

An agent-based model for *Leishmania major* infection

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1. Introduction

Leishmania are protozoan parasites transmitted by bites of infected sandflies. 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. C3HeB/FeJ mice are resistant to *L. major*, but develop chronic cutaneous lesions when infected with another species *L. amazonensis*. The well-characterized mechanism of resistance to *L. major* depends on a Th1 immune response, macrophage activation, and elimination of the parasite [Sacks 2002]. The factors that account for host susceptibility to *L. amazonensis*, however, are not completely understood, despite being generally attributed to a weakened Th1 response [Vanloubbeck 2004].

Computer simulation can provide insight into the differences between these species. Toward this goal, we describe an agent-based model for *L. major* infection and explore the sensitivity of predictions to model parameters. Results indicate that the strength of the Th1 response, resting macrophage speed, and parasite transfer threshold of infected macrophages (which determines when infected macrophages transfer parasite to additional cells) influence time to heal infection, while the timing of the adaptive immune response, macrophage speed, and transfer threshold impact parasite load at the peak of infection.

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

Agent-based models (ABMs) inherently capture the dynamics of complex systems whose properties depend on the collective behavior of the system's 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* that are executed at *discrete time steps*.

We describe an ABM of the immune response to *L. major* infection. The structure of our model follows that of Segovia-Juarez [2004], who explores granuloma formation during infection with another macrophage-tropic parasite, *Mycobacterium tuberculosis*. All model parameters are in Table A.1 of the appendix.

2.1. The environment

Experimental injection of *L. major* in the footpad of mice is a common biological model for studying the immune response to parasite challenge. We model a 2mm x 2mm cross section of footpad as a 100 x 100 grid of square *micro-compartments*. Because we assume the grid is contained within a larger infected area, the environment is *toroidal*, so an object leaving the grid will re-enter at the opposite end. A single micro-compartment can hold up to one macrophage and one T cell, with no restriction on chemokine molecules or parasites. We select four evenly distributed micro-compartments to serve as *source compartments* where new cells enter the system.

We label micro-compartments (i,j) , starting from $(0,0)$ at the bottom left. Define a *Moore neighborhood* of length r at position (x_o, y_o) to be the space

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

Furthermore, define $M_I(x_o, y_o)$ to be the *immediate Moore neighborhood* of a micro-compartment (x_o, y_o) .

2.2. Stages of Infection

2.2.1. Initial Conditions

Mice are infected with *L. major* promastigotes in the experimental infection we study [Vanloubbeeck 2004]. Resident tissue macrophages take up promastigotes, and the parasite changes to the non-motile amastigote form. We start simulating two days post infection to avoid initial infection events, which reduces the number of model parameters and agents. In addition, probably more is known about footpad conditions two days post infection than at initial inoculation. Based on parasite counts at this time [Doug Jones, unpublished observations], we randomly place 105 macrophages on the grid. Fifty parasites infect macrophages, with parasites per macrophage uniform between 1 and P_m . All uninfected macrophages are given random lifespans uniform between 0 and 100 days.

2.2.2. Infection of Macrophages

Macrophages are the primary cells that *Leishmania* parasites infect. We use the term *resting* macrophage to refer to an uninfected, unactivated cell. Intracellular parasites experience logistic growth at rate α_1 with carrying capacity $K_I + 30$. If the macrophage is not activated, intracellular parasites grow until their number exceeds the transfer threshold K_I . Then, the macrophage enters a *dying* state, where it begins transferring parasite to macrophages in its length two Moore neighborhood. In *Leishmania* infection, parasite transmission is thought to be direct, as extracellular parasites are seldom observed [Chang 2003]. Segovia-Juarez [2004] uses a similar model, but allows extracellular parasite and restricts take-up to the length 1 Moore neighborhood. All macrophages that are not dying, including those already infected, can take up parasite. Activated macrophages will eliminate parasite they ingest. A dying macrophage is removed from the system once all of its intracellular parasites are gone.

2.2.3. Chemokines and Cell Movement

Chemokines, chemical attractants that influence cell movement, play an important role in *Leishmania* infection [Roychoudhury 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 (IL-8), an important chemokine involved in early infection.

Cell movement has been described as a biased-random walk in the presence of chemokine [Tranquillo 1988]. Let

$$\begin{aligned} C_{i,j} &= \text{amount of chemokine in micro-compartment } (i,j) \\ C_{M_1(i,j)} &= \text{amount of chemokine in immediate Moore neighborhood } M_1(i,j). \end{aligned}$$

Propose a cell currently in micro-compartment (i,j) moves to micro-compartment $(k,l) \in M_1(i,j)$ with probability $C_{k,l} / C_{M_1(i,j)}$. If the proposed micro-compartment contains no other cells, then the move proceeds. If the current cell is a macrophage (T cell) and the proposed micro-compartment contains a T cell (macrophage), then the cell will move with probability T_{move} . Otherwise, the cell will not move.

2.2.4. Recruitment and the adaptive immune response

During infection, T cells and macrophages are recruited to infected areas. Macrophages are actively recruited around two days post infection with *L. major* [Sunderkotter 1993]. 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. T cells then proliferate and mature into several classes of T cells, including Th1 CD4 cells. In *L. major* infection, Th1 CD4 cells are directed to the infected area and activate infected macrophages. The arrival of T cells takes between 4 and 7 days in the absence of prior pathogen exposure [Janeway 2005], and occurs only after a prolonged period of parasite growth in low dose *L. major* infection [Belkaid 2000]. We assume that the timing of T cell recruitment is related to pathogen load at the infection site, and recruit T cells once a threshold pathogen level, T_{delay} , is reached.

In the ABM, we use source compartments to represent blood vessels where recruited cells enter. At each time-step and at each unoccupied source compartment, macrophages enter with probability M_{recr} and T cells, after T_{delay} is reached, enter with probability T_{recr} . Given recruitment with these probabilities, movement into occupied source compartments is the same as described for movement around the grid.

2.2.5. The role of inflammatory macrophages

During the acute stage of infection, macrophages systematically migrate to the draining lymph node after taking up foreign antigen, apoptotic immune cells, and necrotic tissue. These *inflammatory* macrophages have a shorter tissue lifespan than resident macrophages [Bellingan 1996]. During infection, the macrophage population is a heterogeneous mix of resident, activated, and inflammatory macrophages. For simplicity, and because inflammatory macrophages likely dominate during active infection, we choose to transform all uninfected resident macrophages to inflammatory macrophages when T cell recruitment begins by assigning a lifespan uniform between 2

and M_{ils} days. This rule is consistent with the observed decrease in macrophages seen around the time the T cell response peaks in *L. major* infection [Belkaid 2000]. This decrease cannot be explained merely by the death of infected macrophages, but requires a systematic change such as the proposed phenotype switch [data not shown].

2.2.6. Macrophage activation

All T cells in our model are considered to be antigen specific Th1 CD4 cells that are equally capable of activating infected macrophages. 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 intracellular parasite. M_{ats} days after T cell activation, the macrophage destroys all intracellular parasite, undergoes apoptosis, and is removed from the grid.

2.3. 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. T cells move every 200 time steps (20 minutes). Macrophages move on slower time scales that we allow to vary (Table A.1). Additional update rules, such as the take up of parasite and activation of macrophages, are allowed to occur every *Update* minutes.

3. Sensitivity analysis

3.1. Choices of model parameters

There are 24 parameters in our model. All parameters and ranges are given in Table A.1. For each parameter, we assign ranges biologically consistent with both *L. major* and *L. amazonensis*. References for our choice of ranges are in the table.

3.2. Experimental design

For simplicity we vary only 11 model parameters. Sensitivity to remaining parameters will be explored in later work. Parameter values are chosen from their ranges using Latin hypercube sampling (LHS) as described in McKay [1992]. Each parameter assumes one of 11 possible values during a single simulation. We perform 484 runs using 22 LHS structures and two replicates for each input configuration. We simulate infection for 71 days or until all parasite was cleared.

In the model, two cells of the same type cannot occupy a single micro-compartment. This rule makes it possible for infected macrophages to impede macrophage entry at the source compartments. Although a pathogen may influence the immune response in this way, it is not thought to be the case for *Leishmania*. In order to prevent this unintended effect from biasing our results, we discard runs where at least one infected macrophage occupies a source compartment at the end of any simulation day. A total of 261 runs are used in our final analysis. Of these, 255 infections heal before 71 days.

3.2. Results

We calculated R^2 values for each of our parameters with various simulation output measures as described in McKay [1992]. The R^2 statistic is a measure of the proportion of total variation in the response that can be accounted for by variation in each parameter. These results appear in Table 3.1. Most of the variation in time until clearance is explained by variation in T cell recruitment rate (T_{recr} , 27.88%), the probability that a T cell activates an infected macrophage (T_{actm} , 11.51%), resting macrophage cell speed (M_{rsp} , 11.02%), and transfer threshold (K_I , 9.83%); most variation in peak parasite amount is explained by variation in T_{delay} (55.56%); and most of the variation in the maximum number of infected macrophages is explained by variation in K_I (48.08%).

Param	Time to clearance	# Parasites						Max		
		Max	2 Wks	3 Wks	5 Wks	7 Wks	10 Wks	Mac	T	Mi
α_I	6.14	9.26	30.04	17.60	4.65	3.91	4.02	74.83	3.44	24.63
T_{actm}	11.51	5.40	5.87	15.67	5.10	4.91	4.67	7.00	6.02	10.27
T_{move}	5.83	7.30	9.07	10.41	4.03	3.84	4.11	4.79	16.20	3.63
M_{ils}	6.16	5.51	8.96	3.01	5.09	4.79	4.92	3.89	6.08	3.40
K_I	9.83	20.91	5.30	10.86	8.01	8.34	8.55	9.95	3.89	48.08
M_{rsp}	11.02	21.76	6.11	6.37	11.15	10.41	10.45	16.32	9.58	15.93
M_{asp}	8.56	7.56	8.43	2.06	4.88	4.87	4.92	3.99	10.27	5.75
M_{ils}	7.94	3.62	5.98	8.89	10.18	8.56	8.43	3.35	6.11	2.17
M_{recr}	7.53	6.71	7.33	12.34	3.76	4.09	4.29	12.74	3.49	4.32
T_{recr}	27.88	14.72	20.03	22.53	11.18	8.01	7.90	5.38	83.93	6.35
T_{delay}	7.99	55.56	32.44	13.35	4.74	5.06	5.21	8.32	7.87	13.57

Table 3.1. R^2 values. Percent of total variation accounted for by variation in model parameters for the following responses: time until parasite clearance, maximum amount of parasite, parasite load at 2,3,5,7, and 10 weeks, and maximum numbers of macrophages (Mac), T cells (T), and infected macrophages (Mi). In each column, values in bold indicate R^2 values that exceed the average R^2 values for that column.

3.3 Discussion

A parameter with a high R^2 value indicates that the simulation response is sensitive to the value of that parameter. In the context of *Leishmania* infection, a parameter that has a large impact on infection characteristics indicates that 1) careful characterization of that parameter is necessary for accurate simulation of the infection, 2) differences in this parameter value between *L. major* and *L. amazonensis* infections may account for differences in disease dynamics, and 3) data collected on this response may allow for parameter estimation.

Several parameters are important determinants of time to clearance, including some that may explain the difference between *Leishmania* species. T_{recr} and T_{actm} , which together contribute to the efficiency of the Th1 response, are known to differ between the two species of parasite [Soong 1997; Vanloubbeek 2004]. The parameter M_{rsp} is likely tissue specific and is not expected to vary with pathogen. One reason why our model is sensitive to this parameter is because there is a relatively large amount of

uncertainty about its value. The range of values we use is 0.5-1.5 $\mu\text{m}/\text{min}$, which we center at the value of 1.0 $\mu\text{m}/\text{min}$ used in the model of Segovia-Juarez [2004]. Actual *in vivo* speeds of macrophages could not be determined, though Webb [1996] observes speeds of 0.1–0.5 $\mu\text{m}/\text{min}$ using a Dunn chemotaxis chamber. Sensitivity to these smaller values has not been explored, though current results indicate that using accurate values of this parameter will be important for understanding *Leishmania* infection.

Transfer threshold also has a noticeable, though less substantial, impact on disease severity. In particular both the partial rank correlation between transfer threshold and maximum number of infected cells (-0.768) and transfer threshold and time until infection clearance (-0.123) are negative, indicating that lower transfer threshold is associated with a larger and longer infection. Interestingly, *in vitro* experiments where macrophages are infected with *L. amazonensis* produce more infected cells than similar experiments with *L. major*, despite the fact that initial numbers of macrophages and parasites are the same [Mukbel 2006]. Based on these observations, we hypothesize that pathogen-specific differences in transfer threshold may determine disease outcome, making mice susceptible to *L. amazonensis* but not *L. major*.

An important aspect of computer model evaluation is the efficient estimation of model parameters and assessment of model bias using data observed in the field. Sensitivity analysis is useful in this context since a parameter with a large effect on a response may be estimated using field data of that response. For example, our results indicate that biological data for peak parasite load and peak infected macrophage level can be used to estimate transfer threshold; T_{delay} is best estimated from maximum parasite load or parasite load at 2 weeks post infection; and T_{recr} can be estimated by examining time until clearance. In biological experiments, measurement of peak counts is usually not practical, since it requires frequent sampling. It is standard, however, to measure parasite load at various time points post infection. Computer experiments such as ours can be used to identify time points for data collection that will be most informative about model parameters of interest. Bayesian methods can then be implemented for parameter estimation when ranges or prior distributions for these parameters are known [Higdon 2004; Kennedy 2001].

4. Conclusions

In this work we have described an agent-based model for simulating *Leishmania major* infection. An initial sensitivity analysis indicates that the time until clearance is most sensitive to the T cell recruitment rate and the probability that a T cell activates an infected macrophage, measures of the Th1 immune response that biologists suspect are associated with host susceptibility to *L. amazonensis*. Time until clearance and maximum parasite load are sensitive to the choice of resting macrophage cell speed, highlighting the need for better data about macrophage movement. Time until clearance is also influenced by the transfer threshold of infected macrophages, and differences between *L. major* and *L. amazonensis* that effect this parameter may be partly responsible for the different disease outcomes observed.

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