Cellular Automata Modeling of Pulmonary Inflammation

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Abstract: Better understanding of the acute/chronic inflammation in airways is very important in order to avoid lung injuries for patients undergoing mechanical ventilation for treatment of respiratory problems. Local lung inflammation is triggered by many mechanisms within the lung, including pathogens. In this study, a cellular automata based model (CA) for pulmonary inflammation that incorporates biophysical processes during inflammatory responses was developed. The developed CA results in three possible outcomes related to homeostasis (healing), persistent infection, and resolved infection with high inflammation (inflamed state). The results from the model are validated qualitatively against other existing computational models. A sensitivity analysis was conducted on the model parameters and the outcomes were assessed. Overall, the model results showed possible outcomes that have been seen in clinical practice and animal models. The present model can be extended to include inflammation resulting from damage tissue and eventually to model inflammation resulting from acute lung injury and multiple organ dysfunction syndromes in critical illness and injury.

Keywords: Inflammation, Cellular Automata Modeling, Simulations, Pathogens, Acute Lung Injury

1   Introduction

Inflammation has been recognized as a major integral component for most of the acute and chronic diseases. Inflammation can be initiated within the body as an innate process or by external factors such as infections and trauma. Inflammation is a complex and dynamic process, and involves nonlinearity and stochasticity. In

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response to a pathogen insult, inflammation occurs as necessary responses of the organism. It involves a variety of cell types including immune cells within the injured tissues. Without the inflammation, the harmful stimuli cannot be removed and the healing process cannot occur. However, an over-expression or under-expression of inflammatory responses can lead to severe consequences. Over-expression can continue to occur in absence of the initial stimulus and lead to sepsis and/or Multiple Organ Dysfunction Syndrome (MODS), which is characterized by sequential organ failure. A significant number of sepsis patients develop MODS and is a leading cause of death for intensive care unit patients. The mortality rates for a patient with a single failed organ is 18% and 52% with two or three failed organs. The mortality rate increases as more organs fail [1-3]. Acute lung injury (ALI) is typically one of the first manifestation of MODS. It can be triggered by external stimuli such as pathogens or from inflammatory mediators produced from various other processes ranging from other damaged organs to blood transfusions or even the biomechanical forces of mechanical ventilation itself. Therefore, modeling the acute inflammatory response within the lung is an essential step to understanding the complex dynamics involved in the ALI and MODS.

Many mathematical models have been developed to describe inflammatory responses to pathogens [4-7]. However, there are limitations in those models. The encounter of pathogens and immune cells has been modeled assuming that it occurred uniformly throughout the tissue. In reality the encounter is not uniform for the whole tissue. Some parts of the tissue might have the encounter while some parts do not. A computational model that takes this reality into account would help researchers better understand inflammatory responses in the human body. Thus, developing computational models allowing for spatial simulations may provide powerful tools to assist in our understanding of the complexities of the inflammatory process and to better inform scientists to develop more useful experiments.

In general, there are two approaches to modeling inflammation. The first is the population down approach in which the inflammation process is treated as a dynamical system and is characterized using equations, such as ordinary differential equations (ODE) or partial differential equations (PDE). The second approach is the component up approach involving object/event based and agent based models for inflammation. Recently, Vodovotz et al. [8] nicely summarized the current state and future prospects of modeling inflammation. Due to the complex process of inflammation, several models based on ODE's, PDE's and Agent based Modeling (ABM's) have been developed. ODE models while very successful are completely deterministic and ignore the spatial structure and variability. To overcome this spatial structure deficit, PDE's have been developed to vary over space and time, but do not easily account for stochasticity. In contrast to PDE's methods, ABM's tech-

niques has been developed [8, 9, 17, 18] for computational simulations, in which agents are discrete events, rule-based, and can be stochastic. Recently, Brown et al. [19] developed an agent-based model of inflammation due to particulate exposure in the lung. In this study, a cellular automata based model for pulmonary acute inflammation that incorporates many biophysical processes during inflammatory responses was developed. Here, we model the inflammatory response to a pathogen within the lung. The results from the model are validated against other computational models and the possible outcomes of the results are presented and discussed.

2   Inflammation Model

The inflammation process in the lung begins with an encounter between the pathogens or damaged cells and macrophages. The encounter triggers macrophages to release pro-inflammatory cytokines, signaling proteins that can cause vasodilation and increase the permeability of blood vessels. Vasodilation and increase blood vessel permeability are key components to inflammation [10]. Cytokines also stimulate neighboring cells to secrete chemoattractants of other inflammatory cells; e.g. neutrophils. The activated inflammatory cells release additional cytotoxic mediators that can not only kill the invading pathogen but may also result in further damage to injured innate cells or in damaging previously healthy innate cells such as the alveolar epithelial cells.

The inflammatory response model at the cellular level was developed via the cellular automata (CA) method. The CA model was composed of two species: epithelial cells within the lung and immune cells. The pathogens was not explicitly considered but are modeled as spread directly from one epithelial cell to another. The CA model was constructed on two-dimensional square lattice where each lattice site represented one epithelial cell (see Fig.1). The immune cells were mobile, moving from one lattice to another. Therefore, the square lattice represents the tissue of immobile epithelial cells, which is patrolled by the mobile immune cells. The CA was updated synchronously based on specific rules. The boundary conditions for both epithelial and immune cells were periodic boundary, i.e., an immune cell moving off from one edge of the lattice was reintroduced at the opposite edge and an infectious epithelial cell at one edge can infect a healthy epithelial cell at the opposite edge. Finally, the neighborhood of the lattice was defined as eight closest lattice sites, i.e., Moore neighborhood (see Fig.1).

2.1   CA Rules

Details of the CA rules for each species were derived from the general inflammatory processes in human body and are described below.
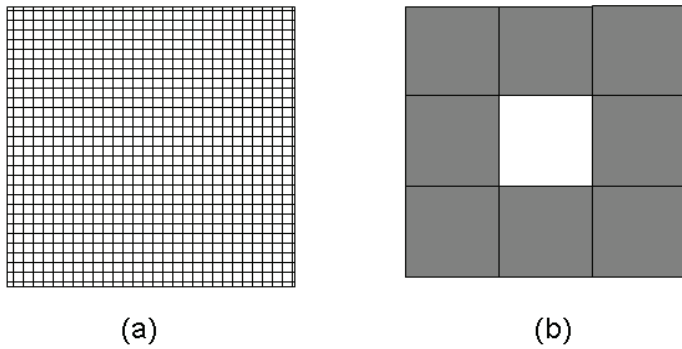
Figure 1: (a) Two-dimensional square lattice used for the inflammatory responses model. (b) Moore neighborhood (dark) of each lattice site (white)
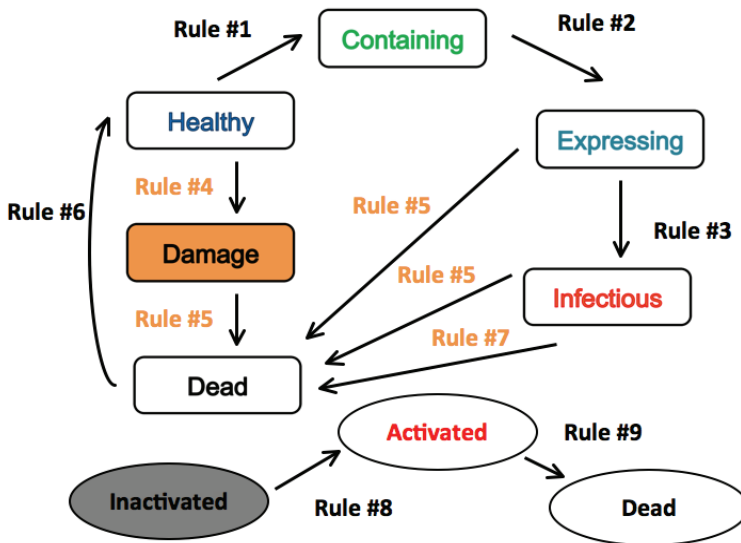

Figure 2: Possible states of an epithelial cells (squares) and immune cells (oval) during inflammatory responses due to pathogen

An epithelial cell can be in any of six states: healthy, containing, expressing, infectious, damage, and dead (see Fig. 2). A containing cell represents a cell that has bacteria within it, but has not presented the bacteria to the immune system. Expressing cells have processed the bacteria and are presenting to the immune system, but the bacteria is unable to spread to neighboring healthy cells. Infectious cells can infect healthy neighbors. Transition of each state occurs as follows:

- Rule 1: A healthy cell becomes a containing cell with probability P_I

  $$P_I = 1 - (1 - P_{1I})^{N_I}$$

  where P_{1I} is a probability that one infectious cell can infect a healthy cell and N_I is a number of infectious cells in the neighborhood.

- Rule 2: A containing cell becomes an expressing cell after being infected for $T_{EXPRESS}$ time steps

- Rule 3: An expressing cell becomes an infectious cell after being infected for $T_{INFECTIOUS}$ time steps

- Rule 4: A healthy cell becomes a damaged cell if there are at least N_D activated immune cells in the neighborhood with probability P_D.

- Rule 5: An expressing, infectious, and damage cell becomes a dead cell with probability

  $$P_P = 1 - (1 - P_{1P})^{N_{AC}}$$

  where P_{1P} is the probability that one activated immune cell can phagocyte the expressing, infectious, or damage cell and N_{AC} is a number of activated immune cell in the neighborhood.

- Rule 6: A dead cell is replaced by a healthy cell with probability

  $$P_H = 1 - (1 - P_{1H})^{N_H}$$

  where P_{1H} is the probability that one healthy cell divides and replaces the dead cell and N_H is the number of healthy cells in the neighborhood.

- Rule 7: The infectious cell is killed by the pathogen with probability P_K after being infected for a minimum of two hours. An epithelial cells will survive for $T_{EXPRESS} + T_{INFECTIOUS} + 1$ (ten hours) before it can die due to the infection. Activated immune cells can damaged the expressing and infectious epithelial cells at any time step and this will lead to death before ten hours.

An immune cell can be in any of three states: inactivated, activated, and dead (*see* Fig. 2). An inactivated immune cell is an immune cell that has no specificity. An activated immune cell is an immune cell that has encountered an expressing, infectious, or damaged cell or has been recruited by another activated immune cell. Immune cells move randomly at every time step. They can move within a 11x11 grid centered at the immune cells current location.

Transition of each state occurs as follows:

- Rule 8: An inactivated immune cell recruits activated immune cells with the recruitment rate θ_R immune cells per neighborhood when it is in the neighborhood of an expressing, infectious, or damage cell. The number of immune cells recruited is determined randomly, between zero and θ_R for each expressing, infectious or damaged cell in the lattice. We are assuming that inactivated cells locally react to the expressing cells and release pro-inflammatory cytokines that activate blood immune cells and attracts these activated immune cells to the local infection site. Therefore, the new cells arrive activated.

- Rule 9: An activated immune cell becomes a dead immune cell when it is older than its lifespan. This lifespan is determined randomly, between zero and L_{AC} for each immune cell.

3   Results and Discussion

3.1   Simulations

The CA rules described above were implemented using MATLAB software. The simulation was performed on a lattice of 100×100 sites which represented a tissue area of 2×2 mm^2 [11]. The initial population of inactivated immune cells was fifty cells. This value represents the normal concentration of immune cells per area in the lung. The initial conditions were a three by three grid of containing cell at the center of the lattice with randomly placed immune cells. Only one immune cell can occupy one lattice site. The periodic boundary conditions were used for all simulations.

The various parameters for the rules are given in Table 1. As it can be seen from this table, there is no set value for P_{1I}. The parameter P_{1I} represents the possibility that the pathogen can affect the epithelial cell and it strongly depends on the pathogen type. For this reason, P_{1I} was varied in the analysis to see the effect on the outcome on inflammatory responses. One simulation time step corresponds to two hours real time.

Table 1: Parameters for the CA model of inflammatory responses due to pathogen

Parameters	Values	Description
$T_{EXPRESS}$	2 h	Delay from containing to express
$T_{INFECTIOUS}$	4 h	Delay from containing to infectious
N_D	4	Minimum number of immune cells that can damage a healthy epithelial
L_{AC}	20 h	An activated immune cell lifespan [20]
θ_R	9	Maximum Number of immune cells recruited
P_{1H}	0.45	Probability that one healthy cell divides and replaces a dead cell
P_{1P}	0.45	Probability that one activated immune cell can phagocyte an expressing, infectious, or damage cell
P_D	0.5	Probability that immune cells damage a healthy cell
P_K	0.6	Probability that an infectious cell is killed by the pathogen

3.1.1 *Inflammatory Responses due to Pathogen*

There are three possible outcomes. These include a return to homeostasis (healing), persistent infection with damaged tissue (labeled infected), and resolved infection but with continued inflammation and damaged tissue (labeled inflamed). In the healthy outcome the infection is eliminated, all epithelial cells which were infected or damaged are replaced by healthy cells. The immune system is no longer stimulated. Therefore, there are only inactive immune cells present. In Figure 3A, time shots from a healthy outcome are shown for times twelve hours, one, four, and seven days. The state of the epithelial determines the color of the lattice square. While lattices occupied by immune cells (inactive or active) are white regardless of the state of the epithelial. In Figure 3B, the transients for various cells types are plotted versus time in days. The infection persists until approximately day five when the immune response is capable of eliminating the infection.

The infected state is characterized by unresolved containing, expressing, infectious, dead and damaged cells. These cells continue to stimulate the inflammatory response. Therefore, both inactivated and activated immune cells are also present. In Figure 4A, time shots are plotted for the infected state. The immune response is unable to eliminate the infection so there are containing, expressing, and infectious cells at day eight, see Figure 4B.

The inflamed state outcome represents an unresolved immune response, despite the elimination of the pathogen. There are no containing, expressing or infectious cells,
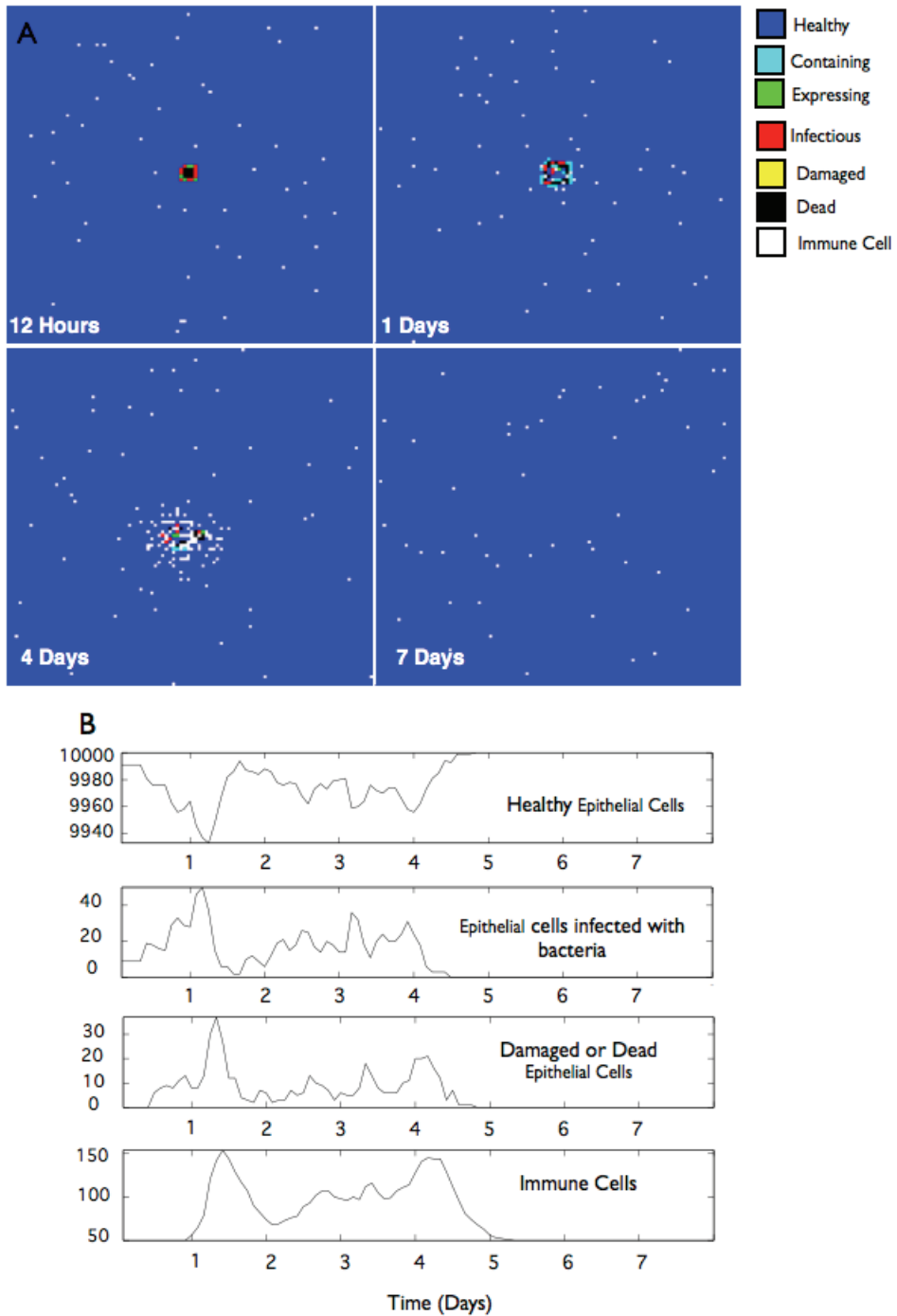
Figure 3: Time shots (A) and transients (B) for the healthy state. $P_{1I}= 0.5$, infection introduced by setting a 3 by 3 square at the center of lattice to containing cells.
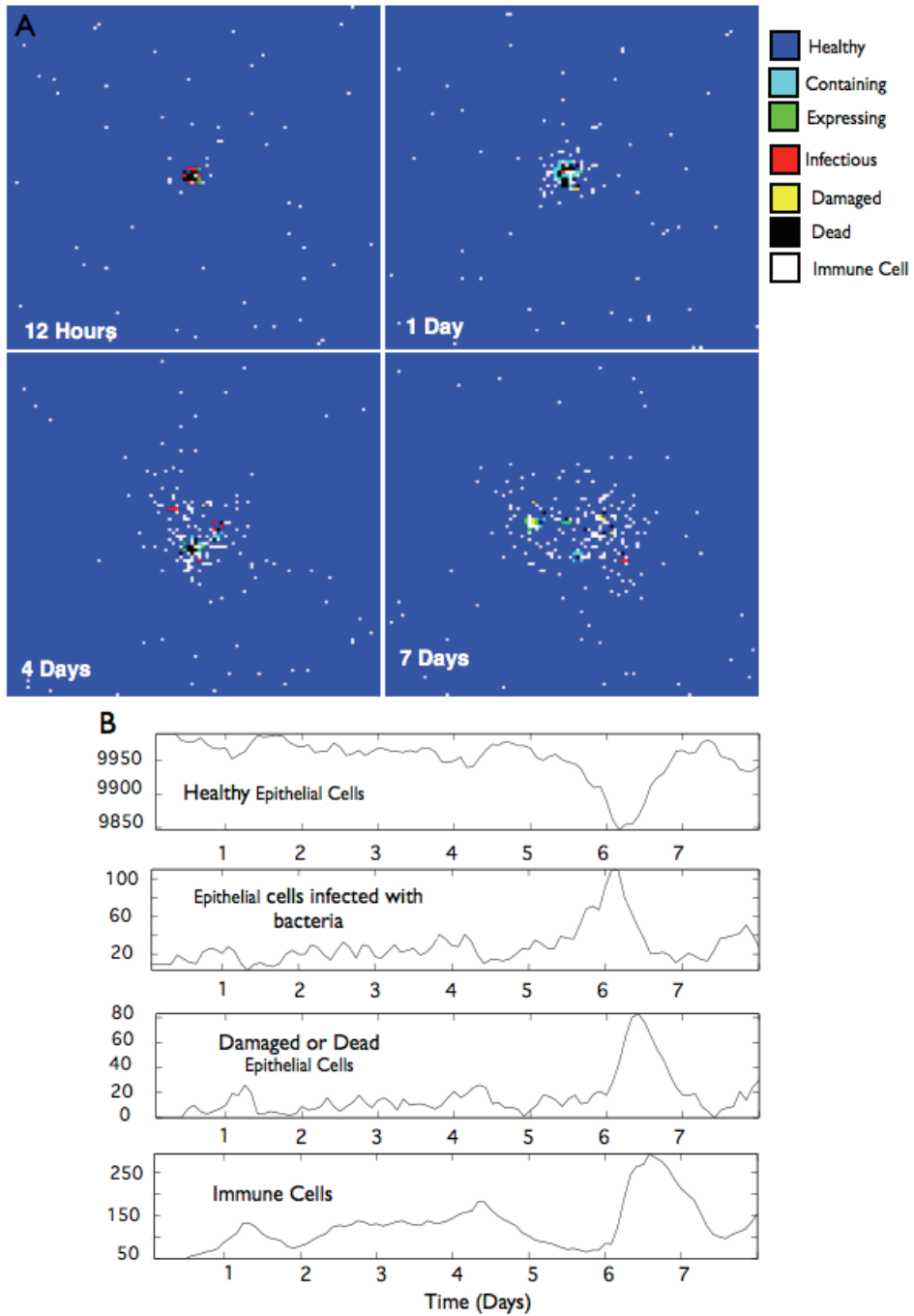
Figure 4: Time shots (A) and transients (B) for the infected state. $P_{1I} = 0.5$, infection introduced by setting a 3 by 3 square at the center of lattice to containing cells.
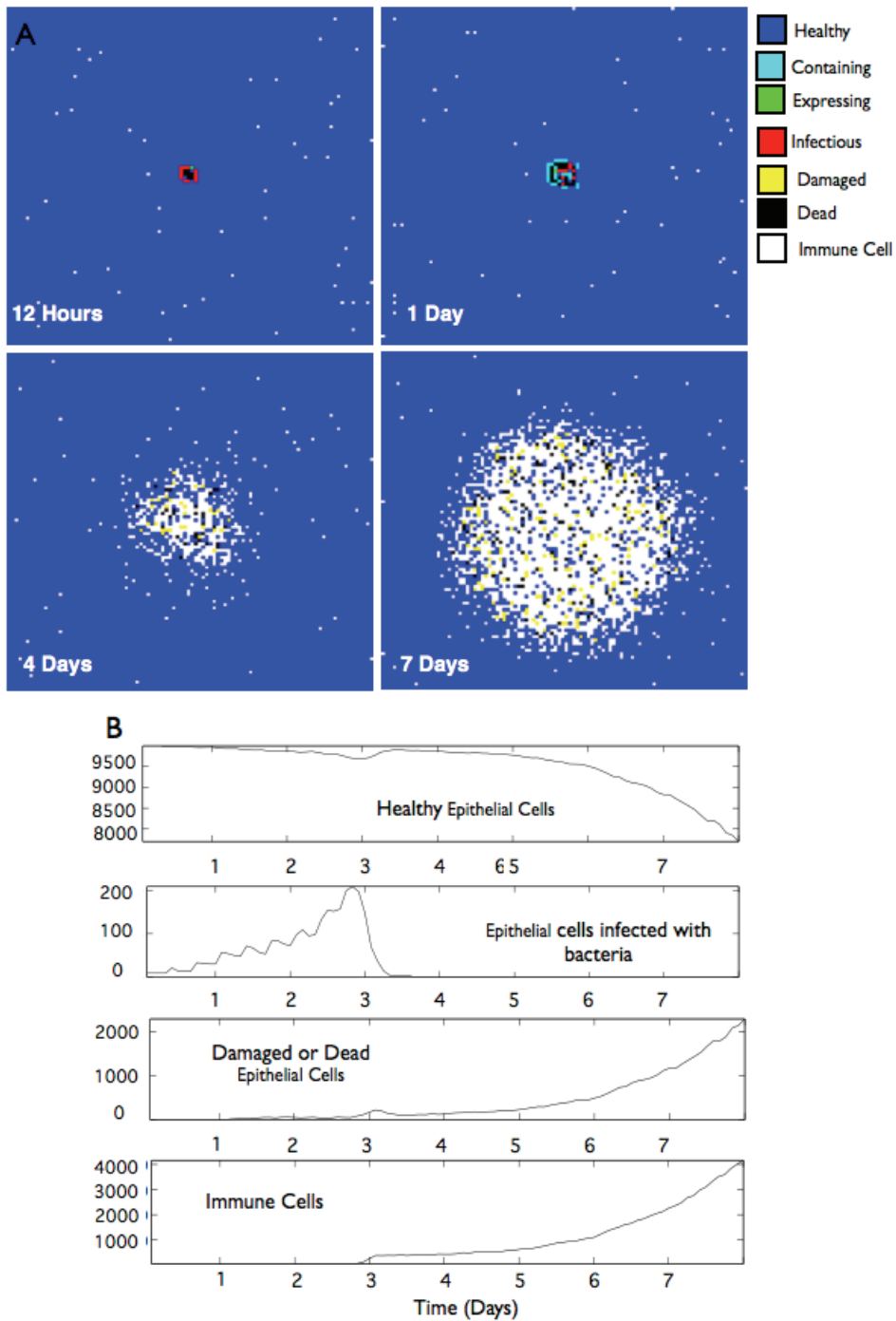
Figure 5: Time shots (A) and transients (B) for the inflamed state. $P_{1I} = 0.5$, infection introduced by setting a 3 by 3 square at the center of lattice to containing cells.

but damage cells are present. The inflammatory response needed to eliminate the infection caused significant levels of tissue damage. This damage is a stimulus to the immune response, which in turn creates more damage. This positive feedback loop leads to spreading inflammation that fills the lattice. In Figure 5 the time shots and transients are plotted for a simulation leading to this state. At day four, we see that the onset of spreading damage has been triggered due to this positive feedback loop and immune cell levels are increasing. These immune cells eliminated the infection by three and a half days.

For most initial immune cell configurations the containing cells are found within the first few time steps given the random movement of the immune cells. However, only in a contrived initial immune cell setup, which forced immune cells to be located a significant distance from the infection would the simulation outcomes be altered. In this scenario the delay in immune cells locating the infection could increase the probability of a persistent infection.

These three outcomes coincide with the three outcomes represented in various ODE models for inflammation, including Reynolds et al. [7]. The Reynolds ODE model has three fixed points representing health, aseptic death and septic death. In the aseptic outcome the infection is resolved, but there is elevated tissue damage and inflammation. This outcome is analogous to the inflamed outcome in the ABM model. The septic death outcome is characterized by persistent infection with high levels of tissue damage and inflammation. Therefore, it is analogous to the infected outcome of the ABM model. In general, the inflammatory responses due to pathogen from the present CA model were similar to those in clinical situations and were qualitatively similar to those from the ODE models of the acute inflammation [4, 6, and 7] and the agent-based model of the acute inflammatory response developed by other researchers [13].

3.2 Sensitivity Analysis

3.2.1 Sensitivity to probability of infection

The ODE model is a deterministic model and the system outcome is by the parameter set and the initial conditions. With the ABM, the same parameters and initial conditions can give rise to different outcomes. Therefore, we run multiple simulations with the same parameters and initial conditions to determine the likelihood of each outcome.

The likelihood for each outcome given a particular parameter set and initial pathogen load was determined by running 800 simulations, all with the same initial conditions with total time eight days. At the end of the eight days an outcome was classified as infected if there was more than ten containing, expressing, or infec-
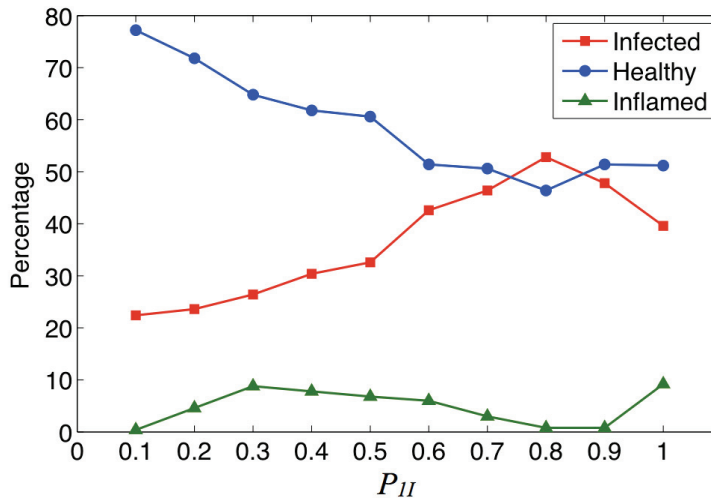
Figure 6: Likelihood of outcomes: P_{1I} ranged between 0.1 and 1. Outcomes were classified as healthy (blue), infected (red), or inflamed (green).

tious epithelial cells. It is classified as inflamed if it was not classified as an infected and there are more than twenty damaged cells. Otherwise the outcome is classified as healthy. Using this classification system with $P_{1I} = 0.5$ and 3 *times* 3 square at the center of lattice of containing cells, 60.6% of the outcome were Healthy, 32.6% infected and the remaining 6.8% were in the inflamed sate. Figure 6 shows the likelihood of outcomes for various P_{1I} with the same initial pathogen load levels. The number of healthy outcomes decreases steadily as P_{1I} increases from 0 to 0.6. Typically, these healthy outcomes are now classified as infected. Above $P_{1I}=0.6$ the likelihood of the healthy outcome remains around 50%. Between $P_{1I}=0.7$ and $P_{1I}=0.8$ the infected outcome continues to increase but instead of the likelihood of healthy outcomes decreasing the inflamed likelihood outcome is decreasing. Above $P_{1I}=0.8$ the likelihood of the infected state decreases and the inflamed outcome is more likely. The quickly expanding infection at these probabilities is more likely to over trigger the immune system leading to the inflamed state.

Using the deterministic ODE model at low pathogen growth rate (analogous to low P_{1I}) the system is bistable between aseptic death and health. The septic death (infected) state does not exist. At low P_{1I} the likelihood of entering the infected is drastically reduced. As the pathogen growth rate is increased in the determin-istic model, septic death comes into existence and is stable. Therefore, with high

pathogen growth the system is tri-stable between all three stats. When we increased significantly P_{1I} the likelihood of a healthy state decreased to about a half and both non-healed states are more likely.

3.2.2   Sensitivity to initial pathogen load

As in the deterministic model the initial pathogen load plays a role in determining the outcome. In order to observe the model outcome dependence on initial pathogen load in this ABM model several simulation were conducted and qualitatively compared to the deterministic model [7]. To increase the number of pathogen containing cell, additional evenly-spaced 3×3 clusters were added to the model lattice with P_{1I}=0.5. As the number of initial containing cells is increased the infected outcome becomes more probable, see Table 2. The likelihood of the healthy state is decreased while the tissue damage state remains low.

Table 2: Increasing the number of containing clusters on the likelihood of model outcomes

Number of Initial Clusters	Healthy	Infected	Inflamed
1	60.6%	32.6%	6.8%
2	30.2%	61.2%	8.6%
4	9.8%	76.2%	14%

In the deterministic model there are two strict thresholds for the initial pathogen load, which determine whether the outcome is health, aseptic death and septic death. Low pathogen results in health, moderate pathogen loads lead to aseptic death, while those above the second threshold result in septic death. Given the stochasticity in the present CA model all outcomes can be reached for all initial pathogen level. The CA outcomes trends are similar to the ODE model. High initial pathogen load typically results in an infected outcome, see Table 2.

Even though the developed ABM of pulmonary acute inflammation was capable of producing outcomes noted in clinical practice and animals models [14, 15, and 16] and there are some limitations. The model does not account for the myriad of discrete and shared biochemical process, such as cytokine production, negative feedback from the immune response, and the specific interactions between the innate and adaptive immune response involved in the inflammatory process as well as many lung tissue and cell properties. As it stands currently the dynamics are not specific to lung tissue. However, the current model is a good first scaffolding step to build upon for the future additions which include the complexities of the inflammatory process and specific lung properties. The model may also lend itself to

multiscaling through connections with other modeling techniques such as the use of ODE models for gas exchange and fluid-solid domain models which take into account the biomechanics of tissue and airflow. This is an initial study that does not account for tissue and cell properties. This will be addressed in future studies. But the three outcomes of this model reflect qualitatively those seen clinically in lung of injured patients, which is a significant first step in modeling lung inflammation. Further validation of the model is required through quantitative comparisons to additional experimental and clinical data in order to utilize the model for practical clinical applications.

4   Conclusions

A cellular automata (CA) based model was developed to simulate the acute lung inflammation. The rules developed were implemented in the CA model consisting of two species: epithelial cells and immune cells. The rules for the CA model were based on previous experiments. The inflammatory responses due to pathogen exposure were investigated. The inflammatory responses produced by the CA model due to pathogen exposure were similar to those in clinical situations and were qualitatively similar to those from the ODE model of the acute inflammation that exist in the literature. The results from sensitivity analysis and dependence on parameters and initial conditions are similar to those seen in previous deterministic models. This is the initial step in developing an agent based model that models the inflammatory response within the lung due to various stimuli. In the future this model can be extended to include the inflammatory response to mechanical stress and strains modeling acute lung injury during mechanical ventilation. This type of multiscale modeling will result in a much more clinical relevance as it pertains to acute lung injury in the setting of critical illness and injury.

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