Mathematical model of a three-stage innate immune response to a pneumococcal lung infection

Amber M. Smith a,*, Jonathan A. McCullers b, Frederick R. Adler c

a Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM 87545, USA
b Department of Infectious Diseases, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA
c Departments of Mathematics and Biology, University of Utah, Salt Lake City, UT 84112, USA

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A B S T R A C T

Pneumococcal pneumonia is a leading cause of death and a major source of human morbidity. The initial immune response plays a central role in determining the course and outcome of pneumococcal disease. We combine bacterial titer measurements from mice infected with Streptococcus pneumoniae with mathematical modeling to investigate the coordination of immune responses and the effects of initial inoculum on outcome. To evaluate the contributions of individual components, we systematically build a mathematical model from three subsystems that describe the succession of defensive cells in the lung: resident alveolar macrophages, neutrophils and monocyte-derived macrophages. The alveolar macrophage response, which can be modeled by a single differential equation, can by itself rapidly clear small initial numbers of pneumococci. Extending the model to include the neutrophil response required additional equations for recruitment cytokines and host cell status and damage. With these dynamics, two outcomes can be predicted: bacterial clearance or sustained bacterial growth. Finally, a model including monocyte-derived macrophage recruitment by neutrophils suggests that sustained bacterial growth is possible even in their presence. Our model quantifies the contributions of cytotoxicity and immune-mediated damage in pneumococcal pathogenesis.

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1. Introduction

Streptococcus pneumoniae (pneumococcus) is a common pathogen associated with community acquired pneumonia, bacterial meningitis, and secondary infections following influenza (World Health Organization, 2008). Furthermore, pneumococcus is the causative agent in an estimated one million deaths worldwide each year among children under 5 years of age (World Health Organization, 2008), particularly in developing countries. Although vaccination and antibiotic use have greatly reduced incidence of disease in all persons, the emergence of resistant strains coupled with an increase in incidence during influenza outbreaks (McCullers and English, 2008) has emphasized a need for novel treatments and a deeper understanding of pneumococcal infection kinetics.

Based on experimental studies in mice, this paper introduces a series of mathematical models that address the factors associated with favorable outcomes from pulmonary pneumococcal infections. These models track three stages of immune cell recruitment to identify how each cell type controls bacterial dynamics and the likelihood of host survival.

1.1. Innate immune response to S. pneumoniae

Pneumococci colonize the nasopharynx in up to 70% of young children (Giebink, 2001) and 40% of healthy adults (Austrian, 1986), and asymptomatic carriage may last up to 6 weeks in an individual (Orihuela and Tuomanen, 2006). However, pneumococci can migrate to the lungs resulting in a more serious infection. Nevertheless, only a small fraction of carriers develop pneumonia. The rarity of pneumococcal pneumonia in healthy individuals reflects the efficiency of host defenses as most disease occurs when pathogen removal systems, such as ciliated epithelium and mucin entrapment, are compromised either due to preceding viral infections or underlying respiratory conditions (Kadioglu et al., 2008). In addition to host characteristics, both the pathogen strain and inoculum size determine whether a pneumococcal population can establish within a host (Kadioglu et al., 2008).

When host barriers are breached, three types of cells arrive sequentially and destroy pneumococci through phagocytosis: resident alveolar macrophages (AMs), neutrophils, and monocyte-derived macrophages (MDMs). Alveolar macrophages play an important role in the defense against airborne pathogens and provide the first line of cellular defense against a pneumococcal lung infection (Dockrell et al., 2003; Gwinn and Vallyathan, 2006; Taylor et al., 2005). These cells phagocytose bacteria that reach the
terminal bronchioles and alveoli (Franke-Ullmann et al., 1996; Jonsson et al., 1985), and coordinate the innate immune response to infection (Kadioglu and Andrew, 2004; Knapp et al., 2003). It is thought that when the ratio of pneumococci to phagocytes does not exceed some critical, although unknown, number, AMs can successfully phagocytose and kill pneumococci (Knapp et al., 2003). However, as this ratio increases for larger initial pneumococcal inoculums, AMs are unable to eradicate all pneumococci. Alveolar macrophages also secrete proinflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α), to initiate the inflammatory response and recruit activated neutrophils into the lung (Monton and Torres, 1998; Zhang et al., 2000).

With a high-dose challenge of pneumococci, neutrophils appear within 2–4 h postinoculation (pi) and dominate the initial inflammatory immune response. A rapid reduction of neutrophil numbers follows a peak 24–48 h pi, even when bacterial titers remain high (Bergeron et al., 1998; Duong et al., 2001; Fillion et al., 2001; Kadioglu et al., 2000). This swift decrease in neutrophils is paralleled by MDM infiltration at 48 h pi (Fillion et al., 2001). Nevertheless, pneumococcal growth can remain unbound causing 100% mortality in mice within 5 days at higher inocula (Bergeron et al., 1998; Duong et al., 2001; Fillion et al., 2001), indicating a limit to the phagocytic capacity of all three types of cells (Clawson and Repine, 1976; Onofrio et al., 1983) and a potential for significant inflammation to occur.

These phenomena suggest that a three-stage phagocytic response is critical for clearance of larger inocula and that skewing of the innate response may have a profound impact on the outcome. Although it is known that effective phagocytosis is not the only factor determining pneumococcal pathogenesis, we aim here to capture some of the complexities of the innate immune response to pneumococcus by developing mathematical models for the three-stage phagocytic response and testing them with data on artificially infected mice.

1.2. Mathematical models of bacterial kinetics

Previously published mathematical models of bacterial kinetics and inflammatory processes present a conceptual framework to examine two phenomena of interest here (Laufenburger, 1985; Pilyugin and Antia, 2000; Reynolds et al., 2006; Rudnev and Romanyukha, 1995): (i) the initial dose threshold, and (ii) the observed variations in outcome (clearance vs sustained growth). These studies (described below) use systems of ordinary differential equations to describe bacterial pathogenesis and often divide phagocytic cells into a variety of different subpopulations. To address these behaviors, one study split the macrophage population into cells that are in a free state (i.e., not associated with bacteria) and cells that are engaged in active phagocytosis of bacteria (Pilyugin and Antia, 2000), and suggested that the “handling” time of bacteria limited the ability of macrophages to fully eradicate a bacterial population.

Other models include two separate subsets of phagocytes, neutrophils and macrophages (Laufenburger, 1985; Rudnev and Romanyukha, 1995), and show that different behaviors are possible with various initial conditions. However, the models did not have these cells arriving sequentially, and the macrophage response included either only recruited macrophages (Rudnev and Romanyukha, 1995) or a single equation combining resident alveolar macrophages and MDMs (Laufenburger, 1985). More commonly, however, models include only one subset of phagocytes. For example, a single type of macrophage was useful in a model that evaluated the importance of prolonged inflammation in the context of sepsis, and emphasized antiinflammatory cytokines and tissue damage as the factors influencing infection outcomes (Reynolds et al., 2006).

Many of these models describe “generic” bacterial dynamics. However, differences among bacterial species render most prior formulations inappropriate for a pneumococcal lung infection. In particular, the succession of neutrophil and MDM accumulation depends on bacterial type and differs between various gram-positive bacteria and gram-negative bacteria (Schluger and Rom, 1997; Toews et al., 1979). For example, an infection with Pseudomonas aeruginosa (gram-negative) is similar to pneumococcus (gram-positive) in that neutrophils are the predominant phagocyte. However, neutrophils may appear simultaneously with MDMs during infections with Klebsiella pneumoniae (gram-negative) and Staphylococcus aureus (gram-positive) (Toews et al., 1979). This variation has been attributed to a distinct, but partially overlapping, set of cytokines induced by different species during bacterial colonization (Schluger and Rom, 1997).

Few existing models have sufficient experimental data for validation and parameterization, and no models specific to S. pneumoniae have been developed. We capitalize on the power and convenience of the mouse model system to compare the kinetics of three bacterial doses over the course of an infection and infer possible interactions between pneumococci and host immune responses. We infected groups of BALB/c mice with three different inocula of S. pneumoniae strain D39 and obtained bacterial measurements from the lungs of individual mice.

Using these data as a guideline, we develop mathematical models that describe the kinetics of each of the three stages. We find a single equation that characterizes the alveolar macrophage response, and use this equation to determine how an initial dose threshold arises. We then extend the model to incorporate proinflammatory cytokine production and the resulting neutrophil recruitment, and establish that two outcomes, clearance or sustained bacterial growth, are possible. Lastly, we examine the possibility of bacterial clearance given an influx of monocyte-derived macrophages. Through each of these models, we gain a better understanding of the extent to which AMs, neutrophils, MDMs, proinflammatory cytokines, and apoptotic cellular material (“debris”) contribute to the initiation and resolution of a pneumococcal lung infection.

2. Experimental data

2.1. Methods and materials

2.1.1. Mice

Adult (6–8 wk old) female BALB/cJ mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed in groups of 4–6 mice in high-temperature 31.2 cm × 23.5 cm × 15.2 cm polycarbonate cages with isolator lids. Rooms used for housing mice were maintained on a 12:12-h light:dark cycle at 22 ± 2 °C with a humidity of 50% in the biosafety level 2 facility at St. Jude Children’s Research Hospital (Memphis, TN). Prior to inclusion experiments, mice were allowed at least 7 days to acclimate to the animal facility. Laboratory Autoclavable Rodent Diet (PMI Nutrition International, St. Louis, MO) and autoclaved water were available ad libitum. All experiments were performed in accordance with the guidelines set forth by the Animal Care and Use Committee at St. Jude Children’s Research Hospital.

2.1.2. Infectious agents and model

S. pneumoniae strain D39 (type 2) was transformed with the lux operon (Xenogen) (McCullers and Bartmess, 2003). For infection experiments, bacteria were diluted in sterile phosphate buffered saline (PBS) and administered at a dose of 10⁴, 10⁵ or 10⁶ colony forming units (CFU) intranasally to groups of six mice lightly anesthetized with 2.5% inhaled isoflurane (Baxter, Deerfield, IL).
in a total volume of 100 μl (50 μl per nostril). Mice were weighed at the onset of infection and each subsequent day for illness and mortality.

2.1.3. Lung titers

Mice were euthanized by CO₂ asphyxiation. Lungs were aseptically harvested, washed three times in PBS, and placed in 500 μl sterile PBS. Lungs were mechanically homogenized using the Ultra-Turrax T8 homogenizer (IKA-werke, Staufen, Germany). Lung homogenates were pelleted at 10,000 rpm for 5 min and the supernatants were used to determine the bacterial titer for each set of lungs using serial dilutions on tryptic soy-agar plates supplemented with 3% (vol/vol) sheep erythrocytes.

2.2. Experimental results

Infecting mice with an initial inoculum of 10⁴ CFU resulted in undetectable bacterial lung titers 4 h pi. We found that a dose above 10⁴ CFU is necessary to induce a pneumococcal lung infection with the type 2 strain D39. The groups of mice given an initial dose of 10⁵ CFU had bacterial titers initially increase exponentially for 8 h, drop briefly at 16 h pi then reach peaks up to 5.8 × 10⁷ CFU/ml lung homogenate at 24 h pi. Bacterial lung titers then declined rapidly and, in all but one sample, became undetectable at 48 h pi. All mice in the 72-h group had completely cleared the infection (Fig. 1).

Mice inoculated with 10⁶ CFU had bacterial titers at significantly high levels by 4 h pi, which reached peaks as high as 2.3 × 10⁸ CFU/ml lung homogenate by 16 h pi. Titers began to decline thereafter; however, clearance slowed between 24 and 48 h pi. The infection was not cleared in these mice, which showed sustained bacterial titers 72 h pi (Fig. 1). Elevated bacterial titers over several days coupled with significant weight loss (data not shown) suggested these mice had severe pneumonia. All but one mouse (72-h group) survived the experiment.

3. Mathematical model

3.1. Methods and materials

Numerical simulation of model equations was done in Matlab using the ordinary differential equation (ODE) solver ode45 for the alveolar macrophage model and the neutrophil model, and using the delay differential equation (DDE) solver dde23 for the monocyte-derived macrophage model. Parameter values used to simulate each model are given in Tables 1–3 and descriptions of the chosen values are given in the text.

3.2. Stage 1: the alveolar macrophage response

Alveolar macrophages are part of the initial host defense that respiratory pathogens encounter when entering the airway. These cells are thought to tightly regulate the growth of small pneumococcal populations but become insufficient with larger initial inocula. This limitation may explain the dependency of initial acquisition on inoculum size, as both we and others have found for a pulmonary pneumococcal infection (Sun and Metzger, 2008).

To investigate the dosage threshold, we developed a mathematical model (Eq. (2)) that depicts the interaction between a pneumococcal population (P) and the resident alveolar macrophages (MA). Pneumococci grow logistically at a rate k per hour with a maximum tissue carrying capacity, Kp CFU/ml, and are phagocytosed at rate γMA f(P, MA) per cell per hour, where

\[ f(P, MA) = \frac{n^2 M_A}{P^n + n^2 M_A^2}. \]  (1)

Eq. (1) represents a decrease in phagocytosis with large pneumococcal population size. The shape parameter x describes the rate of consumption of pneumococci (Fig. 2(a)). The value of n gives the ratio of bacteria to alveolar macrophages, with large values describing a higher phagocytosis rate for a given inoculum size (Fig. 2(b)).

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**Table 1**

The alveolar macrophage model (Stage 1) parameter definitions and values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Units</th>
<th>Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>Bacterial growth rate</td>
<td>h⁻¹</td>
<td>11.3 × 10⁻¹</td>
<td>See text</td>
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<tr>
<td>Kp</td>
<td>Bacterial carrying capacity</td>
<td>CFU/ml</td>
<td>2.3 × 10⁶</td>
<td>See text</td>
</tr>
<tr>
<td>γMA</td>
<td>Bacterial clearance by AMs</td>
<td>cell⁻¹ h⁻¹</td>
<td>5.6 × 10⁻⁶</td>
<td>See text</td>
</tr>
<tr>
<td>n</td>
<td>Maximum number bacteria per AM</td>
<td>(CFU/ml)cell⁻¹</td>
<td>5</td>
<td>Gordon et al. (2000)</td>
</tr>
<tr>
<td>x</td>
<td>Nonlinearity in f(P, Ma)</td>
<td>N/A</td>
<td>2</td>
<td>See text</td>
</tr>
<tr>
<td>s</td>
<td>AM constant supply</td>
<td>cell⁻¹ h⁻¹</td>
<td>1.0–4.2 × 10⁵</td>
<td>Coggle and Tarling (1982), Godleski and Brain (1972), van Oud Alblas and van Furth (1979)</td>
</tr>
<tr>
<td>d</td>
<td>AM death rate</td>
<td>h⁻¹</td>
<td>1.0–4.2 × 10⁻³</td>
<td>See text</td>
</tr>
<tr>
<td>Mₐsteady</td>
<td>AM steady state value</td>
<td>Cells</td>
<td>10⁶</td>
<td>van Oud Alblas and van Furth (1979)</td>
</tr>
<tr>
<td>P₀</td>
<td>Initial number of pneumococci</td>
<td>CFU/ml</td>
<td>10⁵, 10⁶, or 10⁷</td>
<td>See text</td>
</tr>
</tbody>
</table>
Although alveolar macrophages were once thought to locally proliferate rather than derive from blood monocytes (Coggle and Tarling, 1982, 1984; Tarling et al., 1987), recent studies do not support this view (Murphy et al., 2008; Taut et al., 2008). We assume AMs are supplied at a constant rate of \( s \) cells per hour with a clearance rate \( d \), for simplicity. We do not consider pneumococcal-induced death of AMs (Maus et al., 2004) as this effect would likely be minimal in the early stages of infection kinetics. Alveolar macrophage population dynamics are slow compared to bacterial clearance, so we take these cells to be in quasi-steady state such that \( M_A^* = s/d \).

We term this model “the alveolar macrophage model”, which has dynamics described by a single equation for the pneumococcal population,

\[
\frac{dP}{dt} = rP \left( 1 - \frac{P}{K_P} \right) - \gamma_{MA} f(P, M_A)^M_{A^*} P.
\]

### 3.2.1. Parameter identification

We did not use a sophisticated scheme to estimate parameter values because this model is designed to capture only the initial dose threshold and would fit at most 1–2 data points. We instead use parameter values available in the literature where possible and selected others to match the data by tuning values based on biologically feasible ranges (Table 1).

We set the steady-state value of alveolar macrophages to \( M_A^* = 10^6 \) cells, the estimated number that reside in a murine lung (van Oud Alblas and van Furth, 1979). Estimates of the turnover rate of alveolar macrophages range from 10 to 40 days (van Oud Alblas and van Furth, 1979), although recent evidence suggests a longer life-span (Murphy et al., 2008). This corresponds to \( 0.001 \leq d \leq 0.0042 \) implying \( s = 1.0 - 4.2 \times 10^5 \) cells per hour. Based on the maximum bacterial titer measured in our own experiment, we fix \( K_P = 2.3 \times 10^8 \) CFU/ml.

The three initial values of pneumococci \( (P_0) \) were chosen as \( 10^2, 10^4, \) and \( 10^5 \) CFU/ml. The units of these values \( (\text{CFU per ml of lung homogenate}) \) differ from the units of initial inocula \( (\text{CFU}) \) used in the experiments. We assume only a portion of bacteria reach the lungs since some bacteria could be quickly trapped in the airway and removed by mucociliary mechanisms. These values were chosen such that the data could be reproduced and to correspond to the three doses mice were infected with, i.e., \( 10^2, 10^4, \) and \( 10^5 \) CFU.

The doubling time of pneumococcal strain D39 is estimated to be 84 min when injected intravenously in a mouse model of sepsis (Benton et al., 1995). In contrast, the calculated doubling time in blood media is 20–30 min (Todar, 2002). Because we could not establish an exact value of the growth rate in the lung, we explored values between 20 and 84 min to determine which produced the best fit. We chose \( r = 11.3 \times 10^{-1} \) per hour, corresponding to a doubling time of approximately 37 min.

The maximum number of pneumococci associated with an alveolar macrophage \( (n) \) was set to 5 CFU/ml per cell, based on a study that found, on average, five pneumococci bound to each human AM (Gordon et al., 2000) and since the lung homogenate is typically 1–1.5 ml. We fix the degree of nonlinearity \( (x) \) in the function \( f(P, M_A) \) to a value of 2 based on our experimental findings that phagocytosis is decreased for initial inocula greater than \( 10^4 \) CFU (i.e., \( 10^5 \) CFU/ml). Lastly, we found that \( \gamma_{MA} M_A = 5.6 \times 10^{-6} \) per cell per hour (i.e., \( \gamma_{MA} M_A = 5.6 \) per hour) could predict the observed bacterial titers. We find this value reasonable as the estimated killing rate of \( S. aureus \) by neutrophils is 6.9 per hour (Hampton et al., 1994).

### 3.2.2. Results—alveolar macrophage model

We solved Eq. (2) numerically with the parameter values specified in Table 1. For an initial pneumococcal value of \( P_0 = 10^2 \) CFU/ml,
the number of resident alveolar macrophages is large enough that bacterial titers decline immediately (Fig. 3). A dose one log higher \((P_0=10^4 \text{CFU/ml})\) results in a short lag \((-3\text{ h})\) in bacterial growth as AMs initially limit exponential bacterial replication. Shortly after, however, pneumococci overwhelm the AM response and reach a steady-state value of \(P(t)=K_0\). This slowed initial growth phase is not evident with a higher initial value \((P_0=10^5 \text{CFU/ml})\) where immediate bacterial growth occurs.

These results imply that a decreased ability of resident alveolar macrophages to efficiently phagocyte large densities of pneumococci can produce a dosage threshold. Our model predicts that bacterial titers would quickly reach a maximum capacity which could then result in death of the host without additional components of the immune response.

3.3. Stage 2: the neutrophil response

Using the alveolar macrophage model, we found that resident alveolar macrophages could provide protection against a low-dose challenge of pneumococci, partial clearance of an intermediate dose, but no visible effects on a high-dose challenge (Fig. 3). When AMs cannot control bacterial growth through phagocytosis, their main effect switches to modulating the inflammatory response by recruiting neutrophils to the infected lung (Jones et al., 2005; Mizgerd et al., 1996). We build upon Eq. (2) to incorporate proinflammatory cytokine production that induces neutrophil migration and further phagocytosis of pneumococci.

3.3.1. Cytokine production

Pneumonococcal entry into the lung prompts the release of proinflammatory cytokines such as IL-1 and TNF-\(\alpha\) (Bergeron et al., 1998). Alveolar macrophages produce a wide array of cytokines (Monton and Torres, 1998), and alveolar epithelial cells also secrete cytokines following pneumococcal attachment and/or cytotoxic injury (Maus et al., 2004; McRitchie et al., 2000; Stadnyk, 1994). Both IL-1 and TNF-\(\alpha\) have been shown to increase rapidly during a pneumococcal infection, peaking 12 h pi and then declining (Bergeron et al., 1998).

Although these two cytokines have slightly different kinetics (Bergeron et al., 1998), we include both as the single variable \(C\) (Eq. (9)). We divide the alveolar macrophage class into two subsets: \(M_\alpha\), representing AMs in a resting state that are not releasing cytokines, and \(M_c\), representing AMs actively secreting cytokines. Alveolar macrophages in the \(M_\alpha\) class become activated at a rate \(\theta_\beta P\) due to interaction with bacteria and are deactivated at a rate \(\kappa\) per hour. Eqs. (3) and (4) are the subsystem describing this process,

\[
\frac{dM_\alpha}{dt} = s - dM_\alpha - \theta_\alpha PM_\alpha + kM_c,
\]

\[
\frac{dM_c}{dt} = \theta_\alpha PM_\alpha - kM_c - dM_c.
\]

We assume that the total AM population remains constant, \(M_t = M_\alpha + M_c\), and that the activation process is rapid when AMs encounter pneumococci. Thus, the number of alveolar macrophages that release cytokine is

\[
M_c = \frac{\theta_\alpha PM_\alpha}{\phi + \theta_\alpha P}.
\]

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Units</th>
<th>Value</th>
<th>Comments</th>
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<tr>
<td>(\gamma)</td>
<td>Bacterial clearance by neutrophils</td>
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<td>(\theta_\beta)</td>
<td>Activation of cytokine production</td>
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<td>See text</td>
</tr>
<tr>
<td>(\kappa)</td>
<td>Deactivation of cytokine production</td>
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<td>4.2 \times 10(^{-2})</td>
<td>See text</td>
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<tr>
<td>(\nu)</td>
<td>Cytokine production by AMs</td>
<td>(pg/ml) (^{-1}) h(^{-1})</td>
<td>2.9 \times 10(^{-2})</td>
<td>See text</td>
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<tr>
<td>(\omega)</td>
<td>Bacterial attachment to epithelial cells</td>
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<td>(\rho_1)</td>
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<td>1.0 \times 10(^{-3})</td>
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<td>(\rho_3)</td>
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<td>Removal of debris by AMs</td>
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<td>Initial number of neutrophils</td>
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<td>See text</td>
</tr>
<tr>
<td>(D(0))</td>
<td>Initial amount of debris</td>
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<td>0</td>
<td>See text</td>
</tr>
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</table>
incorporate cytokine inhibition from neutrophil (N) effects by reducing production by a factor of $1+k_d N$.

3.3.2. Neutrophil recruitment

The signaling from proinflammatory cytokines recruits neutrophils ($N$, Eq. (10)) at a rate $\eta$ cells per pg/ml per hour which are then cleared at a rate $d_N$ per hour. We do not include a limitation of the phagocytic rate ($\gamma_p$) of neutrophils but we do set the maximum number of neutrophils present in the lung at $N_{\text{max}}$.

Neutrophil exposure to pneumococci results in apoptosis and, at high inocula, necrosis (Zysk et al., 2000). The effect of this process is twofold. First, phagocyte apoptosis following bacterial colonization decreases inflammation and contributes to host recovery (DeLeo, 2004). Alveolar macrophages play an integral role in the clearance of the dead material and debris from the airway (Dockrell et al., 2001, 2003). However, when apoptotic and necrotic material is not efficiently removed, tissue damage increases and bacterial growth may be increased.

We incorporate both the influence of pneumococcal-induced neutrophil apoptosis ($d_{NP}$) and the role of alveolar macrophages in this process. To understand the effect of cell death on pathogenesis, we introduce a state variable to account for the accumulation of debris ($D$, Eq. (11)), which has arbitrary units. In our model, neutrophil death, both natural and bacterial-induced, and the death of epithelial cells translate into debris at rates $r_1$, $r_2$ and $r_3$ per cell, respectively. Alveolar macrophages then eliminate excess debris at a rate $d_D$ per hour. While AMs are engaged in clearing the airway of dead material, we assume their ability to phagocytose pneumococci is inhibited by a factor $1+k_d D_{\text{max}}$.

Fig. 4. Numerical simulation of the neutrophil model, Eqs. (6)–(11), using initial conditions $P_0=10^3 \text{ CFU/ml}$ (dashed-dotted line), $P_0=10^4 \text{ CFU/ml}$ (dashed line), $P_0=10^5 \text{ CFU/ml}$ (solid line), and parameter values in Table 2. (a) Bacteria against titer data (squares—$10^5 \text{ CFU/mouse}$, triangles—$10^6 \text{ CFU/mouse}$), (b) neutrophils, (c) proinflammatory cytokine, (d) debris, (e) susceptible epithelial cells, and (f) epithelial cells with bacteria attached.
Together, these dynamics result in “the neutrophil model” given by

\[
\frac{dP}{dt} = rP \left(1 - \frac{P}{K_P}\right) - \gamma_M P M_A - \gamma_N NP,
\]

(6)

\[
\frac{dE_U}{dt} = -\omega P E_U,
\]

(7)

\[
\frac{dE}{dt} = \omega P E_U - d_E E,
\]

(8)

\[
\frac{dC}{dt} = \alpha E_A + \beta - \frac{\beta_0 P M_A}{(d + \alpha P)(1 + k_D N)} - d_C C,
\]

(9)

\[
\frac{dN}{dt} = \eta C \left(1 - \frac{N}{N_{\text{max}}}\right) - d_N NP - d_N N,
\]

(10)

\[
\frac{dD}{dt} = \rho_1 d_N NP + \rho_2 d_N N + \rho_3 d_E - d_D D_M,
\]

(11)

3.3.3. Results—Neutrophil model

The large number of parameters in our model restricts our ability to fit Eqs. (6)–(11) to the data. Therefore, most parameter values were chosen based on searches in biologically realistic ranges that matched the data, although we fix some values \((d_c\) and \(d_{\text{E}}\)) based on estimates reported in the literature (Gloff and Wills, 1992; Malech, 2007). The initial number of epithelial cells was set at \(10^9\) cells, based on a calculation of the number of cells residing in the murine lung (Stone et al., 1992), and the initial values of the remaining state variables \((C, N, \text{and} D)\) were set to zero. All other parameter values are listed in Table 2.

Simulating Eqs. (6)–(11) with three different initial pneumococcal conditions \((10^3, 10^4\) and \(10^5\) CFU/ml) shows three distinct outcomes (Fig. 4(a)). A low dose, \(P_0 = 10^3\) CFU/ml, is immediately cleared by alveolar macrophages and does not result in noticeable cytokine production or neutrophil recruitment (Fig. 4(b) and (c)). At an intermediate value \((P_0 = 10^4\) CFU/ml), the lag in bacterial growth initially from clearance by alveolar macrophages is reinforced by the appearance of neutrophils in the lung, which extends the delay in exponential bacterial growth from 3 to 6 h (Fig. 4(a)). Proinflammatory cytokine production is initially slow but increases rapidly thereafter and reaches a peak 24 h pi (Fig. 4(c)). These dynamics agree with experimental measurements of both IL-1 and TNF-\(\alpha\) (Bergeron et al., 1998). Paralleling cytokine dynamics, neutrophils increase rapidly and peak 36 h pi before slowly declining over several days (Fig. 4(b)), also consistent with empirical observations (Fillion et al., 2001). The migration of neutrophils into the infection site effectively controls bacterial growth, which peaks 16 h pi. Elimination of bacteria slows between 48 and 72 h pi corresponding to a decrease in neutrophil numbers. At this time, alveolar macrophages are freed up from debris removal and return to phagocytosis of pneumococci. It is unclear whether or not this occurs biologically; however, it is possible that AMs are responsible for the completion of bacterial eradication. During the course of the infection, only a fraction \((\sim 65\%)\) of epithelial cells are killed as a result of bacterial adherence (Fig. 4(e) and (f)).

The neutrophil response is not sufficient to clear a high dose inoculation. For \(P_0 = 10^5\) CFU/ml, bacterial growth slows briefly within 3 h pi in accordance with a sharp rise in both neutrophils and proinflammatory cytokine concentration (Fig. 4(a)–(c)). However, pneumococcal-induced neutrophil apoptosis results in an equally sharp decrease of these phagocytes. At this point, 24 h pi, bacterial titers rise quickly and reach the maximum tissue carrying capacity \((K_P)\). In addition, a significant amount of debris results from both the cytotoxic effects on neutrophils and epithelial cells. In this case, all target cells are killed by pneumococcal attachment.

We next simulated Eqs. (6)–(11) with additional values of \(P_0\) to determine the extent to which outcomes are altered with small changes in the initial condition. As illustrated in Fig. 5, the alveolar macrophage response is sufficient for all initial values.
under $P_0=10^4$ CFU/ml and bacterial titers decline rapidly. However, for initial conditions larger than $P_0=10^4$ CFU/ml, bacteria grow exponentially with an evident lag phase in only a small region around this value. The length of time that bacterial growth is exponential with an evident lag phase in only a small region for initial conditions larger than $P_0$ (Dockrell et al., 2001; Marriott et al., 2004). We also exclude macrophage apoptosis in response to the $P$ values. We exclude macrophage apoptosis in response to the $P$ values.

$\frac{dE}{dt} = \frac{\alpha}{\nu} PE - \gamma_m MP - \gamma_m MP^2$, (12)

$\frac{dD}{dt} = -\gamma_m MP - \gamma_m MP^2$, (13)

$\frac{dA}{dt} = -\gamma_m MP - \gamma_m MP^2$, (14)

$\frac{dC}{dt} = \frac{\theta_m PM^2}{(d + \kappa + \theta_m P) + (1 + \kappa)} - \frac{dC}{dt}$, (15)

Table 3 The monocyte-derived macrophage model (Stage 3) parameter definitions and values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma_m$</td>
<td>Bacterial clearance by MDMs</td>
<td>cell$^{-1}$ h$^{-1}$</td>
<td>$3.2 \times 10^{-5}$</td>
</tr>
<tr>
<td>$M_{max}$</td>
<td>Maximum number of MDMs</td>
<td>Cells</td>
<td>$1.8 \times 10^3$</td>
</tr>
<tr>
<td>$\xi$</td>
<td>MDM recruitment rate</td>
<td>h$^{-1}$</td>
<td>$3.8 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\theta_m$</td>
<td>MDM clearace rate</td>
<td>h$^{-1}$</td>
<td>$1.9 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Time delay before MDM recruit</td>
<td>h</td>
<td>12</td>
</tr>
<tr>
<td>$N(0)$</td>
<td>Initial number of MDMs</td>
<td>Cells</td>
<td>0</td>
</tr>
<tr>
<td>$N(t)$</td>
<td>Number of neutrophils for $-\tau &lt; t \leq 0$</td>
<td>Cells</td>
<td>0</td>
</tr>
</tbody>
</table>

3.4. Stage 3: the monocyte-derived macrophage response

Although neutrophils dominate the early cellular influx of leukocytes (i.e., white blood cells) into sites of pulmonary pneumococcal infections (Mizgerd et al., 1996), these cells are replaced by monocyte-derived macrophages later during inflammation (Fillion et al., 2001). The shift to MDM recruitment is thought to be triggered by the release of soluble factors from neutrophils (Doherty et al., 1988). However, other studies suggest the switch may be the result of MDM response to chemokines produced by tissue cells (Henderson et al., 2003) or regulation by the cytokine IL-6 (Kaplanski et al., 2003). The response to chemokines produced by tissue cells (Henderson et al., 2003), many of which are either influenced by or directly produced by neutrophils (Cassatella, 1995).

$\frac{dN}{dt} = n(C_1 - \frac{N}{N_{max}}) - \delta_N N - dN NP$, (16)

$\frac{dD}{dt} = \rho_1 dN NP + \rho_2 dN N + \rho_2 dE_N - dD M_{N}$, (17)

$\frac{dM}{dt} = \xi N(t - \tau) (1 - \frac{M}{M_{max}}) - d_m M$, (18)

3.4.1. Results—monocyte-derived macrophage model

With parameters in Table 3, this model exhibits the observed behavior of bacterial kinetics for all three initial conditions of pneumococci (Fig. 6(a)). Recruited macrophages (Fig. 6(d)) are evident starting at 24 h pi and peak between 72 and 96 h pi for an intermediate initial value of pneumococci ($10^5$ CFU/ml). This is consistent with experimental findings (Fillion et al., 2001) and corresponds to successful elimination of bacteria, which occurs 24 h earlier with these cells present than with neutrophils and AMs only. Furthermore, the threshold value of $P_0$ such that bacterial eradication occurs is 2.5 times more when macrophages are recruited than with neutrophils and AMs only (9.97 $\times 10^4$ vs 3.98 $\times 10^4$ CFU/ml). For high initial numbers of pneumococci ($10^5$ CFU/ml), these cells continue to increase until a peak is reached 9 days pi (not shown in Fig. 6), reducing pneumococcus numbers without achieving complete clearance. This extended infection with the resulting prolonging of the neutrophil response (Fig. 6(c)) indicates significant inflammation.

The complexity of our model restricts our ability to fully investigate individual parameter values; however, perturbations in many of the model parameters may significantly influence the dynamics of each stage and reflect differences in the host and the pathogen. Performing a differential sensitivity analysis (see Bortz and Nelson, 2004; Eslami, 1994; Frank, 1978) highlights several important aspects of the dynamics (details in the Supplementary File). Changes in bacterial clearance can be the driving force behind a rapid and favorable resolution. As such, altering either of the two of the parameters controlling clearance, $\gamma_m$ and $\gamma_m$, influences the dynamics. The importance of neutrophils in the early stages of bacterial control is confirmed by the differences observed early in the infection with changes in the rate of bacterial clearance by neutrophils ($\gamma_m$) (Supplementary Figs. S1 and S2). Furthermore, this effect is more pronounced and for high initial inocula (i.e., $P_0=10^5$ CFU/ml). On the other hand, changes in the rate of bacterial clearance by macrophages ($\gamma_m$) influences dynamics later in the infection and to a lesser extent (Supplementary Figs. S5 and S6), with little effect when $P_0=10^4$ CFU/ml. In general, parameters related to recruited macrophages ($\gamma_m$, $\xi$ and $\tau$) are relatively insensitive to changes with intermediate or low initial values of pneumococci since these cells only aid bacterial clearance later in the infection when bacteria is low.

Perturbing factors related to inflammation can also result in dynamical differences. One important parameter with the ability to alter infection kinetics is the rate of neutrophil apoptosis due to bacterial cytotoxicity ($d_N$). However, for high initial inocula, the kinetic effect of an increase in this parameter can be balanced with an increase in the rate of neutrophil recruitment ($\eta$) (Supplementary Fig. S1). Other parameters pertaining to inflammation are those which control debris ($\rho_1$, $\rho_2$, $\rho_3$, $d_0$, and $k_0$), but these parameters are insensitive for both intermediate (except $k_0$) and high initial inocula (Supplementary Figs. S7 and S8).

4. Discussion

When a pneumococcal infection overcomes the innate immune defenses, bacterial pneumonia can result. There are
many factors, including virulence of bacterial strain and host defense status, that determine the eventual outcome of a bacterial challenge to the lung. Developing a basic model of acute pulmonary bacterial infection kinetics that could then be expanded or modified to address specific bacterial pathogens would be ideal. However, establishing the appropriate simplifications to be made is challenging and no generalized model may be sufficient given the variability of immune control and modulation by different bacterial species.

We developed a series of models to gain a deeper understanding of how the different layers of host defense in the lower respiratory tract, including physical barriers, resident cells and recruited cells, combine to fight a pneumococcal lung infection and to address the two thresholds observed given the inoculum size: establishment and eradication. Through our experiments and models, we were able to successfully capture the qualitative behavior of the data and identify key steps.

The initial dose threshold was evident through the use of a single equation that took into account a decrease in phagocytosis by alveolar macrophages as bacterial numbers increase, consistent with the view that these cells are sufficient to clear only small densities of pneumococci. Following the first few hours of infection, more complicated dynamics occur. With the neutrophil model, both clearance or sustained growth of bacteria are possible and are dependent on whether alveolar macrophages are engaged in phagocytosis or debris removal, which was correlated to the amount of pneumococcal-induced neutrophil apoptosis. Thus, alveolar macrophages may play a critical role in both establishment and complete eradication of a bacterial infection.

While the neutrophil response is rapid, pneumococcal-induced apoptosis of these cells could result in an equally rapid contraction phase. Normal resolution in the lung includes the apoptosis of neutrophils, but this may also be a significant source of inflammation if the debris is not efficiently cleared. Furthermore, the effects of

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**Fig. 6.** Numerical simulation of the monocyte-derived macrophage model, Eqs. (12)–(18), using initial conditions $P_0 = 10^3$ CFU/ml (dashed-dotted line), $P_0 = 10^4$ CFU/ml (dashed line), $P_0 = 10^5$ CFU/ml (solid line), and parameter values in Table 3. (a) Bacteria against titer data (squares—$10^5$ CFU/mouse, triangles—$10^6$ CFU/mouse), (b) proinflammatory cytokine, (c) neutrophils, (d) monocyte-derived macrophages, (e) debris, (f) susceptible epithelial cells and (g) epithelial cells with bacteria attached.
neutrophil death can be negated with an increase in neutrophil recruitment, but these events combined may still be harmful even though bacterial numbers would not be affected. This effect is further enhanced with the appearance of macrophages, which contribute to both bacterial clearance and respiratory tract inflammation.

Understanding the ways that damage to the respiratory tract epithelium is accrued during an infection has implications on selecting an appropriate antimicrobial for treatment. The strategy of rapid pathogen lysis by antibiotics may work against immune controls by amplifying inflammatory process leading to adverse outcomes. Cell lysis results in the release of bacterial products (e.g., cytokotins, DNA, and cell-wall components) which are recognized by the innate immune system and trigger inflammation (McCullers and Tuomanen, 2001; Orman and English, 2000). Therefore, nonlytic antibiotics may improve the outcome possibly by exhibiting antiinflammatory activities that complement the antimicrobial actions (Ivtić Tkaličević et al., 2006). Although we did not study the implications of antibiotics or the complete feedback of damage to the cellular response, our model could be extended to find alternative treatment strategies that effectively eliminate pathogens without increasing pathogenesis.

The signal triggering the macrophage influx into the lung has yet to be fully understood. Delayed macrophage recruitment following neuphils can predict the behavior of pneumococcus in mice suggesting that an intermediate variable may be responsible. Our model generates consistent results by assuming that the signal originates from neutrophils. However, this is likely an oversimplification and further experiments would be necessary to be sure.

The phagocytic response is essential for successful clearance; however, other factors may contribute to pneumococcal removal. For example, a polysaccharide capsule renders this bacterium resistant to engulfment by phagocytic cells, but antibodies bound to the polysaccharide negate this effect by acting as ligands between the bacteria and the phagocyte, thereby increasing phagocytic efficiency (van Dam et al., 1990). Furthermore, antibodies activate the classical complement pathway (Paterson and Mitchell, 2006), which is thought to be a key step leading to elimination of pneumococci (Kerr et al., 2005). More recent evidence suggests that IL-17-secreting CD4+ T-cells are involved in the neutrophil-mediated immune response and may also help prevent colonization (Lu et al., 2008). However, relatively little is known regarding the influence of these cells, which may have only minimal effects on an initial infection in a naive individual.

Much remains to be discovered concerning the properties of bacterial rise, dissemination, and clearance within a host. Mathematical models provide a means of evaluating relative contributions of individual immune components to the processes during a bacterial infection with pneumococci. Using theoretical analyses to compare host responses in different patient populations will help determine why some individuals never get sick while others are easily colonized. Even models with moderate complexity like ours are important tools for the assessment of host responses. As more data becomes available, model formulations can become more refined and parameter values can be estimated thus facilitating an in depth understanding of the underlying biology of pneumococcal infections.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtb.2011.01.052.

References


