we have primarily focused on analysis and classification of simulated granulomas, one long-term goal of this work is to enable systematic spatial analysis of experimental IHC images of NHP granulomas. Automated quantification of spatial structure for experimental granulomas has not been previously done. Further, we can use this analysis to predict the likely outcomes of experimental granulomas for subjects that were terminated at early time points. For widespread application to experimental granulomas, standardized staining, imaging, and analysis techniques need to be adopted. The current work using GIS shows one step in that direction.

While this work enables us to study simulated granulomas, and even ex vivo granulomas from primates or rabbits, our goal remains to translate to humans. To this end, PET-CT allows time-series tracking of granulomas within NHPs and humans and is increasingly used in TB clinical settings [51]. There is current experimental work to uncover and link factors that lead to elevated inflammation as measured by PET imaging to specific immune and bacterial attributes in TB. Similar work has been done for other types of lesions [52, 53]. Once this link is established for TB, we can add those features to our in silico granulomas and procedures, enabling us to predict the long-term outcomes of in vivo granulomas within NHPs and humans. Using this personalized medicine approach, we could predict individuals that may be candidates for certain drug regimens over others in real time. Our framework, when paired with PET CT imaging, will have the potential to fulfil the promise of personalized medicine and strongly influence therapeutic interventions.

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Part V
Future Perspectives: Translation to Implementation

Chapter 16
The Rationale and Implementation of Model-Based Precision Medicine for Inflammatory Diseases

Gary An and Yoram Vodovotz

This book has been both a compendium and a snapshot of a particularly critical and difficult period in basic and applied research. We have described "tipping points" in terms of the biology of inflammation [1], and have suggested that this concept be applied the socioeconomic dynamics of biomedical research [2]. While the actual monetary amount invested in funding biomedical research is very high, we have noted throughout this book that the successful translation of that research into clinical therapeutics is not keeping up (if not actually decreasing). Furthermore, despite the increased aggregate amount allocated to biomedical research, for the individual researcher the likelihood of actually obtaining funding is near an all-time low [3].

The question in many minds (especially those of taxpayers and legislators faced with fiscal shortages) is: what are we getting for the money invested in research? This question is especially pertinent given that many recent breakthroughs have been met first with tremendous excitement and optimism, often presented in hyperbolic terms, only to have the reality of the translational dilemma result in the equivalent of a market crash in terms of reproducibility and applicability. Is it any surprise then that the current funders of biomedical research are increasingly challenged to choose what to fund and what to not? Adding to the forward feedback loop of uncertainty is the need (perceived and real) that researchers need to "sell" their hypotheses with increasing vigor. This has led to the paradoxical situation in which investigators now must become *advocates* for their hypotheses in order to obtain the means to be able to carry out an investigatory process that is, fundamentally in terms of the philosophy of Science, predicated upon skepticism.

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We assert that the solution to this dilemma is to focus on process (which is, after all, what Science is) in such a way that allows us to be aware of the impermanence and fluidity of "facts." This requires returning the fundamentals of the Scientific Process, of the need to close the iterative loop between correlation and mechanistic causality, with the expressed goal of being able to exercise the skeptical underpinnings of Science and pursue hypothesis falsification [2].

We and others have suggested that mechanistic computational modeling is just this type of process that modeling provides a means for researchers to express putative biological mechanisms more formally as hypotheses and be able to "see them in action" in order to evaluate whether these hypotheses produce plausible and clinically realistic behavior [2, 4, 5]. There has been growing skepticism about the utility of purely data-driven "artificial intelligence"/machine learning methods in the context of Precision Medicine [6]. Used as an exploratory process, integrating researcher intuition and expertise with the increasingly broad data sets generated through new technologies, mechanistic computational modeling is an alternative or complementary approach to pure data-driven modeling (see Chap. 4). Mechanistic modeling may be used to suggest fruitful biological pathways that may be targets for drug development; this is not just the identification of drug candidates, but whether the basis of candidate selection is even a good idea [7]. Dynamic computational modeling may also augment the search for diagnostic biomarkers, by providing the all-important temporal dimension into the characterization of disease/health states [1, 8–10]. Critically, the dynamic component of mechanistic computational modeling may facilitate the identification of paths from health to disease and, hopefully, back; not just in terms of a series of unconnected data snapshots, but rather in a process in which data snapshots are tied to each other by an actualized biological mechanism. Dynamic computational modeling may serve as a "binding knowledge structure," to identify and represent what is it, at a mechanistic and functional level, that ties populations of individuals together [11]. This is true not only for the ability to facilitate the translation of preclinical to clinical situations (i.e., what aspects of mammalian biology can we reliably trust to be similar enough to compare between and translate across species and experimental models), but also to drive personalized medicine, allowing the ability to generate the different characteristics of individuals within a cohort and between cohorts. Finally, mechanistic computational modeling is the core component of model-based predictive control, and we have suggested this approach for rational inflammation reprogramming [5]. We suggest that this approach is the only way to achieve the "$N = 1$" nature of personalized medicine, where specific therapies and interventions are tailored to individual patient properties and disease dynamics [5, 12]. Taken together, these concepts make up model-based Precision Medicine [5].

This book is focused on the acute inflammatory response and its manifestations in infection, sepsis, trauma, and wound healing. The contributors to this book have given their perspectives on systems approaches to these disease states, and have shown specific examples of the translational utility of mechanistic computational modeling. Though this book covers over a decade of progress, these are still early days in this field. The central challenge remains integrating the multi-scale,

multi-system nature of acute inflammation into computational models that drive actionable outcomes. Translational Systems Biology must rise to the challenge of integrating inflammatory, neuro-endocrine, and physiologic processes in order to unravel the multi-dimensional, multi-compartment, and highly dynamic disease landscape [1, 13].

How can these goals be met, especially in the broader context of a biomedical research enterprise that—despite calls for increased use of systems and computational biology—is still overwhelmingly focused on reductionist research focused on single molecules, along with a clinical regulatory infrastructure that—despite similar call for the incorporation of computational modeling—is still focused on single therapeutic targets? We discuss how to implement Translational Systems Biology in the context of the work presented in this book.

An early entry point for Translational Systems Biology was the in silico clinical trial (see Chap. 8). This methodology, in some form or another, is beginning to inform the design of actual clinical trials by the pharmaceutical industry. However, the drugs being tested in silico continue to be developed by some variant or another of a painstaking, slow, and expensive process that has not, at the very least, facilitated the concept of "fail early, fail often, and fail cheaply" that underlies successful drug development [14, 15]. In this context, the value of in silico clinical trials may well rise dramatically if mechanistic computational modeling could be employed at the earliest stages of drug development, ideally being part of the initial process of weeding out the large number of potentially druggable compounds obtained via high-throughput screens. Moreover, this approach—especially incorporating quasi-mechanistic data-driven modeling—has the potential to suggest disease biomarkers simultaneously with drug targets [15]. For example (see Chaps. 4 and 14), statistical analyses, hierarchical and k-means clustering, Principal Component Analysis, and Dynamic Network Analysis suggested the chemokine monocyte chemoattractant protein 1/chemokine (C-C motif) ligand 2 (MCP-1/CCL2) and interleukin-1α (IL-1α) as central coordinators of hypoxia-induced inflammation in mouse hepatocytes, based on a screen of nearly 20 inflammatory mediators assessed over multiple time points. This finding led to the hypothesis the MCP-1 was a central coordinator of hepatic inflammation. In support of this hypothesis, hepatocytes from MCP-1-null mice had altered dynamic inflammatory networks. Importantly, circulating MCP-1 levels segregated blunt trauma survivors from non-survivors. Furthermore, patients with elevated early levels of MCP-1 post-injury had longer total lengths of stay, longer intensive care unit lengths of stay, and prolonged requirement for mechanical ventilation. This study identifies MCP-1 as a main driver of the response of hepatocytes in vitro and as a biomarker for organ damage in a clinical setting, and suggests an experimental and computational framework for discovery of novel clinical biomarkers in inflammatory diseases [16]. We speculate that MCP-1 may serve as a therapeutic target in addition to being a potential diagnostic biomarker. In support of this hypothesis, MCP-1-null mice had lower levels of circulating damage markers following experimental trauma/hemorrhage (Zamora, Vodovotz, and Billiar, unpublished observations). Future mechanistic, equation based computational models (see Chaps. 2, 4, 7, and 8) that incorporate dynamic inflammation

networks driven by MCP-1 may thus serve as a basis for in silico clinical trials of existing and novel compounds targeting MCP-1.

In silico trials and drug development may also be implemented using agent-based models (Chaps. 3, 5, 8, 12, 13 and 15). As in keeping with the progression of the use of modeling and simulation in other fields, there are successive tiers of validation targets, ranging from face validity and determination of plausibility to near-engineering grade simulations that can be used to augment individual trials [15, 17]. However, the concept of an in silico trial need not wait for such high-resolution modeling; as alluded to above the pervasive issue associated with planning control is whether, at a system-level, is a particular pathway even a good idea? As such, the use of agent-based modeling is particularly well suited for dynamic knowledge representation, determination of the sufficiency of existing knowledge structures, and to allow researchers to identify "holes" in their knowledge that can be filled with new experiments and new classes of therapies. The intuitive nature of agent-based modeling makes the use of dynamic computational modeling a bit more accessible to the average biomedical researcher, and facilitates the ability of those researchers to visualize and manipulate these "instantiated thought experiments" on their way to greater translational efficiency [2, 18].

Systems-level insights—some of them obtained from 'omics studies—derive from the study of acute inflammation in sepsis and trauma (Chaps. 2–9) are also likely to guide translational advances in settings such as wound healing (Chaps. 10–13) as well as elucidating key host-pathogen interactions in various other diseases (e.g., tuberculosis [Chap. 15]). Ultimately, these developments will likely impact the study of chronic inflammatory diseases, settings in which the crucial dynamics of the disease processes are even more pronounced and manifest as altered dynamic equilibrium states that are yet more complicated to reset and control [1, 10].

In conclusion, and as noted above, we believe that the state of biomedical research sits at a crucial tipping point where the continued credibility of scientific claims is at stake, driven by the manifest desires of an increasingly wishful public [19] but in a form that may, at times, lead to disappointment [20]. We suggest that that a future path predicated upon returning to scientific fundamentals, with an emphasis on inherently dynamic processes, is a means of backing away from the credibility cliff and onto the ground of rationally grounded expectations and clinically relevant progress.

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