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Mathematical Models of the Inflammatory Response in the Lungs

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MATHEMATICAL MODELS OF THE INFLAMMATORY RESPONSE IN THE LUNGS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Abstract

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By Sarah Minucci, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2017

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Inflammation in the lungs can occur for many reasons, from bacterial infections to stretch by mechanical ventilation. In this work we compare and contrast various mathematical models for lung injuries in the categories of acute infection, latent versus active infection, and particulate inhalation. We focus on systems of ordinary differential equations (ODEs), agent-based models (ABMs), and Boolean networks. Each type of model provides different insight into the immune response to damage in the lungs. This knowledge includes a better understanding of the complex dynamics of immune cells, proteins, and cytokines, recommendations for treatment with antibiotics, and a foundation for more well-informed experiments and clinical trials. In each chapter, we provide an in-depth analysis of one model and summaries of several others. In this way we gain a better understanding of the important aspects of modeling the immune response to lung injury and identify possible points for future research.
Chapter 1

Background

1.1 Introduction

Inflammation in the lungs can occur for many reasons, from bacterial infections to stretch by mechanical ventilation. The immune response to these insults has been widely studied but there is still much that is unknown. Mathematical modeling allows for the organization of biological information, a better understanding of the complex dynamics involved in the immune response, and simulation and prediction of various realistic scenarios. We will give a review of the many mathematical models that have been developed to study the immune response in the lungs. Selected models will be given a more in-depth analysis alongside an overview of several other models and their conclusions.

1.2 Biological background

The immune response can be triggered by invading pathogens or tissue damage in order to protect the body. The response of the immune cells and other mediators give rise to inflammation. The inflammatory process begins with the recruitment of innate immune cells such as neutrophils, macrophages, and other antigen-presenting cells to the
damaged or threatened site. These cells remove the insult and send pro-inflammatory signals to other innate and adaptive immune cells. Adaptive immune cells include T cells and natural killer cells, which are specialized to eliminate the specific insult. Once damaged cells have been cleared and pathogens have been removed, the subsequent anti-inflammatory stage down-regulates the release of pro-inflammatory mediators and suppresses activation of other immune cells. Immune cells secrete pro-resolving cytokines which promotes a resolution of inflammation and return to normal functioning.

Macrophages are innate immune cells found in almost all types of tissue or recruited to the site upon damage and/or infection. They eat pathogens and other substances that are dangerous to healthy cells through a process called phagocytosis. Macrophages are antigen-presenting cells, so they present these pathogens to T cells by displaying the pathogen antigens on their surfaces and carrying them back to the lymph nodes. This helps T cells to develop an adaptive response specific to the pathogen. Neutrophils and dendritic cells are also important in the immune response; neutrophils contain a variety of toxic materials to use in defense against foreign insults. Dendritic cells are another type of antigen-presenting cell.

The innate immune system is typically activated and defends against all pathogens, whereas the adaptive immune response is activated based on the type of damage that occurs. This system includes B and T cells, which fight pathogens that are able to overcome the innate immune system. These immune cells create specific mechanisms that fight against specific pathogens. Most immune responses involve both the innate and adaptive systems, with varying intensities of both.

There are two stages of inflammation: the pro-inflammatory phase and the anti-inflammatory phase. These stages are classified by the cytokines that are produced and up- or downregulate immune cells. Inflammation is first characterized by a pro-inflammatory response, in which damaged cells and/or pathogens are destroyed. NF-κB, a protein involved in DNA transcription, and TNF-α, a cell signaling cytokine, are
upregulated. The second stage is anti-inflammatory, where pro-inflammatory cytokines are downregulated and anti-inflammatory molecules, such as IL-10 and STAT3, promote repair and a return to normal health. The proper shift from a pro-inflammatory response to an anti-inflammatory response is extremely important, and an imbalance in either phase can cause severe complications.

Inflammation specifically in the lungs is caused by a foreign insult or by tissue damage due to stretch or strain. In this work, we will examine the different causes of lung inflammation and how we can use mathematical modeling to understand the inflammatory response in each of them.

### 1.3 Mathematical background

The immune system is a complex system, spanning the organ level down to the molecular level. These networks, both by themselves and together, present positive and negative feedback loops, exhibit highly nonlinear behavior, and can often be quite sensitive to small perturbations in the system. Mathematical modeling is a useful tool in both interpreting the underlying dynamics of these systems and providing predictions and simulations for real-life scenarios and interventions. Figure 1.1 shows the various levels of interactions within the immune system that can be modeled using mathematical techniques.

Specifically, mathematical modeling has several purposes. Cantone et al. describes five: “(a) to inspect and integrate different but complementary types of quantitative experimental and clinical data, (b) to design experiments, (c) to elaborate, analyze and discuss hypotheses, (d) to perform model simulation-based predictions for the course of a disease, or (e) the feasibility of conventional, newly developed or personalized treatments” [6]. According to Eberhardt et al., there are growing expectations that innovations in immunology will provide “new, personalized, and targeted therapeutic options”
Figure 1.1: The immune response can be seen as a system of systems. At the tissue level, immune cells work to fight bacteria and protect the lung tissue. At the cell level, cells interact with one another, sending signaling molecules back and forth. At the intracellular level, receptors on the cell surface bind to molecules which then initiate pathways inside the cell. Figure from Cantone et al. [6]. Reprinted with permission.

[12] for widespread diseases. Because of its quantitative nature, mathematical modeling of immunological systems will be extremely useful in developing and simulating these new treatments.

Various types of mathematical models are used for different biological systems, each with their own advantages and disadvantages. We will give an overview of the most common methods and how they are used.
1.3.1 Boolean networks

Boolean networks are discrete models in which individual units of the model are represented as nodes and the interactions between them are represented as edges. These units, which could be proteins, genes, or complexes, are represented by either a 0 for “unactivated/off” or 1 for “activated/on.” Interactions such as transcription and upregulation are modeled using logic functions such as “and” and “or.”

This type of model allows for the easy incorporation of stochasticity, in which interactions can be assigned a probability of occurring based on data [7]. The network can be validated through -omics data, which consists of large-scale catalogs of molecular information such as that from the Human Genome Project. Interactions can thus be added or removed based on experimental results [6]. Chronological events such as translocation to the nucleus, transcription, and subsequent translation can be modeled by assigning timescales to groups of molecules [29]. This allows stages of reactions to occur at predetermined time steps based on the literature. The drawbacks to Boolean models are that they cannot accurately represent spatial interactions or nonlinearities.

1.3.2 Agent-based models

In biological systems, reactions often occur between cells and even between molecules that are located close to one another. Thus spatial interactions may be helpful in considering intracellular dynamics. In agent-based models (ABMs), molecules or cells are identified as “agents” and move around a defined two- or three-dimensional space based on rules set by the modeler and informed by the behavior of the system.

Agent-based models often replicate dynamics on more than one scale; this is called multi-scale modeling, and it is used for many types of models in addition to ABMs. For example, a model can measure interactions on not only at a cellular level, in which various cells interact with one another, but also a subcellular level, where inside each cell
is a model of interactions between molecules. This is useful in identifying how contact between agents on a small scale can influence interactions on a larger scale and vice versa.

One difficulty with ABMs is that they tend to be computationally intensive and thus difficult to implement. They are very useful for simulations, but currently they cannot be mathematically analyzed [6].

1.3.3 Differential equations

Differential equations are commonly used for modeling biological systems because of their ability to effectively capture nonlinear behavior and demonstrate interactions between numerous variables. Each state variable represents a component of the system, such as a cell, protein, or even a quality like overall damage [24].

Partial differential equations (PDEs) are prevalent in systems where space-dependent dynamics are important, such as air or fluid flow. The equations governing flow are well-studied and well-established in mathematics. Similarly to ABMs, they can also account for spatial components, but PDEs typically account for continuous space rather than discrete steps. However, they can be complex and therefore computationally expensive.

Ordinary differential equation (ODE) models study populations of variables over time and are widely used; thus, there are numerous resources available for solving and analyzing the system as well as estimating and fitting parameters. Stochasticity can also be incorporated into each equation to account for noise in the system. The ODE method of modeling is used most commonly to evaluate the dynamics of overall population sizes of cells or molecules. Spatial features are generally not taken into consideration with ODEs since ODEs assume a well-mixed environment where all elements of a system are evenly distributed throughout the space. This is not necessarily always the case in reality, though including multiple compartments in a model can account for different reactions at different locations, such as the lung and lymph nodes [9, 21].
Another disadvantage for ODEs and PDEs is the lack of experimental data available. Experimental data guides parameter value estimation in these equations; thus, strong parameter-fitting tools are usually needed. We will see that lack of experimental data is a significant limitation for many of the models studied in this review. Therefore, parameter estimation will be valuable in generating useful conclusions.

1.4 Mathematical models for the lungs

In this work, we will focus on mathematical models that examine the immune response caused by lung injury or disease. Many of the mathematical models in this work deal with either pneumonia [11, 23, 31, 33] or tuberculosis [9, 21, 28, 32], which are still leading causes of death throughout the world. The models generally include the pathogen as an input with variables that represent components of the immune response. Several also examine antibiotics or other treatments as a variable in order to study their effectiveness in eradication of the pathogen. The most common types of models for the immune system are Boolean models [3, 28, 37], ABMs [5, 27, 32], and systems of ODEs [9, 10, 11, 13, 15, 19, 21, 23, 24, 25, 31, 33].

It is worth briefly noting that aside from replicating the dynamics of the immune system itself, many models exist which focus on replicating the injuries themselves and perhaps simulating the effect of inflammation on those injuries. Within the context of the lungs, asthma [8, 14, 22, 17, 30] and mechanical ventilation [16, 20] seem to be two of the most commonly modeled conditions. These are certainly related to and cause inflammation, but for this analysis we wish to examine the dynamics of the immune system more directly. These models generally deal more with the mechanics of the airways, including airflow, pressure, and gas exchange.

The following chapters will address mathematical models of various types of lung inflammation in the categories of acute infection, latent versus active infection, and par-
ticulate inhalation. The variety of modeling methods used informs different aspects of inflammation based on the goals of each group of authors. Furthermore, we will be able to compare and contrast the different models and their conclusions. Through an in-depth analysis of one model and summaries of several others in each chapter, we gain a better understanding of the important aspects of modeling the immune response to lung injury and identify possible points for future research.
Infections of the lungs are some of the deadliest in the world [1, 2]. Thus most of the mathematical models focused on the immune response in the lungs are specifically for pathogen-induced inflammation. In this chapter we will examine and compare various mathematical models of the immune system’s response to pneumonia and influenza, two prevalent acute infections. We will also analyze selected models in a more in-depth manner to understand the model-building process and results.

We study pneumonia and influenza together since they both initiate acute lung responses and can be completely eradicated from the body given the proper immune response. They also have a similar ability to mutate, pneumonia becoming resistant to antibiotics and influenza developing new strains. This is a significant motivation for mathematical models to be developed.
2.1 Background & motivation

Influenza is a contagious acute respiratory disease caused by the influenza virus. Over time, the virus goes through adaptive mutations, or even exchanges genetic material with the avian influenza gene pool [15]. It is possible for these mutations to bypass both the innate and adaptive immune systems and cause a pandemic, in which the population has limited or no immunity against the newly mutated virus.

Some notable pandemics were the “Spanish flu” of 1918–19, which killed 50 million people across the world, and the “Asian flu” (1957-58) which caused 70,000 deaths in the US alone [15]. Interestingly enough, Hancioglu et al. noted in 2007 that “many scientists believe that it is only a matter of time until the next pandemics occur.” Then in 2009, H1N1 influenza A virus was named a pandemic, and caused millions of infections [19].

Pneumonia and the bacterium which most commonly causes it, Streptococcus pneumoniae (Sp), cause an estimated one million deaths of children age five and under per year [1] and is especially a problem in developing countries. Co-infection of Sp with other bacteria, most commonly tuberculosis, can also result in life-threatening conditions including sepsis, a complication of infection, and organ failure [11].

Strains of influenza and pneumonia can become resistant to treatment. Thus new vaccines and antibiotics need to be continually developed. Mathematical models are extremely useful in understanding the underlying dynamics of the interactions between these pathogens and the immune system, both innate and adaptive. In addition, they allow for the possibility to simulate the effects of potential treatments. Therefore, these models will aid in a more detailed and robust knowledge of the effects of various strains of these pathogens as well as development of new and personalized treatments for the most susceptible individuals.

Table 2.1 provides a brief summary of the specific motivations of each of the models discussed. This will provide context for subsequent sections describing the methods used and conclusions drawn. In the rest of this chapter, we will give a more in-depth
review of one model then compare the rest of the models together.

<table>
<thead>
<tr>
<th>Model</th>
<th>Motivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al. [3]</td>
<td>Focus on the subcellular pathways and signalling involved in pathogenesis of the influenza virus in order to understand the most important and powerful components of the molecular signature resulting from infection.</td>
</tr>
<tr>
<td>Domínguez-Hüttinger et al. [11]</td>
<td>Use four phenotypes of the host, (i) healthy recovery, (ii) sepsis, (iii) immunological scarring, and (iv) sepsis with immunological scarring, in order to discover the most sensitive aspects of the immune response to pneumonia. They also include antibiotics in their model to evaluate their effectiveness in each situation.</td>
</tr>
<tr>
<td>Hancioglu et al. [15]</td>
<td>Explore the most influential aspects of the immune response to influenza. They also aim to create a model that is as simple as possible while still capturing the overall dynamics, which could be used as the foundation for a susceptible-infected-recovered (SIR) model.</td>
</tr>
<tr>
<td>Schirm et al. [31]</td>
<td>Construct a model of the immune response to pneumonia in order to predict the outcome of various schedules of antibiotic treatments.</td>
</tr>
<tr>
<td>Mochan et al. [23], Manchanda et al. [19]</td>
<td>Build simplified models that could explain the mechanisms behind the varying immune responses due to specific strains of pneumonia and influenza, respectively.</td>
</tr>
<tr>
<td>Smith et al. [33]</td>
<td>Built their model in stages in order to understand the role of each of their three defined stages of inflammation, and therefore predict if the pneumonia bacteria would be cleared or sustain growth within the host.</td>
</tr>
</tbody>
</table>

Table 2.1: Motivation for each model in this chapter.

### 2.2 Analysis of Manchanda et al.

We chose the model by Manchanda et al. [19] of the immune response to the influenza virus to examine more closely. The aim of the model was to study and better understand the dynamics of the immune response to several influenza strains specific to mice.
2.2.1 Model formulation

The three-dimensional system of ODEs (Equations 2.1-2.3) represents the immune response to influenza. The first variable $P$, is the pathogenicity of the virus, i.e. its ability to cause an infection. Then we have $D$, the overall host immune defense, which includes both the innate and adaptive responses. $I$ is inflammation due to the pro-inflammatory response. The equations are as follows:

\[
\frac{dP}{dt} = \alpha P \left( 1 - \frac{P}{k_p} \right) - \beta D \frac{P}{P + 0.01} \\
\frac{dD}{dt} = \gamma P - \theta D \\
\frac{dI}{dt} = \epsilon \left( 1 + \text{tanh} \left( \frac{D - \delta}{\omega} \right) \right) - \rho I
\]

(2.1)

(2.2)

(2.3)

The authors also include a “symptom score,” adapted from Smith et al. [34]. This symptom score is easily calculated and could shed light on the characteristics of an infection. The symptom score $S$ is a measure of how sick the host is, and is calculated by:

\[ S = P + I \]

(2.4)

We can see that pathogenicity ($P$), given by Equation 2.1 grows logistically based on the maximum primary pathogenicity $k_p$ and is also decreased by the strength of the immune defense ($D$). Immune defense growth, the first term in Equation 2.2, is proportional to pathogenicity and the second term accounts for decay of the defense. Equation 2.3 shows the change in inflammation ($I$). It is first characterized by a hyperbolic function which models the defense’s effect on inflammation. The ratio $\delta/\omega$ represents the intensity of the inflammation. The second term in the equation demonstrates the anti-inflammatory response.
2.2.2 Methods and parameter estimation

The original parameter $\beta$ measured the efficiency of the immune response. Using the following transformations, $\beta$ was eliminated:

$$\beta' = 1 \text{ d}^{-1}, \quad D' = \beta D, \quad \gamma' = \gamma \beta, \quad \delta' = \delta \beta, \quad \omega' = \omega \beta$$

For simplicity, the new parameters will drop the prime for the remainder of the analysis. Thus we have only eight parameters, and can divide them into three categories: (i) four parameters that are specific to the virus strain ($\alpha, \gamma, k_p, \epsilon$), (ii) two parameters that are relevant to the biphasic type of infection, in which the infection has two distinct stages ($\delta, \omega$), and (iii) two parameters that are host-specific and thus stay the same for all strains ($\rho, \theta$).

Descriptions of all model parameters and initial conditions are given in Table 2.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>Viral infection rate</td>
</tr>
<tr>
<td>$\kappa_p$</td>
<td>Maximum primary pathogenicity</td>
</tr>
<tr>
<td>$\beta'$</td>
<td>Efficiency of immune response</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Rate of activation of the immune system</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Convalescence rate of immune system; immune decay</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>Rate at which inflammation gets activated</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Triggering threshold value of the defense for inflammation</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Tolerance value of the defense for the chronic inflammation</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Relaxation rate of inflammation/anti-inflammatory response</td>
</tr>
<tr>
<td>$P(0)$</td>
<td>Virus pathogenicity; initial value</td>
</tr>
<tr>
<td>$D(0)$</td>
<td>Antiviral immune defense; initial value</td>
</tr>
<tr>
<td>$I(0)$</td>
<td>Inflammation due to pro-inflammatory response; initial value</td>
</tr>
</tbody>
</table>

Table 2.2: Parameters and initial conditions for model. Table adapted from Manchanda et al. [19]. Reprinted with permission.

To solve the system of equations, the authors used the R-package deSolve, and to estimate parameter values and their sensitivity they used the R-package FME. A Markov Chain Monte Carlo (MCMC) simulation was used to estimate parameter uncertainty. MCMC is a numerical algorithm that uses specific sequential steps to take random sam-
ples of the probability distribution. This process provides information about the distribution, such as uncertainty, without knowing all of the distributions analytical properties [35].

Structural identifiability analysis helps to pinpoint which parameters can be estimated simultaneously. In other words, we would like to know which parameters, when perturbed, cannot be corrected by changing the value of another parameter. The authors used the R function `collin` to calculate the collinearity index of every possible set of parameters. If the collinearity index was above a certain threshold, the set was regarded as “poorly identifiable.” We will examine the most identifiable parameters and their implications in the next section.

### 2.2.3 Results

Upon sensitivity and identifiability analyses, Manchanda et al. found three parameters to be identifiable:

- $\alpha$: viral replication and infection rate
- $k_p$: maximum primary pathogenicity
- $\gamma$: rate of early activation of the immune system

These parameters are also specific to the virus strain, which helps characterize the different virus strains studied.

We can see the fitted results in Figure 2.1. Notice that the first graph shows a unique biphasic response, where there are two clear peaks in symptom score $S$. The parameters $\delta$ and $\omega$ are only estimated for this strain, and the authors found that $\delta$ is highly sensitive. In the biphasic response, $D$ exceeds $\delta$, creating a positive input to the hyperbolic $\tanh$ function, thus allowing the first term in $dI/dt > \epsilon$. This increase along with high values of the parameters $\epsilon$, $\alpha$, $\gamma$ signify the onset of inflammation. However, this is direction for future research because $\delta$, a parameter which together with $\omega$ determines if
inflammation is chronic or acute, is highly sensitive for biphasic profiles while also being non-identifiable for the other four strains.

The authors conclude that the model is able to describe the dynamics of four different virus strains and can thus quantify the severity of each strain. Furthermore, because of its various components, the model can explain why different strains create different outcomes within the host. For example, the biphasic case in one of the viruses is characterized by persistent inflammation, while there was low inflammation in the other three strains studied. The authors stated that it is still unclear whether the second phase of the biphasic case is a result of residual inflammation or caused by mutated virus that has somehow become resistant to the immune response. This would be work for further study.

Manchanda et al. conclude that many important factors in the immune response to influenza can be quantified with only a few parameters, the benefits of a simplified system. The model could also contribute to the development and optimization of virus-specific treatments.
2.3 Other modeling approaches

In this section we will examine several models, along with the previously analyzed model, to see what they have in common and what they do differently. Of the seven models in this chapter, one is a Boolean model and the other six are systems of ODEs. Each model includes several mathematically interesting components which help to describe the pathogenesis and immune response of these infections in novel ways.

2.3.1 A Boolean model

Anderson et al. [3] used Boolean logic rules to examine the network of cellular signaling pathways and transcription factors in dendritic cells upon response to a specific strain of influenza. Using transcriptomics data and other available databases, the authors found that 81 signaling pathways and 9 transcription factors were notably activated upon infection with influenza.

An example of a Boolean logic rule used in the model is that which activates interleukin (IL)-2, a cytokine responsible for differentiation of T-cells. Based on biological knowledge, the presence of transcription factors CREL and NFAT is required for the activation of IL-2 in the presence of one or more other molecules. Therefore, the rule for IL-2 activation is as follow:

\[ IL-2 \text{ ON } = (IRF1 \text{ OR IRF9 OR NFkB OR IL2}) \text{ AND NFAT AND CREL } \]

This model is very different from the other influenza/pneumonia models for two main reasons: (1) it is a discrete dynamics model, and (2) it examines subcellular processes in one type of immune cell. The other models analyze continuous dynamics between several types of immune cells.
2.3.2 ODE models

Though they have many similarities, each of the ODE models studies the immune response to a pathogen using different innovative components. All of them study the intrahost response to the pathogens that are responsible for influenza or pneumonia. Many of them use mass-action kinetics to account for the interactions between cells and the molecules they produce and receive as well as include the effects of antibiotics. See Figure 2.2 for schematics of some of the models examined in this chapter.

Whereas Manchanda et al. chose not to model host cells explicitly, Domínguez-Hüttinger et al. chose to specify host cells by including in their model the epithelial barrier, which is the physical barrier at which the host first interacts with the pathogen, in this case $Sp$. The model, shown in Figure 2.3, quantifies the integrity of this barrier which allows a better understanding of what happens in the immune system when it is weakened or compromised by infection.

Another interesting aspect of this model is the two “switches” which can be described as either on or off, $R$ and $S_v$, also shown in Figure 2.3. The $R$ switch represents the activation of the pro-inflammatory TLR2 pathway inside epithelial cells by $Sp$ bacteria and $S_v$ represents the growth of infiltrated bacteria. When $S_v$ is off, the infection can be cleared by the immune system without treatment, and when $S_v$ is on, treatment is necessary to eradicate the pathogen.

The $R$ switch appears in the equations modeling the neutrophil population and the strength of barrier integrity. If the $R$ is turned on, i.e. the infiltrated bacterial load is above a certain threshold, the TLR2 signaling pathway will be activated. Thus neutrophils will be more prevalent and barrier strength is decreased.

Hancioglu et al. models the effects of the viral load on epithelial cells in a different manner. Instead of evaluating the overall integrity of the epithelial barrier, the epithelial cell population is divided into healthy, infected, dead, or resistant to infection. The number of dead cells is used as a measure of tissue damage. Another interesting aspect
(a) This model focuses on the interferon and cellular components of innate immunity as well as adaptive immunity. Each of these components play a specific role in fighting the pathogen. Figure from Hancioglu et al. [15]. Reprinted with permission.

(b) Model schematic: the pneumococcal population $P$ can cause epithelial cells to be affected ($EA$) or unaffected ($EU$). Also involved are populations of macrophages ($M$) and neutrophils ($N$). Another interesting component of the model is the addition of an antibiotic to eradicate the bacteria. Figure from Schirm et al. [31]. Reprinted with permission.

(c) In this model, overall responses are quantified, including pathogenicity ($P$), antiviral immune defense ($D$), overall inflammation due to pro-inflammatory response ($I$). $S$ is the symptom score calculated based on the three state variables. Figure from Manchanda et al. [19]. Reprinted with permission.

Figure 2.2: Three schematics of the mechanisms involved in the ODE models.
of this model is the variable $S$, defined as antigenic distance. This is a measure of immune memory ranging from 0 (no compatibility between the antibodies and virus strain) and 1 (maximal compatibility). With a higher value of $S$, the adaptive immune response is stronger. In an otherwise healthy individual, antigenic resistance should increase over time, resulting in resistance to the specific strain of influenza.

Similarly, another model (Smith et al.) evaluates damage by calculating the debris $D$, of dead epithelial cells and neutrophils. Through evaluating $D$, we can better understand how pathogenesis of pneumonia is affected by the death of epithelial and other types of cells.

The approach of Smith et al. is also unique in their model-building process. In order to study the effects of each phase of the immune response, they build their model in stages. The first stage is modeling the initial reaction of alveolar macrophages (AMs) to the pathogen. Biologically, this makes sense because AMs are the first to respond to the damage caused by the pneumonia bacterium. In the second stage, pro-inflammatory cytokines produced by AMs recruit neutrophils to further contain the damage and eliminate the pathogen. Therefore, the second model includes neutrophils as well as divides
AMs into resting (not producing cytokines) and active (producing cytokines). The third and final stage of the process adds monocyte-derived macrophages (MDMs), which replace neutrophils in the course of inflammation.

Although all of these models capture the immune response to influenza or pneumonia, each group of authors approaches their models in different ways according to the specific parts of the response they hope to understand. Some of the most prevalent differences are in the model-design process, how epithelial cells are represented, and the ways in which epithelial damage is quantified. In the next section, we will see how these distinctive models were used in generating various results.

2.4 Results and conclusions

The conclusions derived from each model in this review could be divided into two categories: (1) a further understanding of the dynamics of the innate and adaptive immune responses, and (2) recommendations for antibiotic treatments of infection. Here we will discuss the results obtained from the models and determine if any of the models came to similar conclusions.

2.4.1 A deeper understanding of the immune response

Most of the models primarily strive to understand the dynamics of the immune response to influenza and pneumonia, especially among different strains of the pathogen. Innate versus adaptive immunity is also considered.

First of all, a sufficient adaptive immune response is enough to restore the host to health, regardless of the magnitude of the innate immune response as well as the influenza virus itself. Hancioglu et al. were able to determine this by the influence of the parameters $b_{PM}$, $b_A$, and $g_{VA}$, which are the plasma cell production rate, antibody production rate, and neutralization rate of the virus by antibodies, respectively. However, a
stronger innate immune response is important in reducing the duration of infection and amount of tissue damage [15]. Similarly, Manchanda et al. found that adaptive immunity is one of the most impactful factors in the dynamics of influenza. This would result from the inflammation due to pro-inflammatory cytokines, represented by the parameters $\alpha$, $\gamma$ and $\epsilon$, which are the viral replication and infection rate, the rate of early activation of the immune system, and the rate at which inflammation is activated, respectively. Because of the simplified nature of the model by Manchanda et al., the parameters are much more generalized compared to those from the model by Hancioglu et al.

When examining the dynamics in different strains, Mochan et al. found that the innate immune system responds differently to different strains and that the rate at which phagocytosis occurs outside the lungs is also unique to the strain. For example, mice with the strain BALB/c began a strong innate immune response and showed significantly less bacteria within the first day of infection. As the bacteria reached the blood, extrapulmonary phagocytosis, represented by a rate constant and proportional to the pathogen population in the blood, became the most important factor in clearing bacteria.

Smith et al. did not separate the immune response into innate and adaptive, but instead showed which immune cells are most important in the immune response. They found that alveolar macrophages alone can only clear very minor bacterial infections and that neutrophils without monocyte-derived macrophages cannot clear more significant inoculations. The rates of phagocytosis for macrophages and neutrophils were significant in coming to this conclusions, since perturbing the parameters related to these rates resulted in significant changes in dynamics. Thus using parameter sensitivity analysis, they concluded that, similarly to Mochan et al., the rate of bacterial clearance by phagocytes is one of the most important factors in a raid recovery.
2.4.2 Recommendations for treatment

Antibacterial resistance is an issue in treating pneumonia because strains can develop resistance to treatments, rendering antibiotics ineffective. Mathematically, the recommendations for treatment are based on varying the initial bacterial load and intervention time. Schirm et al. used a sum of time-dependent pulse functions in effect to model antibiotic injections. The authors found that, beginning 24 hours after infection with $Sp$, doses of the antibiotic Ampicillin every 12 hours was effective, while every 48 hours was insufficient. Furthermore, they found a threshold of the initial number of bacteria which determined whether or not the immune system by itself could eradicate the infection. This could be useful in determining the least amount of antibiotics necessary to recover from pneumonia, decreasing chances of antibiotic resistance.

Domínguez-Hüttinger et al. also suggest optimal treatments that reduce use of antibiotics. In order to eliminate the pathogen, one or more of the switches needed to be turned off based on the different pathogenic behaviors. For example, for patients with immunological scarring, antibiotics should reduce the bacterial threshold below a certain threshold to turn off the $R$ switch. Results from the model suggest that treatment within 36 hours is extremely important in preventing sepsis; this was consistent with experimental observation. The authors suggest that vaccination, which activates the adaptive immune response, could be used in the prevention of sepsis.

2.5 Assumptions and limitations

The assumptions made in the construction of each model is unique to the goals of each group of authors, but some of the limitations are common to several of these groups, and represent general limitations of mathematical models, especially ODE models within mathematical biology.

Assumptions made by deterministic ODEs begin with the idea that cells are uni-
formly distributed, and that the rate of change of any variable is determined by the present values of these variables [15, 33]. This may not be completely realistic because of the inherent randomness within cell systems, which is not accounted for in any of these models.

Additionally, lack of experimental data is a constant issue for mathematical modelers, especially when estimating parameters. In order to account for this sparsity, models are simplified to capture the overall behavior of the immune system by leaving out some mechanisms. For example, Hancioglu et al. left out intermediate steps in the production of plasma cells and effector cells.

Smith et al. exclude from their model cytokines such as IL-6, which are produced by neutrophils; instead, they assume that monocyte-derived macrophages are recruited proportionally to the number of neutrophils present. Stating that their model is hypothetical, Manchanda et al. include adaptation of the influenza virus to the host environment as a risk factor for cases of influenza with two distinct phases. They include this because there is not enough experimental data to assume otherwise.

### 2.6 Further research

In light of the limitations of the models as explained in the previous paragraphs, many of the authors stated that further work would include making the models more realistic. This could be done by including mechanisms that were previously ignored [23], refining parameter values [11, 15, 31, 23, 19, 33], and combining their models with other existing models [11, 31].

Domínguez-Hüttinger et al. propose that calibrating the model with human data would allow direct translation into clinical settings, helping to identify personalized treatments for patients. Models can also help to understand theoretically why some individuals can clear out pathogens more easily than others [33].
In addition, models that did not initially include the effects of antibiotics or other treatments could add them in future research. Smith et al. assert that they could include a component that would allow them to find treatment strategies that eliminate pathogens without causing further activation of the mechanisms that cause inflammation and infection.
In this chapter we will examine more models of pathogen-induced inflammation. We group tuberculosis and anthrax together because the pathogens that cause these infections are not necessarily fully eradicated in the body. Instead, levels can be so low that they are not acted upon by the immune system and are thus rendered inactive. In the context of tuberculosis, this is called a latent infection, and no symptoms are exhibited. For anthrax, minimal levels of anthrax spores can go undetected by immune cells. Furthermore, these infections are both caused by airborne bacteria. In the same manner as the previous chapter, we will review the model developed by Day et al. [10] and then compare it alongside several other models.

3.1 Background & motivation

Anthrax is a serious infection caused by the bacterium *Bacillus anthracis*. Specifically, inhalation anthrax occurs when anthrax spores are breathed into the lungs. Although it is the rarest form of anthrax [36], it is of the greatest present-day concern due to its
potential as a bioterrorism threat [10]. The immune response to anthrax is not completely known; however, it is known that pathogenesis is significantly different from the immune responses to other bacterial infections [10].

In contrast with the rarity of inhalation anthrax, the bacteria that causes tuberculosis, *Mycobacterium tuberculosis*, is thought to infect around one-third of the world’s population. About 5-10% of these cases actually develop symptoms [9]. With numbers of this magnitude, tuberculosis was responsible for about 1.5 million deaths in 2015 and even more in previous years and is thus one of the top ten causes of death in the world [2]. Although many advances have been made in the prevention and alleviation of tuberculosis, it is clear that this is an area for further investigation.

Table 3.1 shows the motivations stated in each work examined in this chapter. Generally, modelers in this chapter seek to further understand the complex dynamics of these infections which will provide insight for development of treatments.

| Raman et al. [28] | Integrate data into a detailed computational model to understand development of tuberculosis and fill in knowledge gaps. Identify potential ways to combat disease. |
| Day et al. [9] | Explore roles played by different types of macrophages in pathogenesis of tuberculosis and identify when one type becomes more dominant than the other. |
| Segovia-Juarez et al. [32] | Investigate the complex dynamics of granuloma formation in a tuberculosis infection. |
| Marino et al. [21] | Build a two-compartmental model of immune response to tuberculosis, allowing for further and more complex investigations. |

Table 3.1: Motivation for each model in this chapter.
3.2 Analysis of Day et al.

In this section, we will examine the manner by which Day et al. [10] constructed and analyzed their model, as well as provide a summary of their results.

3.2.1 Model formulation

The model developed by Day et al. was constructed in two phases: the first phase replicates immune response to anthrax without any treatment, and in the second phase the model is modified to account for the effects of two different types of antibiotics currently used to combat the infection. It also consists of two compartments: the lung and the thoracic and mediastinal lymph nodes (TMLN). Using a multi-compartmental model allows for an ability to include a spatial component. Equations for each state variable are categorized into compartments so that the model shows which compartment holds a certain population of cells or molecules. Although this spatial concept is not as mathematically descriptive as a PDE or ABM, it provides increased biological realism compared to non-compartmental models. Anthrax spores are first inhaled into the lungs, then make their way to the TMLN. Spores are benign until they interact with a host cell. In this analysis, we will only consider the second stage of the model which includes the effects of antibiotics, which is a system of nonlinear differential equations.

The authors adapt their model from an earlier mathematical model of anthrax infection by Kumar et al. [18]. One adjustment is that instead of assuming a similar immune response for various types of entry of the bacteria, the new model proposes that entry via airway initiates a different immune progression. Furthermore, Day et al. create a simplified model due to gaps in knowledge throughout the literature concerning anthrax. Similar to Manchanda et al., the authors also note the benefit of a simplified model: fewer variables and parameters to estimate. A list of parameters and their descriptions are shown in Table 3.2.
Table 3.2: Parameters for model of anthrax in the lungs. Table adapted from Day et al. [10]. Reprinted with permission.

The equations for the lung compartment are:

\[
\frac{dA}{dt} = -k_2SA + s_A - \mu_AA
\]  
(3.1)

\[
\frac{dS}{dt} = -k_2SA
\]  
(3.2)

\[
\frac{dH}{dt} = \frac{k_2SA}{n_S} - k_3H,
\]  
(3.3)

where variables are alveolar phagocytes \((A)\), accounting for both alveolar macrophages and dendritic cells, spores \((S)\), and host cells \((H)\), which engulf and essentially “host” the bacteria.

Because the phagocytosis of spores \((S)\) by alveolar phagocytes \((A)\) happens so quickly,
the authors chose $A$ to be at a quasi-steady state, where there is no delay in the internalization of spores. Thus by setting $dA/dt$ to be equal to $0$, the equation becomes:

$$A = \frac{s_A}{k_2S + \mu_A}$$

The initial condition represents the number of spores that are first inhaled into the lungs. The authors test various initial spore loads to determine their effects on survival. In the model, spores are inhaled into the lung where they interact with alveolar phagocytes. The phagocytes ingest the spores, thus becoming host cells. These host cells then carry the anthrax to the TMLN, where they cause further damage to their surrounding environment. Figure 3.1 shows a visual representation of what is occurring first in the lungs, then further downstream in the TMLN.

Variables represented in the TMLN are extracellular bacteria, i.e. bacteria that has germinated and been released from the host ($B_e$), neutrophils ($N$), and toxins produced by the bacteria ($T_A$). Furthermore, $B_c$ and $B_d$ represent bacteria affected by antibiotics Ciprofloxacin and Doxycycline, respectively.

As they make their way to the TMLN, spores inside host cells germinate to become bacteria. The amount of time through which spores enter the airway, are ingested by phagocytes, and germinate is denoted the PMG (phagocytosis, migration, germination) period by the authors. This concept is explored later in our analysis. Once the bacteria germinate, they multiply and produce toxins which destroy the host cell. The bacteria thus become extracellular (variable $B_e$) and produce anthrax toxins (variable $T_A$).

These toxins interact with neutrophils (variable $N$) and prevent them from killing bacteria. Anthrax toxins also prevent the ability of neutrophils to mediate bacterial damage through mobility and priming. These interactions cause the inflammation in the mediastinum which characterizes fatal anthrax cases. Inhibition of immune cell efficacy by toxins is shown in the equations through the fraction $\frac{1}{1 + \frac{T_A}{\tau}}$ multiplied by several
Figure 3.1: Schematic for the two-compartment model of anthrax infection. Spores interact with alveolar phagocytes, which translocate to the TMLN. There, spores germinate and are able to destroy cells and release toxins. Figure from Day et al. [10]. Reprinted with permission.

terms in equations for extracellular bacteria \( (B_e) \), bacteria affected by Ciprofloxacin and Doxycycline \( (B_c \text{ and } B_d, \text{ respectively}) \), and neutrophils \( (N) \).

As mentioned above, Day et al. include the effects of two antibiotics in their model. Each antibiotic interacts with anthrax bacteria in a different way: Ciprofloxacin causes bacterial cells to die upon division, while Doxycycline does not kill bacteria, but instead prevents bacterial cells from growing. This allows the immune system to clear out the pathogen naturally without the threat of increasing amounts. Thus the term

\[
-k_5 B_e \left(1 - \frac{B_e + B_c + B_d}{B_{\text{emax}}} \right)
\]
in the $B_c$ equation, which represents death of bacteria due to the antibiotic, is unique to Ciprofloxacin effects. This does not occur for bacteria affected by Doxycycline, $B_d$, and thus $dB_d/dt$ does not include a similar term. The Centers for Disease Control and Prevention (CDC) recommends that individuals are treated with one or the other. Therefore, simulations are run with the effects of either Ciprofloxacin or Doxycycline.

The authors modeled the effects of these antibiotics according to the biological mechanisms they use. In Equation 3.4, antibiotics are included as constants $C$ and $D$. Notice that Equation 3.5 shows three methods of eradicating the bacteria with the effects of Ciprofloxacin included: the second term represents cells killed upon division, the third term represents cells killed by the early immune response, and the fourth term represents cells ingested by neutrophils. Equation 3.6, bacteria affected by Doxycycline, only contains the latter two terms because the antibiotic itself does not kill the bacteria.

Equations for the TMLN compartment are as follows:

\[
\frac{dB_e}{dt} = k_3 n_B H + k_5 B_e \left(1 - \frac{B_e + B_c + B_d}{B_{e \text{ max}}}ight) - k_6 E B_e - \frac{k_8 N B_e}{1 + \frac{T_A}{c_{t1}}} - B_e C - B_e D + k_d B_d
\]  \hspace{1cm} (3.4)

\[
\frac{dB_c}{dt} = B_e C - k_5 B_c \left(1 - \frac{B_e + B_c + B_d}{B_{e \text{ max}}}ight) - k_6 E B_c - \frac{k_8 N B_c}{1 + \frac{T_A}{c_{t1}}}
\]  \hspace{1cm} (3.5)

\[
\frac{dB_d}{dt} = B_e D - k_6 E B_d - \frac{k_8 N B_d}{1 + \frac{T_A}{c_{t1}}} - k_d B_d
\]  \hspace{1cm} (3.6)

\[
\frac{dN}{dt} = k_9 B_e E N_0 \left(\frac{1}{1 + \frac{T_A}{c_{t2}}} + \frac{1}{1 + \frac{T_A}{c_{t3}}}ight) - \mu N N
\]  \hspace{1cm} (3.7)

\[
\frac{dT_A}{dt} = k_4 \frac{B_e}{c_{t_b} + B_e} - \mu T_A T
\]  \hspace{1cm} (3.8)

In the next section we will examine how the authors use this model to obtain interesting results about the immune response to anthrax infection.
3.2.2 Results

Day et al. perform several types of analyses in order to understand on a cellular level what promotes survival in individuals with inhalation anthrax. First, a sensitivity analysis was conducted on 15 of the 23 parameters as well as initial conditions to check which parameters most affected bacterial load and neutrophil population, which are both indicators of the “healthiness” of the individual.

The sensitivity analysis revealed that the most important parameters were those related to the killing of extracellular bacteria $B_e$:

- $k_6$: rate at which resident immune cells in MLN kill $B_e$,
- $E(0)$: initial condition for number of immune cells in MLN, and
- $k_{10}$: rate at which resting neutrophils are activated by already activated neutrophils.

Furthermore, given various initial amounts of anthrax spores, the model was able to show how long antibiotic treatments should last. Figure 3.2 shows the results. The authors determined that a duration of 100 days of treatment with Ciprofloxacin and 70 days with Doxycycline is unrealistic and therefore represents the death of the individual. We can see that with the highest amount of spores, $S(0) = 2 \times 10^7$, there is no chance of survival given the current antibiotics. However, for the other initial levels of spores and intervention times, survival with antibiotic treatment is possible. Furthermore, the authors were also able to conclude that although the two antibiotics target the bacteria through different mechanisms, their effectiveness is about the same.

Another novel aspect of the model was the PMG period. Antibiotics currently used to treat inhalation anthrax assume that germination of the bacteria can be delayed or occur over a longer period of time. By varying the parameter $k_3$ the authors examined the effects of shortening or lengthening the PMG period. The model revealed that a longer PMG period causes survival of more anthrax spores initially. A shorter PMG
period induces faster initial growth of bacteria, which causes antibiotics to activate more quickly, thus requiring less antibiotics in the long run and reducing the duration of the infection. Understanding the effects of PMG period could be extremely useful in determining the effectiveness of antibiotics and informing duration of treatment as well as quantity.

Overall, the model was able to predict the survival of the host based on initial spore load as well as examine which mechanisms of the immune system were most important in eradicating bacteria. It could be further developed to include more information about anthrax as it becomes available as well as inform decisions about antibiotic treatment.

### 3.3 Other modeling approaches

Along with the model by Day et al., we will examine four other models of infection with tuberculosis. Of the five models, three are systems of ODEs, one is an ABM, and one is a Boolean model. Each have varying levels of complexity and different components. In this section, we briefly examine and compare the model-building process and their
components. Figures 3.3 and 3.4 show the mechanisms involved in each mathematical model. Notice the varying levels of complexity and different focuses by which aspects of infection each group decided to investigate.

(a) Three-compartment model studying the interactions between classically and alternatively activated macrophages. Figure from Day et al. [9]. Reprinted with permission.

(b) Diagram for state of macrophages in ABM. Depending on which other cells they interact with, they can fall into several different categories, such as active, resting, or dead. Figure from Segovia-Juarez et al. [32]. Reprinted with permission.

(c) Two-compartment model focusing on role of macrophages. Undifferentiated macrophages eventually take on a phenotype which can result in various outcomes. Figure from Marino et al. [21]. Reprinted with permission.

Figure 3.3: Schematics for three models analyzed in this section.

When comparing the schematics of each model, one of the first things we notice is the level of detail, especially the difference in detail between the Boolean model by Raman et al., shown in Figure 3.4 and the other models, shown in Figure 3.3. We also saw this
Figure 3.4: Schematic for model by Raman et al.; the Boolean model includes molecules from the host and pathogen, cells, cellular processes, and vaccination effects. Figure from Raman et al. [28]. Reprinted with permission.
in Chapter 2 between the Boolean model and the six other ODE models. Boolean models usually include more detail because of their computational nature, the large amount of transcriptomics data available, as well as the benefit of not needing to calculate parameters.

One of the key features of several of the models is multiple compartments. As previously stated, Day et al. included both a lung compartment and a compartment for lymph nodes in their model of anthrax infection. The model by Day et al. for tuberculosis includes both of these components as well as the interstitium, which is the tissue between alveoli and the blood stream. We can also see that in the tuberculosis model, most of the dynamics occur within the lung/airspace whereas in the anthrax model the majority of interactions were in the lymph node. The model by Marino et al. is also a two-compartmental model, accounting for the lung and lymph node.

Three out of the five models also focus heavily on the role of macrophages and their type of activation. Day et al. (2009) and Marino et al. examine the differences between roles of alternatively activated macrophages (AAM) and classically activated macrophages (CAM). Segovia-Juarez et al. examined granuloma, which are collections of macrophages responding to a particular pathogen. Macrophages can be found in several different states, including active, infected, and dead. It is clear that macrophages play an important role in the immune response to tuberculosis and that a greater understanding of its functions could be very useful in determining the best treatments.

### 3.4 Results and conclusions

Similarly to anthrax, response time by the immune system is important in the survival of the host when infected with *M. tb*. Furthermore, activation of macrophages was found to be a sensitive aspect of the mechanisms involved. These are two results that seemed to be prevalent through each model. The models also seek to understand the differences
between latent and active infections.

Including a few parameters in their model to account for events that occur later in time, Raman et al. observed that the adaptive immune response is critical in tuberculosis and that based on their model, an innate immune response may not be enough. The parameter $\delta_{AI}$ represented the delay in the adaptive immune response, with $\delta_{AI} = 0$ representing a vaccinated individual. They also noted that the growth rate of bacteria and subsequent time taken to enter into an active state could be important in determining disease outcome. Bacterial load was also an important point of discussion for Day et al. (2009). In agreement with Raman et al., the authors state that based on model simulations, reducing bacterial populations and the peak bacterial load could be effective treatment strategies rather than focusing on mediators in downstream signaling. In fact, an increase in the parameter $k_{16}$, which controls the ability of infected alternatively activated macrophages to inhibit bacterial growth, decreases bacterial loads but increases the switching time. So an ideal treatment would decrease bacterial loads without influencing later signaling as a result.

Spatial knowledge of the system from an ABM is extremely useful. Through developing a better understanding of granuloma formation, Segovia-Juarez et al. found that bacterial growth was an important factor, as well as recruitment of T cells to the sites of infected macrophages. They conclude that although a certain number of T cells is not sufficient for immune response efficacy, the locations of T cells is important. Ideally, there is a large enough quantity of macrophages to block the spread of bacteria, but not so large that T cells cannot reach bacteria and infected cells. Based on the model, a speedy T cell response results in complete eradication of bacteria.

Marino et al. examine the immune response at a molecular level, in which their model suggests that TNF-\(\alpha\), a pro-inflammatory protein, is more useful in mediating macrophage phenotype than bacterial load. This was determined through virtual depletion, where an element such as TNF-\(\alpha\) can be gradually depleted in the model due to a
simulated treatment. As stated in the previous section, polarization of undifferentiated macrophages towards either classically or alternatively activated is crucial in the development of the infection, and the model shows through sensitivity analysis that this is largely TNF-α-dependent.

Day et al. (2009) proposed a concept for alternatively and classically activated macrophages which they called switching time. This is the time point at which AAM outnumbers CAM, which, as stated previously, is determined by the parameter $k_{16}$, the ability of infected alternatively activated macrophages to inhibit bacterial growth. Marino et al. adapt this approach for their model as well, where AAM immediately become infected when they engulf a bacterium and CAM kills the bacterium. Figure 3.5 shows baseline results from the model by Day et al. (2009). First, AAM outnumber CAM but after 50 days CAM becomes the predominant type of macrophage. In their models, both groups analyzed the effects of decreasing the switching time so that fewer AAM will be infected and more CAM will kill bacteria in a given amount of time.

![Figure 3.5](image.png)

Figure 3.5: Baseline simulation from Day et al. shows the populations of AAM and CAM over 100 days. The switching time, where AAM outnumbers CAM, is shown at 50 days when the two curves intersect. Figure from Day et al. [9]. Reprinted with permission.

Recommendations for treatment as informed by the models would include a faster re-
response by the immune system, which could be achieved through vaccination. Response from classically activated macrophages and T cells are especially significant, which could reduce bacterial loads earlier on in the infection. This was confirmed using different types of models, highlighting the importance of diversity in modeling techniques.

3.5 Assumptions and limitations

Similarly to the models for influenza and pneumonia, those in this chapter are limited by a lack of sufficient experimental data and knowledge about the exact mechanisms employed by the immune system.

Some other assumptions were made for the sake of simplifying the mathematical model and quantifying mechanisms that can be difficult to measure. For example, Day et al. modeled the thoracic and mediastinal lymph nodes as one compartment, although biologically they are two separate entities. Furthermore, since it is not completely known which phagocytes in the lungs actually ingest anthrax spores, the authors added the simplification of generalizing all types of alveolar phagocytes into the $A$ and $H$ variables.

Segovia-Juarez et al. also bring up the limitation of a lack of stochasticity in their model. This and the Boolean network are the only models in this chapter which included stochasticity. Although they include some stochastic rules, such as some related to recruitment of T cells and macrophages and activation of macrophages, not all aspects of the model has stochasticity. They note that the velocity of T cells varies in reality, although it is kept as a constant in their ABM. Furthermore, the probabilistic parameters used in the stochastic rules may not be completely realistic.
3.6 Further research

In addition to adding greater detail to the models as more information becomes available, the authors of each model brought up intriguing areas for further investigation. Raman et al. propose the possibility of creating “virtual patients,” in which host-specific variations in the model could be incorporated in order to determine more individualized interactions and treatments. Segovia-Juarez et al. plan to incorporate more information about the cytokine IFN-γ which is involved in macrophage activation; this could provide new knowledge about the role of IFN-γ and T cell interactions with macrophages. Additionally, all of these models could inform the design of more effective and focused real-life experiments.
Chapter 4

Agent-Based Models of Particulate Inhalation

In this chapter we will examine two ABMs for other types of insults. We will compare the components of each model and the methods used to draw conclusions. We will also present the results of each model and possibilities for future work.

4.1 Background & motivation

Few models have studied the effects of inflammation due to other types of insults besides infectious diseases. As stated in Chapter 1, mathematical models have been developed for asthma and mechanical ventilation, but they do not explicitly model the immune system. In this chapter, we examine how two agent-based models can capture the dynamics of the immune response to asthma and to general particle inhalation.

Asthma is a chronic inflammatory disease with severity varying from patient to patient and can be difficult to manage [27]. Through isolated asthma attacks, constriction of the bronchial tubes occurs and remodeling of the airways can occur through repeated attacks over time [8]. Airway remodeling is also an issue for the inhalation of dan-
gerous particles due to smoking as well as air pollutants and occupational exposure. Depending on the size of the particle and the duration and frequency of the exposure, various immune responses can be elicited [5]. Smoking can lead to Chronic Obstructive Pulmonary Disease (COPD), which is a largely irreversible condition characterized by chronic inflammation [4].

For these conditions, the dynamics of the immune response are still incompletely understood. Pothen et al. [27] and Brown et al. [5] developed models of the immune response to particulate inhalation under different circumstances, in order to further understand the medical conditions.

4.2 Analysis of Pothen et al.: asthma

4.2.1 Model formulation

In the ABM by Pothen et al., the hypothesis of the “inflammatory twitch” is tested in the context of asthma. This inflammatory twitch is a term coined by the same authors in a previous paper [26], in which stimulation of the immune system by an antigen causes inflammation as well as resolution back to normal. They further hypothesize that an asthma attack is characterized by a partial inflammatory twitch; the immune system activates inflammation but is unable to resolve it. The question that the authors hope to answer is, what mechanisms are responsible for the interrupted inflammatory twitch?

For this model, agents are immune cells including mast cells, antigen-presenting cells, helper T cells, and pro- and anti-inflammatory cells. Mechanisms for the immune response to asthma included in the model are shown in Figure 4.1. The response is triggered by breathing in foreign particles.

The basic rules for the model are:

1. Particles are “released” into the system, which mimics an asthmatic response to particle inhalation.
2. Antigen-presenting cells (APCs) move towards particles and release signals while digesting particles.

3. An APC becomes inactive for a user-set amount of time if it encounters a user-set number of particles.

4. Helper T cells move towards APC signals and release pro-inflammatory cytokines for 10 time steps (where 15 time steps equals 1 day).

5. T-regulatory cells move towards pro-inflammatory cytokines; while they move towards pro-inflammatory cytokines, they release anti-inflammatory cytokines.

Similarly to other models we have examined, this ABM has developed a method to quantify health. The rules built into the ABM for the health value are:

1. The scale varies from 0 to 100, where 100 is perfect tissue health with no damage.

2. Each patch has a health value.
3. When a pro-inflammatory cell is on a patch, if the cell moves toward a chemical
signal, subtract 5 from the health of the patch. If it does not, subtract 2.

4. If an anti-inflammatory cell lands on a patch with less than perfect tissue health,
add 1 to the patch’s health.

Now that we have established the components of this model, we will examine those
of the model from Brown et al., then compare their results.

4.3 Analysis of Brown et al.: smoking

4.3.1 Model formulation

This model was developed in order to understand the development of Chronic Obstruc-
tive Pulmonary Disease (COPD), which is often seen in smokers. In COPD, macrophages
respond more intensely to particle inhalation, resulting in a positive feedback loop of
pro-inflammatory response. The mechanisms that make up the model are shown in the
schematic in Figure 4.2.

Figure 4.2: Upon inhalation to particles of different sizes and concentrations,
macrophages react in various ways. Figure from Brown et al. [5]. Reprinted with per-
mission.
Similarly to the ABM by Pothen et al., this one includes a simplified system with abstracted populations of cells. The authors noted that in reality, multiple cells could fall under the category of one variable. In other words, macrophages in the model represent many inflammatory cells including neutrophils and lymphocytes. Furthermore, TNF-\(\alpha\) represents all pro-inflammatory cytokines, and anti-inflammatory cytokines are represented by TGF-\(\beta_1\). With this in mind, the two models have very similar components and interactions. One distinction for the Brown et al. model is the inclusion of collagen, a protein in connective tissue, and fibroblasts, which are cells that maintain the structural integrity of connective tissue.

Additionally, the model was run for 10,000 time steps following exposure. The authors do not specify how these time steps correspond to real time, but conclude that 10,000 iterations of the model is sufficient to determine if the inflammation could be resolved or not.

Rules for the model include:

1. A user-set number of particles are “released” to begin the simulation.
2. Macrophages on the same patch as a particle become activated for 50 iterations.
3. Macrophages are also activated on patches with more than 5 units of collagen.
4. Activated macrophages release pro-inflammatory cytokines and for the last 5 iterations of activation, they release anti-inflammatory cytokines.
5. Fibroblasts move towards and heal damaged tissue by depositing collagenses and increasing the “Tissue Life” value.
6. Particles are degraded by macrophages when the particle’s degradation value is equal to 10; degradation = (number of macrophages on the same patch as the particle)(number of iterations macrophages have been on the patch)
This model quantifies tissue damage similarly to the one by Pothen et al.. Tissue Life is measured on a percentage scale, where 100% represents a perfectly healthy patch of tissue. The presence of pro-inflammatory cytokines (TNF-α) on a patch decreases Tissue Life. This was the first variable investigated by the authors when determining the results of the simulation and the effects of various particle sizes and amounts.

### 4.4 Results and conclusions

With an ABM, the modeler can change the rules in each simulation in order to recreate various scenarios, including inflammation as a result of asthma or smoking.

In the analysis by Pothen et al., the baseline simulation was a normal inflammatory twitch where tissue health returns to normal. Figure 4.3 shows the control simulation along with simulations for various modifications of the model. The most damage done to lung tissue in the model was by increasing the lifespan of pro-inflammatory cells such as neutrophils. The authors concluded, therefore, that a possible therapy option could be increasing apoptosis in neutrophils to decrease the magnitude of the pro-inflammatory response.

For the model by Brown et al., instead of changing the mechanisms of the immune response, different sizes and quantities of particles were chosen to activate the system for each simulation. The authors found that based on the degree and duration of particle exposure, the model elicited three distinct states: (1) self-resolving inflammation, (2) localized tissue damage and fibrosis, and (3) persistent tissue damage and fibrosis. The number of particles as well as the frequency and duration of exposure were all factors that contributed to localized and persistent tissue damage and fibrosis; thus, the immune response resulting in these states are likely a combination of all three influences. However, simulations suggest that the situation causing the most damage would be through increased particulate exposure at higher frequencies, even with short duration.
Figure 4.3: The authors ran several simulations with varying modifications. Each curve represents the average of 100 simulations. The control is a normal inflammatory twitch. Other simulations include: increased pro-inflammatory cell (PIC) duration, mast cell knockout, Th cell knockout, and reduced survival of pro-inflammatory cytokines. Figure from Pothen et al. [27]. Reprinted with permission.

Furthermore, the authors also noted that both localized and persistent tissue damage is characterized by high overall levels of pro-inflammatory cytokines as well as a high number of pro-inflammatory cytokines per macrophage. This is consistent with the findings of Pothen et al. They conclude that the localized and persistent states are likely a combination of these factors with others in the system.

As opposed to a system of ordinary differential equations where it is assumed that all individuals of the population are well-mixed in the space studied, an ABM reveals the spatial components and how locations of cells affect the dynamics of the system. Figures 4.4 and 4.5 show simulations of each model and how considering space can provide useful insights. Brown et al. especially emphasizes the significance of using a spatiotemporal model, supporting the need to include spatial components in future models.
Figure 4.4: Simulations from Brown et al. in a section of lung tissue representing each state: (A) self-resolving inflammation, (B) localized tissue damage and fibrosis, and (C) persistent tissue damage and fibrosis. Darker areas represent damage. Figure from Brown et al. [5]. Reprinted with permission.

Figure 4.5: Simulation from Pothen et al. for a normal immune response. The red area is the capillary, the blue patches are the endothelial barrier, and the gray is the alveolar tissue. Darker areas represent damage. Notice that darker areas (i.e. damaged patches) are clustered together in the bottom middle and top left of the alveolar tissue. Figure from Pothen et al. [27]. Reprinted with permission.
4.5 Further research

The goal of constructing both of these models was to further understand the complex dynamics of the immune response to particle inhalation under different circumstances and potentially provide insight into treatments for asthma and COPD. However, because these insults have not been widely studied in mathematical models, the models by Brown et al. and Pothen et al. are first steps into their computational investigation. Both groups of authors acknowledge the simplification of grouping cells and molecules together as APCs, macrophages, and pro- and anti-inflammatory cytokines. Brown et al. stated that improvements upon the model could be made by distinguishing neutrophils, T cells, eosinophils, and epithelial cells. This would allow for a more detailed understanding of the mechanisms that cause inflammation. Pothen et al. also note that they have not taken full advantage of the spatial aspect of the model, and that it could be further studied in the future.

The two agent-based models studied in this chapter have many similarities and obtain similar results. However, each model examines results differently; Brown et al. chose to focus on three states of increasing damage intensity due to various types of exposure to particulate inhalation. On the other hand, Pothen et al. chose to focus on the isolated mechanisms that could affect the inflammatory twitch, providing possible explanations for asthma-induced inflammation. Both concluded that spatial dynamics are extremely useful in identifying areas of tissue damage, and that it is likely that the presence of pro-inflammatory cytokines is an influential aspect of the positive feedback loop of inflammation.
Chapter 5

Conclusion

In summary, we gave an overview of the biological processes involved in the immune response to lung insults and outline the types of mathematics used to model these systems, each with their own advantages and disadvantages. The greatest motivation for constructing models of the immune response in the lung is to further understand the complex dynamics of immune cells and proteins.

For influenza and pneumonia, unique components of the models included switches that determined important bifurcations in inflammation [11] and building a model in stages [33]. In order to better understand the injury caused to epithelial cells, quantifying damage is important and was achieved using a different method in each model. Two conclusions especially highlighted by several models for the immune response to pneumonia and influenza are (1) that the rate of bacterial clearance through phagocytosis is important, and (2) that the effects of antibiotics can be modeled, providing insight into the issue of antibiotic resistance and overuse.

Understanding the underlying dynamics of the immune response was the most important motivation for the models of tuberculosis and anthrax, since there are many gaps in the knowledge about these infections. Two-compartment models were characteristic in this chapter, whereas they were less of a focus in the models of influenza and
pneumonia. The mechanisms that had the greatest influence on the survival of the host were (1) the response time of the immune system, (2) macrophage activation (CAM vs. AAM), and (3) the bacterial load.

Not many mathematical models focus on the immune response to non-bacterial insults. The two ABMs studied in Chapter 4 examined particulate inhalation under different circumstances. The methods used to measure tissue health were similar, and both models concluded that the most influential factor on tissue health was the effects of pro-inflammatory cytokines. The spatial component of these ABMs, which the two ODE models studied in Chapters 2 and 3 do not include, shows results that could not be observed without accounting for space. This further confirms the necessity of multiple mathematical methods in order to gain more comprehensive knowledge of the immune response to various pathogens.

It is clear that the immune response is unique to each insult and that mathematical models should be individualized for each type of pathogen. Due to the lack of experimental data available and limited biological knowledge, further experimentation is important for developing these models. Fortunately, the models themselves provide useful recommendations for experiments. Furthermore, the benefits of simplified models are highlighted in this review and models such as the three-dimensional one by Manchanda et al. can in fact capture the dynamics of the immune response very well when fit to the available experimental data. Additionally, the quantification of damage using different techniques as well as the focus on distinct states of inflammation separated by manifolds or switches provide insight into the complex biological mechanisms of the immune response, further revealing the usefulness of mathematical modeling of lung inflammation.
Appendix A

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