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Predicting experimental sepsis survival with a mathematical model of acute inflammation

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Abstract

Sepsis is characterized by an overactive, dysregulated inflammatory response that drives organ dysfunction and often results in death. Mathematical modeling has emerged as an essential tool for understanding these complex biological processes. A system of four ordinary differential equations (ODEs) was developed to simulate the dynamics of bacteria, the pro- and anti-inflammatory responses, and tissue damage (whose molecular correlate is damage-associated molecular pattern [DAMP] molecules and which integrates inputs from the other variables, feeds back to drive further inflammation, and serves as a proxy for whole-organism health status). The ODE model was calibrated to experimental data from *E. coli* infection in genetically identical rats and was validated with mortality data for these animals. The model demonstrated recovery, aseptic death, or septic death outcomes for a simulated infection while varying the initial inoculum, pathogen growth rate, strength of the local immune response, and activation of the pro-inflammatory response in the system. In general, more septic outcomes were encountered when the initial inoculum of bacteria was increased, the pathogen growth rate was increased, or the host immune response was decreased. The model demonstrated that small changes in parameter values, such as those governing the pathogen or the immune response, could explain the experimentally observed variability in mortality rates among septic rats. A local sensitivity analysis was conducted to understand the magnitude of such parameter effects on system dynamics. Despite successful predictions of mortality, simulated trajectories of bacteria, inflammatory responses, and damage were closely clustered during the initial stages of infection, suggesting that uncertainty in initial conditions could lead to difficulty in predicting outcomes of sepsis by using inflammation biomarker levels.

1 Introduction

Sepsis is defined as life-threatening organ dysfunction caused by an overwhelming immune response to an infection (1). Sepsis and septic shock often lead to organ failure and are leading causes of morbidity and mortality (1, 2). In the course of a typical infection, pathogens (e.g., Gram-negative bacteria) infect the host, triggering a pro-inflammatory innate immune response targeted at eliminating the pathogen from the system. The body also mounts an anti-inflammatory response that works to maintain homeostasis and prevent overwhelming inflammation to the system. The acute inflammatory response in sepsis becomes detrimental when it can no longer be contained at the locus of infection (where innate immune mechanisms can help eliminate bacteria) and becomes systemic, leading to collateral damage/dysfunction in the surrounding healthy tissue. Thus, a major goal in sepsis research is to determine why the host mounts an overwhelming inflammatory response to infections which leads to sepsis in some cases but not in others (3, 4).

Acute inflammatory responses to infection are further complicated by additional variations among sepsis patients (5), the complex interplay between pro- and anti-inflammatory mediators and the innate and adaptive immune responses (6), and a triphasic distribution of patient deaths that occur days, weeks, and years after initial infection (6). These variations have highlighted the need for patient-specific treatments and novel approaches to sepsis drug design and clinical trials (7-9).

Recent decades have brought about improved sepsis treatment practices, including the rapid administration of antibiotics, infection source control, appropriate choice of fluid (crystalloids) for fluid resuscitation, and administration of vasopressors (norepinephrine) (10). These evolutionary

74 improvements in patient care have reduced in-hospital mortality but have shifted the mortality
75 distribution so that most sepsis-related deaths occur months after treatment (6). While improved care
76 often allows patients to overcome initial septic episodes, it has been speculated that underlying
77 physiologic/biochemical aberrations combined with sepsis-initiated immune dysfunction place
78 patients at long-term risk for sepsis mortality (11). Secondary infections occurring during late stages
79 (> 15 days) were shown to correlate with higher levels of opportunistic fungi and bacteria compared
80 to earlier secondary infections (< 6 days) (12), and impairments in immune function and cytokine
81 secretion were observed in individuals that survived a septic episode (13). Despite evidence of late-
82 stage complications or mortality from sepsis (14), mortality is still common within a week of sepsis
83 onset; interventions in those cases rely on early identification of septic trajectories, which is often
84 difficult.

85

86 Given the complexities of infection and inflammation *in vivo* and *in vitro*, mathematical modeling
87 has been used in an attempt to unravel the complexities of inflammation and the immune response (8,
88 9, 15-17). Multiple mechanistic computational models have described the dynamics of the
89 inflammatory response in the context of a variety of bacterial infections (18-20). These include
90 equation-based (ordinary (16, 17, 21-35) and partial differential equations (36, 37)), agent-based (38-
91 40), and hybrid models (41). Although some models are purely theoretical (16, 17, 21, 37, 40), many
92 were calibrated to experimental data in animals (23-25, 28-30) or attempted to replicate clinical
93 outcomes (26, 38, 39, 42).

94

The current study implemented a mechanistic model of the immune response to an *Escherichia coli (E. coli)* infection and aimed to determine whether early data on both the pathogen and the host response are sufficient to predict a health or disease outcome. A parsimonious system of four ordinary differential equations (ODEs) was used to track changes in bacteria, tissue damage, a pro-inflammatory response, and an anti-inflammatory response following the administration of escalating inoculums of *E. coli* encapsulated in a fibrin clot (an experimental model of Gram-negative peritonitis (43)) in rats. Ultimately, the mathematical model defined in this study was used to evaluate and predict the time dynamics of the bacterial infection, which were observed to vary depending on the initial bacterial dose. The model demonstrated that small changes in the pathogen growth rate or characteristics of the immune response could lead to significantly different mortality times despite identical initial bacterial loads.

2 Materials and Methods

2.1 *Experimental Method*

2.1.1 Preparation and Dose Estimation of *E. Coli*-Impregnated Fibrin Clot

All animal studies were carried out following approval by the University of Pittsburgh Institutional Animal Use and Care Committee (IACUC approval #0807947) and complied with the NIH Guide for the Care and Use of Laboratory Animals. In this study, experimental data were obtained from rats with peritonitis induced by an *E. Coli*-impregnated fibrin clot, similar to the methods established by Ahrenholz *et al*. (43) and modified by Namas *et al.* (44). In brief, the animals were subjected to a varying dose of *E. Coli* (strain ATCC 25922; American Type Culture Collection, Manassas, VA,

USA) inoculum in a fibrin clot introduced into the peritoneum via laparotomy. The *E. Coli* colonies were grown to a specified optical density using a spectrophotometer (DU 530 UV/VIS; Beckman Coulter, Brea, CA, USA) equivalent to a concentration ranging between 1×10^8 to 5×10^8 colony-forming units [CFUs]/clot on the day of bacterial fibrin clot implantation. After the addition of fibrinogen (1%) and Thrombin (15u), the clot was placed in the peritoneum via laparotomy, as described previously (44). Since quantification of bacteria at the time of implantation of the fibrin clot via optical density is highly imprecise, bacteria were quantified after each implantation by limiting dilution plating to obtain a count of implanted *E. coli* in a given rat (44).

We determined that rats that received a fibrin clot inoculum of 1×10^8 to 2.0×10^8 CFUs/clot had a mortality rate of ~40–45% after 48 h of clot implantation while rats that received inocula higher than 2.0×10^8 CFUs/clot had a mortality rate of 80% during the first 24 h of implantation (data not shown). All rats in these experiments exhibited signs of septicemia in the form of lethargy, hypothermia, reluctance to feed, tachycardia, and tachypnea after 24 h of *E. Coli* fibrin clot impregnation into the peritoneum. After closing the abdomen, a topical anesthetic was applied over the surgical wound, and the rats were returned to their cages and allowed food and water *ad libitum*.

2.1.2 Model Calibration and Validation

The experimental studies detailed above generated one experimental data set that was used to calibrate the model and consisted of measurements of bacterial levels at different timepoints in 31 rats injected with increasing doses of bacteria (1.28, 2.48 and 5.05×10^8 bacteria with standard error

of the mean of 1.5×10^7, 2.8×10^7, and 1.2×10^8, respectively). A second (separate) experimental data set was used to validate the model and consisted of time to death (mortality; 24, 48, 72, or 96 h) for 27 rats injected with varying doses of bacteria (1-5 $\times 10^8$ bacteria). We detail the process of model calibration and validation below.

Bacterial levels in the peritoneal cavity measured in individual septic rats, each euthanized at a distinct time point from 24-96 h after being impregnated with $\approx 1 \times 10^8$ to 5×10^8 colony-forming units of *E. coli,* were used to calibrate the model ("calibration data set", see Fig 1A and Table S1). Observed times to mortality for rats injected with $\approx 1.4 \times 10^8$ to 4.8×10^8 colony-forming units of *E. coli* were used to evaluate the predictive capabilities of the ODE model ("validation data set," see Fig 1B and Table S2). These two data sets were derived from two different rat populations.

The calibration data set (Fig 1A and Table S1) was produced using several experiments involving multiple rats in each experiment and three different bacterial levels with mean values of 1.28×10^8, 2.48×10^8, and 5.05×10^8 bacteria. A peritoneal lavage was used to assess the number of bacteria in the peritoneal cavity in each individual animal euthanized at one of the time points indicated in Table S1, as detailed in (44). This produced an estimate for the expected bacterial levels over time in a rat subject to one of the targeted loads, as seen in Fig 1A. The time points were chosen to be closer together for rats injected with higher bacterial loads, since these animals were more likely to die earlier than animals receiving a lower bacterial inoculum.

In the validation data set (Fig 1B and Table S2), the rats were monitored continuously until 96 h, at which time surviving rats were euthanized. If a rat was observed to be dead at a 24-h checkpoint (24, 48, 72, or 96 h), that time point was noted as the "observed mortality time" (OMT) for that rat and the rat was removed from the study. Rats labeled with an OMT = 24, 48, or 72 h either died within the 24 h before their OMT or were euthanized at their OMT (in accordance with IACUC and NIH guidelines) due to being severely unhealthy. Rats labeled with a 96 OMT either died between 72 and 96 h, were severely unhealthy at 96 h, or were relatively healthy at 96 h. Given these distinctions, it is possible that some of the 96 h OMT rats would have survived longer.

Fig 1B depicts the mortality data set (closed circles, ●) collected for 27 rats with the observed mortality time on the *x*-axis and the initial bacterial load on the *y*-axis. A Bartlett test revealed that the variances of the four groups (OMT = 24, 48, 72, 96 h) were not significantly different (*p*-value = 0.689) while ten out of ten normality tests (45) on the deviations in the data from their respective group norms were passed, suggesting that the data in each group are approximately normally distributed. Analysis of variance (ANOVA), which assumes normally distributed data with equal variances across groups, was then performed to show that the initial bacteria levels for the three OMT = 48-, 72-, and 96-h groups with OMT > 24 h were not significantly different from each other (*p*-value = 0.251). More specific delineation of the health and disease status of the rats in the 96-h group may have led to more variance between the OMT = 48-, 72-, and 96-h groups, but those data were not available at the time of this study. ANOVA on all four groups and use of Tukey's procedure with 95% confidence intervals suggested that the OMT = 24-h group had significantly different levels of initial bacterial load compared to the OMT = 48-, 72-, and 96-h groups (*p*-value < 0.0051). The analysis was performed with Matlab (*normalitytest* (45) and Matlab's *vartestn*, *anova1*,

and *multcompare;* Matlab code available via Github, see Supplementary Information), and the resulting conclusions regarding the differences between the groups were consistent with the relationships suggested by the box and whiskers plot shown in Fig 1B. The analysis suggested that there were effectively only two different experimental groups: rats with OMT = 24 h and rats with OMT > 24 h. These two categories were therefore used when assessing the predictive capabilities of the model.

2.2 Model and simulations

2.2.1 Model variables and interactions

The mathematical model developed in this sepsis study was based on previous models of the immune response (16, 21, 36, 46, 47). It was used to predict the dependence of health or disease outcomes on pathogen properties and the immune response. Following the administration of *E. coli inoculum* into the peritoneum, the model was used to predict changes in the number of bacteria in the peritoneal cavity of the animal (B), the level of tissue damage (ε), a pro-inflammatory response (M), and an anti-inflammatory response (A). Tissue damage serves as a proxy for whole-organism health status. *In vivo*, the molecular correlates to tissue damage are damage-associated molecular pattern [DAMP] molecules such as high-mobility group box 1 (HMGB1) (48, 49). Due to limited calibrating data and a desire for model simplicity, the numerous cells and cytokines that generate the pro- and anti-inflammatory responses were grouped into two general populations, as in previous models (16, 46, 50). Specifically, tissue-specific effects of bacterial infection were assumed to be driven by both resident tissue macrophages and inflammatory cells such as neutrophils and macrophages that infiltrate the tissue from the blood (51-54). Nominally pro-inflammatory cytokines (e.g., TNF, IL-1b, IFN-γ, IL-17A) and anti-inflammatory cytokines (e.g., TGF-β1, IL-10, IL-4, IL-13) were not

measured in these experiments, and therefore these cytokines (along with a multitude of chemokines and DAMPs) were grouped into general populations whose effects were assumed independent of inflammatory mediator type.

Fig 2 provides a schematic of the assumed interactions (54) among model populations (schematic adapted from (16)). A time-dependent dosing function, *D(t)*, defines the release of bacteria from the clot into the surrounding tissue. In response, pro-inflammatory cells activate and begin to destroy the bacteria while also causing collateral tissue damage and promoting self-recruitment. Both pro-inflammatory cells and tissue damage trigger an anti-inflammatory response, which in turn inhibits the growth of the pro-inflammatory response and damage levels. These interactions are labeled in Fig 2 with arrow heads indicating upregulation and blunted ends indicating downregulation.

Although it is nearly impossible to state that no other model could account for the variables and interactions depicted in Fig 2 (55), the approach utilized in this study adapted previous models (16, 46) of bacterial infection that were successful at reproducing multiple physiological behaviors. The current model was based on clear assumptions, including ones that stemmed from the experimental technique for bacterial administration into the rats. More specifically, Ahrenholz and Simmons (43) originally used the experimental method implemented in this study to consider the theory that increased fibrin levels decrease the severity of infections during *E. coli* peritonitis. Namas et al. (44) used the same procedure to explore the effectiveness of hemadsorption techniques on treating septic peritonitis. They performed several initial experiments in which they injected rats with fibrin clots containing varying levels of bacteria. The rats usually died or resolved in a relatively short amount

of time, so only the acute immune response was assumed to play a significant role in infection dynamics. Additionally, the experimental technique for bacterial administration introduced bacteria more gradually into the system than instantaneous bacterial injection into the blood. Both observations played a significant role in the modeling choices employed here.

2.2.2 Model equations

The rate of change of the number of bacteria in an animal is governed by Equation 1:

$$\frac{dB}{dt} = D(t) + k_1 B\left(1 - \frac{B}{B_\infty/B_s}\right) - \frac{k_2 s_l B}{\mu_l + k_3 B_s B} - \frac{k_5 B M}{1 + k_A A} \quad (1)$$

The first term, *D(t)*, defines the release of bacteria from the fibrin clot as an exponentially decaying dosing function given by $D(t) = D_1\exp(-k_D t)$. The second term corresponds to logistic growth of bacteria with growth rate k_1. The third term corresponds to a non-specific, local, innate immune response that is assumed to eliminate a small amount of pathogen without activating a full systemic inflammatory response (16). The details of the parameter value derivation for this term are given in (16). The final term defines the elimination of bacteria by the systemic pro-inflammatory response, which is inhibited by systemic anti-inflammation.

As a first-order approximation, all bacteria initially in the clot were assumed to empty into the surrounding tissue without reproducing or dying, which is equivalent to $\int_0^\infty D(t)\,dt$ $=\int_0^\infty D_1\exp(-k_D t)\,dt = B_{source}$, where B_{source} is the initial amount of bacteria in the fibrin clot (B_{source}

250 = 128×10^6, 248×10^6, or 505×10^6 bacteria in the calibration data set). To satisfy this condition
251 for the conservation of clot bacteria, D_1 must equal $k_D B_{source}$. B was also scaled by a factor B_s (see
252 Section 2.3) to account for the difference in bacterial levels between the rat experiments described in
253 this study and the bacteria levels considered in the previous (human) models (16). This factor also
254 converts number of bacteria to concentration of bacteria (bacteria/cm^3) and allows previous
255 parameter values (16, 46) to be used here.

256

257 We note that multiple dosing functions were considered before choosing the exponential decay
258 model. For example, an additional variable tracking the bacterial levels inside the clot was
259 introduced and allowed to experience growth and/or transfer between the clot and surrounding tissue.
260 Despite providing three additional degrees of freedom, this alternate approach did not yield model
261 fits that were significantly better than using a simple exponential function to define the rate at which
262 bacteria emptied into the surrounding tissue. We also explored making parameter k_d a saturating
263 sigmoidal function of B_{source}, since experimental observations suggested that a clot with a small
264 number of bacteria had a slower release rate than a clot containing a large inoculum of bacteria. This
265 assumption, however, also did not lead to improved model fits. Thus, an exponential function with
266 constant rate of decay, k_d, was implemented in the model and yielded reasonable fits to the data.

267

268 Equation 2 defines the rate of change of the systemic pro-inflammatory response (M):

269
$$\frac{dM}{dt} = \frac{\nu_1 f(M,B,\epsilon)}{(\nu_2 + f(M,B,\epsilon))(1+k_A A)} - \mu_M M. \qquad (2)$$

where $f(M, B, \epsilon) = k_M M + k_B B_s B + k_\epsilon \epsilon$. The pro-inflammatory response is increased by pro-inflammatory cells, bacteria, and damage and is inhibited by the systemic anti-inflammatory response. Natural decay of the response occurs at rate μ_M. Recruitment of inactive pro-inflammatory cells occurs at a rate proportional to ν_1, as given in (16, 21). The possible effect of LPS inflicting toxicity (without any bacteria present) has been subsumed into the parameter values for the onset and persistence of inflammation as a consequence of bacterial infection. T helper 1 cells (Th1) and T helper 17 cells (Th17) are also assumed to be included in model population *M*.

The rate of change of the anti-inflammatory response (A) is defined in Equation 3:

$$\frac{dA}{dt} = s_A + \frac{\nu_3(M + k_4\epsilon)}{(\nu_4 + M + k_4\epsilon)(1 + k_A A)} - \mu_A A. \quad (3)$$

The first term accounts for the nonzero background level (s_A) of anti-inflammatory mediators that resides in the body. In the second term, the pro-inflammatory response and damage activate the anti-inflammatory response. The anti-inflammatory response also self-regulates, which is modeled using the ($1+k_A A$) term in the denominator. Natural decay of the response occurs at rate μ_A.

The final model equation (Eq. 4) gives the rate of change of tissue damage levels (ε):

$$\frac{d\varepsilon}{dt} = -\frac{\varepsilon}{\tau} + \frac{[fM - T]_+}{1 + k_A A}. \quad (4)$$

In the first term, damage is repaired at rate $1/\tau$. In the second term, similar to (46), it was assumed that the system can tolerate a certain level of inflammation before pro-inflammatory cells begin causing damage. This assumption is represented using a threshold function:

$$[fM - T]_+ = \begin{cases} fM - T & (fM - T) > 0 \\ 0 & \text{otherwise} \end{cases}. \quad (5)$$

T gives the threshold above which immune cells begin to cause damage in the nearby tissue, and f gives the rate at which the cells cause damage.

2.2.3 Initial conditions

At the onset of each simulation, the rats were assumed to be healthy, corresponding to initial conditions of $B(0) = 0$, $M(0) = 0$, $A(0) = s_A/\mu_A$, and $\varepsilon(0) = 0$. Since animals exhibit background levels of anti-inflammatory mediators (e.g., transforming growth factor-β1 (56)), nonzero initial background levels of anti-inflammatory response mediators were assumed. Parameter B_{source} corresponds to the number of bacteria inserted into the clot for each experiment and controls the magnitude of the dosing function, $D(t)$. Thus, B_{source} was changed in each simulation (i.e., for each rat) to correspond to the experiment being modeled.

For high enough B_{source}, it was observed that the clots were highly saturated to the point that bacteria left the clot nearly instantaneously. To model this, $B(0)$ was set to $B_{source} - B_c$, where B_c is a fitted parameter thought of as the "clot capacity." This assumes the number of bacteria that exceed B_c instantaneously enter the surrounding tissue. With only B_c bacteria assumed to remain in the clot after the initial injection, the dosing function is adjusted to depend only on those bacteria (B_c) remaining in the clot: $D(t) = k_D B_c \exp(-k_D t)$.

2.3 Parameter values

Table 1 provides the values, units, and sources for all twenty-six model parameters. Because of the lumped nature of variables M, A, and ε, general units ("M-units", "A-units", and "ε-units") were used to describe these populations, as treated previously (16, 36, 46). To be consistent with experimental measures, the units for bacteria were given as 10^6 bacteria. Several parameter estimation techniques were utilized to yield maximum parameter estimation efficiency and physiologically realistic results.

The unknown parameter space dimension was decreased by estimating several parameter values using previous studies. Following Reynolds et al. (16), k_A was chosen so that anti-inflammatory inhibition is at most 75% (57), and μ_A was chosen to be significantly smaller than typical cytokine decay rates (58-61) because these cytokines have longer lasting downstream effects in the anti-inflammatory cascade. The quality of the parameter fit varied relatively little (<0.1%) when parameter T was allowed to vary. Choosing T too large made $[fM - T]_+ = 0$ for all simulations while choosing T too small made $[fM - T]_+ \approx fM$, which violates the assumption that the system can tolerate a certain *significant* level of inflammation (46). Setting T to 1 avoided both extremes without affecting fit quality.

Seven parameters remained that still had relatively high levels of uncertainty regarding their values. Those parameters (B_∞, f, k_D, B_s, ν_4, B_c, and k_1) were estimated using a constrained least squares optimization on log-transformed data. The fit was constrained based on qualitative observations regarding the general inflammation paradigm where typically one of three qualitative outcomes occur (54). In the first type of outcome, termed "healthy recovery," individuals are affected by an infection, but their inflammatory response is quick and successfully eliminates the infection. In the

second type of outcome, termed "aseptic death," the inflammatory response eliminates many of the bacteria but overwhelms surrounding healthy tissue until death occurs. In the third type, termed "septic death," large numbers of bacteria survive alongside a mounting inflammatory response and death results. Typical dynamics in other studies (16-18, 21) suggest that lower initial pathogen levels often produce healthy recovery outcomes, mid-level initial pathogen levels often produce aseptic outcomes, and high levels produce septic outcomes. To produce a model capable of similar behavior, the optimization process was constrained so that simulated rats injected with 128×10^6 bacteria experienced healthy recovery outcomes, simulated rats injected with 248×10^6 bacteria experienced aseptic outcomes, and simulated rats injected with 505×10^6 bacteria experienced septic outcomes. Such an approach is supported by the following assumptions: (i) the relatively good fit to the data ($R^2 = 0.70$); (ii) relatively low levels of bacteria ($O(10^6)$) in the 248×10^6 initial bacterial load calibration experiments after 72 h (i.e., probably not septic); (iii) persisting high levels of bacteria ($O(10^7)$) in the 505×10^6 initial bacterial load calibration experiments after 48 h (i.e., probably septic); and (iv) high mortality rates for loads above 200×10^6 bacteria (44). The large range of measured bacterial levels suggested that log-transforming the data was also reasonable. Importantly, the standardized residuals associated with the log-transformed measurements and simulated data (Fig 3B) passed ten out of ten normality tests (45). Similar outcomes were not possible for data that were not log-transformed.

In the optimization procedure, the following squared residuals of the log-transformed data were minimized:

$$\sum_{i=1}^{N_m} (\log(y_i) - \log(\hat{y}_i))^2 \qquad (6)$$

where y_i corresponds to all experimentally measured bacterial levels at the time points and initial bacterial loads listed in Table S1, $\hat{y}_i$ corresponds to the model-predicted bacterial levels at the same time points and initial bacterial loads, and $N_m = 31$ is the total number of measurements. Although a range of values for k_2 and ν_1 are later used to assess their effects on system dynamics (see Fig 8), during the optimization, and by default unless otherwise stated, k_2 and ν_1 were set to 0.6 1/*M*-units/h and 0.08 *M*-units/h, respectively, to be consistent with the values used in Reynolds et al (16). Parameter k_1 was fit during the optimization procedure to yield its default value of $k_1 = 1.27$/h; this value was used throughout the study except when assessing the impact of varying this parameter (Fig 8). Multiple standard optimization techniques were used during the process to ensure the finding of a reasonable optimum, including the Nelder-Mead simplex method, the steepest descent algorithm, and random restarts (Matlab code available via Github, see Supplementary Information). The resulting parameter estimates are given in Table 1.

2.4 Sensitivity calculation

Due to the high dimension of parameter space (26 parameters) and the relatively small amount of data (18 independent time points), only a small subset of the parameters should be optimized at any given time to avoid excessive computations and identifiability issues. Identifiability is discussed later in this section but briefly, the larger the number of fitted parameters, the more likely we will encounter an unidentifiable parameter set where multiple sets of parameter values can be used to produce the exact same optimal fit quality. Having no unique optimal parameter set can cause traditional optimization techniques to fail. Here, seven parameters were chosen to be fit based on their associated levels of uncertainty. It is possible that improved fits could be obtained by choosing a different parameter set (e.g., by fixing some of the fit parameters to other estimates found in the

383 literature (16)). In addition, while calibrating the model to a given data set will limit the general
384 dynamics that a model may exhibit, it is possible that multiple optimal parameter sets exist whose
385 dynamics significantly differ.

386

387 To explore (i) the suitability of the current model, (ii) the set of fit parameters, and (iii) the possible
388 effects of varying other parameters, a local sensitivity analysis around the current best fit was
389 performed. The relative sensitivities, s_{ij}, of the i^{th} simulated measurement ($\hat{y}_i$) with respect to the j^{th}
390 parameter (p_j) were estimated using the relative change in $\hat{y}_i$ divided by the relative change in the
391 parameter value:

392 $$s_{ij} = \frac{\left(\frac{\hat{y}_i(p_j+p_j\delta_j)-\hat{y}_i(p_j)}{\hat{y}_i(p_j)}\right)}{\left(\frac{(p_j+p_j\delta_j)-p_j}{p_j}\right)} = \left(\frac{\hat{y}_i(p_j+p_j\delta_j)-\hat{y}_i(p_j)}{\delta_j\hat{y}_i(p_j)}\right) \quad (7)$$

393

394 In Eq. 7, the magnitude of δ_j is chosen to be the square root of machine epsilon. For some
395 parameters, differently signed δ_j's would alter the original simulation outcomes (healthy recovery,
396 aseptic, septic). While such changes can happen in reality, this results in significant jumps in
397 simulated measurement values and complicates the analysis. A complete analysis should consider
398 behavior both when such jumps occur and when they do not and average the results. To include all
399 parameters in the jump data analysis, δ_j would need to be altered so that all parameters experience
400 such jumps. Since the jumps are typically of approximately the same size, an alternate strategy
401 would need to be developed to compare these similarly sized jumps. Because of such complications,
402 only sensitivities with no outcome behavior changes were considered here. At the same time, we

note that larger calculated values of $|s_{ij}|$ (calculated in the absence of outcome changes) correlate well with increased likelihood of these more significant outcome changes. To gauge the general sensitivity of all measurements to particular parameters and to understand how much a given parameter may affect outcomes, the root mean squared sensitivity (RMSS, S_i) was calculated:

$$S_i = \sqrt{\frac{1}{N_m}\sum_{i=1}^{N_m} s_{ij}^2} \tag{8}$$

where $N_m = 31$ gives the number of measurements made in the calibrating data set. This sensitivity value (S_i) is provided in Table S3 and provides an estimate for the relative importance of each parameter in the model fitting process with higher sensitivities corresponding to parameters that affect the fit and corresponding dynamics more. Better and quicker fits tend to result when parameters are more sensitive. For any given set of parameters, a sum of their RMSSs can give an estimate to how sensitive the measurements are to that specific parameter set.

Identifiability is another important consideration when assessing how parameter values affect system dynamics. As an example, model parameters k_2 and s_1 are not identifiable from the data because increasing k_2 by 1% will produce the exact same change in the variable outputs as increasing s_1 by 1%. This is because they only appear in the model as ($k_2 \cdot s_1$), never separately elsewhere. In such scenarios, it is impossible to identify the values of such parameters without additional outside information, as was the case in (16). Studying the identifiability of a large set of parameters with an associated nonlinear system can be difficult. While standard linear algebra methods can be used for linear systems (62, 63), nonlinear systems require more specialized and often more computationally and theoretically intensive approaches such as methods utilizing rational functions (64), methods focusing on local expansions or transformations (63), and methods centering on observability (65).

Here we employed a local analysis, including linearization about the current model's parameter values, to consider the issue of identifiability. In the local analysis, identifiability is investigated by considering the collinearity of the parameters, where the effects of changing one parameter can be reproduced by a linear combination of changes in the other parameters. In such cases, parameters can be redundant, with their variations capable of being reproduced by changing the other parameters. The idea of collinearity can be written in terms of the previously mentioned sensitivities: $s_{ik} = \sum_{j \neq k} \alpha_j s_{ij} \; \forall i$. A corresponding collinearity index can be defined as: $CI_k = 1/\sigma_{min}$ where σ_{min} is the smallest singular value of the associated sensitivity matrix defined by components s_{ij} (66). Lower collinearity indices indicate more weakly correlated parameters that better describe the local parameter space and typically produce better overall fits and corresponding calibrated models. Such analysis identifies parameters sets that should not be used for fitting (high CI_k values) because of the possible presence of redundant parameters. The index can also be used to identify sets of parameters that may produce better fits.

3 Results

3.1 Bacterial infection model dynamics reproduce key sepsis outcomes

The bacterial infections simulated in this study led to three possible outcomes: healthy recovery, aseptic death, or septic death (16). In this model, a healthy recovery outcome corresponds to a return to a steady state where pathogenic bacteria are fully eliminated ($B = 0$) and tissue damage levels return to baseline pre-infection levels ($\varepsilon = 0$). Aseptic death occurs when the bacteria are cleared ($B = 0$) but damage levels remain elevated ($\varepsilon > 0$). Septic death results when bacteria levels ($B > 0$) and damage ($\varepsilon > 0$) remain elevated at steady state.

In Fig 4, all three outcomes were predicted to occur given the same level of initial bacterial dose (B_{source} = 128 × 10^6 bacteria) but for three different levels of pathogen growth rate, k_1. For a low pathogen growth rate (k_1 = 1.2/h), the pro-inflammatory response eliminated the bacteria, and healthy recovery (B/B_{max}, M/M_{max}, $(A\text{-}A_{healthy})/A_{max}$, and $\varepsilon/\varepsilon_{max}$ all less than 1%) was predicted to be restored by 37 h (cyan curve). For a slightly higher pathogen growth rate (k_1 = 1.3/h), the pro-inflammatory response also eliminated the bacteria, but caused an elevated and sustained inflammatory response corresponding to aseptic death (blue curve) due to a forward-feedback loop of inflammation → damage → inflammation (44). For a high pathogen growth rate (k_1 = 1.4/h), the pro-inflammatory response was incapable of eliminating the bacteria, so bacterial levels, damage, and the anti-inflammatory response all remained elevated at the steady state (corresponding to septic death, red curve).

3.2 Sensitivity analysis supports choice of initial model parameters

Calculating local collinearities and sensitivities allowed for the identification of other parameter sets that are better (from a sensitivity and collinearity point of view) than the best fit identified in this paper. As an example, k_D, B_s, B_c, k_1, k_2, μ_I, and s_A have a sum of RMSSs of 137 and a collinearity index of 17 while the fit used in this paper has a sum of RMSSs of 62 and a collinearity of 55 (less sensitive and more collinear). Despite worse metrics, optimization of this alternative parameter set (originally suggested by our local sensitivity and collinearity analyses) did not significantly improve the overall parameter fit (R^2 still approximately 0.70). This suggests that the parameters currently

chosen for the model fitting are reasonable despite lower sensitivities and higher collinearities compared to other parameter sets.

The values of RMSS (Table S3) showed that the model results are most sensitive to local immune response parameters (i.e., s_I, k_2, μ_I and k_3) and second-most sensitive to pathogen and dosing related parameters (i.e., k_1, k_D, B_s, B_{inf}). The other parameters affecting the inflammatory response and damage displayed lower sensitivities.

3.3 Comparison of model simulations and experimental data suggest a high mortality prediction capacity

Prior studies utilizing reduced models of sepsis-induced inflammation were not docked to data, especially with regard to the level or trajectory of the damage variable that equated with death (16, 17, 21). To address this shortcoming, we compared the behavior of the damage variable in our model to mortality observations in an experimental model of Gram-negative bacterial sepsis.

In Fig 5A, the ability of the model to use damage levels to predict deaths occurring within 24 h (OMT = 24 h) and deaths occurring after 24 h (OMT > 24 h) was quantified by comparing damage-based predictions of the mathematical model with the experimental observations in the validation data set. Using the mathematical model, the time of death (i.e., mortality time), t_c, for a simulated rat was assumed to be the model-predicted time at which the value of the damage variable, ε, reached a critical damage level (ε_{crit}). If $t_c \leq 24$ h, the rat was predicted to die within 24 h and to belong to the

493 OMT = 24-h group. Similarly, if $24 < t_c \leq 48$, the model-predicted value for the OMT was 48 h and
494 the rat was predicted to belong to the OMT > 24-h group. The initial levels of bacteria given to each
495 rat from the validation data set (27 total) were used as inputs to the calibrated model to yield model
496 predictions for survival time (OMT = 24 h or OMT > 24 h) for each rat in the study. Model accuracy
497 was defined as the ratio of correct predictions to total number of predictions. Both the predictions
498 and model accuracy are functions of ε_{crit}, as shown in Fig 5A. A relatively high level of accuracy (>
499 80%) was found for a wide range of critical damage levels ($49.2 \leq \varepsilon_{crit} \leq 123.4$ ε-units). A maximum
500 accuracy of 96% was attained for ε_{crit} values between 49.8 and 55 ε-units.

501

502 As an additional assessment tool of the predictive capability of the model, an area under the curve-
503 receiver operating characteristics (AUC-ROC) curve was constructed (Fig 5B). In this figure, a
504 positive outcome or identification corresponds to death occurring within 24 h. As such, 'true
505 positive' corresponds to a model prediction agreeing with an experimental observation of a rat that
506 dies within 24 h, while 'false positive' corresponds to a model prediction of a rat dying within 24 h
507 when the experiment indicated that the rat did not die within 24 h. The recall/sensitivity/true positive
508 rate = (number of true positives)/(number of correct predictions) and the false positive rate = (number
509 of false positives)/(number of correct predictions). These rates are calculated for $0 \leq \varepsilon_{crit} \leq 200$ ε-
510 units to produce the AUC-ROC curve in Fig 5B. An area under this curve that is close to 1 indicates
511 a model that can classify outcomes correctly. Here, the area under the curve was 0.98.

512

513 Since the experimental data in this study suggest significant differences between the OMT = 24-h and
514 the OMT > 24-h groups, simulations were conducted to determine whether the model could reveal
515 additional differences that may exist between these two groups. In Fig 6, a simulation was run for each

of the 27 different bacterial loads (B_{source}) administered to the rats in the validation data set (Fig 1B) assuming that ε_{crit} = 53 ε-units (corresponding to the maximum accuracy determined in Fig 5A). Simulations that correspond to a predicted OMT = 24 h are shown in blue and those corresponding to an OMT > 24 h are shown in red. As observed in Fig 6, the OMT 24-h group was predicted to have significantly higher bacteria, inflammatory, and damage levels. While there was a notable divide between model-predicted septic and aseptic cases in terms of bacterial levels (high vs. near zero levels) after approximately 36 h, there was no particularly notable divide between the predicted OMT = 24-h (blue) and the septic members of the OMT > 24-h (red) groups.

The model was also used to give possible explanations for the lack of statistical difference among the three OMT > 24-h groups. As shown in Fig 1B and Table S2, some rats with similar levels of initial bacterial loads exhibited significantly different outcomes. For instance, Rats 11 and 20 received similar initial bacterial loads, but Rat 11 died within 48 h while Rat 20 lived until 96 h. The model was used to investigate these large discrepancies by varying model parameters and evaluating their impact on system outcomes. For instance, these investigations showed that small changes in pathogen growth rate (k_1) had a substantial effect on the predicted outcomes of the system. Fig 7 shows the model-predicted impact of decreasing this parameter slightly, given similar initial bacterial loads of 163×10^6 bacteria in Rat 11 (red) and 172×10^6 bacteria in Rat 20 (cyan). Specifically, k_1 = 1.27/h for Rat 11, and k_1 = 1.2/h for Rat 20. With these parameter values, the model predicted that Rat 11 died (i.e., its damage levels crossed the critical damage threshold of ε_{crit} = 53 ε-units) at approximately 34 h (corresponding to OMT = 48 h). Rat 20, however, was predicted to be healthy at 96 h (OMT = 96 h) with a low steady state value of bacteria and zero steady state value of damage (since pro-inflammatory levels never rise high enough to cause damage to the surrounding tissue). Similar changes in model

539 predicted outcomes were observed when other parameters, such as those governing the immune
540 response, were varied (see Section 3.4 and Fig 8).

541

542 ***3.4 Outcome dependence on parameter values***

543 Fig 8 summarizes the dependence of stable outcomes (health is cyan, asepsis is blue, and sepsis is
544 red) on the number of bacteria in the fibrin clot (B_{source}), pathogen growth rate (k_1), strength of the
545 local immune response (k_2), and pro-inflammatory activation rate (v_1). As k1 was increased or as k_2
546 was decreased, the number of bacteria that the system could handle and still recover decreased (Fig
547 8A and 8B). The dashed lines in the figures indicate the three levels of B_{source} that were administered
548 to the rats in the calibration data set. These multiple parameter bifurcation plots suggest that a
549 healthy recovery outcome was not possible for many (high) levels of B_{source}. For lower levels of
550 B_{source}, the model predicted that regions of healthy recovery become aseptic and then septic as the
551 pathogen growth rate was increased (Fig 8A) or the strength of the local immune response was
552 decreased (Fig 8B). As B_{source} was decreased, the width of the aseptic region decreased and
553 completely disappeared when $B_{source} = 6 \times 10^6$ bacteria. This suggests that, regardless of the value of
554 k_1 or k_2, an aseptic outcome is not possible for a small enough initial bacterial load.

555

556 Parameter v_1 is the maximum activation rate of the pro-inflammatory response. As shown in Fig 8C,
557 there was a very narrow region (199×10^6 bacteria $< B_{source} < 201 \times 10^6$ bacteria) in which the model
558 predicted that increasing v_1 causes a change in stable outcome from septic death to healthy recovery
559 and then to aseptic death. This suggests that increasing the recruitment of pro-inflammatory cells and
560 mediators first helps then overwhelms the system. For higher levels of B_{source} ($B_{source} > 258 \times 10^6$
561 bacteria), a moderate activation rate ($v_1 = 0.08$ *M*-units/h) divides the septic region ($v_1 < 0.08$ *M*-

562 units/h) from the aseptic region ($\nu_1 > 0.08$ *M*-units/h), suggesting that a highly responsive pro-
563 inflammatory activation rate leads to aseptic outcomes.

564

565 The nearly equal split between septic and aseptic cases shown in Fig 8C for $B_{source} > 258 \times 10^6$ can be
566 explained by the interactions between the bacteria and inflammatory response. The interaction term
567 involving *BM* in the bacteria equation (Eq. 1) was initially very small since not enough *M* had been
568 recruited. Thus, early dynamics for the bacteria equation were governed primarily by the remaining
569 non-interaction terms (which are almost entirely unrelated to ν_1). If *B* values increased above a
570 (relatively low) threshold, the non-interaction terms became large (and positive) enough to outweigh
571 any of the mounting contributions from the interaction term. This means that resulting high values of
572 *B* depended on initial dynamics when *M* values were low and ν_1 had only a minor impact. In
573 addition, if $B_{source} > 258 \times 10^6$ bacteria, bacterial levels approached a high steady state level,
574 regardless of the size of the interaction term. So, if $\nu_1 < 0.08$ *M*-units/h (right boundary of septic
575 region in Fig 8C), the pro-inflammatory levels never grew large enough to outweigh the non-
576 interaction terms in Equation 1. If $\nu_1 > 0.08$ *M*-units/h, however, then the pro-inflammatory levels
577 caused the interaction term in Equation 1 eventually to outweigh the non-interaction terms, and the
578 bacteria were eliminated. This elimination took place after the system attained high bacterial levels
579 and was independent of B_{source} so long as $B_{source} > 258 \times 10^6$ bacteria.

580

581 ## 4 Discussion

582 In this study, a mathematical model of the immune response to a bacterial infection was developed
583 from previous inflammation models (16, 36, 46) and applied to an experimental study in which
584 variable doses of *E. Coli* were administered to rats. The model was calibrated to bacterial levels in

585 the rats at multiple time points following implantation of the *E. coli* fibrin clot. The model
586 successfully predicted outcomes of observed mortality times in 27 rats given several different initial
587 bacterial loads. The differences in model-predicted levels of bacteria, pro-inflammatory response,
588 anti-inflammatory response, and damage for rats with OMT = 24 h and rats with OMT > 24 h,
589 however, were not as large as expected. Thus, the model accurately predicts observed outcomes
590 when initial conditions are well-known, but predicting final outcomes based on data at early time
591 points is challenging when large uncertainty surrounds the appropriate initial conditions to use for
592 model or statistics-based predictions. The model also showed that variability in experimental
593 outcomes can result from variability in the pathogen growth rate, strength of the local immune
594 response, or maximum activation rate of the pro-inflammatory response, since small changes in these
595 parameter values generated large changes in system outcomes.

596

597 An important goal for mathematical models of disease is to yield accurate patient-specific outcomes.
598 Ideally, given early time course data on bacterial levels and cytokine levels in a patient, a model
599 could be used to predict whether that patient will follow a healthy recovery (resolving), septic, or
600 aseptic outcome. Then, depending on the forecasted outcome, appropriate countermeasures could be
601 prescribed, allowing for an efficient and accurate use of resources. In many studies, pure statistics
602 are used to predict the likely status or outcome of a given patient. Applying pure statistics to the
603 validation data set in this study (e.g., using a predictor that predicts death within 24 h if the initial
604 bacterial load exceeds a certain amount) would produce similar successful predictions but would not
605 be able to predict septic vs. aseptic outcomes, explain failed predictions, or reveal the underlying
606 system dynamics. While more complex statistics using more data per patient can help to improve the
607 accuracy of statistical methods, ultimately such methods still do not identify the mechanisms leading
608 to observed outcomes as mechanistic mathematical models do. An optimal theoretical approach for

609 understanding disease should include a combination of mathematical modeling and statistical
610 methods (67, 68). The study presented here provides an example of such a combined mathematical
611 and statistical approach.

612

613 ***4.1 Mathematical and statistical predictions***

614

615 Statistical analyses applied to the validation data set suggested no significant difference between the
616 bacterial levels in rats with observed mortality times of 48, 72, and 96 h. Therefore, this study
617 considered only two groups from the data set: rats that died before 24 h (OMT = 24 h) and rats that
618 died after 24 h (OMT > 24 h).

619

620 No clear separation among bacteria, inflammatory, or damage levels was predicted by the model for
621 the OMT = 24-h and OMT > 24-h groups. Interestingly, the model predicted very similar trajectories
622 for the pro- and anti-inflammatory response. For example, there was a tight grouping of the pro- and
623 anti-inflammatory response in the OMT = 24-h group. As a result, statistical models for this system
624 would have difficulty predicting whether a septic or aseptic outcome should be expected especially
625 due to the uncertainty associated with detecting when the infection begins to take hold. These model
626 predictions imply that that pro-inflammatory, anti-inflammatory, or bacterial levels at early time
627 points (e.g., 8 h) cannot be used to predict outcomes from a statistical perspective, thereby motivating
628 the need for a combined statistical and mechanistic modeling approach.

629

630 Modeling helps to determine the relative importance (and validity) of proposed mechanisms and
631 potential targets for successful interventions. For instance, the sensitivity analysis in this study
632 showed that the local immune response is important. Obtaining patient specific parameters for the
633 local immune response using well-designed measurements could improve parameter estimation,
634 mitigate uncertainty in initial conditions, and enable more accurate patient-specific model-generated
635 predictions regarding potential septic outcomes, corresponding dynamics, and proposed
636 treatments. Such measurement planning and usage rely on well-chosen statistical techniques and
637 could include both patient-specific measurements and/or general population measurements. In the
638 case of the latter, maps of parameter spaces for general populations could be generated and then a
639 few well-chosen measurements could be used to estimate the location of a specific patient in that
640 space and their patient-specific corresponding parameter values. In addition, future models could
641 make similar improvements in predictions by including mechanisms that involve specific (rather than
642 general) cytokines (25) or those involving spatial organization of the system (36).

643

644 The validation data set showed that similar bacterial loads led (in some cases) to significantly
645 different mortality times in rats. Just as previous models have demonstrated the impact of
646 stochasticity on model outcomes (34, 40), the current model demonstrated that experimental or
647 clinical variability in sepsis outcomes can be explained by very small differences in parameters
648 governing bacteria or the host response. Additionally, the different outcomes in two rats with nearly
649 identical bacterial loads could result from small differences in initial conditions due to underlying
650 stressors at the beginning of the experiment. As a result, in order to enable patient-specific care,
651 different parameter sets and/or initial conditions must be used for different individuals to capture
652 variability among patients.

4.2 Comparison to prior mathematical modeling studies of sepsis

Several sepsis modeling studies have been described previously. Kumar et al. (17) used a model similar to the model developed in the current study to predict that sepsis may have multiple negative outcomes (e.g., septic and aseptic death) that may require different treatment approaches. However, their study did not explicitly incorporate any experimental data. Yamanaka et al. (69) created a model founded on clinical data focused solely on septic shock, an important subset of sepsis. McDaniel et al. (42) introduced a "whole-body" sepsis model (via the BioGears Physiology Engine) to be used as an *in silico* septic simulator. While these and several other sepsis models (21, 23-25, 31, 33, 38-40, 70) have offered many useful insights, the present study emphasized the acute inflammatory dynamics that take place during early sepsis development using a relatively simple and novel modeling framework that can be used to identify the mechanisms that underly vastly different outcomes despite nearly identical initial conditions. Notably, our results using experimental sepsis induced in genetically identical rats support those of Cockrell et al. (40), who used an agent-based model of human sepsis to suggest that mortality could occur under diverse conditions and influences which in turn would defy stratification based on inflammation biomarkers.

4.3 Limitations

As is necessary in theoretical modeling, the model presented in this study applies simplifying assumptions to make predictions about the average health status in a system. For example, several immune system mediators are grouped together into two general populations for the pro- and anti-inflammatory responses. Also, the model considered only virulent bacteria; non-virulent (e.g.,

675 probiotic) bacteria (46) were not included. Although additional model components could improve
676 predictions, the simplicity of this model allows for very useful analysis of the impact of parameters
677 and interactions on the health of a rat. The simplicity of the model, however, does limit the ability to
678 capture more complicated underlying dynamics. For instance, a single variable, ϵ_{crit}, is used to
679 predict outcomes based on system damage when outcomes likely depend on additional factors.
680 Similarly, differences between species are subsumed into a single parameter (B_s) when converting
681 bacteria-associated quantities from the human system developed by Reynolds et al. (16) to the rat
682 system considered in this study.

683

684 The model was calibrated using both quantitative data (bacterial levels at multiple time points) and
685 qualitative observations (coexistence of healthy recovery/septic/aseptic steady states for some
686 parameter values). The data used in this study did not include measurements of cytokine levels, and
687 thus using data sets with both bacterial and cytokine levels could help to improve model calibration
688 and design. In addition, the validation data only included time points of 24, 48, 72, and 96 h. There
689 is probably a more continuous change in mortality outcomes, and thus a more accurate threshold for
690 death could be obtained given additional time point data. Finally, the model has been calibrated to
691 peritoneal injections that cause sepsis in rats. More experimental data would allow for more general
692 insights.

693

694 ***4.4 Concluding remarks***

695

With complicated processes such as inflammation where hundreds of molecules, cells, and other factors play roles, mathematical models are essential for providing a mechanistic understanding of the system, as in the current sepsis study. This is especially true when very small differences in initial conditions or parameter values in complex systems can have a major impact on outcome, as illustrated in this study. Thus, model reduction is needed to facilitate analysis and interpretation. The parsimonious model of sepsis presented here, after calibration, reproduced experimental results, identified an inherent level of uncertainty associated with experimental data and associated predictions, predicted trends as bacterial load, pathogen growth rate, strength of the local immune response, and activation rate of the pro-inflammatory response were varied, and provided a simplifying paradigm that can be used to understand the life vs. death outcomes for septic individuals. As recent studies in blunt trauma (71), traumatic brain injury (72), and pediatric acute liver failure (73, 74) have shown, a dichotomy in patient outcomes was observed as soon as measurements were taken, suggesting that inflammation, regardless if it results from trauma, disease, or sepsis, exhibits the same features illustrated in this work. Using mathematical modeling provides an understanding of possible mechanisms that could explain such dichotomies in outcomes, in contrast to methods solely based on statistical assessments of clusters or outcomes.

720 **Figure Captions**

721

722 **Fig 1. Experimental data sets used for model calibration and validation.** (A) The calibration
723 data set is depicted using closed circles (●) for each measured bacterial level. Bacterial
724 measurements were obtained for the following three initial bacterial loads: 1.28×10^8 bacteria
725 (cyan), 2.48×10^8 bacteria (blue), and 5.05×10^8 bacteria (red). Minimum and maximum bars and
726 lines connect the geometric means of the measurements at each time point. (B) The validation data
727 set includes the observed mortality time for twenty-seven rats injected with varying levels of bacteria
728 via an *E. coli*-impregnated fibrin clot (each measurement is represented with a ●). The bottom and
729 top of the box-and-whiskers plot indicate the edges of the first and third quartiles, respectively, and
730 the whiskers extend to the smallest and largest values in each group that are less than 2.7 within-
731 group standard deviations from the mean. The data points for Rat 11 and Rat 20 (Section 3.3) are
732 denoted by a red and cyan ●, respectively.

733

734 **Fig 2. Schematic illustrating bacteria-immune interactions among the four model populations.**
735 The model schematic shows the interactions between bacteria (B), pro-inflammatory response (M),
736 anti-inflammatory response (A), and damage levels (ε). The external infection is denoted by $D(t)$.
737 The arrow heads correspond to upregulation, and blunted (flat) arrow heads indicate downregulation.
738 This figure was adapted from (16).

739

740 **Fig 3.** Validity of fitting the mathematical model to log-transformed data. (A) Calibrating data set
741 (●) plotted with model-predicted bacterial dynamics over the experimental times considered (solid
742 curves). The following three initial bacterial loads were provided in the data set: 128×10^6 bacteria
743 (cyan), 248×10^6 bacteria (blue), and 505×10^6 bacteria (red). (B) Plot of the standardized residuals

744 for the given fit to log-transformed data. These plots demonstrate the validity of using log-
745 transformation and fitting via least squares.

746

747 **Fig 4.** Predicted dynamics for healthy recovery, septic, and aseptic outcomes as pathogen growth rate
748 is varied. Model predictions of bacteria (A), pro-inflammatory (B), anti-inflammatory (C), and damage
749 (D) levels as pathogen growth rate (k_1) was varied. Three different outcomes were predicted for an
750 initial bacterial infection of $B_{source} = 128 \times 10^6$ bacteria: healthy recovery ($k_1 = 1.2$/h, cyan), asepsis
751 ($k_1 = 1.3$/h, blue), and sepsis ($k_1 = 1.4$/h, red).

752

753 **Fig 5. Demonstrated accuracy of model predictions of health or disease outcomes.** (A) Model
754 prediction accuracy. The number of correct model predictions divided by the total number of
755 predictions (i.e., model prediction accuracy) is shown as a function of the critical damage level (ε_{crit}).
756 Relatively high levels of accuracy are attained for a large range of critical damage levels. (B) Area
757 under the curve-receiver operator characteristics curve. An area under this curve close to 1 indicates
758 the model is capable of classifying outcomes correctly. The area under the curve is 0.98.

759

760 **Fig 6.** Similarities in time dynamics predicted despite varying bacterial loads and mortality data for
761 27 rats. The model-predicted dynamics of the bacteria (A), pro-inflammatory (B), anti-inflammatory
762 (C), and damage (D) levels are shown for each of the rats from the validation data set. Blue curves
763 correspond to rats that had a predicted observed mortality time of 24 h, and red curves correspond to
764 rats that were predicted to survive for more than 24 h. The clustering of many of the trajectories

765 suggests that small uncertainties in the initial state of the system could lead to significantly different
766 systemic predictions.

767

768 **Fig 7**. **Small variations in system parameters produce significantly different outcomes**
769 **consistent with experimental measurements.** Comparison of the predicted dynamics for the
770 bacteria (A), pro-inflammatory (B), anti-inflammatory (C), and damage (D) dynamics for Rat 11
771 (red) and Rat 20 (cyan) using different parameter values. Parameters for Rat 11: $B_{source} = 163 \times 10^6$
772 bacteria, $k_1 = 1.27$/h. Parameters for Rat 20: $B_{source} = 172 \times 10^6$ bacteria, $k_1 = 1.2$/h. Despite similar
773 parameter values, the model predicts significantly different OMTs for Rat 11 (OMT = 48 h) and Rat
774 20 (OMT = 96 h). These OMTs are the same as those observed in the validation data set (Fig 1B). A
775 horizontal dashed line is included in panel D and corresponds to the critical damage level, $\varepsilon_{crit} = 53$ ε-
776 units.

777

778 **Fig 8.** Varying bacterial and immune response parameters significantly impacts model predicted
779 outcomes. (A) Increasing the pathogen growth rate (k_1) turns healthy (cyan) outcomes into aseptic
780 (blue) and then septic (red) outcomes. (B) Increasing the strength of the local immune response (k_2)
781 turns septic outcomes into aseptic then healthy outcomes. (C) Increasing the pro-inflammatory
782 activation rate contributes to more aseptic outcomes and lowers the probability of a healthy outcome.
783 Dashed lines correspond to initial bacterial levels that were used in the experiments. The three
784 yellow ◊'s in panel A indicate the three values of k_1 (1.2, 1.3, 1.4/h) that were simulated in Fig 4.
785 The two yellow ○'s in panel A indicate the two values of k_1 (1.27 and 1.2/h) that were simulated in
786 Fig 7 for Rats 11 and 20.

787

788

789 **Table 1: Model parameter values, units, definitions, and sources.**

Parameter	Value	Unit	Description	Source
k_1	1 – 1.5	1/h	pathogen growth rate, 1.27 fitted/default	estimated
B_∞	0.737	10^6 cells/cm^3	pathogen carrying capacity	estimated
B_s	0.00083	1/cm^3	rescaling factor for bacterial levels	estimated
k_2	0.4 - 0.7	1/M-units/h	rate at which non-specific local response eliminates pathogen, 0.6 default	(16)
s_l	0.005	M-units/h	source of non-specific local response	(16)
μ_l	0.002	1/h	decay of non-specific local response	(16)
k_3	0.01	1/(10^6 cells/cm^3)/h	rate at which the non-specific local response is exhausted by pathogen	(16)
k_5	1.8	1/M-units/h	rate at which pro-inflammatory response consumes pathogen	(16)
k_A	7.08	1/A-units	inhibition rate of the anti-inflammatory response	Adapted from (16, 21)
v_1	0 - 0.16	M-units/h	maximum activation rate of pro-inflammatory response , 0.08 default	(16, 21)
v_2	0.12	1/h	half-saturation of pro-inflammatory response	(16, 21)
k_M	0.01	1/M-units/h	self-activation of pro-inflammatory response	(16, 21)
k_B	0.1	1/(10^6 cells/cm^3)/h	activation of pro-inflammatory response by pathogen	Adapted from (16, 21)
k_ε	0.02	1/ε-units/h	activation of pro-inflammatory response by damage	(16, 21)

μ_M	0.05	1/h	decay of pro-inflammatory response	(16, 21)
s_A	0.0125	*A*-units/h	source of anti-inflammatory response	(16, 21)
v_3	0.04	*A*-units/h	maximum production rate of anti-inflammatory response	(16, 21)
v_4	3640	*M*-units	Half-saturation of anti-inflammatory response	estimated
k_4	48	*M*-units/ε-units	relative effectiveness of pro-inflammatory response and damage inducing the anti-inflammatory response	(16, 21)
μ_A	0.05	1/h	decay of the anti-inflammatory response	Comment in (16, 21)
τ	24	h	rate of recovery from damage	(46)
f	213	ε-units/*M*-units/h	maximum rate of damage produced by the pro-inflammatory response	estimated
T	1	ε-units/h	threshold for damage	Comment in (46)
k_D	0.0344	1/h	decay of bacterial population in the clot (released into body)	estimated
B_C	407	10^6 cells	Number of bacteria a clot can hold	estimated
ε_{crit}	53	ε-units	critical damage level	estimated

Conflict of Interest

Yoram Vodovotz is a co-founder of, and stakeholder in, Immunetrics, Inc.

Author Contributions

JA and YV both equally contributed as senior authors, with JA supervising the modeling components and YV supervising the experiments. RN performed experiments and collected and compiled the experimental data set. JA, JB, TB, AC and AT developed the model equations and analyzed model behavior. JB finalized all model calibrations to experimental data and statistically analyzed model fits and subsequent predictions. JB, JA, YV, and RN wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Data Availability Statement

All relevant data are plotted within figures in the manuscript and available in spreadsheet form from supporting information files. All code used for producing simulations for this study may be found on Github:

https://github.com/jarobarb/code_for_sepsis.git.

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