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Parameter estimation and sensitivity analysis in an agent-based model of *Leishmania major* infection

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Abstract

Computer models of disease take a systems biology approach toward understanding host-pathogen interactions. In particular, data driven computer model calibration is the basis for inference of immunological and pathogen parameters, assessment of model validity, and comparison between alternative models of immune or pathogen behavior. In this paper we describe the calibration and analysis of an agent-based model of *Leishmania major* infection. A model of macrophage loss following uptake of necrotic tissue is proposed to explain macrophage depletion following peak infection. Using Gaussian processes to approximate the computer code, we perform a sensitivity analysis to identify important parameters and to characterize their influence on the simulated infection. The analysis indicates that increasing growth rate can favor or suppress pathogen loads, depending on the infection stage and the pathogen's ability to avoid detection. Subsequent calibration of the model against previously published biological observations suggests that *L. major* has a relatively slow growth rate and can replicate for an extended period of time before damaging the host cell.

1 Introduction

Leishmania are protozoan parasites that are transmitted by bites of infected sandflies. The macrophage is the primary host cell. Over 20 species of *Leishmania*, endemic in 88 countries, are capable of causing human disease. Disease is either cutaneous, where skin ulcers occur on exposed surfaces of the body, or visceral, with near certain mortality if left untreated. In mice that are able to control *L. major* infection, the resistance mechanism is well understood: secretion of IL-12 by dendritic cells promotes a CD4⁺ Th1 response, Th1 cells activate macrophages through IFN- γ production, and activated macrophages clear the parasite.

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However, there are other species of the *Leishmania* parasite, such as *L. amazonensis*, which cause chronic disease in mice (McMahon-Pratt & Alexander, 2004), and the development of successful vaccines for any *Leishmania* species has remained elusive (Vanloubbeeck & Jones, 2004).

Computer models of disease take a systems biology approach toward understanding adverse or inefficient immune responses by integrating multiple sources of knowledge about host-pathogen interactions and immune cell function in order to study the collective, emergent behavior of a population of immune cells, i.e., the immune response. Such computer models have been used to gain insight into a variety of diseases. For example, in a model of *Mycobacterium tuberculosis* infection, Segovia-Juarez *et al.* (2004) identify chemokine diffusion rates and the arrival time, location, and macrophage activation efficiency of T cells as important factors in granuloma formation. In a model of systemic inflammatory response and multiple organ failure, An (2002) reproduces outcomes of unsuccessful clinical trials involving blockage of proinflammatory mediators. In a model of influenza A infection, Beauchemin (2006) shows how infection dynamics depend on the spatial structure of initially infected cells. In a model of Epstein-Barr virus, Shapiro *et al.* (2008) identify lytic reactivation of B cells as an important parameter that determines disease outcome. Finally, in a model of antigen escape in HIV infection, Bernaschi & Castiglione (2002) find that escape mutants with low transcription rate can explain the long-term asymptomatic phase of disease. For a recent review of computational immune system models, see Forrest & Beauchemin (2007).

A challenge faced when working with computer models is the need to choose values for model parameters. Typically, plausible choices for parameters are determined through literature searches and expert consultation, yet often the best available information are plausible ranges for model parameters rather than single values. In order to validate the model, one must ultimately fit the computer output to field data, choosing parameter values that yield the best match between simulation output and biological observations. Model calibration has proven to be difficult in practice, especially since most computer models are high dimensional, non-linear, and resource-intensive. As a result, computer modelers traditionally employ *ad-hoc* approaches to parameter estimation (Kennedy & O'Hagan, 2001), where model validation is based on the qualitative comparison of model predictions with field data. In the field of population ecology, this approach is known as pattern-oriented modeling (reviewed in Grimm *et al.*, 2005).

Nevertheless, data-driven model calibration is a fundamental and necessary step for making meaningful inference on model parameters, assessing the validity of a model, and performing model comparisons (Bayarri *et al.*, 2002). The calibration process first requires the specification of the relationship between the computer model output and data that is observed in the field. When the computer model is expensive to run, the process then entails 1) approximation of the computer code through a surrogate statistical model (i.e., a Gaussian process), and 2) estimation of computer model parameters via the surrogate model. The Gaussian process approximation also facilitates sensitivity analysis which identifies important model parameters and the relationships between parameters and simulation output. Schonlau & Welch (2006), for example, use a Gaussian process emulator of a computer model of global human development to identify important parameters and characterize their effects. These sensitivity analysis tools are particularly useful for computer models, such as agent-based and cellular automata models, where it is not clear how the computer code itself can be exploited, without running the model, to quantitatively understand the system.

Computer model calibration through a Gaussian process intermediate has been demonstrated successfully in several disciplines. For example, Kennedy & O'Hagan (2001) calibrate a computer model of radionucleotide exposure following a chemical accident; Bayarri *et al.*

calibrate a vehicular suspension system model (2006), and a vehicle collision model (2002); Higdon *et al.* (2004) calibrate a spot welding model; and Heitmann *et al.* (2006) calibrate a cosmological model. A recent formulation of the computer model calibration and validation approach is provided in Bayarri *et al.* (2007).

In this paper we describe the sensitivity analysis and calibration of an agent-based model of *Leishmania major* infection. A model of macrophage loss triggered by necrotic tissue production is proposed for explaining macrophage depletion after peak infection. We find that pathogen growth rate and host cell carrying capacity both affect macrophage levels early in infection, though not independently. Increasing parasite growth rate can both augment and paradoxically, suppress parasite loads, depending on the stage of infection and the ability of the pathogen to avoid detection. Furthermore, the ability of the pathogen to evade the adaptive immune response has a large effect on macrophage levels at 6.5 wpi. We verify that parameter estimation using the Gaussian process intermediate is accurate by calibrating the computer model using simulated field data, and then calibrate our model using field data from Belkaid *et al.* (2000). Parameter estimates suggest that intracellular pathogen replicates extensively before spreading to additional cells, a finding consistent with observations of *Leishmania* growth in cultured macrophages (Chang *et al.*, 2002). Finally, model comparison supports our proposal of explicit macrophage loss in response to necrotic tissue production.

The rest of this paper is organized as follows: in Section 2 we describe an agent-based model of *Leishmania* infection in mice; in Section 3 we describe the statistical methods used in Gaussian process computer model approximation (3.1), sensitivity analysis (3.2), calibration (3.3), implementation via Markov Chain Monte Carlo (3.4), and model comparison (3.5); in Section 4 we report results obtained by applying these statistical methods to the agent-based model; and in Section 5 we discuss our findings.

2 An agent-based model of *Leishmania major* infection

Agent-based models (ABMs)² capture the dynamics of complex systems whose properties depend on the collective behavior of their interacting components. An ABM contains distinct entities, or *agents*, that inhabit a *spatial environment*. A simulation visualizes agents as they move and interact according to *update rules* executed at *discrete time steps* (Wooldridge, 2002).

We describe an ABM of the immune response to *L. major* infection in the ear of a C57BL/6 mouse, a system where experimental data are available (Belkaid *et al.*, 2000). Our computer model is stochastic, so that multiple simulations will give different results even when the same model parameters and starting conditions are used. The current model is an extension of an earlier ABM of *L. major* infection (Dancik *et al.*, 2006), and is largely based on the work of Segovia-Juarez *et al.* (2004), who explore granuloma formation during infection with another macrophage-tropic parasite, *Mycobacterium tuberculosis*. All model parameters are listed in Table C1 of the Supplement.

2.1 The model environment

We model a 2mm × 2mm cross section of the ear as a 100 × 100 lattice of square *micro-compartments*. This lattice represents an internal portion of a uniform infection area, and is therefore *toroidal*, so an object leaving the lattice will re-enter at the opposite end. We select four evenly distributed micro-compartments to serve as *source compartments* where new cells enter the system.

²Abbreviations used in the paper: Agent-based model (ABM), weeks post infection (wpi), Gaussian process (GP), sensitivity analysis (SA), principle components (PC), functional analysis of variance (FANOVA)

We label micro-compartments (i, j) , starting from $(0, 0)$ at the bottom left. Define a *Moore neighborhood* of length r at position (x_0, y_0) to be the local collection of points in the lattice given by

$$M_r(x_0, y_0) = \{(x, y) : |x - x_0| \leq r \text{ and } |y - y_0| \leq r\}.$$

Furthermore, define $M_1(x_0, y_0)$ to be the *immediate Moore neighborhood* of a micro-compartment (x_0, y_0) .

2.2 Stages of infection

2.2.1 Initial conditions—We take the starting point of our simulation to be 3.5 weeks post infection (wpi) of the experiment described in Belkaid *et al.* (2000), near the beginning of the dramatic phase of parasite growth. Data for the number of infected macrophages at this time point are not available. However, because pathogen load dynamics during the dramatic phase of parasite growth are similar to pathogen load dynamics following high dose infection (Lira *et al.*, 2000), we assume that conditions at the start of our simulation match conditions just two days post high dose infection. Based on parasite and expected cell counts observed two days post high-dose infection (Doug Jones, unpublished observations), we randomly place 105 macrophages on the lattice. A random number of parasites between 1 and P_m infect randomly selected resting macrophages until total pathogen load on the lattice exceeds $50 - P_m$. A final, random resting macrophage receives $50 - P_m$ parasites, so the initial pathogen load is 50. All uninfected macrophages are given random life spans uniform between 0 and 100 days (Furth *et al.*, 1973).

2.2.2 Infection of macrophages—Macrophages are the primary cells that *L. major* parasites infect (Belkaid *et al.*, 2000) and the only host cells that support pathogen replication (Naderer *et al.*, 2006). We use the term *resting* macrophage to refer to an uninfected, unactivated cell. We assume that intracellular parasites experience logistic growth at rate a_I with carrying capacity $k_I + 30$. If the macrophage is not activated, intracellular parasites grow until their number exceeds the lethal parasite density k_I . Then, the macrophage enters a *dying* state, where it begins transferring parasite to macrophages in its length two Moore neighborhood. Since extracellular parasites are seldom observed in *Leishmania* infection (Chang *et al.*, 2002), parasite transmission is thought to occur when nearby macrophages phagocytize dying host cells. Segovia-Juarez *et al.* (2004) use a similar model, but allow extracellular parasite and restrict parasite uptake to macrophages in their length 1 Moore neighborhood. All macrophages that are not dying, including those already infected, can take up parasite. A dying macrophage is removed from the system once all of its intracellular parasites are consumed by surrounding macrophages.

2.2.3 Chemokines and cell movement—Chemokines, chemical attractants that influence cell movement, play an important role in *Leishmania* infection (Roychoudhury & Roy, 2004). We include one generic chemokine as an attractant for both macrophages and T cells. Its diffusion and decay properties are based on interleukin-8, an important chemokine involved in early infection. Infected and activated macrophages release c_I units of chemokine per time step. In addition to directing cell movement toward infected areas (see below), chemokine also increases the rate of macrophage recruitment (Section 2.2.4).

Cell movement has been described as a biased-random walk in the presence of chemokine (Tranquillo *et al.*, 1988), and we model this random walk on the ABM lattice. Let

$C_{i,j}$ =amount of chemokine in micro – compartment (i, j)

$C_{M_1}(i, j)$ =amount of chemokine in immediate Moore neighborhood $M_1(i, j)$

A cell currently in micro-compartment (i, j) moves to micro-compartment $(k, l) \in M_1(i, j)$, $(k, l) \neq (i, j)$ with probability $\frac{C_{k,l}}{C_{M_1}(i,j)-C_{i,j}}$. All cells in our model move in this way, but at different speeds which depend on cell type and state (Section 2.2.7 and Table C1).

2.2.4 Recruitment and the adaptive immune response—T cells and macrophages are recruited to infected areas during infection. In the ABM, source compartments represent blood vessels where recruited cells arrive. At each time-step, macrophages enter with probability based on source compartment chemokine levels (see below), and T cells, after a delay, enter with constant probability p_{Trecr} .

Macrophage recruitment in response to chemokine: Macrophages are actively recruited around two days post infection following high-dose *L. major* inoculation (Sunderkotter *et al.*, 1993), and after a delay in low dose infection (Belkaid *et al.*, 2000). This recruitment is modulated by chemokines such as macrophage chemotactic and activating factor (Badolato *et al.*, 1996). Certain chemokines trigger the activation of endothelial cells and upregulation of their monocyte receptors, which facilitate extravasation of cells from the circulatory system into infected tissue. Other chemokines, such as granulocyte-macrophage colony-stimulating factor, upregulate macrophage production from the bone marrow (Janeway *et al.*, 2005). Therefore, chemokines collectively increase the amount of circulating macrophages as well as the rate of influx of these macrophages into the tissue.

We use a logistic function to model macrophage recruitment in response to the generic chemokine in our model. This function captures initial exponential increase in macrophage numbers due to macrophage production and influx, as well as the eventual loss of chemokine potency at high chemokine concentrations due to the saturation of chemokine receptors (Rajotte *et al.*, 1997). Let

$$p_{Mrecr,i}(x) = \begin{cases} \frac{m_{Mrecr} \times \exp(\alpha_{Mrecr} + \beta_{Mrecr} \times x)}{1 + \exp(\alpha_{Mrecr} + \beta_{Mrecr} \times x)} & x > C_M \\ 1.74 \times 10^{-5} & x \leq C_M \end{cases} \quad (1)$$

where $p_{Mrecr,i}(x)$ is the probability that a macrophage is recruited when there are x units of chemokine at source compartment i . The parameter m_{Mrecr} represents the maximum recruitment probability, α_{Mrecr} determines the minimum probability of recruitment as $x \rightarrow 0$, and β_{Mrecr} determines how sharply recruitment increases in response to chemokine. This *active* macrophage recruitment mechanism is used as long as x is above a chemokine threshold, C_M ; otherwise we use a base level of recruitment that will maintain macrophage equilibrium in the absence of infection. In the experimental infection we are modeling, macrophage levels are initially constant (i.e., due to a base level of recruitment), then dramatically increase after 3.5 wpi as active recruitment begins (see Belkaid *et al.*, 2000, Fig. 1B). Because the starting point of our simulation is 3.5 wpi of the experiment described in Belkaid *et al.* (2000), we set C_M dynamically to correspond to source compartment chemokine levels after one day of simulation. On average, C_M is assigned a value of approximately 12,000 chemokine units.

The adaptive immune response: During infection, antigen-presenting cells take up pathogen from the site of infection, migrate to the draining lymph node, and present antigen (processed

pathogen) to naïve T cells. Naïve T cells, in response, proliferate and mature into several classes of T cells, including CD4⁺ Th1 cells. In *L. major* infection, CD4⁺ Th1 cells migrate to the infected area and activate infected macrophages. The initiation of the adaptive immune response requires a threshold level of antigen (Janeway *et al.*, 2005) and occurs only after a prolonged period of parasite growth in low dose *L. major* infection (Belkaid *et al.*, 2000). We assume that this threshold level of antigen is related to pathogen load at the infection site, and recruit T cells once a threshold pathogen level, $T_{threshold}$, is achieved. The threshold is a one way trigger so T cells will continue to enter at each time step and at each source compartment with probability p_{Trecr} as long as source compartment chemokine levels are greater than a threshold of C_T chemokine units.

2.2.5 Macrophage emigration following uptake of necrotic tissue—In *L. major* infection, there is a sharp decrease in macrophage levels near the time that the T cell response reaches its peak (Belkaid *et al.*, 2000). In our model, this decrease cannot be explained merely from the death of infected and activated macrophages (data not shown). We propose a model of macrophage loss based on an experiment which shows that as the inflammatory response resolves, macrophages systematically migrate to the draining lymph node (Bellingan *et al.*, 1996), an event known to occur following macrophage uptake of foreign antigen, apoptotic immune cells, and necrotic tissue (Winchester *et al.*, 1984). In the ABM, macrophage emigration is triggered by the production and uptake of necrotic tissue, a side effect of macrophage activation (Billack, 2006). Subsequent to macrophage activation, A_{necr} units of non-diffusive necrotic tissue are released. Uninfected macrophages in the length two Moore neighborhood consume one unit of this tissue and disappear from the lattice, representing rapid migration to the draining lymph node.

2.2.6 Macrophage activation—All T cells in our model are considered to be antigen specific CD4⁺ Th1 cells that are equally capable of activating infected macrophages. Macrophage activation is cell-mediated (Sypek *et al.*, 1984), and T cells within the immediate Moore neighborhood of an infected macrophage activate it with probability T_{actm} . In *L. major* infection, macrophage activation is sufficient for elimination of intra-cellular parasite (Wei *et al.*, 1995). M_{als} days after T cell activation, the macrophage finishes destroying all intracellular parasite, undergoes apoptosis, produces A_{necr} units of necrotic tissue, and is removed from the lattice.

2.2.7 Time scales—Each time step in the ABM is equal to approximately six seconds of real time. Chemokine diffusion and decay as well as parasite growth occur each time step. Following Segovia-Juarez *et al.* (2004), T cells move every 200 time steps (20 minutes) and macrophages move on slower time scales that vary according to macrophage state (see Table C1). Additional update rules, such as the take up of parasite and activation of macrophages, occur every 10 minutes.

2.3 Implementation

We program the ABM using C++ to encode the spatial environment, rules, and entities described above. All parameters are fixed or allowed to vary according to Table C1. Starting with the initial conditions described in Section 2.2.1, the ABM proceeds to simulate *L. major* infection according to the above rules. With initial conditions representing 3.5 wpi, we simulate infection until 8.5 wpi. Each simulation takes approximately 15 minutes on an Intel Xeon 3.00 GHz workstation.

3 Statistical methods

Our primary objective is to estimate the computer model parameter vector $\theta = (\theta_1, \dots, \theta_p)$ (p parameters from Table C1) given a vector y_F of biological field observations. The calibration process (see Section 3.3) requires a large number of computer model evaluations to explore the parameter space. Although we limit our attention to the agent-based model described in Section 2, the methods we describe are appropriate for the analysis and calibration of a wide range of mathematical and computational models, including ordinary differential equation models. For models that are expensive, in money or time, the number of evaluations is limited, and calibration demands a fast and accurate emulator of the computer code. The type of emulator that we use is the Gaussian process (GP). In addition to its role in parameter calibration, the GP emulator is utilized in sensitivity analysis to explore the influence of parameters on the simulated response. A schematic overview of GP prediction and calibration is given in Fig. 1, and a listing of available GP-based analyses is given in Table 1.

In this section we give a brief, mostly conceptual overview of the statistical methods we use for GP prediction of scalar computer model output, sensitivity analysis, computer model calibration, and model comparison, with particular attention paid to notions relevant to our ABM. Throughout, we refer the reader to subsections in Supplement A for additional details. The reader is also encouraged to consult the cited references for more background and theory.

3.1 The Gaussian process as a computer model surrogate

GPs are flexible and useful tools for approximating computer models when the computer model response surface of interest is a smooth function of the parameter space (Sacks *et al.*, 1989; Kennedy & O'Hagan, 2001; Bayarri *et al.*, 2002). We start by obtaining a set of m known scalar computer model outputs, for example macrophage counts or pathogen load at a particular day in the disease progression, by running the model at known parameter settings $\theta^{(1)}, \dots, \theta^{(m)}$ selected by a maximin Latin Hypercube design (Morris & Mitchell, 1995). Call this data $z_{\text{known}} = (z(\theta^{(1)}), \dots, z(\theta^{(m)}))$. These are scalar data because there is a single output for each parameter vector input. The GP approximation is built by fitting a multivariate normal distribution to z_{known} , assuming the correlation structure depends on the parameter vector θ . Computer model output at an untried parameter $\theta^{(\text{new})}$, conditioned on z_{known} , is predicted using standard multivariate normal distribution theory (Renchner, 2002). The predicted output $\hat{z}(\theta^{(\text{new})})$ is given by $E[z(\theta^{(\text{new})})|z_{\text{known}}]$ while $\text{Var}[\hat{z}(\theta^{(\text{new})})] = \text{Var}[z(\theta^{(\text{new})})|z_{\text{known}}]$ quantifies uncertainty in the prediction (Figs. 1B, 2). A simple example of GP prediction of scalar output is provided in Fig. 2. If the computer model is *deterministic*, then the predictor will interpolate the known output z_{known} (Fig. 2A). If the computer model is *stochastic*, as is the case with our ABM, the GP includes an extra variance term, called a *nugget*, so the predicted output interpolates *and smooths* the known output z_{known} (Fig. 2B). For details see Supplement A.1. The accuracy of the GP model is assessed via a cross-validation procedure, described in Supplement A.2. More on the choice of parameter vector inputs $\theta^{(i)}$ is discussed in Supplement A.7.

For models more complex than the sin wave of Fig. 2, computer output is typically multivariate. It is possible to fit one GP to each component of the data, but this solution becomes impractical for high dimensional functional data such as time series. For example, we can record the pathogen load every day of our simulation, a total of 35 measurements between 3.5 and 8.5 wpi. GP prediction of functional data involves using singular value decomposition to reduce the dimensionality of the output and fitting GPs to the most important principle component (PC) weights. In practice, we fit GPs to the most important PC weights that together account for at least 99% of the functional variance. For details on GP prediction of functional output, see Supplement A.3.

3.2 Sensitivity analysis using the Gaussian process

Sensitivity analysis (SA) includes a broad range of statistical techniques for measuring a parameter's ability to influence the output of a model. Typically, sensitivity analysis is used in *factor screening* methods for identifying the most important parameters of a system, as well as for assessing *how* the response of a model depends on its input parameters (Saltelli *et al.*, 2000). Some of these methods have been adapted to ABMs (Marino *et al.*, 2008), but here, we focus on sensitivity analysis techniques that capitalize on the functional GP approximation, following Schonlau & Welch (2006). Of interest is how the computer model parameter vector $\theta = (\theta_1, \dots, \theta_p)$ influences computer model output. Inferences are based on a prior distribution $\pi(\theta)$, meant to capture lack of knowledge about model parameters, and the observed computer model output vector z_{known} . In our SA we use independent Unif(0,1) priors on all components of θ scaled from the ranges given in Table C1.

For an appealing, but relatively qualitative visual assessment of computer model parameters on output, one can estimate the *main effect* of each parameter. First, we discuss main effects on scalar computer output. For θ_k , the k^{th} component of the parameter vector, the *main effect* $g(\theta_k)$ is the expected computer model output, $E[z(\theta)|z_{known}, \theta_k]$, averaged over the joint prior distribution $\pi(\theta_{-k})$ for all components of θ except the k^{th} . Two-way interaction effects $g(\theta_k, \theta_l)$ are defined similarly, except integration is with respect to a joint prior on all parameter vector components but the k^{th} and l^{th} , $k \neq l$. A main effects graph plots $g(\theta_k)$ against θ_k , thereby illustrating the effect of varying the single parameter θ_k on the simulation output. Two-way interaction effects are visualized through 3D surfaces based on the two effects and expected computer model output. For functional computer model output, like pathogen load or macrophage counts over time, a main effects plot displays expected *functional* computer model output for a fixed θ_k . Varying θ_k will produce multiple plots that can be compared to reveal the main effect of θ_k . For details see Supplement A.4.

The GP is a functional approximation of the computer model and therefore lends itself to functional analysis of variance (FANOVA) decomposition when marginal priors on the individual components of θ are independent (Schonlau & Welch, 2006). In FANOVA decomposition, the total functional variance of the GP is decomposed into variance due to the main and interaction effects of the parameters. The percentage of the total functional variance accounted for by a particular effect provides a measure of the importance of that effect. We report the percent of total functional variance that is accounted for by main effects and two-way interactions. Details can be found in Schonlau & Welch (2006).

3.3 Computer model calibration using field observations

Computer model calibration is the process of estimating the unknown computer model parameter vector θ , based on a vector of field observations $y_F = (y_{F,1}, \dots, y_{F,n})$, so that output from the calibrated computer model is (hopefully) consistent with field data (see the schematic provided in Fig. 1). Typically, the relationship between a computer model and reality is defined according to

$$y_{F,i} = y_M(\theta) + b + \varepsilon_i, \quad i=1, \dots, n,$$

where $y_{F,i}$ denotes a scalar field observation, $y_M(\theta)$ the corresponding computer model output evaluated at the true value θ of computer model parameters, b is a bias term, and ε_i are observation errors (Higdon *et al.*, 2004). For simplicity, we assume that the computer model is an accurate, unbiased representation of reality, so $b = 0$. As is typical, we assume measurement errors are independent and identically distributed (iid) as $N(0, \sigma_F^2)$, so that all

field observations are normally distributed. Finally, because $y_M(\theta)$ is not readily available, we replace it with the GP approximation $z(\theta)$. Then the field data conditional on the computer output z_{known} follow a normal distribution with density $f(y_F | \theta, z_{known})$. Details are in Supplement A.5.

With the likelihood of the data y_F defined, estimation of θ can proceed. Here we perform estimation of θ in a Bayesian context. To reflect knowledge or uncertainty before data collection, a prior distribution $\pi(\theta)$ is specified, in our case the same product of independent uniforms used for SA. After observing the field observations y_F , calibration yields updated knowledge about θ in the form of a posterior distribution

$$\pi(\theta | y_F, z_{known}) \propto f(y_F | \theta, z_{known}) \pi(\theta).$$

The posterior distribution is estimated using Markov Chain Monte Carlo (see next section). It incorporates various sources of uncertainty, including measurement error, uncertainty in the GP approximation to the computer model, and prior uncertainty about θ . For dealing with multiple scalar computer outputs and other details, see Supplement A.5.

3.4 Markov Chain Monte Carlo

Markov Chain Monte Carlo (MCMC) is a sampling method used to sample from a probability distribution that is known up to a multiplicative constant (Gilks *et al.*, 1998). In computer model calibration, the goal is to obtain the posterior distribution $\pi(\theta | y_F, z_{known})$, for the computer model parameter vector θ , given a prior $\pi(\theta)$ on computer model parameters. Using the MCMC sample, we estimate the marginal posterior distributions of each $\theta_k, k = 1, \dots, p$, by constructing a histogram. We take the posterior median as a point estimate and use the 90% posterior credible interval to quantify uncertainty in the estimate. The 90% posterior credible interval of the parameter θ_k is the interval (a, b) , where a is the 5% quantile of θ_k and b is the 95% quantile of θ_k from the posterior distribution; 90% prior credible intervals are defined similarly, but using quantiles of the prior distribution. An example of a 90% posterior credible interval is given in Fig. 1D. For details about sampling from the posterior distribution, see Supplement A.6.

3.5 Bayesian model comparison

Bayes factors (BFs) are commonly used for assessing the evidence in favor of one model against an alternative (Kass & Raftery, 1995). In our case, we are interested in assessing the evidence in favor of model M_1 , which allows necrotic tissue parameter A_{necr} to be 1, 2, 3, 4, 5, or 6, against the null model M_0 , where A_{necr} is identically 0. The full parameter vector for both models includes additional parameters, but A_{necr} is the only parameter altered in the comparison.

For observed data y_F , the *Bayes factor* BF_{10} in favor of model M_1 against M_0 is the ratio of the marginal likelihood of the data under M_1 to the marginal likelihood of the data under M_0 . Equivalently, the Bayes factor is the ratio of the posterior odds in favor of M_1 to its prior odds,

$$BF_{10} = \frac{\frac{\pi(M_1 | y_F, z_{known})}{\pi(M_0 | y_F, z_{known})}}{\frac{\pi(M_1)}{\pi(M_0)}},$$

where $\pi(M_i)$ is the *a priori* probability assigned to M_i , $i = 0, 1$, with $p(M_0) = 1 - p(M_1)$. If the two models are *a priori* equally likely, which is what we assume, then the Bayes factor is the posterior odds in favor of M_1 . In our analysis, we estimate the probabilities $\pi(M_i|y_F, z_{known})$, $i = 1, 2$ from the posterior distribution of the computer model parameter vector θ , which includes A_{necr} , that we obtain using MCMC.

4 Results

4.1 Experimental design and computer model output

Ideally we would like to calibrate all 25 parameters in our computer model, but because field data is limited we will focus our attention on the five parameters we believe to be the most interesting and the most relevant given the field data available. Thus, we focus on parameter vector $\theta = (\alpha_I, k_I, \beta_{Mrecr}, p_{Trecr}, T_{threshold})$ whose true value we assume exists somewhere in the hypercube formed by allowing each parameter to vary in the ranges specified in Table C1 of the Supplement. The remaining parameters are fixed at values given in the table. Note that the parameter A_{necr} is currently fixed at 2; we vary this parameter in Section 4.5. The parameters α_I, k_I , and $T_{threshold}$ are pathogen-dependent parameters which determine growth rate, the lethal parasite density in infected macrophages, and the threshold level of pathogen that triggers the adaptive immune response. β_{Mrecr} is a macrophage recruitment parameter and p_{Trecr} determines the rate of T cell recruitment. We choose all parameter values and ranges to be consistent with *L. major* infection, and display references for these choices in Table C1.

In order to construct the GP approximation to the computer model, we must obtain computer model output for m representative choices of the parameter vector θ . In the absence of informative prior information about the true value of θ , the desired approach is to choose a space-filling design that adequately covers the parameter space. We choose 50 design points using a maximin Latin Hypercube design (Morris & Mitchell, 1995) and run two replicates at each design point. Output from the simulation consists of total macrophage and parasite counts on the lattice over time (Fig. 3, black dashed lines). Variation in macrophage counts and pathogen load is evident in the sizes and locations of the peaks. Peak macrophage levels range from 112 to 1903 macrophages, with peak locations ranging from 4.2 to 6.9 wpi. Peak pathogen load ranges from 3263 to 15610 (57.1 and 125 on the scale of Fig. 3B), with peak locations ranging from 4.5 to 7.0 wpi. In all of our simulations, infection is cleared by approximately 8 wpi.

4.2 Fitting the Gaussian processes

Our goal is to fit a GP approximation to the computer model output given in Fig. 3. The calibration will consider the following field data: macrophage counts at $t_1 = 5$, $t_2 = 5.5$, $t_3 = 6.5$ and $t_4 = 8$ wpi; and parasite load at $t_5 = 4.5$ and $t_6 = 6.5$ wpi (Fig. 3, solid black circles). Therefore, our GP emulators must be able to predict computer model output at these six time points. Let $z_j^*(\theta^{(i)})$ be the *untransformed* computer model output for simulation i at time point t_j , and define

$$z_{known,j}(\theta^{(i)}) = \begin{cases} \log z_j^*(\theta^{(i)}), & j=1, \dots, 5 \\ \log(z_j^*(\theta^{(i)})+1), & j=6. \end{cases} \quad (2)$$

The simulation output is adjusted at t_6 to prevent numerical issues for simulations where the infection is cleared by 6.5 wpi. We fit independent GPs to each vector $z_{known,j}$ as described in Section 3.1 for the six time points using the software PERK (Santner *et al.*, 2003). Using independent GPs is a significant assumption, especially for time series data on single subjects,

but the calibration data we use are independent because distinct mice were sacrificed for each observation (Belkaid *et al.*, 2000). Accuracy, as determined by cross-validated predictions and standardized residuals of the GP predictors, is good (see Supplement B.1); subsequent calibration uses these fitted GPs.

4.3 Sensitivity analysis

In order to quantify the importance of computer model parameters on simulation output and to characterize their effects, we perform sensitivity analysis as described in Section 3.2. While we performed many different SA's, we report here three results that both illustrate the GP-based techniques and important dynamics of the model. Specifically, we study the main effects of model parameters on the time course of pathogen load, use FANOVA to find important parameters and interactions at all time points where data are available, and study these effects through main and interaction effects plots.

To characterize the qualitative effect of each parameter on pathogen load over time, we fit GP emulators to the six most important PC weights of the pathogen load time course. These six components explain over 99% of the variation in output. Figure 4 illustrates the main effect of growth rate, (α_I), the most important computer model parameter on pathogen load over time, where we assess parameter importance by calculating the variability of each main effect as described in Supplement A.4. Interestingly, high growth rate favors pathogen load early in infection but not at later time points. When growth rate is high, there is a sharp increase in pathogen load and a higher peak, but a faster resolution of infection. Infection is prolonged when growth rate is low, and the peak is lower, but the infection is still eventually cleared.

Because we are interested in calibrating the computer model to time points $t_1, \dots, t_6$, we perform sensitivity analysis on the GP predictors for those time points. The percentage contributions of main and important two-factor interaction effects are provided in Table 2. Together, main effects and two-factor interactions account for more than 97% of the total functional variance of the GP emulators for all time points. The most important main effects at t_1 , for parameters α_I and k_I are plotted in Fig. 5A. The most important main effect at t_5 and t_6 , which are both for α_I , are plotted in Fig. 5B. The two largest interaction effects, $\alpha_I \times k_I$ and $\alpha_I \times T_{threshold}$, are plotted in Fig. 6.

The most influential parameter for any time point is pathogen growth rate (α_I), which accounts for 93.5% of the total functional variance for the GP predictor at t_5 . Growth rate is also the most important parameter at t_1 and t_6 (Table 2). For (scaled) values of $\alpha_I > 0.2$, the relationship between pathogen growth rate and macrophage levels at 5.5 wpi (t_1) is positive, whereas lethal parasite density (k_I), the second most important parameter at t_1 , is negatively related to the response (Fig. 5A). However, these effects are not independent; the $\alpha_I \times k_I$ interaction accounts for 11.3% of the total variance of the t_1 GP predictor (Table 2), with k_I showing a slight positive effect when α_I is below 4×10^{-4} /minute, but a negative effect otherwise (Fig. 6A). In agreement with the α_I main effect plot (Fig. 4), the effect of α_I on pathogen load changes over time (Fig. 5B). The parameter α_I has a positive effect on pathogen load at 4.5 wpi but a negative effect on pathogen load at 6.5 wpi (t_5 and t_6 , respectively). At t_6 , there is an interaction between α_I and $T_{threshold}$, which accounts for 4.5% of the total functional variance of the GP predictor (Table 2). $T_{threshold}$ is positively and strongly correlated with pathogen load, but only when α_I is low (Fig. 6B).

4.4 Calibration using field data

Belkaid *et al.* (2000) provide data plots for parasite and macrophage counts over time following low dose *L. major* infection in the mouse ear, but the amount of field data available for calibration is limited (e.g. macrophage data are *representative* of 10 experiments; Belkaid *et*

al., 2000), and we do not have accurate information about field error. Therefore, in order to verify that accurate calibration is possible using the type of data available, we first apply the calibration approach to simulated field data, generated assuming a randomly chosen parameter vector $\theta^{(\text{sim})}$. In the absence of field-estimated error, we assume that σ_F^2 is equal to the sample variance of observed computer model output, which we calculate by using the ABM to generate three independent observations for each time point and fixing $\theta = \theta^{(\text{sim})}$. The simulated field data yielded substantial and accurate information about the five calibration parameters, particularly α_I and k_I . See Supplement B.2 for details and discussion.

We next calibrate the model using the mouse data (Belkaid *et al.*, 2000). Because we are modeling the *center* of the infection, and not the entire ear, we scale field observations by a factor of 71^{-1} , based on expected macrophage counts at two days post high dose infection (i.e., the start of our simulation), and log transform these observations following Eq. (2). We continue to assume the field variance σ_F^2 estimated during testing with simulated field data. A summary of the resulting marginal posterior distributions is given in Table 3, and the distributions for α_I , k_I , and $T_{\text{threshold}}$ are plotted in Fig. 7. Using posterior medians as point estimates, we estimate a pathogen growth rate (α_I) of 7.73×10^{-4} /minute (a doubling time of 15 hours), and a lethal parasite density (k_I) of 332 parasites. The posterior distributions of $\beta_{M_{\text{regr}}}$ and $p_{T_{\text{regr}}}$ are relatively non-informative (Table 3 and data not shown). To determine whether our calibrated model could accurately reproduce field observations, we ran 100 simulations using parameter vectors drawn from the posterior distribution of θ . Graphs of mean computer model output, ± 1 standard deviation, along with the observed field data, are given in Fig. 8. All field observations are within one standard deviation of mean computer model output from the calibrated computer model.

4.5 Comparison of alternative models of macrophage behavior

In the previous model, we arbitrarily fixed the parameter A_{necr} at a value of two in an attempt to capture the decrease in macrophage counts between 5.5 and 8 wpi that Belkaid *et al.* (2000) observe. We next want to estimate the value of A_{necr} and to assess whether or not a model with necrotic tissue fits the data better than a model without necrotic tissue. Formally, we consider the alternative model M_1 , where A_{necr} is 1, 2, 3, 4, 5, or 6, against the null model M_0 , where A_{necr} is identically 0. Because the posterior distributions for α_I , k_I , and $T_{\text{threshold}}$ had posterior distributions concentrated at the high end of their ranges (Fig. 7), we update our prior ranges for the parameter vector $\theta = (\alpha_I, k_I, \beta_{M_{\text{regr}}}, p_{T_{\text{regr}}}, T_{\text{threshold}})$ by shifting the range of each parameter such that its 99.99% highest posterior density credible interval is centered in the new range. This shift did not effect the parameters $\beta_{M_{\text{regr}}}$ and $p_{T_{\text{regr}}}$. The width of the ranges were kept the same in order to maintain the same level of uncertainty in our priors. Updated posterior ranges for α_I , k_I , and $T_{\text{threshold}}$ and the range for A_{necr} , are given in Table C2 of the Supplement.

We use a 70 point maximin Latin Hypercube design to vary the updated computer model parameter vector $\theta = (A_{\text{necr}}, \alpha_I, k_I, \beta_{M_{\text{regr}}}, p_{T_{\text{regr}}}, T_{\text{threshold}})$, again with two replicate runs per design point. Because A_{necr} is discrete, we map its scaled values from the Latin Hypercube design $\{\frac{1}{70}, \dots, 1\}$ to the discrete values $\{0, \dots, 6\}$ so that each possible value of A_{necr} is observed 10 times in the design. For time points $t_j, j = 1, \dots, 6$, we fit independent GPs to $z_{\text{known},j}$ and calibrate our computer model as before. A sensitivity analysis shows the sharpest decrease in macrophage levels after 5.5 wpi occurs when A_{necr} is high, while the other computer model parameters mostly affect the peak height, rather than steepness of the decline (Fig. B3 of the Supplement and data not shown).

To calibrate A_{necr} and other parameters to the available data, we assume that the two models are *a priori* equally likely (i.e., $\pi(M_0) = \pi(M_1) = \frac{1}{2}$), specifically

$$\pi(A_{necr}) = \begin{cases} \frac{1}{2} & \text{if } A_{necr}=0 \\ \frac{1}{12} & \text{if } A_{necr}=1, \dots, 6, \end{cases} \quad (3)$$

and A_{necr} is independent of the other parameters. The remaining (scaled) parameters are again given independent Unif(0,1) priors. A summary of each marginal posterior distribution is given in Table 4, and the posterior distribution of A_{necr} is given in Fig. 9. Using posterior medians as point estimates, we estimate a pathogen growth rate (α_I) of $(7.38 \times 10^{-4})/\text{minute}$ (a doubling time of 15.7 hours), a transfer threshold (k_I) of 317 parasites, and the production of three units of necrotic tissue (A_{necr}) following macrophage activation. The Bayes factor for M_1 against M_0 is $\text{BF}_{10} = 7.3$ which indicates ‘substantial’ evidence in favor of the model with necrotic tissue-induced macrophage loss, using the scale of Kass & Raftery (1995).

5 Discussion

In this paper, we have described an agent-based model of *L. major* infection, fit Gaussian process approximations to computer model outputs of pathogen load and macrophage counts at various time points, performed sensitivity analysis on the GP emulators to identify and characterize the effects of important model parameters, and calibrated the computer model using the data of Belkaid *et al.* (2000). GP diagnostics confirm the accuracy of the GP emulators (details in Supplement B.1), and tests with simulated field data indicate accurate model parameter estimates are possible despite the limited field data (details in Supplement B.2). Our most interesting findings are: (1) pathogen growth rate naturally enhances pathogen load early in infection but counterintuitively suppresses pathogen load later, (2) the macrophage recruitment parameter is *not* the greatest determinant of macrophage levels when these cells first infiltrate the infection site (5 wpi), (3) *L. major* can replicate extensively before destroying the host cell, indicative of an immune hiding phenotype, and (4) accelerated macrophage loss in the presence of necrotic tissue provides a substantially better fit to the data. Sensitivity analysis, calibration with field data, and model comparison are discussed in detail in the following sections.

5.1 Sensitivity analysis

The FANOVA decomposition (Table 2) summarizes how variability in computer model *output* is attributed to uncertainty in computer model *input*, specified through prior distributions. Using the language of Kennedy & O’Hagan (2001), the prior distribution may represent 1) current belief about the true value of a parameter (*parameter uncertainty*), or 2) environmental variability in a parameter that cannot be controlled (*parametric variability*). Our choices of priors are primarily based on uncertainty, although we also expect variability to be present. We also expect calibration to provide the most information about parameters with large FANOVA contributions. Indeed, the credible interval for α_I , which was the greatest across-the-board contributor to variation (Table 2), decreased the most after calibration (Table 3). In Supplement B.3 we discuss the idea of using sensitivity analysis to guide data collection so that model calibration is more efficient. Here, we discuss how the estimated main and interaction effects (Figs. 4–6) help illustrate the complex relationship between pathogen behavior and host response.

Pathogen persistence is associated with slow pathogen growth and immune avoidance—A fast-growing pathogen peaks early and quickly disappears, while a slow-growing pathogen peaks late and typically lower (Fig. 4). These patterns are also apparent in the main effects of growth rate (α_I), which has a positive slope for pathogen load at 4.5 wpi (time point t_5) but a negative slope at 6.5 wpi (time point t_6 ; Fig. 5B). The paradox that high

growth rate favors early survival but is long-term detrimental to the pathogen results because a rapidly replicating pathogen risks early detection by the adaptive immune response. In the model, the adaptive response is triggered once pathogen load crosses a threshold ($T_{threshold}$). Looking at the last pathogen time point t_6 (Fig. 6B), expected pathogen load decreases as growth rate increases, but the loss of pathogen can be partially abrogated by increasing $T_{threshold}$. Together, pathogens that grow slowly and without early detection by the adaptive immune response persist at the highest levels late in infection.

There have been biological and model-based observations about pathogen immune evasion and growth rate related to our findings. Parasites use many mechanisms to escape immune detection (reviewed in Damian, 1997). For example, *L. major* is known to inhibit antigen presentation in infected macrophages (Fruth *et al.*, 1993), and *Toxoplasma gondii* downregulates MHC class II expression, which is required for antigen presentation (Luder *et al.*, 1998). Both are slow-growing pathogens that produce latent or persistent infection. Others have noted that high growth rates are not always favorable to pathogens. In their model of *Mycobacterium tuberculosis*, Segovia-Juarez *et al.* (2004) report that the sign of the partial rank correlation coefficient between growth rate and extracellular bacteria changes from positive to negative over time. From an epidemiological perspective, Ewald (1994) notes that virulent pathogens cannot survive if they kill or immobilize their hosts before transmission to a new host occurs, so high growth rate is detrimental without efficient transmission, for example via vectors.

Many pathogen and host parameters influence the amount of macrophages at 5.5 and 6.5 wpi (time points t_1 and t_3 , respectively)—One surprising result is that the macrophage recruitment parameter β_{Mrecr} is not the most influential at either time point (Table 2), despite being the only parameter that *directly* influences macrophage levels. The large uncertainty ranges for some input parameters, such as α_I , explain some of the corresponding variation in macrophage counts, but there are also unanticipated system dynamics at work. At macrophage time point t_1 , growth rate (α_I) is the most important parameter. As the doubling time increases above 30 hours ($\alpha_I > 0.2$ in Fig. 5A), the number of macrophages is expected to increase sharply, probably because rapid growth promotes spread of the infection among macrophages, which each release macrophage-recruiting chemokine. The upward curve in the main effect of α_I near its lower range is likely an artifact of the stochasticity of the ABM and our use of only two replicates per design point when fitting the GP. $T_{threshold}$, the pathogen level that triggers the T cell response, accounts for the most functional variance of the GP predictor at time point t_3 . Although the primary role of T cells is macrophage activation, activated macrophages are short-lived and leave behind necrotic tissue that triggers the departure of additional resting macrophages. Also, as T cells successfully resolve the infection, cytokine levels decrease, reducing macrophage recruitment. In combination, pathogen and T cell actions, and therefore α_I and $T_{threshold}$, have a significant effect on macrophage numbers.

Like α_I , lethal parasite density k_I in macrophages should affect infection spread and consequently, macrophage recruitment. As the value of k_I decreases, increased pathogen transmission leads to increased macrophage recruitment and higher macrophage counts (Fig. 5A). However the role of parameters α_I and k_I on macrophage recruitment is complex, and their two-way interaction accounts for 11.3% of the total functional variance of the t_1 GP predictor. Interestingly, k_I has a positive effect on macrophage counts when α_I is low but a negative effect when α_I is high (Fig. 6A). We suspect that low α_I prevents much spread of infection by t_1 regardless of k_I , so large k_I simply means more infected macrophages have survived until t_1 . However, when α_I is high, rapid spread of infection, enhanced as k_I declines, induces massive macrophage recruitment.

5.2 Calibration using field data

In Supplement B.2, we show that calibration with simulated data, designed to mimic the available field data, yields accurate and informative estimates of computer model parameters. Nevertheless, we cautiously interpret calibrated model parameters (for an important discussion of this issue, see Kennedy & O'Hagan, 2001). The essential point is that since no model is correct, the 'true' value of a parameter almost certainly differs from its calibrated value. Our intent is to use the calibrated computer model as a measure of our systems-level understanding of *L. major* infection. If our prior parameter ranges are reasonable and the model is correct, then the posterior distributions reflect, to the best of our knowledge, the 'true' values of model parameters. Surprising or interesting posterior estimates may warrant special attention and should be further analyzed through biological experiments. Lethal parasite density k_I is such a parameter in our model. Relatively little is known about its *in vivo* value. What follows is a discussion of the calibration results of Section 4.4, where we let θ vary according to Table C1 and fix A_{necr} at 2.

Despite the limited amount of field data used, we were able to learn information about most of the computer model parameters we estimated (Table 3). The high posterior median for lethal parasite density ($k_I = 332$) suggests that *L. major* is capable of extensive intracellular replication without destroying the host cell, while the median growth rate (15 hour doubling time), is relatively slow. As discussed earlier, these properties lead to longer term pathogen persistence and lower peak pathogen loads. They are further believed critical to the microbial virulence of *L. amazonensis* (Chang *et al.*, 2002), and may partially explain the silent phase of *L. major* growth observed by Belkaid *et al.* (2000), although our calibration results did not consider this slow phase.

In the calibration process, we have assumed that field measurements are unbiased and, because the raw replicated field data were not available, that the field variance equals the computer model variance. Importantly, the assumption of unbiased field measurements may not hold for certain types of biological data, such as parasite counts, that are estimated through serial dilution assays. Typically, biologists use *ad hoc* methods to estimate concentrations in this manner, but more precise estimates with corresponding standard errors can be obtained through formal statistical treatment of the data (Gelman *et al.*, 2004; Mehrabi & Matthews, 1995). Although Belkaid *et al.* (2000) do not provide the raw data, they do provide error bars for the two pathogen time points in their Fig. 1. Not surprisingly, since variability observed by Belkaid *et al.* (2000) includes measurement error and biological variation (Wong *et al.*, 2005), the computer variance appears to underestimate the plotted error bars. Thus, underestimation of field variance implies our posterior distributions may be misleadingly narrow. With replicate field observations available, field variance could be estimated directly, or handled in a Bayesian context as part of a more accurate calibration process. In fact, because our model is a stochastic ABM, if measurement error is assumed constant, replicate observations would allow us to calibrate the model not only by matching the field data with model output means, but also the field variance with model output variances.

Our model does not capture the late, persistent stage of *L. major* infection, and our calibrated k_I seems to contradict the low pathogen loads measured during persistence. Although Belkaid *et al.* (2000) provide data for up to 10.5 wpi, we have chosen not to use any data after 8 wpi in our analysis. After 8 wpi, despite the fact that the infection is controlled, low levels of parasite persist (Belkaid *et al.*, 2000). It is now known that regulatory T cells are required to maintain this low-level parasite persistence, which also provides host immunity to re-infection (Belkaid *et al.*, 2003). Without modeling regulatory T cells, the computer model achieves complete parasite elimination (Fig. 3B), an outcome consistent with regulatory T cell depletion studies following *L. major* infection (Belkaid *et al.*, 2003). We expect that inclusion of regulatory T cell function into our model will decrease the rate of parasite clearance, achieve a better fit at

the last parasite time point t_6 , and capture the low level parasite persistence. Nevertheless, the nature of pathogen persistence is curious in light of our high estimate for lethal parasite density k_I . Belkaid *et al.* (2000) observe the persistence of 100–10,000 parasites in the skin, which combined with our k_I estimate suggests that pathogens survive in a very small population of infected macrophages (between 1 and 30) at the site of infection. Although Bogdan *et al.* (2000) report pathogen persists in lymph node fibroblasts, it remains perplexing to us how parasites survive at the infection site, where it appears that only macrophages are infected. The *in vivo* value of k_I is not known, and it is unclear whether k_I changes as the infection progresses, but Belkaid *et al.* (2000) observe “heavily infected macrophages” during the persistent stage of infection.

5.3 Model comparison and macrophage loss

When mean computer model behavior is considered, predictions from the calibrated computer model are accurate for most time points, but appear biased low at 5.5 wpi and high at 8 wpi for macrophages (time points t_2 and t_4) (Fig. 8). Together, these results suggest that at least one component of macrophage loss following infection resolution is missing or incomplete. Indeed, *in vivo* experiments involving peritonitis and the adoptive transfer of macrophages show that these macrophages are no longer detectable 96 hours following the resolution of inflammation (Bellingan *et al.*, 1996). The exact signals and mechanisms for this rapid macrophage loss are unclear, but macrophage loss could be triggered by long-range signals, such as decreasing cytokine levels, in addition to local phagocytotic events. In an earlier version of the model that assumed a constant macrophage recruitment rate, we modeled this behavior by arbitrarily assigning macrophages a short life span during the adaptive immune response (Dancik *et al.*, 2006).

In our current model, we have proposed that macrophage activation results in the production of A_{necr} units of necrotic tissue, and that macrophages leave the site of infection following uptake of necrotic tissue. This mechanism of macrophage loss is supported by the high Bayes factor ($BF_{10} = 7.3$), indicating ‘substantial’ evidence in favor of a model where macrophage behavior is altered in the presence of necrotic tissue. Our posterior estimate of A_{necr} is three (Fig. 9), which is one unit higher than the value of A_{necr} used in the original calibration. The large amount of uncertainty in our posterior estimate of A_{necr} (Table 4) is the result of assuming that both models are *a priori* equally likely, i.e., $\pi(A_{necr}=0)=\pi(A_{necr} \in \{1, \dots, 6\})=\frac{1}{7}$. Using a uniform prior $\pi(A_{necr})=\frac{1}{7}$, $A_{necr} \in \{0, \dots, 6\}$ gives a 90% posterior credible interval of (1,4) and has little effect on the Bayes factor (data not shown).

Our proposed model of macrophage loss following uptake of necrotic tissue is one possible mechanism for explaining the observed decrease in macrophages. Although it is possible to use other macrophage recruitment functions than Eq. (1), the decrease in macrophage numbers cannot be explained even by *entirely ceasing* recruitment after the macrophage peak (data not shown). While we imagine macrophage loss is via emigration to the draining lymph, loss could also be through death. Another explanation is that altered cytokine environments bias monocyte differentiation toward the dendritic cell type (Mohamadzadeh *et al.*, 2001; Chomarat *et al.*, 2003). However, if this differentiation takes place prior to monocyte influx into the tissue, it would be another recruitment effect. Furthermore, it cannot explain the rapid decrease in macrophages observed by (Belkaid *et al.*, 2000), who distinguish between macrophages and dendritic cells in their collection assays. Interestingly, macrophage take-up and presentation of necrotic tissue (i.e., self-antigen) may partially explain the increase in natural regulatory T cells observed at the infection site during resolution of the infection (Cozzo *et al.*, 2003; Mendez *et al.*, 2004). Although *L. major*-specific natural regulatory T cells have been observed (Suffia *et al.*, 2006), it is unclear why regulatory T cell levels do not increase until the infection is nearly resolved (Mendez *et al.*, 2004).

Mathematical models of disease generally assume that the average lifespan of host cells is constant. A notable exception are models attempting to explain CD4⁺ T cell depletion in HIV (Perelson *et al.*, 1992; Bernaschi & Castiglione, 2002; Yates *et al.*, 2007). Our results indicate that in *L. major* infection, the average lifespan of a resting macrophage at the infection site is *not* constant, and our proposed mechanism of macrophage loss is not specific to *L. major* (Bellingan *et al.*, 1996). We would expect similar macrophage behavior in other infections where necrotic tissue or, perhaps more generally, self antigen is present. It may be important to consider this non-constant macrophage behavior when modeling infection of other macrophage-tropic organisms, such as *M. tuberculosis* and *T. gondii*.

6 Conclusions and future work

In this work we described an agent-based model of *L. major* infection, used a GP to approximate and analyze the computer model, and estimated six immunological and pathogen-related parameters using field data. Simulations from the calibrated model are generally consistent with biological data, although more field data is needed in order to test the effects of the 19 parameters we did not investigate. In addition, our current model neither captures the long, slow, early phase of low dose infection nor the long-term persistence of *L. major* (Belkaid *et al.*, 2003). We await proposals for specific biological mechanisms that explain these observations. Working within the dynamics of mid-stage infection, there is no question that our findings are influenced by the limited set of parameters we chose to vary. Nevertheless, our findings motivate interesting questions that may yield testable hypotheses. Some questions can be addressed *in silico*, for example whether the presence of necrotic tissue can explain the timing of regulatory T cell recruitment. Others require further biological investigation. For example, the high estimate of lethal pathogen density k_I within infected macrophages may imply that non-macrophage cellular reservoirs (e.g., fibroblasts) fuel the persistence of pathogen at the *infection site*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- An G. Agent-based computer simulation and SIRS: building a bridge between basic science and clinical trials. Shock 2002;16:266–273. [PubMed: 11580108]
- Badolato R, Sacks DL, Savoia D, Musso T. *Leishmania major*: infection of human monocytes induces expression of IL-8 and MCAF. Exp Parasitol 1996;82:21–26. [PubMed: 8617327]
- Bayarri M, Berger JO, Cafeo J, Garcia-Donato G, Liu F, Palomo J, Parthasarathy RJ, Paulo R, Sacks J, Walsh D. Computer model validation with functional output. Technical Report Number 165, National Institute of Statistical Sciences 2006;70:102.
- Bayarri, M.; Berger, JO.; Higdon, D.; Kennedy, M.; Kottas, A.; Paulo, R.; Sacks, J.; Cafeo, J.; Cavendish, J.; Tu, J. A framework for the validation of computer models. In: Pace, D.; Stevenson, S., editors. Proceedings of the Workshop on Foundations for V&V in the 21st Century; San Diego, CA: Society for Modeling and Simulation International; 2002.
- Bayarri M, Berger JO, Paulo R, Sacks J, Cafeo JA, Cavendish J, Lin CH, Tu J. A framework for validation of computer models. Technometrics 2007;49:138–154.
- Beauchemin C. Probing the effects of the well-mixed assumption on viral infection dynamics. J Theor Bio 2006;242:464–477. [PubMed: 16650441]

- Belkaid Y, Mendez S, Lira R, Kadambi N, Milon G, Sacks D. A natural model of *Leishmania major* infection reveals a prolonged “silent” phase of parasite amplification in the skin before onset of lesion formation and immunity. *J Immunol* 2000;165:969–977. [PubMed: 10878373]
- Belkaid Y, Piccirillo CA, Méndez S, Shevach EM, Sacks DL. CD4+CD25+ immunoregulatory T cells control *Leishmania major* persistence and the development of concomitant immunity. *Nature* 2003;420:502–507. [PubMed: 12466842]
- Bellingan GJ, Caldwell H, Howie SEM, Dransfield I, Haslett C. In vivo fate of the inflammatory macrophage during resolution of inflammation. *J Immunol* 1996;157:2577–2585. [PubMed: 8805660]
- Bernaschi M, Castiglione F. Selection of escape mutants from immune recognition during HIV infection. *Immunol Cell Bio* 2002;80:307–313. [PubMed: 12067418]
- Billack B. Macrophage activation: role of Toll-like receptors, nitric oxide, and nuclear factor kappa B. *Am J Pharm Educ* 2006;70:102. [PubMed: 17149431]
- Bogdan C, Donhauser N, Döring R, Rölinghoff M, Diefenbach A, Rittig MG. Fibroblasts as host cells in latent leishmaniasis. *J Exp Med* 2000;191(12):2121–2129. [PubMed: 10859337]
- Chang K, Reed SG, McGwire BS, Soong L. *Leishmania* model for microbial virulence: the relevance of parasite multiplication and pathoantigenicity. *Acta Trop* 2002;85:375–390. [PubMed: 12659975]
- Chomarat P, Dantin C, Bennett L, Banchereau J, Palucka AK. TNF skews monocyte differentiation from macrophages to dendritic cells. *J Immunol* 2003;171(4):2262–2269. [PubMed: 12928370]
- Cozzo C, Larkin JI, Caton AJ. Cutting edge: self-peptides drive the peripheral expansion of CD4+CD25+ regulatory T cells. *J Immunol* 2003;171:5678–5682. [PubMed: 14634074]
- Damian RT. Parasite immune evasion and exploitation: reflections and projections. *Parasitology* 1997;115:169–175.
- Dancik GM, Jones DE, Dorman KS. An agent-based model for *Leishmania infection*. *Interjournal of Complex Systems* 2006:1853.
- Ewald, P. *Evolution of Infectious Disease*. USA: Oxford University Press; 1994.
- Forrest S, Beauchemin C. Computer immunology. *Immunol Rev* 2007;216:176–197. [PubMed: 17367343]
- Fruth U, Solioz N, Louis JA. *Leishmania major* interferes with antigen presentation by infected macrophages. *J Immunol* 1993;150:1857–1864. [PubMed: 8436821]
- Furth RV, Dulk MDD, Mattie H. Quantitative study on the production and kinetics of mononuclear phagocytes during an acute inflammatory reaction. *J Exp Med* 1973;138:1314–1330. [PubMed: 4762549]
- Gelman, A.; Carlin, JB.; Stern, HS.; Rubin, DB. *Bayesian Data Analysis*. London: Chapman & Hall; 2004.
- Gilks, WR.; Richards, S.; Spiegelhalter, DJ. *Markov Chain Monte Carlo in practice*. London: Chapman & Hall; 1998.
- Grimm V, Revilla E, Berger U, Jeltsch F, Mooij WM, Railsback SF, Thulke HH, Weiner J, Wiegand T, DeAngelis DL. Pattern-oriented modeling of agent-based complex systems: lessons from ecology. *Science* 2005;310:987–991. [PubMed: 16284171]
- Heitmann K, Higdon D, Nakhleh C, Habib S. Cosmic calibration. *ApJ* 2006;646:L1–L4.
- Higdon D, Kennedy M, CCJ, Cafeo JA, Ryne RD. Combining field data and computer simulations for calibration and prediction. *Siam J Sci Comput* 2004;26:448–466.
- Janeway, CA.; Travers, P.; Walport, M.; Shlomchik, MJ. *Immunobiology: The Immune System in Health and Disease*. Vol. 6. New York: Garland Science Publishing; 2005.
- Kass, RE.; Raftery, AE. Bayes Factors; *Journal of the American Statistical Association*. 1995. p. 773–795. URL citeseer.ist.psu.edu/539880.html
- Kennedy MC, O’Hagan A. Bayesian calibration of computer models. *JR Statist Soc B* 2001;63:425–464.
- Lira R, Doherty M, Modi G, Sacks D. Evolution of lesion formation, parasitic load, immune response, and reservoir potential in C57BL/6 mice following high- and low-dose challenge with *Leishmania major*. *Infect Immun* 2000;68:5176–5182. [PubMed: 10948141]
- Luder CK, Lang T, Beuerle B, Gross U. Down-regulation of MHC class II molecules and inability to up-regulate class I molecules in murine macrophages after infection with *Toxoplasma gondii*. *Clin Exp Immunol* 1998;112:308–316. [PubMed: 9649196]

- Marino S, Hogue IB, Ray CJ, Kirschner DE. A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J Theor Biol* 2008;254(1):178–196. [PubMed: 18572196]
- McMahon-Pratt D, Alexander J. Does the *Leishmania major* paradigm of pathogenesis and protection hold for New World cutaneous leishmaniasis or the visceral disease? *Immunol* 2004;201:206–224.
- Mehrabi Y, Matthews JNS. Likelihood-based methods for bias reduction in limiting dilution assays. *Biometrics* 1995;51:1543–1549.
- Mendez S, Reckling SK, Piccirillo CA, Sacks D, Belkaid Y. Role for CD4+CD25+ regulatory T cells in reactivation of persistent leishmaniasis and control of concomitant immunity. *J Exp Med* 2004;200:201–210. [PubMed: 15263027]
- Mohamadzadeh M, Berard F, Essert G, Chalouni C, Pulendran B, Davoust J, Bridges G, Palucka AK, Banchereau J. Interleukin-15 skews monocyte differentiation into dendritic cells with features of langerhans cells. *J Exp Med* 2001;4(4):1013–1020. [PubMed: 11581322]
- Morris MD, Mitchell TJ. Exploratory designs for computational experiments. *J Stat Plan Infer* 1995;43:381–402.
- Naderer T, Ellis MA, Sernee MF, De Souza DP, Curtis J, Handman E, McConville MJ. Virulence of *Leishmania major* in macrophages and mice requires the gluconeogenic enzyme fructose-1,6-bisphosphatase. *PNAS* 2006;103:5502–5507. [PubMed: 16569701]
- Perelson AS, Kirschner DE, Boer RD. Dynamics of HIV infection of CD4+ T cells. *Math Biosci* 1992;114:81–125. [PubMed: 8096155]
- Rajotte D, Cadieux C, Haman A, Wilkes BC, Clark SC, Hercus T, Woodcock JA, Lopez A, TH. Crucial role of the residue R280 at the F-G loop of the human granulocyte/macrophage colony-stimulating factor receptor chain for ligand recognition. *J Exp Med* 1997;185:1939–1950. [PubMed: 9166423]
- Rencher, AC. *Methods of Multivariate Analysis*. Vol. 2. New York: Wiley; 2002.
- Roychoudhury K, Roy S. Role of chemokines in *Leishmania* infection. *Curr Mol Med* 2004;4:691–696. [PubMed: 15357217]
- Sacks J, Welch WJ, Mitchell TJ, Wynn HP. Design and analysis of computer experiments. *Stat Sci* 1989;4(4):409–435.
- Saltelli, A.; Chan, K.; Scott, EM. *Sensitivity Analysis*. New York: Wiley; 2000.
- Santner, TJ.; Williams, BJ.; Notz, W. *The Design and Analysis of Computer Experiments*. New York: Springer; 2003.
- Schonlau, M.; Welch, W. Screening the input variables to a computer model via analysis of variance and visualization. In: Dean, A.; Lewis, S., editors. *Screening: Methods for Experimentation in Industry, Drug Discovery, and Genetics*. New York: Springer; 2006. p. 308–327.
- Segovia-Juarez JL, Ganguli S, Kirschner D. Identifying control mechanisms of granuloma formation in *M. tuberculosis* infection using an agent-based model. *J Theor Biol* 2004;231:357–376. [PubMed: 15501468]
- Shapiro M, Duca KA, Lee K, Delgado-Eckert E, Hwkins J, Jarrah AS, Laubenbacher R, Polys NF, Hadinoto V, Thorley-Lawsome DA. A virtual look at Epstein-Barr virus infection: Simulation mechanism. *J Theor Bio* 2008;252:633–648. [PubMed: 18371986]
- Suffia IJ, Reckling SK, Piccirillo CA, Goldszmid RS, Belkaid Y. Infected site-restricted Foxp3+ natural regulatory T cells are specific for microbial antigens. *J Exp Med* 2006;203(3):777–788. [PubMed: 16533885]
- Sunderkotter C, Kunz M, Steinbrink G, Meinardus-Hager M, Goebeler HB, Song C. Resistance of mice to experimental leishmaniasis is associated with more rapid appearance of mature macrophages in vitro and in vivo. *J Immunol* 1993;151:4891–4901. [PubMed: 8409447]
- Sypek JP, Panosian CB, Wyler DJ. Cell contact-mediated macrophage activation for antileishmanial defense. *J Immunol* 1984;133:3358–3365. [PubMed: 6208278]
- Tranquillo RT, Lauffenburger DA, Zigmond SH. A stochastic model for leukocyte random motility and chemotaxis based on receptor binding fluctuation. *J Cell Bio* 1988;106:303–309. [PubMed: 3339093]
- Vanloubbeeck Y, Jones DE. The immunology of *Leishmania* infection and the implications for vaccine development. *Ann NY Acad Sci* 2004;1026:267–272. [PubMed: 15604504]

- Wei XQ, Charles IG, Smith A, Feng GH, Huang FP, Xu D, Muller W, Moncada S, Liew FY. Altered immune response in mice lacking inducible nitric oxide synthase. *Nature* 1995;375:408–411. [PubMed: 7539113]
- Winchester G, Sunshine G, Nardit N, Mitchison A. Antigen-presenting cells do not discriminate between self and nonself. *Immunogenetics* 1984;19:487–491. [PubMed: 6610634]
- Wong A, Gottesman I, Petronis A. Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Hum Mol Genet* 2005;14(Review Issue 1):R11–R18. [PubMed: 15809262]
- Wooldridge, M. *Introduction to Multiagent Systems*. New York: John Wiley & Sons, Inc; 2002.
- Yates A, Stark J, Klein N, Antia R, Callard R. Understanding the slow depletion of memory CD4+ T cells in HIV infection. *Plos Med* 2007;4(5):e177. [PubMed: 17518516]

Figure 1. Overview of Gaussian process-based computer model prediction and calibration

A. The computer model is evaluated at m representative computer model parameters $\theta^{(i)}$, $i = 1, \dots, m$, to produce the output vector z_{known} . B. Using the GP predictor, which is fit to z_{known} , the predicted value at untried input $\theta^{(new)}$ is given by $\hat{z}(\theta^{(new)})$ while $Var[\hat{z}(\theta^{(new)})]$ quantifies uncertainty in the prediction. C. Calibration is the process of estimating the computer model parameter (vector) θ so that computer model output is consistent with the field data vector y_F . D. Calibration of a single computer model parameter θ is shown. The calibration process yields a posterior distribution $\pi(\theta | y_F, z_{known})$ that reflects current knowledge about θ based on the field data vector while considering various sources of uncertainty, including measurement error, computer model approximation using the GP predictor, and the prior distribution $\pi(\theta)$. The posterior median (dotted line) is used as a posterior estimate of θ while 90% posterior credible intervals (dashed lines) quantify uncertainty in the posterior estimate.

Figure 2. Gaussian process prediction and uncertainty

Gaussian process prediction (solid black lines) of the function $z(\theta^{(i)}) = \sin(\theta^{(i)}) + \varepsilon_i$, where ε_i are iid $N(0, 0.02)$ residuals. To prepare these plots, we used the fitted f_{GP} to make predictions at seven equally spaced values $\theta^{(new)}$ from -2 to 4 . A. Without a nugget term, the GP predictor interpolates known computer model output (black circles). B. With a nugget term, the GP predictor interpolates and smooths the known data set. In either case, GP prediction of output at the parameter $\theta^{(new)}$ is given by $\hat{z}(\theta^{(new)}) = E[z(\theta^{(new)}) | z_{known}]$, which takes the unconditional mean μ (horizontal dotted line) and adds a correction term (vertical dotted line) that accounts for the correlation between outputs. Uncertainty in the GP prediction is quantified using $\text{Var}[z(\theta^{(new)}) | z_{known}]$. In the figure, uncertainty bounds (dashed lines) correspond to predicted output ± 3 standard deviations. For details about GP prediction, see Supplement A. 1.

Figure 3. Simulated output over time (black dashed lines) and the field data (solid black circles) used in calibration

A. Simulated macrophages; field data for time points t_1 through t_4 . B. Square root of simulated pathogen load; field data for time points t_5 and t_6 . Simulated data was obtained from a 50 point Latin Hypercube design with two replicates per design point over the parameters α_I , k_I , β_{Mrecr} , p_{Trecr} , $T_{threshold}$. Outputs from all 100 simulations are plotted.

Figure 4. Main effects of growth rate (α_I) on simulated pathogen load over time

Main effects are calculated using low (l) and high (h) values of α_I , with (l,h) equal to

$$(2.41, 9.63) \times \frac{10^{-4}}{\text{min}}$$

Figure 5. Main effects of selected parameters

A. Log macrophages at 5.0 (t_1) as a function of α_I and k_I . B. Similarly, log pathogen load at 4.5 (t_5) and 6.5 (t_6) wpi as functions of α_I . The biological ranges of model parameters, corresponding to (0,1), are as follows: α_I , $(2.41, 9.63) \times \frac{10^{-4}}{\text{min}}$; k_I , (50, 400) parasites.

Figure 6. Two-way interaction plots

A. Log macrophages at 5 wpi (t_1) for parameters α_I and k_I ; B. Log pathogen load at 6.5 wpi (t_6) for parameters α_I and $T_{threshold}$. Units are rate/minute for α_I and parasites for k_I and $T_{threshold}$.

Figure 7. Posterior distributions of α_I , k_I , and $T_{threshold}$ following calibration using field data
 Frequency histograms representing posterior distributions of α_I , k_I , and $T_{threshold}$ are obtained from three chains of 200,000 samples each, including a 10,000 sample burn-in. Dashed black lines correspond to 90% posterior credible intervals; the median value of each parameter is represented by a thick black dotted line. The solid black lines that surround the histograms indicate the *a priori* parameter ranges, according to independent prior distributions:
 $\alpha_I \sim \text{Unif}(2.407, 9.632) \times \frac{10^{-4}}{\text{min}}$, $k_I \sim \text{Unif}(50, 400)$ parasites, and $T_{threshold} \sim \text{Unif}(1500, 7000)$ parasites.

Figure 8. Posterior predictive distribution of macrophage counts (A) and parasite load (B)
 One hundred simulations were run using randomly selected parameter vectors from the posterior distribution of θ . Solid black lines, mean computer model output ± 1 standard deviation; solid black circles, field data (A, time points t_1 through t_4 ; B, time points t_5 and t_6) used during calibration; vertical dashed black lines indicate ± 1 standard deviation corresponding to estimated field variance.

Figure 9. Posterior distribution of A_{necr} following calibration using field data

A frequency histogram representing the posterior distribution of A_{necr} obtained from three chains of 200,000 samples each, including a 10,000 sample burn-in. Prior distribution of A_{necr} is $\pi(A_{necr}) = \frac{1}{2}$ if $A_{necr} = 0$ and $\pi(A_{necr}) = \frac{1}{6}$ if $A_{necr} \in \{1, \dots, 6\}$. BF_{10} , Bayes factor of $M_1: A_{necr} \in \{1, \dots, 6\}$ against $M_0: A_{necr} = 0$.

Table 1
Gaussian-process based analyses

The fitted GP is used in sensitivity analysis, which identifies important parameters through FANOVA decomposition and characterizes parameter effects on model output through main and interaction effects plots; and in computer model validation, which involves calibration (i.e., parameter estimation) and model comparison. For each type of analysis, we provide references in our manuscript where methods are described.

	Analysis Methods	Method Description
Sensitivity analysis	FANOVA decomposition main effects plots interaction effects plots	Section 3.2 Section 3.2, Supplement A.4 Section 3.2
Computer model validation	calibration model comparison	Section 3.3, Supplement A.5 Section 3.5

Table 2

FANOVA decomposition

For each time point, the percentage contribution of main effects and two-way factor interaction effects to the total functional variance of the Gaussian process predictor. Only effects accounting for > 1% of the total variance for at least one time point are shown, with ‘-’ indicating < 1%.

Effect	Time point					
	$t_1(5)$	$t_2(5,5)$	Macrophage (wpi) $t_3(6,5)$	$t_4(8)$	$t_5(4,5)$	Pathogen (wpi) $t_6(6,5)$
a_I	54.13	24.74	1.32	3.08	93.47	54.61
k_I	18.68	29.73	36.65	33.53	5.09	12.44
β_{Mrecr}	9.76	6.48	6.09	8.44	-	1.68
p_{Trecr}	-	-	-	-	-	2.46
$T_{threshold}$	4.15	17.48	39.25	32.08	-	23.32
$a_I \times k_I$	11.27	11.30	5.42	5.04	1.31	-
$a_I \times T_{threshold}$	1.43	5.48	-	-	-	4.47
$k_I \times T_{threshold}$	-	2.50	-	15.12	-	-
Total	99.42	97.72	97.30	97.40	99.88	98.98

Table 3
Posterior summary of computer model parameters (with $A_{necr}=2$) following calibration using field data

All parameters are scaled back to their biological values and are reported in the units indicated; α_I is reported on two scales, as a rate of growth and as a doubling time. The last column reports the percent decrease in the width of the 90% posterior credible interval relative to the 90% prior credible interval.

Parameter	Posterior estimates		Units	% decrease in credible interval
	Median	90% credible interval		
α_I	7.73	(7.24, 8.88)	/min. ($\times 10^{-4}$)	74.73
	1.50	(1.30, 1.60)	doubling time ($\times 10$ hrs)	
k_I	3.32	(2.70, 3.84)	parasites ($\times 10^2$)	63.79
β_{Mrecr}	2.31	(2.03, 2.81)	/unit chemokine ($\times 10^{-4}$)	13.58
p_{Trecr}	1.93	(1.54, 2.43)	%/time step ($\times 10^{-2}$)	0.79
$T_{threshold}$	5.57	(3.92, 6.82)	parasites($\times 10^{-4}$)	41.46

Table 4
Posterior summary of computer model parameters, including A_{necr} , following calibration using field data

All parameters are scaled back to their biological values and are reported in the units indicated; α_I is reported on two scales, as a rate of growth and as a doubling time. The last column reports the percent decrease in the width of the 90% posterior credible interval relative to the 90% prior credible interval.

Parameter	Posterior estimates		Units	% decrease in credible interval
	Median	90% credible interval		
α_I	7.38	(6.97, 8.07)	/min ($\times 10^{-4}$)	83.05
	1.57	(1.43, 1.66)	doubling time (10 hrs)	
k_I	3.17	(2.26, 3.89)	parasites ($\times 10^2$)	48.38
β_{Mrecr}	2.58	(2.06, 2.94)	/unit chemokine ($\times 10^{-4}$)	2.38
p_{Trecr}	1.86	(1.53, 2.41)	%/time step ($\times 10^{-2}$)	2.81
$T_{threshold}$	5.27	(2.53, 6.80)	parasites ($\times 10^{-4}$)	13.61
A_{necr}	3.00	(0.00, 6.00)	units of necrotic tissue	0.00