

## **A model for the control of a bacterial infection by the non-specific immune response and antibiotics**

Model by B.R. Levin, R. Antia

Motivating experimental work by T. deRouin, J. Bull, R. Zappala, C. Bloch and N. Walker and B.R. Levin

The mathematical model and computer simulations described in this web page are intended for primarily heuristic purposes. Their role is to illustrate how, and attempt to convince the reader that, a quantitative consideration of the within-host population dynamics of the interactions between a proliferating population of bacteria and the host defenses is essential for a comprehensive understanding of the course of bacterial infections, their pathogenesis and their control with antibiotics. The model developed here is a mathematical version of that presented in diagram in Figure 1 of our perspective (Science, ..., 2001).

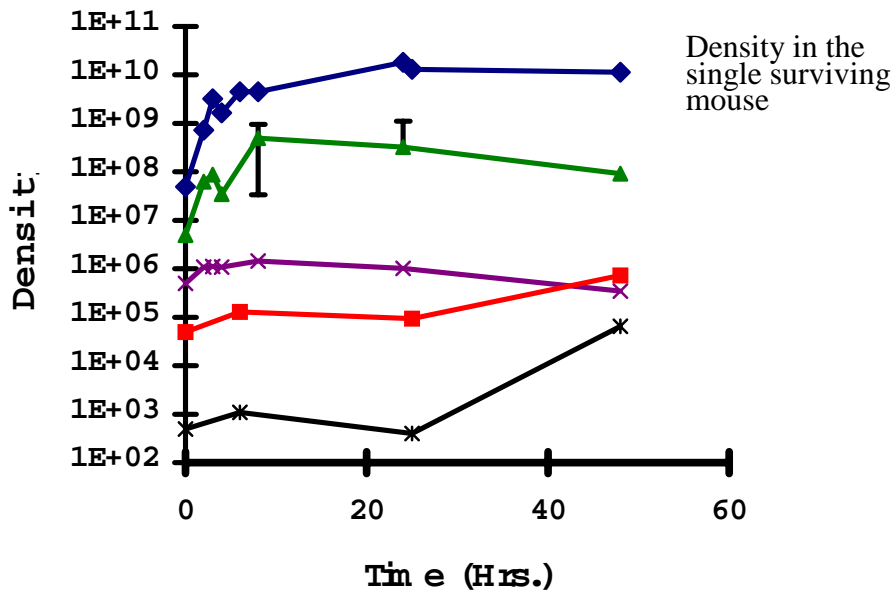
While motivated by an experimental result, we consider this model as only the first step in the development of a realistic (empirically-based and tested) model of the within-host dynamics of a bacterial infection. The role of this model is primarily to guide the experimental studies of the population dynamics of bacterial infections by identifying and evaluating the relative contribution different host-, bacteria- and antibiotic processes and properties have in determining the course and outcome of an infection. Although this web-site is filled with words, equations, and figures, it should still be considered to be “under construction”. The model presented here will, doubtless, be modified as more experimental work is done.

### **Empirical Motivation:**

This model was developed specifically to explore, in a quantitative way, hypotheses to account for the results of experimental infections with a *E. coli* (O18:K1:H7) in laboratory mice (ICR outbred mice purchased from Harlin). The design of these experiments were almost identical to those employed by H. William Smith and M. B. Huggins in their experimental study of phage and antibiotic therapy (Smith and Huggins 1982). When  $5 \times 10^7$  or more of these capsulated *E. coli* from stationary phase Luria Bertani broth cultures are injected into the thighs of laboratory mice, within 30-40 hours, more than 95% of the mice so inoculated die of a rampant sepsis. The bacteria at the site of the infection grow rapidly reach densities approaching  $10^{10}$  per gram (as measured from CFUs from homogenized tissue) and disseminate to other tissues.

In our experiments of this sort, we found that when the inoculation densities of these capsulated *E. coli* was lower than  $10^7$  cells, the mice almost invariably survive the infection and there was relatively little dissemination of bacteria through the body of the mouse. When the mice were inoculated with less than  $10^6$  bacteria, from only a few mice were only able to recover from the blood and spleen. Moreover, in the samples from whence these bacteria were recovered, their densities never exceed  $10^2$  per ml in the blood or  $10^3$  per gram in the spleen. On the other hand, when the mice were inoculated with more than  $10^6$  *E. coli* O18:K1:H7, these bacteria were recovered from all samples

taken from the blood (as many as  $10^4$  per ml for the highest density inoculum,  $10^8$ ) and from the spleen (as many as  $10^7$  per gram of spleen).



**Figure 1 – Estimated densities of *E. coli* 018:K1:H7 at different times after inoculation. Unless otherwise noted, the densities reported at each time are the mean CFU estimates from three mice. The points at time 0 are the estimated densities of inocula, rather than those recovered from the mice. The single mouse injected with  $5 \times 10^7$  bacteria from whence the 50 hour sample was taken was the only one in this study that survived for more than 42 hours with an inoculation of that high a density.**

How can we account for these dynamics? Why, when they are inoculated at these lower densities, don't the bacteria grow more rapidly or die more rapidly, as would be anticipated from immune control models like that employed in (Levin and Bull 1996). Why is there a threshold inoculation density above which the bacterial population grows rapidly, disseminates and kills the mouse and below which there is little dissemination of bacteria and the mouse survives? Why, when a mouse is inoculated with a normally lethal density of bacteria does the rate of mortality depend on the time antibiotic treatment is initiated?

## The Basic Model

To explore the different processes that could account for these observations, we developed the model depicted in the diagram presented in Figure 1 of Levin and Antia (Reference to the SCIENCE perspective ). In the basic form of this model, there is one population of free bacteria of  $B$  and two populations of phagocytic cells<sup>1</sup>,  $M$  and  $MB$ , for those that have yet to take up bacteria and those that have engulfed one or more viable bacterium, respectively. with designations and densities  $M$  and  $MB$ , respectively. These variables,  $B$ ,  $M$  and  $MB$  are both the designations and densities of these populations at the site of the infection.

We assume that in the absence of any phagocytes or antibiotics, the bacteria proliferate at the site of the infection at a rate proportional to their intrinsic rate of increase  $r$  and the carrying capacity of the tissue,  $K_B$ , via a logistic function,  $r(1-B/K_B)$ , where the parameter,  $K_B$  is the maximum number of bacteria that can be maintained at that site.

Free bacteria are taken up by  $M$  and  $MB$  phagocytes at rates proportional to their densities at the site of the infection, and rate parameters,  $\gamma_M$  and  $\gamma_{MB}$ . By taking up bacteria phagocytes of the  $M$  type are converted into  $MB$ . We assume that the capacity of phagocytes to take up bacteria is limited and individual phagocytic cells can become satiated (saturated) and unable to take up additional bacteria.. For this we assume that average each phagocyte is able to take up  $Z$  bacteria. When  $B < Z(M+MB)$ , the rate of uptake of free bacteria by phagocytes is maximal,  $(\gamma_M M + \gamma_{MB} MB)$  per bacteria. When  $B \geq Z(M+MB)$  free bacteria are taken up at that declines with the density of bacteria,  $(\gamma_M M + \gamma_{MB} MB) ((M+MB)*Z)/B$ . We assume that at a rate  $x$  per hour,  $MB$  phagocytes become free of viable bacteria and return to the  $M$  state. New phagocytes cells,  $M$ , enter the site of the infection at a rate directly proportional to the density of the bacteria at that site. For this we assume a hyperbolic function of the sort employed by Monod for the relationship between the rate of growth and the concentration of limiting resources, (Monod 1949),  $V_M (B/(B + K_M))$ , where  $V_M$  cells per hour is the maximum number of  $M$  cells that can enter the site per hour and  $K_M$  the number of bacteria at the site at which they enter at half that maximum rate. We further assume that the site can only hold  $M_{MAX}$  phagocytes, so that at any given time the input of phagocytes into the site of the infection declines in a logistic-like manner so that  $M_{MAX}$  is the total number of phagocytes that could be present at that site.

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<sup>1</sup> The phagocytic cells in this model include neutrophils, monocytes, macrophages and other host cells with the capacity to take-up bacteria. This simple version of the model does not separately consider the populations of these different types of phagocytes and the temporal changes in their level of activation.

With these definitions and assumptions, the rate of change in the density of the B, M and MB populations at the site of the infection is given by the coupled differential equations,<sup>2</sup>

$$dM/dt = V_M * B / (B + K_{MB}) * (1 - (M + MB) / M_{MAX}) - dM - F(\gamma_M M)B + xMB$$

$$dMB/dt = F(\gamma_M M)B - dMB - xMB$$

$$dB/dt = B[ r(1 - BK_B) - F(\gamma_M M + \gamma_{MB} MB) ]$$

where

$F=1$  when  $B < Z(M + MB)$ ,

$F= ((M + MB) * Z) / B$  when  $B \geq Z * (M + MB)$

and  $d$  is the rate at which phagocytes die or disseminate from the site of the infection.

### Simulation results

At this time, we have not formally (a euphemism for mathematically) analyzed the properties of this model and have restricted our exploration of its properties to numerical solutions of these differential equations. For this computer simulation we used a constant step size (0.001 hour) Euler method. The simulation is programmed in Fortran (77) and will be made available to interested readers in either or both a form that can be executed on a Windows (DOS) computer or as code that can be converted into a executable program with a Fortran compiler.

The value of the parameters and initial values of the variables used in these simulations have been chosen to illustrate how the previously described in mouse-o observations with *E. coli* K1 infections could be accounted for by the natural control of the infection by saturatable populations of phagocytic cell population.

In Figure 2, we present the results of simulations of the population dynamics of the control of a bacterial infection by phagocytic cells present and migrating into the site of the infection. In Figure 1a, the initial density of bacteria is  $10^6$  and the infection is controlled. The density of free bacteria rapidly declines while that of free phagocytes and phagocytes that have engulfed bacteria, M and MB, respectively declines slowly. With the same parameters and an initial density of  $10^7$  bacteria in the site of the infection, the population of free bacteria proliferates and reaches its maximum value and then levels off as does that of the M and MB phagocyte populations.

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<sup>2</sup> In this version of the model we have not considered the effects of complement and other chemicals in inhibiting the growth and killing bacteria, as we have in figure 1. We can incorporate these compounds the model by adding another differential equation for the changes in the concentration of these growth inhibiting bacterial toxic compounds, C. In accord with the diagrammatic form of this model, the rate of increase in C would increase with the density of bacteria and the growth rate of the bacteria would decline (and could become negative) with the concentration of these compounds, C.

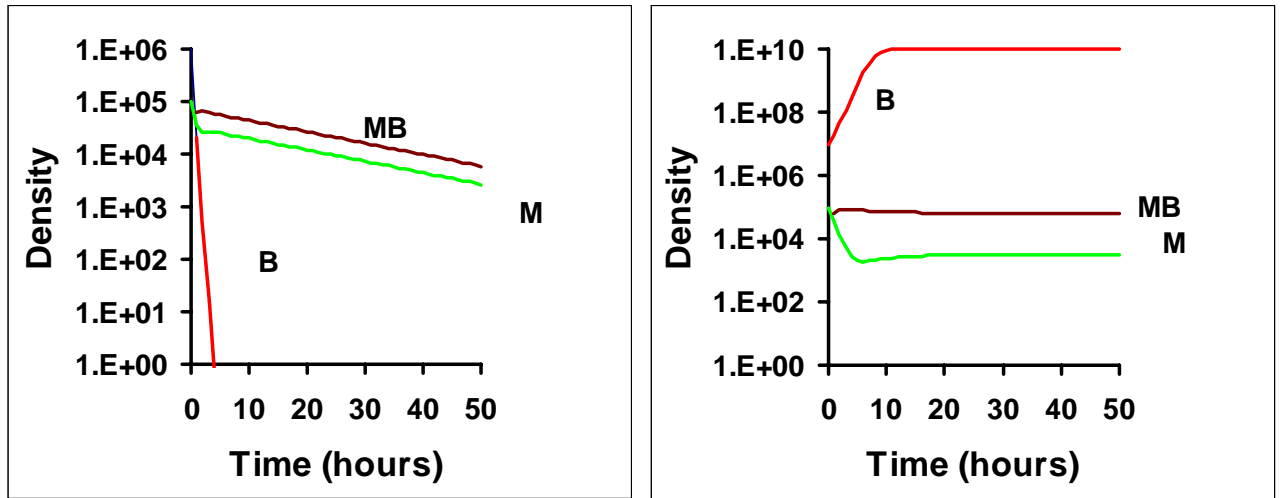


Figure 2: Simulation results control of a bacterial infection by phagocytic cell. Parameter values,  $r = 1$ ,  $K_B = 10^{10}$ ,  $d = 0.05$ ,  $Z = 10$ ,  $V_M = 10^4$ ,  $M_{MAX} = 10^7$ ,  $\gamma_M = \gamma_{MB} = 10^{-5}$ ,  $x = 0$ . (a) Initial densities,  $B = 10^6$ ,  $M = 10^5$ ,  $MB = 0$ , (b)  $B = 10^7$ ,  $M = 10^5$ ,  $MB = 0$ ,

## Antibiotics and antibiotic resistance.

### 1- Adding antibiotic inhibition of bacterial growth to the model

Antibiotic can be present at the site of the infection at a concentration of  $A$  ug and at intervals of  $D$  hours,  $A_{IN}$  ug of that antibiotic is introduced into that site. The antibiotics inhibit the growth and kill sensitive bacteria at rate proportional to their concentration,  $A$ . For this antibiotic inhibition and killing function we use a quadratic equation  $F(A) = c_1 A + c_2 A^2$  and assume that the effective concentration antibiotic decays at a rate of  $d_A$  per hour. In addition to the bacterial population that is sensitive to the antibiotic, we allow for a second population of bacteria of density  $B_A$  that is completely resistant to its action, i.e., its rate of growth and survival are unaffected by the antibiotic. We further assume that these antibiotic resistant bacteria have the same sensitivity to the phagocytes as antibiotic sensitive bacteria, but that resistance can engender some reduction in their rate of replication (Andersson and Levin 1999). For this fitness cost we assume that the rate of replication of the antibiotic resistant bacterial population,  $B_A$ , is  $(1-s)$  as great as that of the antibiotic sensitive bacteria, where  $0 \geq s \leq 1$ .

With these definitions and assumptions, the rates of change in the densities of the bacterial and phagocyte populations and concentration of antibiotics are given by,

$$dM/dt = V_M * B / (B + K_{MB}) * (1 - (M + MB) / M_{MAX}) - dM - F(\gamma_M M)B + xMB$$

$$dMB/dt = F(\gamma_M M)B - dMB - xMB$$

$$dB/dt = B[ r(1 - BK_B) - F(\gamma_M M + \gamma_{MB} MB) - (c_1 A + c_2 A^2) ]$$

$$dBA/dt = B[ r(1 - BK_B)(1 - s) - F(\gamma_M M + \gamma_{MB} MB) ]$$

$$dA/dt = -d_A A$$

where

$F=1$  when  $B < Z(M + MB)$ ,

$F = ((M + MB) * Z) / B$  when  $B \geq Z * (M + MB)$

And every  $D$  hours,  $A_{IN}$  ug of the antibiotic enters the site of the infection and augments the concentration of the antibiotic at that site by that amount.

### Simulation Results

In Figure 3 we present the antibiotic inhibition function used in our simulation experiments, i.e. the relationship between the exponential growth (or death) rates of the bacteria at different concentrations of the antibiotic. With these parameter values, the bacteria are able to grow, when the concentration of the antibiotic at the site of the infection is less than  $\sim 30$  ug, the MIC (minimum inhibitory concentration). Above that concentration, they are killed at a rate that increases with the concentration of the antibiotic.

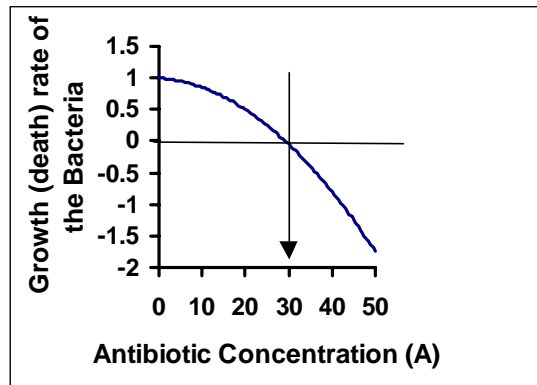


Figure 3 – Antibiotic inhibition function,  $r=1$ ,  $c_1=0.005$ ,  $c_2=0.001$ .

In Figure 4, we consider a situation where there are no phagocytes and the growth of the bacteria is limited solely by the antibiotics and resources. In Figure 4a, resistant bacteria are not present and in Figure 4b resistant bacteria they are present at an initial

present at a density of 10 at the site of the infection. While the density of sensitive bacteria increases when the concentration of the antibiotic falls below its MIC

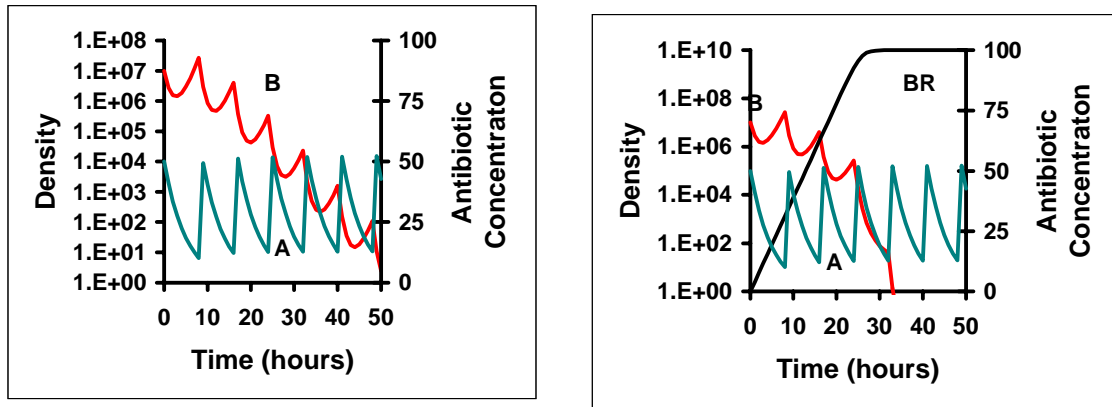
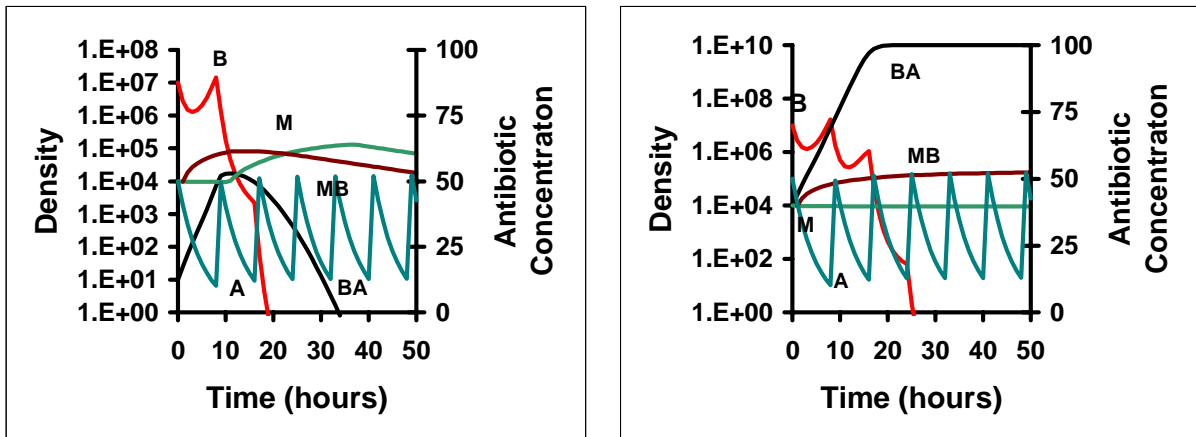


Figure 4. Simulation results: control of a bacterial infection by antibiotics. Parameter values,  $r=1$ ,  $K_B = 10^{10}$ ,  $d = 0.05$ ,  $Z=10$ ,  $V_M=0$ ,  $M_{MAX} = 0$ ,  $\gamma_M = \gamma_{MB}=10^{-5}$ ,  $x=0$ ,  $s=0.10$ ,  $c_1=0.005$ ,  $c_2=0.001$ . (a) Initial densities and concentrations,  $B=10^7$ ,  $BA=0$ ,  $M=0$   $MB=0$ ,  $A=50$ ,  $d=8$ ,  $A_{IN}=50$  (b) ,  $B=10^7$ ,  $BA=10$ ,  $M=0$   $MB=0$ ,  $A=50$ ,  $d=8$ ,  $A_{IN}=50$ .

the net rate of bacterial growth is negative and, in the absence of resistant bacteria, the antibiotic is able to clear the infection without functional host defenses. When resistant bacteria are present, however, the antibiotic sensitive population is cleared while that of the initially rare resistant bacteria ascends to the resource-limited equilibrium.

With phagocytic cells contributing to the demise of the bacteria, the effects of resistance on the efficacy of antibiotic treatment depends on the density of resistant bacteria at the time of treatment. If resistant bacteria are initially rare, in combination with the antibiotics, the phagocytes can clear an infection of both antibiotic susceptible and resistant bacteria (Figure 5a). If, however, the resistant population is relatively common at the time antibiotics are introduced, the phagocyte population can become saturated and the infection will not be cleared (Figure 5b). Treatment will fail because of resistance.

Figure 5 Simulation results: control of a bacterial infection by antibiotics and phagocytic



cells. Parameter values,  $r=1$ ,  $K_B = 10^{10}$ ,  $d = 0.05$ ,  $Z=10$ ,  $V_M=10^4$ ,  $x=0$ ,  $M_{MAX} = 10^7$ ,  $\gamma_M = \gamma_{MB}=10^{-5}$ ,  $s=0.10$ ,  $c_1=0.005$ ,  $c_2=0.001$ . Initial densities and concentrations,  $B=10^7$ ,  $M=10^5$ ,  $MB=0$ ,  $A=50$ ,  $d=8$ ,  $A_{IN}=50$ . In Figure 5a, the initial density of resistant cells is  $10^1$ . In Figure 5b, the initial density of resistant cells is  $10^4$ .

### Predictions

The model makes three primary predictions about the dynamics of the control of bacterial infection by phagocytes and antibiotics.

- (1) If the proliferation of the bacteria is controlled by a phagocyte population as well as by resources limitations, there would be an inoculation density, a threshold, above which bacteria inoculated into a mammalian host will be able to proliferate and only stop growing when its population becomes limited by resources. When introduced at densities below this threshold, the proliferation of free bacteria will be controlled by the phagocytes before their densities reach the level of resource limitation. The level of this threshold depends on the density of phagocytes present at the site of the infection and the rate at which they migrate into that site, the number of bacteria they can take up and the rate at which these bacteria are phagocytized and the rate at which the phagocytes die and/or are removed from the site of the infection.
- (2) If the proliferation of an infecting bacterial population is controlled by phagocytes, the vast majority of bacteria recovered after the growth of free bacteria ceases, will be bacteria in phagocytes.
- (3) When inoculated sufficiently frequently and at high-enough concentrations, if resistant bacteria are not present, antibiotics can control a bacterial infection without an effective host defense via phagocytes. If resistant bacteria are present, however, effective control of the infection can however be achieved by phagocytosis if the total density of bacteria at the site of the infection remains below the threshold at which the phagocyte population becomes saturated.

For each of these predictions the model specifies the quantitative conditions under which each of these outcomes will obtain.

### **Caveats, Limitations and Excuses:**

As noted in the introduction to this web site, we consider the present model and simulation only a first step in the development of a realistic model of the within-host dynamics of a bacterial infections and their control by host defenses and antibiotics. The functions employed in this model and the values of the parameters and variables used in the simulations, although in an arguably a realistic range, were made up rather than independently determined and estimated in the experimental system under consideration. The present model is certainly far simpler than the laboratory mouse – *E. coli* 018:K1:H7 infection system it was developed to mimic and is even simpler than the model depicted in our Science perspective. As noted, we have not considered complement and other chemical defenses and have lumped the various different types of phagocytic cells into a single class. During the course of the infection, the distribution of the different types of phagocytes will change as will the parameters governing their rates of survival, rates of migration to and from the site of the infection, rates which they engulf bacteria, the number of bacteria they can take up, and the rate at which those bacteria are killed. For example, as the course of the infection proceeds the parameters that govern the rates at which bacteria,  $\gamma_M$  and  $\gamma_{MB}$ , up by phagocytic cells will increase as those phagocytes become activated. For capsulated bacteria,  $\gamma_M$  and  $\gamma_{MB}$  will increase profoundly after the specific immune system kicks in and those bacteria are opsonized by circulating antibody.

We can readily make this model more complex and extend it to overcome all of the above-described and other limitations and, in that sense, make it more realistic. Although increasing the numbers of variables and parameters would make the model even less amenable to a mathematical analysis, it would still be possible to analyze its properties numerically (by computer simulation). At this juncture, however, we believe these extensions would be premature and unnecessary. The purpose of the model presented here (and models in general) is not to generate a quantitatively precise analog of the within-host population dynamics of a specific bacterial infection, but rather identify and determine the magnitude of the contribution of different biological and human-mediated processes contributing to the course of a bacterial infection in mammalian hosts. In particular, we wish to explore the hypothesis that the population dynamics of the infection depicted in Figure 1 can be attributed to the saturation of the phagocyte cell population. The present model makes very specific qualitative and quantitative predictions about what to anticipate were this hypothesis valid. The model also makes specific predictions, hypotheses, about when the presence of an antibiotic resistant population of bacteria will prevent the control of the infection in mice with and without an effective population of phagocytes. These hypotheses can be tested with the laboratory mouse – *E. coli* 018:K1:H7

### **Testing the hypothesis:**

If the phagocyte saturation hypothesis accounts for the population dynamics depicted in Figure 1 the following predictions should hold. (1) When the bacteria are introduced at sublethal densities, as the course of the infection proceeds, there should be relatively few free bacteria in the site of the infection. The majority of the bacteria recovered would be viable cells within neutrophils and other phagocytic cells. (2) When the bacteria are

introduced at lethal densities, the majority of bacteria recovered after the period of exponential growth should be free, in the intercellular fluids. (3) Without an effective phagocyte population, the presence of any resistant bacteria should preclude the control of the infection with antibiotics. The resistant population should ascend and dominate the community. (4) With an effective phagocyte population, whether the presence of resistant bacteria will preclude effective treatment will depend on the density they achieve during the course of the infection. As long as density of the combined population of sensitive and resistant bacteria remains below the phagocyte saturation threshold, the infection will be controlled.

These predictions (hypotheses) can be tested with the mouse thigh – *E. coli* O18:K1:H7 model described above and is in the process of being tested by Renata Zappala, an MD/PhD student at Emory. In addition to more qualitative predictions of this model, Renata is also testing the quantitative predictions by a combination of in vitro and in vivo experiments. A number of the parameters of this model can be independently estimated in vitro, e.g. see (Hampton et al. 1994; Hampton and Winterbourn 1999) for the estimation of the phagocytosis parameters. Estimates of the densities and changes in the densities of bacteria, neutrophils and other phagocytes at the site of the infection can be obtained directly from infected mice. And at least rough estimates of the relative frequencies of phagocytes with and without engulfed bacteria can also be obtained. Additional tests of the quantitative and qualitative predictions of this model can and will be obtained indirectly. For example: 1) By using chemicals like cyclophosphamides or phagocyte cell-specific antibody it is possible to control the numbers and rates of proliferation of neutrophils and other phagocytic cells. 2) By using different antibiotics it is possible to manipulate the relative extents which extracellular (free) bacteria and bacteria within phagocytic cells are killed. 3) By using genetically marked, unencapsulated strains of this bacterium O18:K1:H7, (Bloch et al. 1989), which proliferate but do not kill the mice (Smith and Huggins 1982), it should be possible to saturate the phagocytic cells with decoys and follow the proliferation of the virulent, encapsulated strain. In accord with the phagocyte saturation model, the threshold density for the virulent, K1+ strain to kill the mouse should be reduced by saturating the phagocytes with avirulent decoys.

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