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A mathematical model of intrahost pneumococcal pneumonia infection dynamics in murine strains

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Abstract

The seriousness of pneumococcal pneumonia in mouse models has been shown to depend both on bacterial serotype and murine strain. We here present a simple ordinary differential equation model of the intrahost immune response to bacterial pneumonia that is capable of capturing diverse experimentally determined responses of various murine strains. We discuss the main causes of such differences while accounting for the uncertainty in the estimation of model parameters. We model the bacterial population in both the lungs and blood, the cellular death caused by the infection, and the activation and immigration of phagocytes to the infected tissue. The ensemble model suggests that inter-strain differences in response to streptococcus pneumonia inoculation reside in the strength of nonspecific immune response and the rate of extrapulmonary phagocytosis.

Keywords

Immune response model; *Streptococcus pneumoniae* infection; Ensemble modeling; Bacterial dynamics

1. Introduction

Pneumonia is the most significant cause of death of children worldwide, killing over one million children under the age of 5 every year, mostly in developing countries (World Health Organization, 2013), and is the most prevalent infectious disease in the elderly in developing countries (Kaplan et al., 2003), often complicating respiratory viral infections. Pneumonia is an inflammatory condition of the lung affecting primarily the alveoli, caused

by bacterial or viral infection, or to a lesser extent other microorganisms or chemicals. *Streptococcus pneumoniae*, also known as pneumococcus, is the most common cause of bacterial pneumonia (World Health Organization, 2013). Pneumococcus is estimated to permanently colonize the upper respiratory system of about 10% of adults and 20–40% of children (Bogaert et al., 2004). While the bacteria remain in the nasal cavities, carriers do not show signs of infection and are not considered to be ill. Pneumonia begins when bacteria move from the upper to lower respiratory tract and grow, which may or may not be contained by the ensuing immune response.

The first line of defense against microbes in the upper respiratory tract is the mucociliary layer, which can mechanically clear bacteria via directed mucus flow (Van der Poll and Opal, 2009) or the production of antimicrobial peptides (Bals and Hiemstra, 2004). Mucociliary flow is rather slow (about 2 $\mu\text{m/s}$), and furthermore, pneumococcal strains have been found to impair the flow by disrupting the F-actin cytoskeleton of epithelial cells (Fliege et al., 2013), so larger bacterial challenges can overwhelm the mechanical defenses. Once pneumococci reach the lower respiratory tract, they will adhere to the epithelial cell surface via platelet-activating factor receptor (Cundell et al., 1995), which is quickly internalized by the cell together with the bacteria. The bacteria damage the host epithelial cells primarily via the action of pneumolysin, a pore-forming cytotoxin that is lytic to the cells at sufficiently high concentrations (Van der Poll and Opal, 2009). A pneumolysin-deficient pneumococcus mutant is less likely to cause lethal infection than wild type and reaches overall lower levels in both lung and blood (Hirst et al., 2004). Once the epithelial cell wall is disrupted, the bacteria can cross into the bloodstream.

Alveolar macrophages are the first immune cell type to recognize and phagocytose low numbers of pneumococci (Gordon et al., 2000; Marriott and Dockrell, 2007). If the pneumococcal population grows quickly, macrophage phagocytic activity can be overwhelmed. Macrophages also orchestrate the inflammatory response by producing pro-inflammatory cytokines such as IL-1 or TNF- α , which are involved in the recruitment of neutrophils and other phagocytic cells (Marriott and Dockrell, 2007). Neutrophils are the primary phagocytic agent for clearing the infection (Mizgerd et al., 1996). They are activated in the blood and then move toward the infected tissue, and though they will digest bacteria found in the bloodstream along the way, most of their work will be performed in the tissue. Once in the bloodstream, pneumococcus can cause bacteremia and spread to other tissues, a particularly deadly complication of localized infection. Extrapulmonary phagocytosis in the blood is an important factor in determining survival or nonsurvival in response to infection (Fang et al., 1990). Once bacteria are cleared from the lung, newly recruited macrophages enter the alveoli to clear neutrophils and cellular debris (Knapp et al., 2003; Fillion et al., 2001). Macrophages themselves then apoptose after phagocytosis (Dockrell et al., 2003).

The purpose of our study is to use a model-based approach to provide insight into why distinct murine strains elicit distinct phenotypes in response to the same load of an identical strain of pneumococci. It has been observed, for example, that when infected by D39 strain, CBA/Ca and MF1 mice succumb to the infection after 1–2 days, while the BALB/c and C57BL/6 mice are generally able to overcome the infection quickly (Kadioglu et al., 2000,

2011; Gingles et al., 2001) Moreover, the progression of infection in the lung differs from a mild increase (CBA/Ca), to a decline followed by sharp increase (MF1), to a constant level followed by a decline (BALB/c), to a sharp decline (C57BL/6). In particular, the origin of a sharp increase following the decline in MF1 strain has not yet been satisfactorily explained (Hirst et al., 2004).

Previous work in the intrahost response to pneumonia has led to several ordinary differential equation (ODE) models. Smith et al. (2011) used models of increasing complexity to model the intrahost immune response of BALB/c mice to three initial doses of pneumonia. Another study used ODEs to study the amount of energy required by the human body to clear an infection (Romanyukha et al., 2006). Pilyugin and Antia (2000) studied the influence of the handling time, or time required to complete ingestion of bacteria, of phagocytic cells. The equations and parameters of their model are not specific to any species but provide guidance as to how to incorporate this handling time into simple ODE models. These studies focus on different aspects of the immune system and provide parameters based on experimental data for a single combination of bacterial and mouse strains. Thus, characterizing distinctive immune response across strains using a model-based approach has not been previously attempted. None of the models differentiate between bacteria in the lung and bacteria in the blood, a feature that we find especially important for description of various types of responses.

Using data obtained from the literature, we fit the ODE model to lung and blood pathogen data from four strains of mice: CBA/Ca, MF1, BALB/c, and C57BL/6 (Kadioglu et al., 2000, 2011; Gingles et al., 2001). Each strain has a distinct phenotypic response to the bacterial infection. Using an ensemble modeling approach, we compare differences in the distributions of parameters for each strain. Variations in the parameters characterizing the innate immune response are sufficient to provide the range of interstrain phenotypic variations observed in experimental data.

2. Results

2.1. Overview of modeling strategy

The novel ODE model of intrahost pneumococcal pneumonia utilized in this study follows the time-evolution of 4 variables representing the bacterial populations in both the lung (P_L) and blood (P_B) compartments, damage to the epithelial cells lining the lung (D), and total activated phagocytic cells levels (N). The model consists of 4 differential equations with terms that depend on 17 parameters. In order to evaluate uncertainty in estimation of parameter values we use Bayesian inference approach coupled with Metropolis–Hastings Monte Carlo sampling to compute a posterior distribution of parameter sets in parameter space. Our knowledge of the system parameters is then encapsulated in a model ensemble, a large collection of parameter sets compatible with the data and the biological heuristic constraints, coupled with the biological interactions captured by the differential equations. Since we are comparing four strains of mice, we generate four distinct parameter distributions, each of which describes the immunological response of a particular mouse strain to the infection.

Four of the model parameters are designated as (mouse) strain-independent, meaning these parameters vary between mouse strains and are largely responsible for the dissimilarity seen in the data. The strain-dependent parameters are those that most control the immune response to infection, and they have been shown in literature to vary between murine strains during bacterial infection. These parameters include ν , the rate at which nonspecific immunity can clear pathogen from the lungs (Rossum et al., 2005); h , the rate at which phagocytes are activated due to the presence of lung bacteria (Corteling et al., 2002; Darville et al., 2001; Craig et al., 2009; Eruslanov et al., 2005; Mandel and Cheers, 1980); and ξ_{nl} and ξ_{nb} , the rate at which phagocytes clear pathogen from lung tissue and blood, respectively (Gingles et al., 2001; Corteling et al., 2002). All other parameters are strain-independent, meaning any strain of mouse would have parameters in this range, regardless of immune strength. We have made analogous studies assuming all parameters are strain dependent and found all but the four selected parameters had little variation between the strains (results not shown).

We compared posterior distributions between strains to determine what factors of the immune system lead to each of the four phenotypes. In summary, the largest differences between the strains exist in the rate of phagocytosis in blood. The C57BL/6 and BALB/c mice, both of which survive the experiments, were able to control blood pathogen levels efficiently, lowering their chances of sepsis. CBA/Ca and MF1 mice, however, were unable to keep blood bacteria in check, leading to death within the first 48 h post-inoculation. The detailed results that led to these conclusions are outlined below.

2.2. Strain-specific ensembles

Model ensembles for each strain comprised two million parameter sets. Fig. 1 shows the ensemble trajectories for each of the four strains. The solid black line represents the median value, at a given time point, of all trajectories computed from parameter sets comprising the ensemble, not any particular or best fitting trajectory. The darker shadowed area corresponds to the interval between 25th and 75th percentile of trajectory values, and the lighter outside shadows the interval between 5th and 95th percentile. The data and their standard deviations are also displayed.

The most variation seen in the CBA/Ca mice tends to occur in the overall level of damage. The other trajectories are tight, as the bacteria levels will rise quickly and settle at the carrying capacity by day 2. The model predicts that these mice should show the most damage to the epithelium, as would be expected given their high morbidity. In the first 12 h, pathogen levels remain relatively constant as a result of the nonspecific immunity. High pathogen levels in tissue encourage movement to the blood, where bacteria reproduce rapidly. Simultaneously, pathogen increases in the lung due to the infiltration through the damaged epithelium, leading to high, sustained pathogen levels in both compartments. Since phagocyte levels are proportional to lung pathogen, the phagocyte trajectories are also tight and held essentially constant over the 7-day simulation. In experiments, CBA/Ca mice were found moribund within 24 h post-inoculation.

MF1 mice are also killed by pneumococcus, but infection evolved more slowly following inoculation. We see an initial decay in P_L levels as the nonspecific immunity in the lung

fights the initial dose of bacteria and the remaining bacteria invade the tissue and the blood. Then, as P_B levels rise, the pathogen will infiltrate back into the lungs and will enter a phase of uncontrolled reproduction. The phagocytes are not strong enough to overcome the infection, and mice die, despite the high level of activated phagocyte levels.

The BALB/c and C57BL/6 mice were both able to overcome the infection. BALB/c mice controlled the blood pathogen levels during the full course of the infection. The peak of P_B is several orders of magnitude lower for BALB/c than either the MF1 or CBA/Ca. The majority of P_L and P_B trajectories generated for the BALB/c ensemble will decay to zero eventually, though some will stay at a chronic level. Endpoints of the trajectories are largely influenced by the strength of parameters directing movement between tissue and blood in these populations; those with strong tendencies for infiltration will tend to be unable to clear the infection. Lung bacteria populations are also largely dependent on blood bacteria, given that tissue infiltration is the major contributing factor to P_L levels. BALB/c mice also show little damage since the bacterial levels are brought under control so quickly.

The C57BL/6 trajectories show little variation, given the few data points available for this strain. The data show lung bacteria clearance by 24 h post-inoculation, and because of this rapid clearance, there is no appreciable blood bacteria and no tissue damage. Phagocytes are the only factor showing variation in the ensemble. Phagocyte levels are proportional to lung pathogen by a factor of h . The width of this ensemble reflects the width of the posterior distribution on h for C57BL/6 mice.

2.3. Posterior distributions explain four phenotypes

2.3.1. Full marginal distributions—The marginal posterior distributions describing the uncertainty in the determination of all 17 parameters are shown in Figs. 2 and 3. The strain-independent parameter distributions are the same across all four strains. The strain-dependent parameters (Fig. 3) show how the four phenotypes can be generated by changing just four parameters in our model. Parameter h denotes the rate at which phagocytes are activated in response to lung pathogen presence. The C57BL/6 mice tend to have a higher rate of phagocyte influx, while the other three strains all have similar h distributions. Gingles et al. (2001) report a higher rate of neutrophil influx in BALB/c than CBA/Ca. Our distributions show an average value of 3.4×10^6 for BALB/c and 2.1×10^6 for CBA/Ca.

The rate of mechanical clearance of lung pathogen is quantified by ν . Posterior distributions for ν divide the strains into two groups: two with higher ν (MF1 and BALB/c) and two with lower ν values (CBA/Ca and C57BL/6). MF1 mice exhibit strong nonspecific immunity in the first 12 h to begin fighting the infection and therefore have high nonspecific immune strength. BALB/c mice also have control over bacteria in the first 12 h post-inoculation, again showing that nonspecific immunity is an important factor in their ability to clear bacteria. C57BL/6 mice have the lowest overall ν values, despite their strong fight of the lung pathogen.

The distribution of ξ_{nl} , the phagocytosis rate in the lung tissue, divides the strains again into two sets of two. BALB/c and CBA/Ca mice both tend towards the lower end of the spectrum, while MF1 and C57BL/6 have high phagocytosis rates. The phagocytosis rate in

the blood, ξ_{nb} , is lower for CBA/Ca mice and higher for BALB/c mice. MF1 and C57BL/6 mice show no discernible patterns in this space.

2.3.2. Two-dimensional distributions—We also analyzed the two-dimensional correlations of these parameters in the posterior distribution (Fig. 4). CBA/Ca mice exhibit almost no correlations of interest, as the parameters for this strain tend to exist only in the lower half of the parameter space.

The other three strains show more interesting trends. A negative correlation exists between ξ_{nl} and h , which is most clearly visible in the C57BL/6 parameter space. Thus, if the actual activation rate of phagocytes is high and more will be brought to the infected tissue, the strength of the phagocytosis does not need to be as high as if there were fewer phagocytes present to fight the infection.

There is also a negative correlation between ξ_{nb} and h in both MF1 and BALB/c mice. C57BL/6 mice do not show this trend, as the distribution for ξ_{nb} spans the entire allowable range. Again, if more phagocytes are brought in to eliminate bacteria, their individual efficacy does not need to be as high.

2.4. Principal component analysis describes trajectory sensitivity to parameters

We performed a principal component analysis on the ensembles to identify factors that describe the most sensitive directions in a parameter space. The eigenvalues associated with each of the 17 principal components (Fig. 5, left panel) show a significant difference between principal components 16 and 17, denoting a sharp increase in sensitivity at the last principal component (Toni et al., 2009). Therefore, principal component 17 characterized a very stiff direction in parameter space, indicating that parameters contributing large coefficients to this principal component are particularly sensitive.

The makeup of this principal component is shown in pie charts in Fig. 5 (right panel). CBA/Ca mice show the most sensitivity to the activation rate of phagocytes and the nonspecific clearance rate. If either of these factors was increased, the CBA/Ca mice would have a better chance of fighting off the infection and the phenotype would be altered. The posterior distributions for these parameters tend to include low rates for both of these parameters, however, keeping the CBA/Ca mice from being able to clear pathogen from the lungs.

MF1 mice are most sensitive to the nonspecific immunity, with some contribution from the activation rate of phagocytes. Nonspecific defenses are responsible for the initial decline in lung pathogen numbers in these mice, and thus are the primary factor to creating this phenotype.

The variation in the trajectories of BALB/c mice is most sensitive to the blood pathogen phagocytosis rate and the nonspecific immunity. BALB/c mice are primarily able to resist the infection because they can keep the blood bacteria levels under control, and are therefore able to limit movement back into the lungs and survive the infection. The initial decline in

lung pathogen levels is driven by nonspecific clearance, accounting for the large contribution of ν to the sensitivity.

C57BL/6 mice are most sensitive to the lung pathogen phagocytosis and the phagocyte activation rate. Since these mice clear all bacteria from the lungs before there can be any significant movement into the blood component, the variation in this phenotype will be predominantly subject to the lung phagocytosis parameters. We thus conclude that the stiff directions identified in the strain-specific ensembles point to the biological drivers for each of the phenotypes.

2.5. The model can predict other phenotypes

To ensure the model is not overfit to these data, we validate our model on other data obtained from literature searches. Smith et al. (2011) published data for BALB/c mice infected intranasally with D39 strain pneumococcus at three different inocula: 10^4 CFU/ml, 10^5 CFU/ml, and 10^6 CFU/ml. At the low inoculum, the mice clear the bacteria within a few hours, but at a higher inoculum there are appreciable bacteria levels over the first few days. We simulated this system with all parameter sets previously generated for BALB/c but with the three initial conditions listed (Fig. 6a). The mice clear bacteria within 2 days post-inoculation for both 10^4 CFU/ml and 10^5 CFU/ml. At 10^6 CFU/ml the predicted trajectories are those originally generated by the BALB/c mice ensemble (Fig. 1c). Given the large standard deviations in the data, the ensemble fits these data generally well, except on the first day, as our predicted trajectories all decrease immediately after inoculation, contrary to these observations. There may be an uncertainty in the actual starting inoculation levels due to different technique of delivery in the Smith et al. experiments compared to those used for calibration of the model.

We also validated predictions in MF1 mice on data from Manco et al. (2006) in which MF1 mice were infected with a higher dose of D39 strain pneumococcus (10^8 CFU/ml). Only lung pathogen data is reported for this testing set, obtained over the first 24 h post-inoculation. Qualitatively, the data look similar to our training set, with a smaller initial dip in lung pathogen populations but the same general timeframe of events. At this higher inoculum, innate immunity will not be as effective, and so the initial clearance is not as noticeable as in the training data. Yet, the ensemble of trajectories fit the data well, again validating our ensemble as biologically relevant (Fig. 6b).

Kadioglu et al. (2011) provided data for female C57BL/6 mice after challenges of both 10^5 and 10^6 CFU/ml. We train our set on the lower inoculum, and then validate on the higher inoculum (Fig. 6c). We have only four data points for lung bacteria levels, but the trajectories generally fit these points well. Gingles et al. (2001) provided a count of blood pathogen 24 h post-inoculation for the C57BL/6 mice, estimating about 2–3 log CFU/ml. This estimate aligns with our simulated fit as well, also validating predictions from the ensemble. Mean survival time given in the Gingles et al. study was about 72 h post-inoculation for this strain. Most of the trajectories at this higher initial condition will cause sustained high lung pathogen levels, but a small percentage of the trajectories will be able to clear within a few days. In this situation, the strength of our inference is limited by available data: without late data, it is impossible to discern how many of these trajectories that predict

bacterial clearance would, in reality, be associated with dead mice. However, following trends seen in the training set for shapes of trajectories and mean time to death, this test again seems to support that inferences using our approach is robust.

3. Discussion

We developed an ensemble model of intrahost immune dynamics of pneumococcal pneumonia in four murine strains, where each strain is represented as a model ensemble, a large collection of parameter sets compatible with experimental data. With only four equations and 17 parameters, the model expresses phenotypic differences across strains as differences in parametric distributions across the corresponding ensembles. These differences in immune response crystalize into four strain-dependent parameters governing different aspects of the immune response. Variance in the phenotypes can also be explained biologically through the principal component analysis described in Fig. 3.

Two of the strains studied in this work, BALB/c and C57BL/6, were resistant to the pneumococcal infection and able to survive the inoculum, but the model suggests that their immune systems handled the infection in different ways. BALB/c mice first appear to control lung pathogen levels through a strong nonspecific immunity, showing a sharp decay in bacteria levels in the first day. This component of immunity may include mechanisms such as mucociliary clearance, a specific characteristic of respiratory epithelial cells conveying resistance to bacterial attachment, alveolar macrophage activity, or the presence of other participants to mucosal immunity such as defensins (Gomez and Prince, 2008). As infiltration into the blood increases, blood and extrapulmonary pathogen clearance become the strongest influence on the ability of bacteria to propagate infection. C57BL/6 mice, on the other hand, control bacterial populations by recruiting phagocytes quickly and maintaining a high level of efficacy of phagocytosis in tissue.

We see separate trends for the strains that do not survive the infection. CBA/Ca mice have low bacterial clearance rates both by leukocytic phagocytosis and by nonspecific immunity, explaining their tendency to die soon after inoculation with a high pathogen load. MF1 have strong nonspecific immune defenses, but phagocytes cannot effectively control excess pathogens and therefore do not overcome the infection.

We have also shown that our model does not overfit the four experimental datasets used to compute the strain-specific ensembles, as predictions generated from those ensembles produced good fit of testing data, both qualitatively and quantitatively. Important conclusions from literature, such as survival times and strength of cytokine signaling, can be validated with the parameter ensembles, supporting the biological and mathematical relevance of our model.

It is also clear that containing the infection to the lung, containing blood and extrapulmonary bacteria to low levels is vital to surviving the infection. Several studies suggest that resistant mice have a higher rate of influx of phagocytes than the susceptible mice, corresponding to our parameter h (Craig et al., 2009; Eruslanov et al., 2005; Mandel and Cheers, 1980). The ensembles show the highest rates of influx in C57BL/6 and MF1 mice, and the lowest rates for CBA/Ca mice. In the future, flow cytometry could be performed to test the number of

activated phagocytes in the blood of each of these strains. We could then determine if this difference in phagocytosis rates is due to an increase in the number of phagocytes in the blood compartment, or whether the same number of phagocytes is more efficient in clearing bacteria in the BALB/c mice. We would predict that, as phagocyte number is driven by bacterial counts, higher phagocyte counts would be associated with worse outcomes. Of course, profound immune failure associated with extremely low blood phagocytes would also be associated with a poor outcome. Support for these predictions can be found in several reports, where successful containment of infection was associated with a strong immune response at the site of infection, with limited expansion of the inflammatory response beyond this site (Alves-Filho et al., 2006; Kovach and Standiford, 2012). Our model explains the data for all testing and training sets well and provides biological mechanisms associated with these varying phenotypes. Our equations greatly simplify the dynamics of the system. We summarize the immune system with only three components: one term for the nonspecific removal of pathogen, one term encompassing all phagocytosis in the lung tissue, and one term for the phagocytosis in blood. In reality, these mechanisms are more complex, involving macrophages, neutrophils, plasma cells and other adaptive immune components, and many cytokines and chemokines for signaling. The model could be adapted to include more of these components explicitly. Incorporating more mechanistic details could allow the models to further elucidate the biological underpinning of phenotypic differences. Such enhancements provide a path for future improvements but may be limited by available experimental data.

Mice are typically sacrificed whenever data were collected, although methods to collect data longitudinally in smaller animals are expanding. Extending the model-based approach to truly longitudinal data would improve the robustness of inference. The ensemble methodology is not an attempt to construct a synthetic population of individuals. Rather, it simply attempts to reflect data sparsity and uncertainty into model uncertainty, and therefore prediction uncertainty (Zenker et al., 2007). The posterior distribution, because it is computed from a limited number of individuals, represents an underestimate of population variability. Whether access to longitudinal data would impose more constraints on this distribution remains an open question. In the context of precision medicine, comparison of human phenotypes could be possible with simple models as well. Differences in human susceptibility to pneumonia and other lung diseases can be partially attributed to differences in the inflammatory response (Lommatzsch et al., 2007; Harford and Hara, 1950). Our model can distinguish between phenotypes with high and low neutrophil recruitment, which may lend insight to mechanisms distinguishing severe from mild infections, for comparable pathogen loads.

Traditionally, contrasting phenotypes is accomplished through data analysis, from which speculative mechanisms explaining differences are inferred (Gingles et al., 2001; Lommatzsch et al., 2007; Prince et al., 2006; Ashman and Papadimitriou, 1992; Day et al., 2009; Ludewick et al., 2011; Buckley et al., 1997). Our ensemble-based approach determines putative mechanisms *a priori*, and quantifies their relative importance in explaining various phenotypes through distributional differences in phenotype-specific ensemble. Our approach has two main limitations. Potential mechanisms can only be

included a priori, and mathematical models have limited complexity owing to data sparsity. If quantification of some of the mechanisms can be supported by data, a model-based approach would also offer more detailed and robust insight. Both approaches, data-first and model-first, are in fact complementary. The richness of the data should determine the level of detail with which individual mechanisms can be modeled. Yet, a model-based approach may lead to mechanistic insight more naturally, and provide direct intuition as to the structure of the ensuing experimental agenda.

4. Conclusions

In summary, we provided a small model of pneumococcal pneumonia that can explain multiple phenotypes in murine pneumonia data. Our model demonstrates the importance of a strong immune response both by phagocytosis in blood and by the innate immunity. We have also offered a model-based method of contrasting phenotypic responses to infectious challenges, potentially broadly applicable in heterogeneous populations and experimental challenges.

5. Materials and methods

5.1. Experimental data

We calibrate the model to literature data (Kadioglu et al., 2000, 2011; Gingles et al., 2001) obtained for female mice of four strains, CBA/Ca, MF1, BALB/c, and C57BL/6, infected intranasally by *S. pneumoniae* strain D39, which is a particularly virulent serotype often employed in murine models (Lanie et al., 2007). All studies provided data for bacteria populations in lung, and all but one (C57BL/6) provided data for blood pathogen and neutrophil levels as well.

BALB/c is an inbred albino mouse strain mainly used for hybridoma development, monoclonal antibody production and infectious disease studies. The CBA/Ca strain is commonly used for brain development, neurochemistry, and behavioral studies. The MF1 strain is suitable for general use in toxicology, pharmacology, physiology, and behavioral science. C57BL/6 strain is used for diet-inducing obesity, transgenic/knockout model development, safety and efficacy testing, and immunology. All four strains have very different origins, and it is therefore not surprising that they should exhibit contrasting phenotypes under challenges they were not specifically engineered to withstand (Casellas, 2011). CBA/Ca are the most susceptible to infection, as they succumb to the infection about 24 h post-inoculation and exhibit no appreciable decrease in bacteria levels in either the lung or blood compartment. MF1 are moderately susceptible mice. In the first 12 h, these mice are able to mount a defense in the lung tissue through nonspecific immunity, but the blood pathogen levels rise too quickly for the phagocytes to control. MF1 mice are known to die about 48 h post-inoculation.

C57BL/6 and BALB/c mice are resistant to the infection at these inocula. Female C57BL/6 mice are able to keep lung pathogen levels under control immediately, clearing the infection within 24 h post-inoculation. Only lung pathogen data was available for this strain, and we

have only 48 h of data from the experiment. BALB/c mice are also able to survive their challenge to the end of the 7-day experiment.

5.2. Mathematical model

The equations developed for the pneumonia model are shown in Eqs. (1)-(4). We model populations of four variables: the concentration of pathogen in the lungs (P_L), concentration of pathogen in the blood (P_B), concentration of activated phagocytic cells (primarily neutrophils) (N), and a variable describing the damage to the epithelium (D). Blood phagocytosis is an abstraction for extrapulmonary phagocytosis, as bacteria, once they escape the lung, will migrate to several other tissues through hematogenous spread. Pathogen is first found in the lungs, where it begins to damage the lining of the epithelium. Damage facilitates the migration of bacteria to blood and extrapulmonary tissues and vice-versa. The bacteria's presence signals phagocytes to enter the tissue and phagocytose the pathogen. As immune cells travel to the lung, they can attack bacteria they encounter along the way.

5.2.1. Bacteria—The pneumococcal population in the lungs (P_L) is modeled in Eq. (1). We take the growth of bacteria in the lungs to be at a constant rate of k_l . There is no need to use logistic term as lung bacterial populations never approach carrying capacity in *in vivo* experimental setups. We use saturating term to express the rate at which the innate immune system initially controls the bacteria in the lung, including mechanical clearance via cilia and mucus and phagocytosis by alveolar macrophages. These dynamics, modeled with parameters ν and μ , are the first line of defense against the pathogen. The innate response can clear low levels of pathogen without the need for phagocyte influx, but if the pathogen is allowed to reproduce quickly, additional phagocytes will be recruited to the infected tissue to phagocytose bacteria. Immune cells (N) phagocytose the pathogen at a rate ξ_{nl} .

The last two terms in the equation describe the effects of exchange between the lung and blood pathogen populations. Parameter f represents the volumetric difference between the blood and lung compartments. We utilize two modes of movement between compartments: damage-dependent motion and damage-independent motion. Some proportion of the blood bacteria population (P_B) moves back into the lungs. Parameter b represents the permeability of the membrane to the bacteria, which is determined by the level of damage (D) present in the epithelial tissue. Parameter a represents the damage-independent motion of the blood bacteria into the lung compartment. Bacteria circulating in the bloodstream exhibit a tendency to attach to capillary walls in the lung tissue and concentrate at this location (Spencer, 1967). The final term ($-bDP_L$) denotes the movement of the bacteria in the lung to the bloodstream, which is dependent on the level of damage and permeability of the membrane.

$$\frac{dP_L}{dt} = k_l P_L - \frac{\nu P_L}{\mu + P_L} - \frac{\xi_{nl} N P_L}{1 + \xi_2 P_L} + f[(bD + a) P_B - bDP_L] \quad (1)$$

Blood pathogen (P_B) grows logistically at a rate k_b , limited by the carrying capacity K and the Allee threshold ε . Blood pathogen is removed by phagocytes in the blood at a rate ξ_{nb} ,

proportional to the pathogen population. Like the previous equation, the final terms describe damage-dependent and damage-independent motion of bacteria between the two compartments.

$$\frac{dP_B}{dt} = k_b P_B \left(\frac{P_B - \varepsilon}{P_B + \varepsilon} \right) \left(1 - \frac{P_B}{K} \right) - \frac{\xi_{nb} N P_B}{1 + \xi_2 P_B} - (bD + a) P_B + bD P_L \quad (2)$$

5.2.2. Damage—Eq. (3) represents the increase in damage to the epithelium of the lungs. As the bacteria enter the epithelial cells, they release pneumolysin and other toxins; in response the cells apoptose, creating cellular debris. Damage increases at a rate q , which is proportional to the amount of bacteria present in the lung. The body is able to repair damage at a rate c . The damage varies only between 0 and 1, as it is meant to represent a percentage of the epithelial tissue damaged by the pathogen.

$$\frac{dD}{dt} = q P_L (1 - D) - cD \quad (3)$$

5.2.3. Immune cells—Eq. (4) denotes the change in the lung population of activated phagocytes. The dynamics of phagocytes occurs on time-scale τ_N . Activation is proportional to the population of lung pathogen. We assume that the activation and influx of the phagocytes is initially proportional to the lung pathogen level with rate constant h , but saturates with a half-maximum value at n_h . This activation is mediated by cytokine signaling that is not modeled explicitly.

$$\frac{dN}{dt} = \left(-N + \frac{h P_L}{n_h + P_L} \right) \left(\frac{1}{\tau_N} \right) \quad (4)$$

5.2.4. Initial conditions—We assume that initially there is no damage to the epithelium, no activated phagocytes present in tissue, and no blood pathogen. Since we only account for activated phagocytes, for the purposes of model fitting we subtract the baseline level of phagocytes (i.e., the level at $t=0$) from each data point so N starts at zero. Lung bacteria levels start at 10^6 CFU/ml for CBA/Ca, MF1, and BALB/c, and 10^5 CFU/ml for C57BL/6, in accord with experimental conditions.

5.3. Model parameters

The parameters of this model can be divided into two subcategories: mouse-strain-independent parameters and mousestrain-dependent parameters. We define four parameters to be strain-dependent: h , the rate at which phagocytes migrate to lung tissue; ν , the rate at which pathogen is cleared from the lungs by non-specific immunity, and ξ_{nl} and ξ_{nb} , the rate at which phagocytes clear pathogen from lungs and blood, respectively. These four parameters are different for each of the four strains of mice, as they are defined to be the parameters primarily responsible for phenotypic differences.

The strain-independent parameters are those that would not necessarily vary between strains of mice. These include parameters inherent to the bacteria (k_i , k_b , K), the time to phagocyte activation (τ_N), steady-state and threshold parameters (ε , μ , n_b , ξ_2) and the parameters related to bacterial infiltration (b , f , a , q , c).

Bounds for these parameters were defined in biological ranges where literature values could be found, and approximated for the other parameters. Rates of bacterial multiplication were estimated from doubling times in the lungs and blood (Jay et al., 1976; Todar, 2008). Phagocyte activation rates were also estimated from literature (Corteling et al., 2002). Most parameters ranged over at least two orders of magnitude, and sampling was performed in log space. Strain-dependent parameters were given larger ranges so as to fully understand differences in these distributions. Full details of parameter meanings and bounds are summarized in Table 1.

5.4. Markov chain Monte Carlo sampling

As in Battogtokh et al. (2002) we use Bayesian inference to estimate the likely values of the parameters of the system. If $\mathbf{p}$ represents the list of all parameters and $\mathbf{y}$ represents the vector of the data, then the posterior distribution $\rho(\mathbf{p}|\mathbf{y})$, reporting on the probability that the system has parameters $\mathbf{p}$ given the data $\mathbf{y}$, is given by the Bayes formula $\rho(\mathbf{p}|\mathbf{y}) = Q^{-1} L(\mathbf{y}|\mathbf{p})\theta(\mathbf{p})$ where $L(\mathbf{y}|\mathbf{p})$ is the likelihood of observing $\mathbf{y}$ for a model with parameters $\mathbf{p}$, $\theta(\mathbf{p})$ is the prior distribution based on information about the parameter distribution available before the data is collected, and $Q = \int L(\mathbf{y}|\mathbf{p})\theta(\mathbf{p})$ is the normalizing constant. The likelihood function used in the generation of posterior distribution is the exponential of the negative sum of squared errors, i.e.

$$L(Y|\mathbf{p}) = \exp \left(- \sum_{i,j} \frac{(\bar{y}_{i,j} - y_{i,j})^2}{2\sigma_{i,j}^2} \right) \quad (5)$$

where $\bar{y}_{i,j}$ is the mean of variable j at time i , and $\sigma_{i,j}^2$ is its standard deviation. For prior distribution $\theta(\mathbf{p})$ we choose a product of uncorrelated bounded Jeffrey's prior distributions for P_i which are equivalent to uniform distributions for $\log(p_i)$ between X and Y times a biologically reasonable baseline value.

Three heuristics were also added to the likelihood function to ensure biologically relevant solutions were found. First, we require that the system is stable with no pathogen, meaning the healthy state is stable. We also require complete clearance of bacteria at a low inoculum of 10^2 CFU/ml within 2 days post-inoculation. Lastly, for the BALB/c mice, the system must be able to clear lung pathogen within 2 days post-inoculation at an inoculum of 10^5 CFU/ml. Smith et al. (2011) showed this to be true experimentally, so we use this requirement as well. If a parameter set does not fulfill any of these heuristics, a large penalty is added to the overall cost, essentially eliminating any chances of this parameter set being accepted.

A sample of parameter sets that represents the posterior distribution $\rho(\mathbf{p}|\mathbf{y})$ over the parameter space was found by using the Metropolis–Hastings Monte Carlo method

(MHMC) (sometimes also called Markov Chain Monte Carlo). We generate parameter sets for all strains of mice simultaneously, with $\mathbf{p}$ consisting of 13 strain-independent parameters that are applied to all 4 strains' parameter vectors, and four sets of 4 strain-dependent parameters, one for each strain. This results in a total of 29 parameters generated at every step of the simulation. The error is calculated for each strain's fit, and the likelihood $L(\mathbf{y}|\mathbf{p})$ is taken to be the product of likelihoods of individual strain fits.

To allow for full sampling of the 29-dimensional space, we use a parallel tempering technique (Metropolis et al., 1953; Hastings, 1970; Earl and Deem, 2005), in which chains move through parameter space in steps analogous to their assigned energies, with larger steps taken at higher energies. We generated two million parameter sets and tested convergence of the chains with a Gelman–Rubin diagnostic (Gelman and Rubin, 1992) and a Geweke test (Geweke, 1992).

5.5. Data analysis

The parameter ensembles were analyzed to elucidate the properties of the posterior distribution. Fig. 2 shows the marginal distributions for each of the model parameters, obtained by integrating $\rho(\mathbf{p}|\mathbf{y})$ over all but one parameter value. These distributions define the probabilities of the parameters taking on particular values within their pre-defined bounds. We provide distributions for both the strain-independent (Fig. 2a) and straindependent (Fig. 2b) parameters.

Principal component analysis (PCA) was performed on the computed ensemble for each strain to ascertain correlations between parameter values and eigenvalues, and principal components of the sample were computed. The smallest eigenvalue corresponds to the stiffest direction, providing a quantitative measure of the sensitivity of the ensemble to parameters which contribute significantly to the corresponding eigenvector.

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HIGHLIGHTS

- We developed a small ODE model of intrahost immune response to bacterial pneumonia.
- We fit and compare data for bacteria and phagocyte levels from four strains of mice.
- We quantify interstrain differences in the mice in terms of immune strength.
- Strong nonspecific defenses are not sufficient to overcome infection.
- Extrapulmonary phagocytosis is key to survival of pneumonia.

Fig. 1.

Ensemble of fits of each strain to data for lung pathogen (P_L), blood pathogen (P_B), damage to epithelium (D), and activated phagocytes (D). The black line represents the median trajectory, the inner dark gray area represents the 50% confidence interval, and the outer light gray envelope represents 90% of the trajectories (see text for details). Trajectories are simulated over 1 week starting immediately after pathogen enters the lungs. Data are shown with the triangles with standard deviations noted on each.

Fig. 2. Fully marginalized (one-dimensional projections) parameter distributions of strain-independent parameters. These distributions apply to all four strains used in this study.

Fig. 3.

One-dimensional parameter distributions of strain-dependent parameters. Each column represents one parameter, and each row represents one strain. Distributions for CBA/Ca are in green, MF1 in blue, BALB/c in red, and C57BL/6 in magenta. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4.

Two-dimensional parameter correlations of the strain-dependent parameters. Each dot represents one pair of parameters in the ensembles, revealing correlations between parameter pairs. (A) CBA/Ca, (B) MF1, (C) BALB/c, (D) C57BL/6.

Fig. 5. Principal component analysis of the parameter ensembles for four parameter populations: (A) eigenvalues of each population. Small eigenvalues denote stiff directions in parameter space, explaining which directions are associated with the highest levels of sensitivity in the population. (B) Composition of the principal component associated with the smallest eigenvalue for each mouse strain. The larger the fraction represented by a parameter, the more sensitive the population is to that parameter.

Fig. 6. Validation on other data for BALB/c, MF1, and C57BL/6 mice: (A) Ensemble fits to Smith et al. (2011). The data includes lung pathogen counts at three inocula: 10^4 CFU, 10^5 CFU, and 10^6 CFU. (B) Ensemble fits to MF1 data from Manco et al. (2006). (C) Ensemble fits of C57BL/6 mice simulations to Kadioglu et al. (2011) at a higher initial challenge of 10^6 CFU.

Table 1

Biological interpretation of parameters of the model.

Parameter	Meaning	Units	Bounds
τ_N	Time to activation of phagocytes	h	0.01–10
n_h	Steady-state value of phagocyte population	cells	10^2 – 10^4
μ	Michaelis–Menten rate of nonspecific immunity	CFU/ml	10^5 – 10^7
ξ_2	Inhibition of phagocyte phagocytosis	ml/CFU	10^{-13} – 10^{-11}
h	Activation of phagocytes due to P_L population	cells/CFU/ml	10^5 – 10^8
K	Carrying capacity of blood pathogen	CFU/ml	10^7 – 10^8
k_l	Growth rate of pathogen in lung tissue	h^{-1}	0.1–5
k_b	Growth rate of pathogen in blood	h^{-1}	20–40
q	Rate of increase of damage due to P_L	h^{-1}	10^{-9} – 10^{-7}
ε	Threshold value of P_B	CFU/ml	1–10
a	Directionality of movement from P_B to P_L	h^{-1}	0.1–10
f	Volumetric ratio	dimensionless	10–50
b	Effect of D on movement rates	h^{-1}	0.01–10
ν	Rate of clearance of P_L by nonspecific immunity	CFU/ml/h	10^4 – 10^9
ξ_{nl}	Rate of phagocyte phagocytosis in lung tissue	$\text{cell}^{-1} \text{h}^{-1}$	10^{-9} – 10^{-4}
ξ_{nb}	Rate of phagocyte phagocytosis in blood	$\text{cell}^{-1} \text{h}^{-1}$	10^{-9} – 10^{-4}
c	Rate of repair of damage	h^{-1}	0.01–1