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A Low Dimensional Dynamical Model of the Initial Pulmonary Innate Response to Infection

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Abstract

In order to gain a deeper understanding of the onset and progression of pulmonary infections we present and analyze a low dimensional, phenomenological model of infection and the innate immune response in the lungs. Because pulmonary innate immunity has features unique to itself, general mathematical models of the immune system may not be appropriate. The differential equations model that we propose is based on current knowledge of the biology of pulmonary innate immunity and accurately reproduces known features of the initial phase of the dynamics of pulmonary innate system as exhibited in recent experiments. Further, we propose to use the model as a starting point for interrogation with clinical data from a new noninvasive technique for sampling alveolar lining fluid.

1 Introduction

Pulmonary infections are a common and costly health care complication. We refer consistently to pneumonia, but most everything holds for pulmonary infections in general. Pneumonia is the second most common nosocomial infection of critically ill patients [23], affecting 27% of these patients [43]. The diagnosis of pneumonia remains problematic [23]. Using clinical criteria, a diagnosis of pneumonia is typically not made until an infection is well established. This leaves physicians in the position of fighting an established infection that:

1. Is much harder to control than a nascent one.
2. Causes more collateral damage to the patient.
3. Costs the patient, the hospital and society more money, resources, time and effort.

There exist numerous risk scores that are used to aid in the diagnosis of pneumonia. While these are of enormous value they are based on point estimates and none, that we are aware of, focus on time series data and the predator-prey like dynamics between the pulmonary immune response and the infectious agent.

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In [21] the authors introduce a new inexpensive, direct and non-invasive method to monitor the presence and levels of pathogens of intubated patients (connected to a ventilator). We use PCR (polymerase chain reaction) technology to detect pathogens from aerosolized droplets of alveolar lining fluid (ALF) that collect in a standard filter in the ventilator circuit. This method can produce time series data of exactly the type required for phenomenological models such as that studied in this work. We describe the method and some results in Appendix A.

The origin of this work is the desire to develop models of pulmonary infection/immunity dynamics that can be used in conjunction with the new monitoring techniques to understand, diagnose and control ventilator associated pneumonia (VAP).

In this manuscript we begin with a review of some of the known features of the immune response in the lungs. We then develop a low-dimensional differential equations model of the interaction between infection and the pulmonary innate response. We incorporate many of the properties of the innate system that are specific to the lungs. We analyze the dynamics of the model and numerically compare the model to recent experimental findings.

2 The Immune System and the Lungs

2.1 General information

The human immune system is an extremely complicated system with hundreds or thousands of components that interact with each other and with other physiological processes via perhaps tens of thousands of interactions. Some of these interactions are relatively well-understood, some are barely understood and most have not been investigated.

It is conventional to subdivide the immune system into two parts; the innate and adaptive systems. The table below summarizes the characterization. These two systems are not independent but interact in various ways.

Innate immune system	Adaptive immune system
Response is non-specific	Pathogen and antigen specific response
Immediate/rapid response	Delay between exposure and response
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

Under ideal conditions bacterial pathogens can divide and grow *much* faster than marrow and hematopoiesis can produce mature immune cells of the adaptive system. Given this and the fact that we are all exposed to bacterial pathogens on a constant basis, a fundamental question is: why aren't we sick all the time? The answer seems to lie in the innate system, that provides surveillance and a "first response" to invasion by pathogens (see for example [10]). In the lungs, the innate system has components that are always present in alveoli and alveolar lining fluid and also components that are recruited and activated very rapidly and this system is sufficient to clear away small levels of exposure to most pathogens.

2.2 Pulmonary Innate Immunity

Many cell types contribute to innate immunity including macrophages, neutrophils, dendritic cells, and the epithelial cells [12]. These cells can secrete multiple chemical agents that play

various roles in detecting, marking, inhibiting and killing pathogens, and signaling to other components of the immune system [20, 32, 46, 51].

In broad strokes the alveoli are patrolled by alveolar macrophages that are capable of phagocytizing small numbers of bacteria. Macrophages are large cells that inhabit the healthy lung and play a wide variety of roles that include killing of pathogens and signaling both the innate and adaptive immune responses. When the alveolar macrophages detect a threat that they themselves cannot contain, they can recruit other components of the immune system.

In humans, it is known that the largest population of intravascular phagocytes are the *polymorphonuclear leukocytes* (PMN's) [28, 47, 51]. Also known as *neutrophils*, PMNs are more effective in their killing capacity than macrophages [37, 47]. Estimates suggest that as much as 40% of the body's PMNs are contained in the lung capillary bed [33, 51].

Another seemingly important aspect of the lungs is *compartmentalization* [36]. Most of the chemical signaling agents that are associated with the immune response in the lungs do not migrate into the bloodstream during a bacterial challenge [20]. For example, signals for the recruitment of PMNs into the alveolar space seem to go only as far as the interface between the epithelial cells and the capillaries. Only a few types of innate signaling agents, such as G-CSF, migrate into the bloodstream en route to the bone marrow and lymph nodes [20].

2.3 Dynamic Nature of the Innate System in the Lungs

It has been long recognized that the immune system is inherently dynamical (e.g. [44, 48]) and dynamical modeling of different aspects of the system has been a fruitful endeavor for many years (e.g. [29, 26, 39]). Here we summarize some of the dynamical aspects of the innate system in the lungs that have been learned from experimental and clinical studies.

Alveolar spaces are normally a nearly sterile environment. Other epithelial surfaces, such as the upper respiratory tract or the digestive track, are in constant contact with pathogens and the innate systems in those areas are in a constant state of activation. In contrast, the innate system in the alveolar space is normally at a low level of activity, but can be rapidly induced into action [51, 5], and once activated it remains more sensitive to repeated insults as we will discuss below.

A small number of PMNs can be found in a healthy lung [8], but they enter the lungs in large numbers after a bacterial challenge is encountered and chemical signals are emitted to recruit PMN's to the site of the challenge.

In the lungs of mice it has been shown that PMN's are the dominant effectors of the innate response to bacterial invasions [10, 51]. It was shown that mice having either deficient numbers of PMN's or deficient PMN recruitment mechanisms are much more likely to experience severe bacterial infections and death when exposed to only small amounts of two different pneumonia causing bacteria [51]. In animal studies, healthy lungs contain only a very small amount of PMNs, but within 3–4 hours after introduction of an intrapulmonary challenge by bacteria, PMNs constitute 60% to 80% of the cells recovered from samples of alveolar fluid [36].

Human PMNs normally live about 8 hours and are constantly being replenished by the bone marrow. PMNs that are activated to fight infection have a longer life span, but also die after ingesting and destroying several bacteria cells [19]. It is known that bacterial invasion in the lungs stimulates further production and release of PMNs by the bone marrow, presumably to replace those recruited into the air sacs. It is also known that PMNs are subject not only to

recruitment, but also priming, activation and down-regulation. These processes are complex and seem to be very important in protecting the lungs from damage.

In a series of experiments [5] mice were exposed to one strain of a bacteria by microaspiration, then 4 hours later exposed to a nearly identical second strain. After 24 hours, the amounts of each strain were measured and the first order growth rate constants of each strain were calculated. The results show that the second strain proliferated much more slowly than the first (which strain was introduced first did not matter). Further, the growth constant of the second strain was inversely related to the amount of the first strain introduced. The growth constant of the first bacteria was mostly unaffected by the size of its initial load.

The main conclusion of these “two-hit” experiments is that the pulmonary innate system is dynamic. It quickly becomes activated by the introduction of the first strain. Thus when the second strain is introduced the conditions for growth are much less favorable than in the quiescent state.

3 A Low-dimensional ODE Model of the Immune System

Pugliese and Gandolfi [40] introduced the following simple equations to model the interaction between a pathogen and a host immune response:

$$\begin{aligned}x' &= \alpha x - \frac{mx}{1+\beta_u x} - \frac{x}{1+\beta_s x} y \\y' &= -y + \frac{x}{1+\gamma x} y + \eta.\end{aligned}\tag{3.1}$$

Here $x(t)$ is the density of the pathogen load, and $y(t)$ is the density of cells of the adaptive system. The pathogen is exponentially replicating with rate α . Elimination of the pathogen due to the adaptive system is captured by the term $-xy/(1 + \beta_s x)$. Production of y is stimulated by presence of the pathogen as represented by the term $xy/(1 + \gamma x)$. The small term η represents the adaptive system’s base level production of cells (needed to keep y from going to zero). These parts of the equations represent a very standard type of predator-prey modeling. What is remarkable about the work is the inclusion of the term $mx/(1 + \beta_u x)$ that models the elimination of the pathogen by the innate immune system. This term is immediate, but diminishing in its effect. Thus the innate system is included, but the equations are still only two dimensional. This allows P-G to do a complete analysis of the equations. One easy-to-see property is that if $\alpha < m$, then the equilibrium with $x = 0$ is locally stable, i.e. the model correctly reproduces the phenomenon that the innate system can eliminate small pathogen loads.

An obvious shortcoming of the model (3.1) is that the interaction terms are very specific. In a phenomenological model of a complicated system with little understanding of the first principles of the interactions, there is no justification for any specific functions appearing in the model. In [11] d’Onofrio generalized the model (3.1) by writing and analyzing the equations:

$$\begin{aligned}x' &= x(\alpha - M(x) - \phi(x)\pi(y)) \\y' &= -y + P(x)y + \eta,\end{aligned}\tag{3.2}$$

where the functions $M(x)$, $\phi(x)$, $\pi(y)$ and $P(x)$ are taken to be in large classes of functions defined only by certain biologically motivated assumptions, such as $M'(x) < 0$. For this model d’Onofrio is able to reproduce most of the results that were proved in [40]. While this generalization is useful, it is not surprising, since the dynamical behaviours exhibited by the

equations (3.1) are generally of types that are structurally stable, i.e. also exhibited by all equations that are close to the original ones (in the sense of norms on vector fields) [1].

The models (3.1) and (3.2) (PGO models) do not accurately model the action of innate immunity in the lungs since in those equations the action of the innate system is immediate and decreases with the bacterial load. In reality the most effective agents of the pulmonary innate system (PMNs) start at very low levels, but increase rapidly after the introduction of the pathogen. All of this leads to an important challenge: to correctly add the action in the lungs of PMNs and other rapid (but not immediate) innate effectors to PGO models. Of particular interest, the PGO models cannot predict the results of the two-hit experiment [5]. A new model should reproduce this phenomenon.

In this manuscript, as in [40] and [11], we focus on low-dimensional phenomenological models and on properties peculiar to the lungs.

4 A low-dimensional model of the innate response in the lungs

We begin our modeling by noting that the pulmonary innate system has components, such as the macrophages, that are ever present and immediately effective. According to our knowledge, the interaction as modeled in [40]:

$$x' = \alpha x - \frac{mx}{1+bx} \quad (4.1)$$

has support as a model for the bacterial load and the part of the pulmonary innate immune response that is immediate. It is evident from experiments that these initial effectors are limited in their capacity to clear larger bacterial loads. This is accurately reflected in the model since the “effective growth rate” of x ,

$$\alpha - \frac{m}{1+bx},$$

will be positive for x sufficiently large. From the experimental evidence, we require that

$$\alpha < m \quad (4.2)$$

in order to reflect the fact that very small pathogen loads are effectively eliminated by the immediate part of the system. With this assumption the equation (4.1) will serve as our model for this part of the innate pulmonary response.

Now we turn our attention to the parts of the innate system of the lungs that are not immediate, but are “rapid” in their deployment, focusing primarily on our knowledge of PMNs. Let z denote the level of active PMNs and other rapidly responding elements of the innate system inside the lungs. From our physiological knowledge of the system, the “background” state of z should be low, but not zero, and z should increase in response to a bacterial attack on a time scale of a few hours. We could also include, in addition to z , a variable representing the level of PMNs waiting in the pulmonary capillaries, however this source of PMNs is constantly being renewed on a time scale of a few hours and we would not expect this variable to experience large fluctuations, so we will treat it as a constant. Thus we will model the influx of PMNs into the alveolar sacs as dependent only on the bacterial load x for small z . We expect the activation of the variable z by x to be self-

limiting. Taking all of these considerations into account, we propose the following equation to govern z :

$$z' = \delta x - \gamma' xz + \eta - \mu z \quad (4.3)$$

In this equation η and $-\mu z$ represent the small baseline influx and natural death of PMNs respectively. In the absence of bacteria, the equations maintain a z level of η/μ . Here η is small and μ is on the order of $1/8\text{hr}^{-1}$. Since we expect the presence of bacteria to recruit significant numbers of PMN's, we should have that $\gamma' \ll \delta$.

Finally, we add to the model the phagocytic action of the PMNs. We will model this simply by the mass-action term $-\beta xz$ since this interaction should be approximately proportional to both x and z . Since PMNs die after ingesting and destroying several pathogens [13, 19], we add a similar interaction term $-\beta' xz$ to the equation for z as well, where $\beta' \ll \beta$. Using the notation $\gamma = \gamma' + \beta'$, the full model becomes:

$$\begin{aligned} x' &= f(x, z) \equiv \alpha x - \frac{mx}{1+bx} - \beta xz \\ z' &= g(x, z) \equiv \delta x - \gamma xz + \eta - \mu z \end{aligned} \quad (4.4)$$

Generally, since we know that large bacterial loads will overcome the innate system we expect that α will be larger than βz for z not too large. Since z has an “effective carrying capacity” of δ/γ for x large, this leads to a constraint:

$$\frac{\alpha}{\beta} > \frac{\delta}{\gamma}. \quad (4.5)$$

Other constraints on the parameters should include the requirement:

$$\frac{\eta}{\mu} < \frac{\delta}{\gamma}.$$

That is, the background level of PMNs is much less than the effective carrying capacity.

We have written the interactions as specific equations. We know that this lacks justification. However, as long as the behaviours exhibited by the equations are relatively “simple”, then we know that these qualitative behaviours are shared by all nearby equations. The dynamics we encounter are all of the simplest possible class known as “Morse-Smale”. In short, a system is Morse-Smale if (1) its non-wandering set consists of a finite number of hyperbolic equilibria or hyperbolic periodic orbits and (2) any intersections of stable and unstable manifolds are transversal [1, p. 79]. Morse-Smale systems are structurally stable, i.e. all nearby equations share the same dynamical characteristics.

5 Analysis of the Equations

5.1 Fixed point at $x = 0$

First we observe that the system has an equilibrium at $(0, \eta/\mu)$. At this point the linearization of the vector field is represented by the matrix:

$$DF|_{(0,\eta/\mu)} = \begin{pmatrix} \alpha - m - \frac{\beta\eta}{\mu} & 0 \\ \delta - \frac{\gamma\eta}{\mu} & -\mu \end{pmatrix}.$$

It is easily seen that the eigenvalues of this linearization are both negative provided

$$\alpha < m + \frac{\beta\eta}{\mu}. \quad (5.1)$$

As stated in the previous section we assume that $\alpha < m$ (4.2) and so under our assumptions, the equilibrium $(0, \eta/\mu)$ is a stable node and thus small bacterial loads are eliminated.

5.2 Other equilibria

Next we turn our attention to the possibility of additional equilibria in the model. The nullcline associated with $x' = 0$, $x \neq 0$ is

$$\alpha - \frac{m}{1+bx} - \beta z = 0,$$

or, the graph of a function z_1 :

$$z_1 = \frac{1}{\beta} \left(\alpha - \frac{m}{1+bx} \right).$$

Note that this curve is negative at $x = 0$ under our assumptions, it is monotone increasing and is asymptotic to α/β as x goes to infinity. The nullcline associated with $z' = 0$ is given by:

$$z_2 = \frac{\delta x + \eta}{\gamma x + \mu}.$$

This is also an increasing curve. It emanates from the equilibrium at $(0, \eta/\mu)$ and is asymptotic to δ/γ as $x \rightarrow +\infty$.

Graphically, it is clear that if $\alpha/\beta > \delta/\gamma$ then the two nullclines must cross. In the other case, the curves might not cross, or they might cross an even number of times (excluding tangencies). Equating the formulas for the nullclines we obtain:

$$\frac{1}{\beta} \frac{\alpha + \alpha bx - m}{1+bx} = \frac{\delta x + \eta}{\gamma x + \mu}.$$

Simplifying, we have a rational equation in x :

$$\frac{b(\alpha\gamma - \beta\delta)x^2 - (\beta(\delta + \eta b) + \gamma(m - \alpha) - \alpha\mu b)x - \mu(m - \alpha) - \beta\eta}{\beta(1+bx)(\gamma x + \mu)} = 0. \quad (5.2)$$

The denominator is non-zero for $x > 0$ and the numerator is a quadratic

$$Ax^2+Bx+C=0,$$

where

$$\begin{aligned} A &= b(\alpha\gamma - \beta\delta) \\ B &= \beta(\delta + \eta b) + \gamma(m - \alpha) - \alpha\mu b \\ C &= -\mu(m - \alpha) - \beta\eta. \end{aligned} \tag{5.3}$$

Since we assume $m > \alpha$ we see that $C < 0$. This implies that there are two main cases, Case 1:

$$\alpha\gamma > \beta\delta$$

which implies that there always exists a unique positive root, and, Case 2:

$$\alpha\gamma < \beta\delta$$

which implies that either (a) there are no real roots, (b) one real root, or (c) two real roots of the same sign. Case 1 is consistent with the constraint (4.5). For completeness we will also consider Case 2 in an Appendix.

5.3 Case 1

In Case 1, the x value of the unique intersection of the nullclines is given by:

$$x^* = \frac{-B + \sqrt{B^2 - 4AC}}{2A}.$$

We will show the following result.

Theorem 5.1 *In Case 1, the unique intersection of the nullclines is a saddle equilibrium. The stable manifold of the equilibrium divides the phase space into two parts. It is a graph of a function $x = \phi(z)$. All solutions with initial conditions to the left of the manifold are asymptotic to the equilibrium $(0, \eta/\mu)$ and solutions with initial conditions to the right of the manifold are unbounded in x as $t \rightarrow \infty$.*

Proof: To examine the stability of this fixed point, we use a geometric argument. Note that the two nullcline equations, z_1 and z_2 , corresponding to $x' = 0$ and $z' = 0$, cross at the roots of the quadratic in the numerator of (5.2). In the case that there are two real roots, the roots of the quadratic must be non-degenerate. This implies that we have $z_1' > z_2'$ at the crossing. Now consider that

$$z_1' = \frac{f_z}{f_x} \quad \text{and} \quad z_2' = \frac{g_z}{g_x},$$

where f and g denote the components of the nonlinear vector field F in the x and z directions. This and $z_1' > z_2'$ imply

$$f_x g_z - g_x f_z = \det(DF) < 0,$$

Now since the eigenvalues of DF are the solutions to $\lambda^2 - \text{Tr}(DF)\lambda + \det(DF) = 0$ there must be two real eigenvalues at the equilibrium, one positive and one negative. So the unique equilibrium in Case 1 is a saddle and therefore must have a stable manifold and an unstable manifold.

Consider that the nullclines subdivide the 1st quadrant into 4 sectors, which we denote by Roman numerals from I to IV as in Figure 1. Recall that both nullclines are the graphs of increasing functions. Note from the equations (4.4) that above the $x' = 0$ nullcline the x component of the vector field is negative and below the nullcline the x component is positive. Above the $z' = 0$ nullcline, the z component of the vector field is negative, and below the nullcline it is positive. Thus in sector I the vector field points in the “southeast” direction, in sector II it points “southwest”, in sector III it points “northwest” and in sector IV it is “northeast”. See again Figure 1.

Now consider the local stable and unstable manifolds of the equilibrium which are smooth by standard results. First note that neither of the nullclines can be invariant under the flow. Thus the local stable and unstable manifolds cannot coincide with either nullcline.

Next suppose that either a stable or an unstable manifold were contained in sector I. Because the nullclines both have positive slope, a local stable or unstable manifold in sector I would have to have positive slope near the equilibrium. Since the stable and unstable manifolds are flow invariant, the flow along those manifolds must be either to the northeast (unstable) or to the southwest (stable). However, this is not consistent with the fact that the flow points in the southeast direction everywhere in this sector. So we conclude that neither the local stable or local unstable manifolds can be in sector I. It follows immediately from the smoothness that the invariant manifolds also cannot be inside sector III.

Thus the stable and unstable manifolds in a neighborhood of the equilibrium must have components contained in sectors II and IV. In sector IV the flow is toward the northeast. Invariance implies that W^s and W^u must have positive slopes in sector IV. In sector II the flow is to the southwest and this implies that W^s and W^u must both have positive slope throughout this sector. It follows that locally W^s and W^u must be arranged as in Figure 2.

Now let us consider W^s and W^u globally. First consider the component of W^s that locally intersects sector IV. Since it has positive slope, and there are no other fixed points, W^s must intersect either the x axis or the nullcline $x' = 0$. However, since the flow on this nullcline along the boundary between sector IV and sector III is upward, the latter case cannot happen. Thus the component of W^s in sector IV terminates at the x axis as in Figure 3.

Next consider the component of W^s that is in sector II. Since its slope is positive it must either continue indefinitely in the northeast direction in sector II, or, it might intersect $x' = 0$. But, the vector field along $x' = 0$ between sectors I and II is pointing downward. We conclude that the component of W^s in sector II continues indefinitely in sector II and the global arrangement of W^s is as in the diagram Figure 3. and the theorem.

By similar arguments, the unstable manifold W^u is the graph of a function $z = \psi(x)$ with positive slope. One component is contained entirely in sector II and terminates at the stable node $(0, \eta/\mu)$, while the other component is contained in sector IV and it asymptotic to $z = \delta/\gamma$ as $x \rightarrow \infty$.

Theorem 5.1 gives a complete picture of the dynamics of the system in Case I. The stable manifold of the saddle equilibrium serves as a dynamic threshold beyond which the innate system as modeled cannot eliminate a pathogen. This threshold is not simply a fixed value, but depends on the level of activation of the innate system at the time of the challenge.

6 Comparison with the two-hit experiments

6.1 The model with two nearly identical pathogens

Suppose that the pulmonary innate immune system is attacked by two nearly identical strains of a bacteria as in the two-hit experiments [5]. Let x_1 and x_2 represent the bacterial loads of each strain. According to our model, the dynamics of the infection could be studied using the equations:

$$\begin{aligned}x_1' &= f(x_1, z) \equiv \alpha x_1 - \frac{mx_1}{1+b(x_1+x_2)} - \beta x_1 z \\x_2' &= f(x_2, z) \equiv \alpha x_2 - \frac{mx_2}{1+b(x_1+x_2)} - \beta x_2 z \\z' &= g(x_1+x_2, z) \equiv (\delta - \gamma z)(x_1+x_2) + \eta - \mu z\end{aligned}\quad (6.1)$$

Since the two strains are nearly identical, we do not assume any interaction between them other than what would be expected were they a single population of size $x = x_1 + x_2$. For two different bacterial species, this assumption might not be valid.

In the next sections we simulate the two-hit experiment using the most reasonable values of the parameters available and show that all features are accurately reproduced. Further we use the data from the two-hit experiment to estimate certain model parameters.

6.2 Simulation with estimated parameter values

The accurate estimation of parameter values in any detailed model is difficult and in this low-dimensional model it is already somewhat problematic. A few of the parameter values are known, some are estimated based on our best information and others are chosen arbitrarily within the constraints of Case 1 of the model. We have not attempted to optimize the values with respect to any principle such as metabolic efficiency or with respect to the experiments to which we compare the model. Actual values of the parameters would vary somewhat from host to host and would vary, perhaps greatly, for different species of pathogen.

The bacteria *Klebsiella pneumoniae*, used in the experiments in [5], has a doubling time on the order of one hour, so we let $\alpha = 1$. Since neutrophils are known to be very effective phagocytes, the value of β is also on the order of 1. We take it to be 0.2 or on the low side. We expect the response rate of the PMNs (the time scale for their response is about 4 hrs.) and other rapidly deploying immunological cells to be significantly lower than the bacterial growth rate, and we use values near $\delta = 0.04$ and $\gamma = 0.01$. The parameter μ is known to be approximately 0.087, and we let $\eta = 0.04$ so that the background rate of immune activity is low (0.5). The parameters representing the immediate part of the immune system m and b are taken to be 4 and 3.7, respectively, so that the effect of this part of the system becomes small for x in the range of the experiments. With this choice of parameters, the system belongs to Case 1 described in section 4.3, above.

We use a second-order modified Euler's Method to integrate the system (6.1) on the time interval $t \in [0, 12]$, with a time step of .01. The initial condition is $x_1(0) = d_1$, $x_2(0) = 0$, $z_1(0) = \eta/\mu$. When $t = 4$, x_2 is set to $d_2 = 5$ CFU and then allowed to progress normally. This corresponds to the second inoculum administered after 4 hours. As in [5], we vary the first

dose d_1 from 5 CFU to 15 CFU. At $t = 12$, first order growth rates are determined for the two strains via the formula

$$k_i = \frac{\ln \frac{x_i(12)}{d_i}}{T_i}$$

where $T_1 = 12$ and $T_2 = 8$.

Both initial conditions are well to the right of the stable manifold, and accordingly both strains are seen to display positive growth. The calculated growth constants k_1 and k_2 are plotted in Figure 4. They match the measured values in [5] quite well; first, we see that $k_1 > k_2$ and in particular, $k_1 - k_2 \approx 0.25$, whereas the median observed difference in the experiments was 0.22. Second, the actual values for k_1 and k_2 match closely with experimental observations. Third, the graph shows that k_2 is inversely related to the size of the initial inoculum, while k_1 is much less strongly affected, again matching up with the experimental results. The conclusion is that our model is adequate to accurately reproduce this experimentally observed phenomena in the pulmonary immune system.

6.3 Further comparison of the model with the two-hit experiment

From the two-hit experiments we have data for x_1 and x_2 , but not z . However, with reasonable simplifications we can compare the data with the model.

During the first four hours of the experiment we need only consider x_1 . Since the inoculate $x_1(0)$ is large enough that we are past the significant influence of the term $mx_i/(1 + bx_1)$, we ignore this nonlinear term. Further, at least initially the level of z is very low (since the response time is on the order of 4 hr as noted earlier). We take it to be zero throughout this interval. This leads to a linear equation for x_i with an exponential solution $x_i(t) \approx x_i(0) \exp(\alpha t)$.

At hour 4 the inoculate $x_2(4)$ is introduced and during the next 20 hours both strains grow, but under the negative influence of z . We will make the simplifying assumption during this period that $z \approx \delta/\gamma$, the limiting value of z , corresponding to full activation (on the order of 4 hrs.). This gives two linear equations with solutions:

$$\begin{aligned} x_1(t) &= x_1(0) e^{4\alpha} e^{(\alpha - \beta\delta/\gamma)(t-4)} \\ x_2(t) &= x_2(4) e^{(\alpha - \beta\delta/\gamma)(t-4)}. \end{aligned} \quad (6.2)$$

The experimental data is summarized by:

$$x_1(24) = x_1(0) e^{24k_1} \quad \text{and} \quad x_2(24) = x_2(4) e^{20k_2}.$$

Comparison of the data with the simplified model gives:

$$\begin{aligned} \alpha - \frac{5\beta\delta}{6\gamma} &= k_1 \\ \alpha - \frac{\beta\delta}{\gamma} &= k_2. \end{aligned} \quad (6.3)$$

Immediately from the second equation we have:

$$\alpha = \frac{\beta\delta}{\gamma} + k_2 > \frac{\beta\delta}{\gamma},$$

in other words, we confirm that the system is operating in Case 1 as we supposed.

Second, we may solve the linear system:

$$\alpha = 6k_1 - 5k_2 \quad \text{and} \quad \frac{\beta\delta}{\gamma} = 6(k_1 - k_2).$$

Unfortunately, these equations are mildly ill-conditioned and so results derived from them should be considered as approximate and their precision should be confirmed by other experiments. The chief factor leading to the ill-conditioning is the disparity in time intervals (4 hr and 20 hr) and a repeat of the experiment using a shorter second period would be useful. The authors of the two-hit experiment reported the median apparent growth constant for the initial inoculum was $k_1 = 0.34h^{-1}$ and $k_2 = 0.11h^{-1}$ for the later inoculum. This yields approximations:

$$\alpha \approx 1.49 \quad \text{and} \quad \frac{\beta\delta}{\gamma} \approx 1.38.$$

Note that the estimated value of α corresponds well to known values. Note also that we have not specified units for z and so the second ratio must be considered as non-dimensional and scaled to the proper units if used.

7 Discussion

7.1 Summary of results from the model

Research in pulmonary immunity reveals that the innate system contains components that are immediate in their effect and components that are not immediate, but dynamic. The system of differential equations (4.4) incorporates basic known properties of both immediate and rapidly responding parts of pulmonary innate immunity in a 2-dimensional model. We have analyzed the dynamics of this model completely for the biologically relevant parameter values (Case 1). In this case we showed that there is a unique non-zero equilibrium that is a hyperbolic saddle - it possesses both stable and unstable manifolds. The stable manifold subdivides the phase space of the model into two parts, defining a dynamical threshold for the outcome of an initial condition. For infection densities less than the threshold, the innate system returns the patient to the zero equilibrium (resolution of the infection). For infection densities greater than the threshold, the infection will grow unbounded. Of course this model is only reasonably valid for the initial phase of an infection since it does not take into account the adaptive system or even the fine details of the innate system. However, we do find that the simple model with parameters chosen in Case 1 is adequate to explain the dynamical nature of the innate response found in the two-hit experiments [5]. We also used data from the two-hit experiments to estimate the parameter α and the ratio $\beta\delta/\gamma$ in our model. To make further estimates, more data is needed. An experiment that could be completed in the murine model would be to give very low level inoculates. These could be used to estimate m and b for the specific host and pathogen. Also note that δ/γ might be directly measured as the concentration of neutrophils recovered in the murine model after subjection to a large inoculate and a sufficient period of response time.

7.2 The model in the context of modeling immunity and infection

In this article we have only explored low-dimensional, or *phenomenological* modeling. There is a long history phenomenological model of both immunity and infection and these methods, though by definition simple, have proven to be extremely helpful in furthering our knowledge of biological processes, at least in some cases. Modeling of HIV and other viral infections have been the subjects of particularly intense and productive studies in recent years [29, 35, 39, 2] and, for instance, led to experiments that revealed the high reproduction and mutation rate of HIV. Studies of models for immunity and tumor growth have led to insights into possible therapies [26, 22, 15, 4], and embedding of immunity models in epidemiological models has increased the accuracy of forecasting in that field [17, 31].

Much of the effort in modeling of immunity has been placed on long term dynamics and the adaptive system [3, 50, 2] and there has been limited focus on the onset of infection and initial response (for one such study see [34]). Further, we find very little mathematical modeling of the immune response in the lungs, except for an extensive and rich literature on M. tuberculosis infections (see [16, 30]). The peculiar nature of the TB infection makes comparison with other types of infections tenuous.

Many studies have considered immune models that incorporate delays [29, 15, 50, 4, 14]. Delays may be an important underlying cause of oscillatory behavior in chronic states of disease. It appears that delays are not needed in our model because there is no evidence of delays in the part of the immune system under study.

Detailed modeling of components of the immune system and their interactions has also been a subject of intense activity [16, 42, 25, 41, 30]. A good example of this can be found in [24], where the *complement system*, a subsystem of the innate system consisting of enzymes (such as C3) rather than cells, is investigated with as much detail as possible. This leads to dozens of coupled differential equations relating the levels of the various component enzymes. According to the authors: “The principal difficulty that hinders the modeling effort is the absence of the measured values of kinetic constants of many biochemical reactions forming the CS.” Several different approaches – some heuristic, some practical, some theoretical – have been developed to deal with the influence of parameters. The authors of [24] address the problem by adjoining an optimality condition that asserts that the parameters are the ones that optimize metabolic efficiency. While this has some philosophical appeal and has been shown to work in some applications, it has also been shown to fail in other problems and an optimality condition cannot be assumed to be literally true. Another approach is to study whole sets of parameter values. This however quickly runs into the curse of dimensionality. If more than a few values are unknown, then simulating with sets is not feasible. Another approach is to consider “typical” behaviour of systems of the class being modeled, or to discover structural properties of the equations that confer some universal behavior that manifests itself, in part or in whole, independent of the parameters or initial conditions. Finding universality in detailed models is rare and precious. Even in our low-dimensional model, the number of unknown parameters is minimized, but still several constants arise that can only be approximated at best.

Also, note that the complex three dimensional structure of the lungs impacts the process of pulmonary infection and the innate immune response. Spatial considerations can be modeled [18], but we eschew such considerations as important but beyond the scope of this manuscript, since our new sampling technique can only give a picture of the state of the lungs averaged over its entire spatial extent.

We choose to explore “coarse-grain” modeling but emphasize that all types of modeling lead to understanding and that there is cross-fertilization between high dimensional detailed component models on the one hand and minimalist phenomenological models on the other.

7.3 The model and newly available data

While the models and modeling are in and of themselves interesting, their utility lies in the ability to describe and categorize the trajectories of human subjects and data is required to bring this utility to fruition. Our motivation for this model is that it should serve as a starting point for comparison with patient data from a new sampling technique being developed by the authors (see Appendix A). We have proposed and conducted preliminary investigations into a noninvasive technique of sampling the alveolar lining fluid (ALF) from intubated patients using accumulated fluid in ventilator circuit filters and PCR (polymerase chain reaction) technology [21] for identification of the pathogens. Initial studies have concluded that the new procedure is just as effective as the standard BAL (bronchoalveolar lavage) in detecting bacterial pathogens in patient ALF. Further, we have shown that many immune system signals, such as cytokines, can also be measured in the fluid.

Together, direct measurement of both specific bacterial load and components of immune response promise to give clinicians a patient’s pulmonary-specific state in unprecedented detail. The new sampling technique promises to provide, for the first time, direct inpatient time series of the onset and progress of a pneumonia and the accompanying immune response. This opens up the possibility of developing a new protocol for the early detection of VAPs in which dynamical models play a role.

Much more work is needed to validate the sampling method and correlate data with models. The model proposed here, since it is based on our current phenomenological understanding of the pulmonary infection/innate immune response, should serve as a reference point for further investigations. The ultimate goals should be to aid physicians to develop new protocols for diagnosis and treatment of VAP and to help advance clinical practice from point estimates to process-based intervention. The initial challenge will be to estimate model parameters from patient data for various common pathogens.

From a wider point of view, the new aerosol sampling technique may prove to be a useful tool not only for clinical diagnosis, but also for progress in the general understanding of the immune system. Since the data is inexpensive and non-invasive, it should be possible to collect large amounts of data from many different regimes of infection, thus facilitating comparison and validation of this and other models. The technique, combined with model interpretation of the data, provides the possibility for ethical, direct, and accurate monitoring of details of the pathogen-immune interaction within human subjects. It is hoped that the combination of measurement and modeling will provide a powerful tool to help unravel the dynamics of the human immune system.

Highlights

- > A system of 2 differential equations is derived that models aspects of innate immunity specific to the lungs.
- > The system incorporates both immediate and quickly activated components.
- > The system predicts a dynamic threshold between infections eradicated and those not controlled by the innate system.
- > The system accurately predicts experimental dynamics in a Murine model.

References

1. Afraimovich, VS.; Arnol'd, VI.; Il'yashenko, YS.; Shil'nikov, LP. Translated in Encyclopedia of Mathematical Sciences. Vol. Vol. 5. Springer Verlag; 1994. The Theory of Bifurcations, in Modern Problems of Mathematics, Fundamental Directions, Vol. 5, VINITI, Moscow, Nauka (1986) [Russian].
2. Althaus CL, Ganusov VV, de Boer RJ. Dynamics of CD8 T cell responses during acute and chronic lymphocytic choriomeningitis virus infection. *J. Immunol.* 2007; 179:2944. [PubMed: 17709509]
3. Antia R, Bergstrom CT, Pilyugin SS, Kaech SM, Ahmed R. Models of CD8+ responses. 1. What is the antigen-independent proliferation program? *J Theor. Biol.* 2003; 221:585–598. [PubMed: 12713942]
4. Banerjee S, Sarkar RR. Delay-induced model for tumour-immune system interaction and control of malignant tumour growth. *Bio. Systems.* 2008; 91:268–288.
5. Ben-David I, Price S, Bortz D, Greineder C, Cohen S, Brauer A, Jackson T, Younger J. Dynamics of Intrapulmonary Bacterial Growth in a Murine Model of Repeated Microaspiration. *Amer. J. Respir. Cell Molec. Biology.* 2005; 33:476–482.
6. Bodini A, DOrazio C, Peroni D, Corradi M, Folesani G, Baraldi E, Assael B, Boner A, Piacentini G. Biomarkers of Neutrophilic Inflammation in Exhaled Air of Cystic Fibrosis Children With Bacterial Airway Infections. *Pediatric Pulmonology.* 2005; 40:494–499. [PubMed: 16229003]
7. Brauer, F.; Castillo-Chávez, C. *Mathematical Models in Population Biology and Epidemiology.* Vol. 40. Springer-Verlag, TAM; 2001.
8. Cohen AB, Rossi M. Neutrophils in normal lungs. *Am. Rev. Respir. Dis.* 1983; 127:53–59.
9. Corhay JL, Hemelaers L, Henket M, Sele J, Louis R. Granulocyte chemotactic activity in exhaled breath condensate of healthy subjects and patients with COPD. *Chest.* 2007; 131:1672–1677. [PubMed: 17565017]
10. Craig A, Mai J, Cai S, Jeyaseelan S. Neutrophil Recruitment to the Lungs during Bacterial Pneumonia. *Infect. Immun.* 2009; 77:568–575. [PubMed: 19015252]
11. D'onofrio A. On the interaction between the immune system and an exponentially replicating pathogen. *Math. Biosci. Bioengineering.* 2010; 7:579–602.
12. Evans S, Xu Y, Tuvim M, Dickey B. Inducible Innate Resistance of Lung Epithelium to Infection. *Ann. Rev. Physiol.* 2010; 72:413–435. [PubMed: 20148683]
13. Fleischmann J, Goldie DW, Weisbart RH, Gasson JC. Granulocyte-Macrophage Colony-Stimulating Factor Enhances Phagocytosis of Bacteria by Human Neutrophils. *Blood.* 1986; 68:708–711. [PubMed: 3488777]
14. Forys U. Stability and bifurcations for the chronic state in Marchuks model of an immune system. *J. Math. Anal. Appl.* 2009; 352:922–942.
15. Galach M. Dynamics of the tumorimmune system competition: The effect of time delay. *Int. J. Appl. Math. Comput. Sci.* 2003; 13:395–406.
16. Gammack D, Segovia-Juarez JL, Ganguli S, Marino S, Kirschner D. Understanding the Immune Response in Tuberculosis Using Different Mathematical Models and Biological Scales. *SIAM J. Multiscale Modeling Simulation.* 2005; 3:312–345.
17. Gilchrist M, Sasaki A. Modeling hostparasite coevolution. *J. Theor. Biol.* 2002; 218:289–308. [PubMed: 12381431]
18. Grant A, Restif O, McKinley T, Sheppard M, Maskell D, Mastroeni P. Modelling within-Host Spatiotemporal Dynamics of Invasive Bacterial Disease. *PLoS Biol.* 2008; 6:757–770.
19. Hamers M, Bot A, Weening R, Sips H, Roos D. Kinetics and Mechanism of the bactericidal action of human neutrophils against, *Escherichia coli*. *Blood.* 1984; 64:635–641. [PubMed: 6087960]
20. Happel K, Bagby G, Nelson S. Host Defense and Bacterial Pneumonia. *Seminars in Respir. Crit. Care Medicine.* 2004; 25:43–51.
21. Isaacs RJ, Debelak K, Norris PR, Jenkins JM, Rooks JC, Young TR, May AK, Boczek EM. Non-Invasive detection of pulmonary pathogens in ventilator-circuit filters by PCR. In Revision.
22. Kirschner D, Panetta JC. Modeling immunotherapy of the tumor - immune interaction. *J. Math. Biol.* 1998; 37:235–252. [PubMed: 9785481]

23. Koenig S, Truitt J. Ventilator-associated pneumonia: diagnosis, treatment, and prevention. *Clinical Microbiology Reviews*. 2006; 19:637–657. [PubMed: 17041138]
24. Korotaevskiy A, Leonid G, Khanin G, Khanin MA. Non-linear dynamics of the complement system activation. *Math. Biosci.* 2009; 222:127–143. [PubMed: 19854207]
25. Kumar R, Chow CC, Bartels J, et al. A mathematical simulation of the inflammatory response to anthrax infection. *Shock*. 2008; 29:104–111. [PubMed: 18157069]
26. Kuznetsov VA, Makalkin IA, Taylor MA, Perelson A. Nonlinear dynamics of immunogenic tumors: Parameter estimation and global bifurcation analysis. *Bull. Math. Biol.* 1994; 56:295–321. [PubMed: 8186756]
27. Larson K. Inflammatory markers in COPD. *Clin. Respir. J.* 2008; 2:84–87. [PubMed: 20298355]
28. Lehrer RI, Ganz T, Selsted ME, Babior BM, Curnutte JT. Neutrophils and host defense. *Ann. Intern. Med.* 1988; 109:127–142. [PubMed: 3289431]
29. Marchuk GI, Petrov RV, Romanyukha AA, Bocharov GA. Mathematical model of antiviral immune response. I. Data analysis, generalized picture construction, parameters evaluation for hepatitis B. *J. Theor. Biol.* 1991; 151:140.
30. Marino S, El-Kebir M, Kirschner D. A hybrid multi-compartment model of granuloma formation and T cell priming in Tuberculosis. *J. Theor. Biol.* 2011; 280:50–62. PMID: 21443879, NIHMSID: 292232. [PubMed: 21443879]
31. Martcheva M, Pilyugin S. An epidemic model structured by host immunity. *J. Biol. Syst.* 2006; 14:185–203.
32. Martin TR, Frevert CW. Innate Immunity in the Lungs. *Proc. Am. Thorac. Soc.* 2005; 2:403–411. [PubMed: 16322590]
33. Mizgerd JP. Acute Lower Respiratory Tract Infection. *N. Engl. J. Med.* 2008; 358:716–727. [PubMed: 18272895]
34. Mohtashemi M, Levins R. Transient dynamics and early diagnosis in infectious disease. *J. Math. Biol.* 2001; 43:446–470. [PubMed: 11767206]
35. Müller V, Marée AFM, de Boer RJ. Small variations in multiple parameters account for wide variations in HIV-1 set-points: a novel modelling approach. *Proc. R. Soc. Lond. B.* 2001; 268:235–242.
36. Nelson S, Bagby GJ, Bainton BG, Wilson LA, Thompson JJ, Summer WR. Compartmentalization of Intraalveolar and Systemic Lipopolysaccharide-Induced Tumor Necrosis Factor and the Pulmonary Inflammatory Response. *J. Infect. Dis.* 1989; 159:189–194. [PubMed: 2644368]
37. Ozaki T, et al. Role of alveolar macrophages in the neutrophil-dependent defense system against *Pseudomonas Aeruginosa* infection in the lower respiratory tract. *Am. Rev. Respir. Dis.* 1989; 140:1595–1601. [PubMed: 2513762]
38. Pereira Gomes JC, Pedreira WL Jr, Araújo EM, Soriano FG, Negri EM EM, Antonangelo L, Tadeu Velasco I. Impact of BAL in the management of pneumonia with treatment failure: positivity of BAL culture under antibiotic therapy. *Chest*. 2000; 118:1739–1746. [PubMed: 11115467]
39. Perelson A. Modeling viral and immune system dynamics. *Nat. Rev.* 2001; 2:28–36.
40. Pugliese A, Gandolfi A. A simple model of pathogen-immune dynamics including specific and non-specific immunity. *Math. Biosci.* 2008; 214:73–80. [PubMed: 18547594]
41. Riviere B, Epshteyn Y, Swigon D, et al. A simple mathematical model of signaling resulting from the binding of lipopolysaccharide with Toll-like receptor 4 demonstrates inherent preconditioning behavior. *Math. Biosci.* 2009; 217:19–26. [PubMed: 18950647]
42. Reynolds A, Rubin J, Clermont G, et al. A reduced mathematical model of the acute inflammatory response: I. Derivation of model and analysis of anti-inflammation. *J. Theor. Biol.* 2006; 242:220–236. [PubMed: 16584750]
43. Richard MJ, Edwards JR, Culver DH, Gaynes RP, et al. Nosocomial infections in medical intensive care units in the united states. *Crit. Care Med.* 1999; 27:887–892. [PubMed: 10362409]
44. Romball CG, Weigle WO. A cyclical appearance of antibody-producing cells after a single injection of serum protein antigen. *J. Exp. Med.* 1973; 138:1426–1442. [PubMed: 4586979]
45. De Rose V. Mechanisms and markers of airway inflammation in cystic fibrosis. *Eur. Respir. J.* 2002; 19:333–340. [PubMed: 11866015]

46. Semenzato G, Adami F, Muschio N, Agostini C. Immune mechanisms in interstitial lung diseases. *Allergy*. 2000; 55:1103–1120. [PubMed: 11117267]
47. Sibille Y, Reynolds HY. Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am. Rev. Respir. Dis.* 1990; 141:471–501. [PubMed: 2405761]
48. Stark J, Chan C, George AJT. Oscillations in the immune system. *Immun. Reviews*. 2007; 216:213–231.
49. Whitman A, Ashrafiun H. Asymptotic theory of an infectious disease model. *J. Math. Biol.* 2006; 53:287–304. [PubMed: 16733728]
50. Wodarz, D. *Mathematical and Computational Approaches to Immunology*. Springer; 2007. *Killer Cell Dynamics*.
51. Zhang P, Summer W, Bagby G, Nelson S. Innate immunity and pulmonary host defense. *Immun. Reviews*. 2000; 173:39–51.

A Appendix

A Non-Invasive Sampling Technique

Clinical suspicion of pneumonia in an intubated patient is currently based on three measures: infiltrate in a chest x-ray, elevated white blood cell count and changes in body temperature. Of these, the latter two are non-specific, and the three combined effectively measure only the adaptive system response to a developed pneumonia, not the bacterial or viral agent causing the pneumonia.

Samples of alveolar lining fluid (ALF) from the focus of infection contain information regarding both the status of the bacterial load and the immune response (e.g. [45, 6]). Until recently, obtaining samples of ALF from human subjects has only been possible through BAL (bronchoalveolar lavage) and similar procedures that are invasive and expensive. Data from the electronic medical record indicate that, in the vast majority of cases, samples of ALF, such as those obtained through BAL, are only sought after an infection is strongly suspected [38]. The tests are ordered to provide confirmation of suspicion.

We have developed and explored an inexpensive, rapid and noninvasive method that can detect pathogens from the aerosolized droplets of ALF that collect in ventilator circuit filters [21]. In the majority of ventilated patients, the ventilator circuit contains a hygroscopic condenser humidifier (HCH) filter that is designed to limit moisture loss from the patient, minimize heat loss, and filter bacteria that may be present in the tubing outside of the HCH filter. The HCH unit is situated in the ventilator circuit between the Y-piece and the endotracheal tube, just external to the patient. Condensed vapor and aerosols from the breath of intubated patients visibly collect within this filter unit and are retained there until the unit is exchanged – every 8 to 12 hours as part of routine circuit maintenance.

We have proposed detection, identification and measurement of pathogens in the fluid that collects in the filter using standard PCR (polymerase chain reaction) amplification techniques. PCR provides a rapid, quantitative and inexpensive way to profile the HCH fluid for bacterial, fungal and viral pathogens. Preliminary data comparing a convenience sample of 17 HCH filters retrospectively with available culture data demonstrated a marked correlation that could not be discounted to chance alone [21].

Based on the encouraging preliminary results, we undertook a designed pilot study directly comparing matched samples of ALF and BAL taken from the same patient at the same time. The results of the matched samples trial indicate that the quantitative detection of alveolar pathogens from ALF is accurate and most likely superior to BAL. We also demonstrated that ALF samples from the filters contain immune biomarkers such as IL-2 and IL-10 cytokines. These and other chemical signals can be detected in alveolar lining fluid, but

because of compartmentalization, pulmonary levels of these compounds are not readily measurable in the blood. Serial sampling of the bacterial load and biomarkers together reveal a highly correlated interaction. Compelling evidence that the ALF samples contain real information is the observed correlation between the changes in bacterial growth rate and cytokine spikes. Serial sampling of the bacterial load reveals the within host growth and decline of pathogenic organisms over the course of a patient's mechanical ventilation. The data indicate potential physiological signatures that may presage the onset of VAP.

In Figure 5 we show the time series of bacterial and immune component levels recovered from HCH filters in [21]. We note that this is the first time-series that we have recovered and that it is incomplete. Notice the temporal coincidence of the cytokine spikes with the bacterial growth features. The IL-1 β and G-CSF levels spike as the bacteria begin to amplify. IL-6 spikes at the peak of the *S. aureus* growth and presumably is related to the subsequent dip in bacterial growth. But both bacterial species recover and begin to regrow! A BAL is not performed till the double arrowheads. It is clear that the sampling of ALF from the ventilator filters provides evidence of and information about an infection long before that point.

At this stage, we have not attempted to fit the patient time series data to our model. First, our model is only intended to have validity at the onset of a pneumonia. The time series we thus far have does not capture this part of the infection. Secondly, since PMNs, the most effective agents of the innate system, are relatively large we do not expect to find them in the aerosolized ALF. Thus, work needs to be done to determine which cytokine levels might serve as proxies for activation of the rapidly responding parts of the innate system, a question that has already begun to be investigated [6]. Finally, our clinical studies thus far have emphasized pair-wise point comparisons of the new technique with BAL measurement and only two incomplete patient time-series are available at this time. Far more time series data would be needed for validation of the model and meaningful parameter estimation. We hope that these obstacles will be overcome in the next round of clinical trials.

B Appendix

Analysis of Case 2

Case 2a. No intersection of the nullclines.

The only equilibrium is the stable node at $(0, \eta/\mu)$.

Proposition B.1 *In Case 2a all solutions tend to the unique equilibrium $(0, \eta/\mu)$.*

Proof: In this case the entire first quadrant is partitioned into 3 parts: below the graph of z_1 , between z_1 and z_2 and above z_2 . We denote these regions by Roman numerals I, II, and III, respectively. See Figure 6.

In Region I, note that z' is positive and bounded below since the region is bounded away from $z' = 0$ and since $z' \rightarrow (\delta - \gamma z)x$ as $x \rightarrow +\infty$. (Even along $z = 0$ the z component is bounded away from zero.) Note also that Region I is bounded above by $z = \alpha/\beta < \delta/\gamma$. Thus any initial condition in this region must leave the region in finite time.

Next consider Region II. At the lower boundary of this region ($x' = 0$), the z component is positive and bounded away from 0. Thus any initial condition in a neighborhood of $x' = 0$ will leave that region in a fixed time interval. Further, any initial condition above a neighborhood of $x' = 0$ will move to the northwest with negative x velocity bounded uniformly away from 0. Since there are no equilibria in the interior of this region, all such

solutions must eventually either intersect the graph of z_2 or the z axis. The latter can only happen at the equilibrium $(0, \eta/\mu)$. Because the x component of the vector field is negative everywhere along $z' = 0$, all orbits reaching $z' = 0$ will enter Region III.

Finally all orbits in region III must travel southwest. These orbits cannot reach $z' = 0$ and cannot reach the z axis except at the equilibrium. Thus we conclude that all orbits in Region III must be asymptotic to $(0, \eta/\mu)$.

We find, then, for parameter values satisfying Case 2a that the innate immunity system as modeled is adequate to eliminate any pathogenic load. This is obviously not a realistic model for the interaction with most bacterial pathogens.

Case 2b. One intersection of the nullclines.

This case involves a bifurcation since in Case 2a there are no nonzero fixed points and for Case 2c there are two. Case 2b is clearly the transition between these cases and generically we expect the bifurcation to be a saddle-node bifurcation i.e. the linearization of the equations at this point has one zero eigenvalue and one non-zero eigenvalue.

We see that indeed one of the eigenvalues is zero since at the point of intersection of the two smooth nullclines we must have a tangency, $z'_1 = z'_2$, and this implies that $f_z/f_x = g_z/g_x$ at the intersection and thus we must have $\det(DF) = 0$. Therefore one of the eigenvalues of the characteristic equation for DF is zero. It follows also that the other eigenvalue is given by $\text{Tr}(DF)$.

Consider the linearization:

$$DF = \begin{pmatrix} \alpha - \beta z - \frac{m}{(1+bx)^2} & -\beta x \\ \delta - \gamma z & -(\gamma x + \mu) \end{pmatrix}.$$

The characteristic polynomial for this matrix is:

$$\lambda^2 + \left[\gamma x + \mu - \alpha + \beta z + \frac{m}{(1+bx)^2} \right] \lambda + (\gamma x + \mu) \left(-\alpha + \beta z + \frac{m}{(1+bx)^2} \right) + \beta x (\delta - \gamma z) = 0.$$

Since we are only concerned with the linearization at equilibria and the $x' = 0$ nullcline is given by

$$\alpha - \beta z = \frac{m}{1+bx},$$

the polynomial simplifies to:

$$\lambda^2 + \left[\gamma x + \mu - \frac{mbx}{(1+bx)^2} \right] \lambda - (\gamma x + \mu) \frac{mbx}{(1+bx)^2} + \beta x (\delta - \gamma z) = 0.$$

Similarly, substituting for the remaining z by the equation for the $z' = 0$ nullcline gives us:

$$\lambda^2 + \left[\gamma x + \mu - \frac{mbx}{(1+bx)^2} \right] \lambda - (\gamma x + \mu) \frac{mbx}{(1+bx)^2} + \beta x \frac{\delta \mu - \gamma \eta}{\gamma x + \mu} = 0.$$

Here we see that the roots of this equation depend non-trivially on the equilibrium. The middle coefficient, which is $-\text{Tr}(DF)$, is given by

$$\text{Tr}(DF) = -\gamma x - \mu + \frac{mbx}{(1+bx)^2},$$

where x in this formula is the x -coordinate of the equilibrium. For biologically relevant parameter values, we expect the third term to be insignificant and thus we expect the trace to be negative and the saddle-node to have a stable direction as in Figure 7.

Case 2c. Two intersections of the nullclines.

In this case the local analysis of the first equilibrium is the same as in Case 1. It is also a saddle with a stable and an unstable manifold. Globally, by the same arguments as in Case 1, the stable manifold of the saddle equilibrium again subdivides the first quadrant into two parts. All orbits to the left of the manifold must be asymptotic to the equilibrium 28 at $(0, \eta/\mu)$. Orbits to the right of the stable manifold cannot cross over the manifold and thus the model predicts that a bacterial infection will be persistent, never returning to $(0, \eta/\mu)$.

The second equilibrium in this case is more difficult. At this intersection of the nullclines we have $z'_1 < z'_2$. This implies that $\det(DF)$ at this point is positive. This gives the possibilities that the eigenvalues are either complex or real with the same sign. Thus the equilibrium must either be a stable or unstable node or a stable or unstable focus. The stability depends entirely on the term $\text{Tr}(DF)$. In the case $\text{Tr}(DF)$ is zero, then the point will be non-hyperbolic.

From our observations in Case 2b, near to the saddle-node bifurcation (if $\text{Tr}(DF) < 0$), this second equilibrium must be a stable node. Far from the bifurcation parameters the second equilibrium may lose stability. Since no other equilibria exist, stability may only be lost via a Hopf bifurcation.

See Figure 8 for an illustration of Case 2c. In this case the second fixed point is a stable node.

Figure 1.
Local flow at the nonzero equilibrium in Case 1.

Figure 2.
Arrangement of the local stable and unstable manifolds at the saddle equilibrium in Case 1.

Figure 3.

Phase plane showing the features of Case 1. In this plot the parameter values were set for demonstration to $\alpha = 1.0$, $\beta = 0.2$, $\gamma = 0.2$, $\delta = 0.5$, $\eta = 0.087$, $\mu = 1.0$, $m = 2.0$, $b = 0.5$. There is a saddle equilibrium at the intersection of the nullclines at $\approx (2.9, 1.0)$. All orbits to the left of the stable manifold W^s of the saddle are asymptotic to the stable node at $(0, \eta/\mu)$. All orbits to the left of W^s are unbounded in x and approach δ/γ in z .

Figure 4. First order growth rates of the first and second strains vs. the initial dose of the first strain calculated in the simulation of the two-hit experiment. The values k_1 and k_2 are the calculated first order growth constants for the first and second strains respectively.

Figure 5.

Incomplete HME fluid time series from an intubated patient [21]. The figure shows the growth of *S. aureus* in dashed brown. The growth of *P. aeruginosa* in dashed black. The axes labels describe the units for the bacteria measurements. The cytokine profiles have been scaled in height only, so that they may appear on the same graph. The IL-1 β and G-CSF signals are virtually superimposable and appear as the thin red curve. The IL-6 curve is shown in thin blue. A BAL was performed at the double arrowheads.

Figure 6.

In the Case 2a there is no intersection of the nullclines and thus no equilibrium other than the “zero equilibrium” at $(0, \eta/\mu)$, which is globally attracting. In this plot the parameter values were set to $\alpha = 1.0$, $\beta = 0.4$, $\gamma = 0.2$, $\delta = 1.0$, $\eta = 0.087$, $\mu = 0.5$, $m = 2$, $b = 0.5$.

Figure 7.

Case 2b. The nullclines intersect at a tangency which implies that the equilibrium there is non-hyperbolic, the linearization has at least one eigenvalue with zero real part. In this plot the parameters were set to $\alpha = 1.0$, $\beta = 0.4$, $\gamma = 0.2$, $\delta = 1.0$, $\eta = 0.087$, $\mu = .67$, $m = 2.0$, $b = .5$. For these values we have that $\text{Tr}(DF) < 0$ so the other eigenvalue is negative.

Figure 8.

Case 2c. The first of the two intersections of the nullclines is a saddle equilibrium. Its stable manifold separates the phase space into two parts. All orbits to the left of the manifold are asymptotic to the stable equilibrium at $(0, \eta/\mu)$. In this plot the parameter values were set to $\alpha = 1.0$, $\beta = 0.4$, $\gamma = 0.2$, $\delta = 1$, $\eta = 0.087$, $\mu = .89$, $m = 2$, $b = 3$. The second intersection is a stable node for these parameters.